U.PORTO
FACULDADE DE DESPORTO
UNIVERSIDADE DO PORTO

# Potential Role of High Voltage Monophasic Pulsed Current on the Healing of Chronic Tendinopathy

**Beshoy Adel Abdalla Girgis**

Porto, 2024

U.PORTO
FACULDADE DE DESPORTO
UNIVERSIDADE DO PORTO

# Potential Role of High Voltage Monophasic Pulsed Current on the Healing of Chronic Tendinopathy

**Beshoy Adel Abdalla Girgis**

Thesis submitted in fulfillment of the requirements for the degree of Doctor in Physiotherapy by the Faculty of Sport of the University of Porto, under the Decree-Law n.° 74/2006 of March 24th.

Supervisor: Professor Doctor José Alberto Ramos Duarte

Porto, 2024

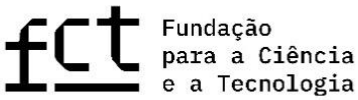
Girgis, B. (2024) *Potential Role of High Voltage Monophasic Pulsed Current on the Healing of Chronic Tendinopathy*. Porto: B. Girgis Dissertação de Doutoramento em Fisioterapia apresentada à Faculdade de Desporto da Universidade do Porto.

**Keywords:** Tendinopathy; tendon injury; electrical stimulation; pressure ulcers; failed healing response.

**Funding sources**

The experimental work of the present scientific thesis was funded by the Portuguese Foundation for Science and Technology, under the project SFRH/BD/144090/2019.

This dissertation was conducted at CIAFEL, Research Center of Physical Activity, Health and Leisure, a Health Science Research Unit housed at the Faculty of Sport, University of Porto, Portugal, supported by the Portuguese Foundation for Science and Technology (UID/DTP/00617/2020).












**Dedication**

This work is dedicated to Edyla Maria Porto Freitas Camelo, Abdalla Girgis Alexander, and Henna Alexander Mina, who passed away during the time period of its conduction. May their souls rest in peace.

**Acknowledgments**

Professor José Alberto Duarte, thank you so much for all the support and kindness throughout these years. You were a role model to me and will always look up to you. Your dedication and guidance were essential to this work, and I am certain that it would have been impossible to do this work without your help. I really cannot thank you enough. Thanks a lot, to Professor Jorge Mota for being forever supportive and for providing the necessary means to carry out this work. Many thanks to Prof. José Oliveira for the continued support and inquisitive discussions which contributed to this work. I would like to thank Dona Celeste Resende a lot for your technical assistance, teaching, and saving the day. You are a very kind hearted person and it has been a pleasure to learn from you.

I would also like to thank Prof. Hélder Fonseca a lot for your care and advice that saved this work repeatedly. A big thank you to Prof. Daniel Moreira-Gonçalves for your kindness, support and advice that were always at the right time. So many thanks to Prof. Paula Alexandra Martins de Oliveira for guidance and support that indeed saved this work. Thank you so much, Prof. Ana Isabel Martins Novais Padrão for your continued support, kindness and cheerfulness. A big thank you to Prof. Manuela E. Gomes for your kindness, support, guidance and welcoming at 3Bs. Many thanks to Dr. Márcia T. Rodrigues for your mentorship and the long brainstorming discussions which greatly contributed to this work. Also, many thanks to Dona Teresa Oliveira for technical guidance and constructive opinions that helped the conduction of this work. I would like to thank all the administrative support team at 3Bs for their help and cooperation; Liliana G. Gomes, Paula Cristina Coutinho Sol, Claudia M.Costa, and Ana Sofia Silva.

Thank you very much, Prof. Rui Corredeira for your help, kindness, and enabling the implementation of this work. Thanks a lot, to Prof. José Ribeiro for your support and teaching all the time. Many thanks to Prof. João Paulo Vilas-Boas for your help and pedagogy. Thank you, a lot, Prof. Maria Paula Santos for your teaching and kindness. Thank you very much, Ms. Maria de Lurdes Domingues for your continued care, support and kindness.

Thanks a lot, to Dona Luzia Oliveira and Dona Júlia Ferreira for your kindness. Many thanks to Sr. Rui Biscaia and Sr. Serafim Pereira for your continued support. So many thanks to Sr. Coimbra Pereira, Sr. Sambade, Sr. Peixoto, and Sr. Rui for your help and support. I would also like to thank all workers at the faculty's restaurant; Amélia, Manuela, Carla Dias, Carla Pereira, Jesus, Jorge, Natália, Daniela, Hélder Costa, Graça.

I would also like to thank Prof. Dr. Jorge Machado and Dra. Rosália Fonte for your huge help, which I would not have been here without. Thank you so much Sr. Daniel Braz, Sr. Fernando Gonçalves, and Prof. Nuno Gaspar, from the Library and Faculty of Psychology, for your immense kindness and help to have a great environment in which a large part of this work was conducted. Many thanks to Paula Santos Cunha, David Davies, Freddy David Davies, and Dona Maria Augusta for your support and kindness during this time.

Moreover, I would like to thank my friends and colleagues whom have helped tremendously in performing this work. My friends, Manuel Ephraim, Mário Esteves, Giorjines Boppre, Florêncio Diniz-Sousa, Toni Bovolini, Lucas Veras, Ana Catarina Resende Coelho, Beshoy Ghaly, Beshoy Akmal, Kerolos George, Mina Ibrahim, Maurílio T. Dutra, Leonardo Yung Maciel, Suiane Lima Cavalcante, Andrea Bezerra, André Melo, Maluseu Tapaeko, William Young, Laura Freitas, Sara Nobre Maia, Grace Fernanda, Nilton Rolim Filho, Patrícia Santos, Ana Filipa Reis Mendes, Albina Franco, Rita Quinteira, Daniela Cruz-Moreira, Ana Filipa Martins de Almeida, João Paredes, Rúben Domingues, Fatine Hamza, Sara Ponte, Qiaoqiao Chen, Teresa Inácio and the Fantastic Seven; Marta Pereira, Marta Correia, Maria Emília Assunção, Paula Pinho, Susana Martins, Diana Miranda, and Iolanda Giordano. Your support was absolutely essential to this work and please accept my apologies for not giving back as much as I took.

**Table of Contents**

**Abstract** ..... i

**Resumo** ..... iii

**Summary of findings** ..... vi

**List of abbreviations** ..... ix

**Chapter I** ..... 1

***General introduction*** ..... *1*

*Tendon injury: terminology* ..... *3*

*Tendinopathy* ..... *3*

*Risk factors* ..... *4*

*Pathogenesis theories and their historical progress* ..... *6*

*Pathophysiology* ..... *11*

***Rationale*** ..... *12*

*Mechanosensing* ..... *12*

*Mechanosensitive and voltage-gated calcium channels* ..... *15*

*High-voltage monophasic pulsed current (HVMPC)* ..... *16*

*Adverse reactions* ..... *22*

*Precautions* ..... *23*

***Objectives*** ..... *25*

**Dissertation articles** ..... 49

**Chapter II** ..... 51

***State of the art*** ..... *51*

**Chapter III** ..... 180

***Original manuscripts*** ..... *180*

**Chapter IV** ..... 537

***General discussion*** ..... *537*

**Chapter V** ..... 542

***Main conclusions*** ..... *542*

***Appendix*** ..... 551

**Abstract**

Tendinopathy is a common clinical condition characterized by pain and dysfunction which may become chronic or recurrent. An umbrella review of systematic reviews, that were published in the last decade, showed that current physical therapy interventions for tendinopathy may be supported by insufficient evidence. The role of high-voltage monophasic pulsed current (HVMPC) as a potential treatment for tendinopathy was, consequently, thought to be evaluated.

Dermal ulcers, as possible examples of failed healing response and possibly the most common application of HVMPC, were chosen to determine and quantify the effects of HVMPC. Level I, II and IV evidence studies indicated that HVMPC plus standard wound care (SWC) were more effective than SWC alone or SWC plus sham HVMPC in treating stage II-IV pressure ulcers (PrUs). HVMPC plus SWC were found to reduce wound surface area by 12.39% per week; 95% CI, [10.43,14.37] compared with 6.96% per week; 95% CI, [5.56, 8.38] for SWC alone or SWC plus sham HVMPC. Moreover, HVMPC plus SWC likely increased the probability of complete wound healing of PrUs compared with sham/no HVMPC plus SWC; relative risk (RR) 2.08; 95% CI: [1.42, 3.04], p = 0.0002; $I^2$ = 0%, p = 0.61; eight studies, 358 ulcers.

In order to investigate whether HVMPC effects on PrUs could be transferred to chronic tendinopathy, an animal model of overuse-induced tendon injury was developed, to which the influence of HVMPC intervention was examined. Reproducible injury of Achilles tendons, on cellular and genetic levels, was sustained for at least 15 and 30 days following the end of protocol, respectively, and up to 60 days with administration of dexmedetomidine injections. The results might provide an insight into the changes that may delineate overuse-induced tendon injury, and possibly precede tendinopathy.

HVMPC intervention might have augmented insertional ground-substance content compared with sham HVMPC; at 30-day timepoint; difference between medians= -0.8300; Hodges-Lehmann= -0.8200; Sham HVMPC= 0.7600, n= 43; HVMPC= 1.590, n= 44; U= 433.5; P= <0.0001, and at 45-day timepoint; difference between medians= 0.5300; Hodges-Lehmann= 0.6600; Sham HVMPC= 1.520, n= 55; HVMPC= 2050, n= 57; U= 1220; P= 0.0430. Nonetheless, a non-statistically significant difference was

found at the 60-day timepoint post overuse tendon injury; difference between medians= -0.1700; Hodges-Lehmann= -0.4300; Sham HVMPC= 1.630, n= 59; HVMPC= 1.800, n= 59; U= 1440; P= 0.1057. In addition, HVMPC intervention might have increased calcification compared with sham HVMPC at both the 30-day and the 45-day timepoints. These results collectively suggest that HVMPC might have a potential beneficial role in treating chronic tendinopathy.

Keywords: Tendinopathy; tendon injury; electrical stimulation; pressure ulcers; failed healing response.

**Resumo**

A tendinopatia é uma condição clínica comum caracterizada por dor e disfunção que pode se tornar crônica ou recorrente. Uma revisão abrangente de revisões sistemáticas, publicada na última década, mostrou que os intervenções fisioterapêuticas atuais para tendinopatia podem ser apoiadas por evidências insuficientes. O papel da corrente pulsada monofásica de alta voltagem (HVMPC) como um tratamento potencial para tendinopatia foi, consequentemente, pensado para ser avaliado.

Úlceras dérmicas, como possíveis exemplos de falha na resposta de cura e possivelmente a aplicação mais comum do HVMPC, foram escolhidas para determinar e quantificar os efeitos do HVMPC. Estudos de evidências de nível I, II e IV indicaram que o HVMPC mais o tratamento padrão de feridas (SWC) foram mais eficazes do que o SWC sozinho ou o SWC mais o HVMPC simulado no tratamento de úlceras por pressão (PrUs) em estágio II-IV. Descobriu-se que HVMPC mais SWC reduziram a área de superfície da ferida em 12,39% por semana; IC 95%, [10,43, 14,37] comparado com 6,96% por semana; IC de 95%, [5,56, 8,38] para SWC isoladamente ou SWC mais HVMPC simulada. Além disso, HVMPC mais SWC provavelmente aumentou a probabilidade de cicatrização completa de feridas de PrUs em comparação com HVMPC simulada/sem HVMPC mais SWC; risco relativo (RR) 2,08; IC 95%: [1,42, 3,04], p = 0,0002; $I^2$ = 0%, p = 0,61; oito estudos, 358 úlceras.

A fim de investigar se os efeitos da HVMPC nas PrUs poderiam ser transferidos para a tendinopatia crônica, foi desenvolvido um modelo animal de lesão do tendão induzida por uso excessivo, para o qual foi examinada a influência da intervenção da HVMPC. A lesão reprodutível dos tendões de Aquiles, nos níveis celular e genético, foi sustentada por pelo menos 15 e 30 dias após o final do protocolo, respectivamente, e até 60 dias com a administração de injeções de dexmedetomidina. Os resultados podem fornecer uma visão sobre as alterações que podem delinear a lesão do tendão induzida pelo uso excessivo e possivelmente preceder a tendinopatia.

A intervenção HVMPC pode ter aumentado o conteúdo de inserção da substância fundamental em comparação com o HVMPC simulado; no período de 30 dias;

diferença entre medianas= -0,8300; Hodges-Lehmann= -0,8200; Sham HVMPC= 0,7600, n= 43; HVMPC= 1.590, n= 44; U= 433,5; P= <0,0001, e no período de 45 dias; diferença entre medianas= 0,5300; Hodges-Lehmann= 0,6600; Sham HVMPC= 1,520, n= 55; HVMPC= 2.050, n= 57; U= 1220; P = 0,0430. No entanto, uma diferença não estatisticamente significativa foi encontrada no período de 60 dias após a lesão do tendão por uso excessivo; diferença entre medianas= -0,1700; Hodges-Lehmann= -0,4300; Sham HVMPC= 1,630, n= 59; HVMPC= 1.800, n= 59; U= 1440; P = 0,1057. Além disso, a intervenção HVMPC pode ter aumentado a calcificação em comparação com a HVMPC simulada nos momentos de 30 e 45 dias. Estes resultados sugerem coletivamente que o HVMPC pode ter um papel potencialmente benéfico no tratamento da tendinopatia crônica.

Palavras-chave: Tendinopatia; lesão no tendão; estimulação elétrica; úlceras de pressão; resposta de cura falhada.

**Summary of findings**

- A literature review was conducted to identify whether common pathological features and findings characterize pressure ulcers and tendinopathies, particularly that may lead to a failed healing response, which provided the basis for the rationale. Several factors were found to be influential, namely; mechanical overload, aging, and sustained inflammatory state.

- A systematic review and meta-analysis was performed to evaluate and quantify the efficacy and safety of high-voltage monophasic pulsed current (HVMPC) in treating stage II-IV pressure ulcers. The study concluded that existing clinical studies, with evidence levels I, II and IV, consistently indicated the efficacy of this intervention in enhancing the probability of complete healing, and reducing worsened healing probability. Rare adverse reactions were reported.

- A systematic review and meta-analysis was done to examine the effect of HVMPC on diabetic ulcers and their potential pathophysiologic factors, and confirm its safety. Conclusive evidence regarding the effect of HVMPC on diabetic ulcers was not found, however, direct evidence, with moderate certainty, may support its efficacy in treating pressure ulcers. Few adverse reactions were reported.

- An umbrella review of systematic reviews and meta-analyses was implemented to summarize evidence in the last decade regarding the efficacy of physical therapy interventions to treat tendinopathy, as a single disease entity. The study found moderate-quality evidence that may support low-level laser therapy, extra-corporeal shockwave therapy, and exercise therapy.

- A systematic review was accomplished to determine the effectiveness of physical therapy interventions in treating chronic lateral elbow tendinopathy (LET) as assessed using pain-free grip strength and/or patient-rated tennis elbow evaluation questionnaire as outcome measures. It was concluded that many of the current physical therapy interventions for chronic LET were supported by insufficient evidence.

- An animal model of tendinopathy was developed in rats through inducing overuse tendon injury based on a treadmill running protocol. The results may suggest a reproducible cellular injury of Achilles tendons was sustained for at least 15 days following the end of protocol and up to 60 days with administration of dexmedetomidine injections. Reduction in cellular function and/or number, due to senescence and/or apoptosis, might explain reduced ground substance synthesis, particularly of carboxylated and sulfated glycosaminoglycans. These changes might represent precursory alterations that may precede tendinopathy and might correlate with the “lag phase” of tensile strength recovery. Moreover, the “rebound phenomenon”, which might correlate with reactive hyperemia, was not observed throughout the entire study period; this might suggest that complete recovery did not occur. Calcification was reproducibly induced by overuse tendon injury, and its resorption may occur spontaneously with rest.

- An animal study to assess the role of HVMPC in treating overuse tendon injuries. HVMPC might improve cell activity and function, and might enhance calcification under certain conditions. These responses, moreover, occurred despite slower healing, possibly due to restraint and/or injections, which might correlate with lower body weight and dietary intake.

## List of abbreviations

| | |
|---|---|
| mGLUT | Metabotropic glutamate receptor |
| PGP 9.5 | Protein Gene Product 9.5 |
| NPY | Neuropeptide Y |
| JNK | c-Jun N-terminal kinases |
| MAPKs | Mitogen-activated protein kinases |
| RTKs | Receptor tyrosine kinases |
| IP3 | Inositol trisphosphate |
| IGF-I | Insulin-like Growth Factor-I |
| TRP | Transient Receptor Potential |
| TM | Transmembrane |
| $K_v$ | Voltage-gated potassium channels |
| $K_{ca}$ | Calcium-activated potassium channels |
| $K_{ir}$ | 2 TM domain inwardly rectifying channels |
| $K_{2P}$ | 4 TM domain ‘‘two pore domain’’ channels |
| HLA-E | Human Leukocyte Antigen- alpha chain E |
| NF-κB | Nuclear Factor kappa B |
| IFNγ | Interferon gamma |
| PTGES1 | Prostaglandin E Synthase |
| TXB2 | Thromboxane B2 |
| COX-2 | Cyclooxygenase-2 |
| PGE2 | Prostaglandin E2 |
| TSPCs | Tendon Stem/Progenitor Cells |
| SASP | Senescence-associated secretory phenotype |
| SA-β-Gal | Senescence-associated beta-galactosidase |
| VEGF | Vascular endothelial growth factor |
| bFGF | Basic Fibroblast Growth Factor |
| ADAMTs | A Disintegrin and Metalloproteinase with Thrombospondin motifs |
| TIMPs | Tissue inhibitor of metalloproteinases |

| | |
|---|---|
| MMP | Matrix metalloproteinase |
| Col1 | Collagen type I |
| Col3 | Collagen type III |
| TNMD | Tenomodulin |
| DCN | Decorin |
| SCX | Scleraxis |
| MKX | Mohawk |
| MPa | Megapascal |
| LET | Lateral elbow tendinopathy |
| HIF-1α | Hypoxia-inducible factor-1 alpha |
| PIEZO1 | Piezo Type Mechanosensitive Ion Channel Component 1 |
| HVMPC | High-voltage monophasic pulsed current |
| IP | Intra-peritoneal |
| IM | Intra-muscular |
| V | Volts |
| HZ | Hertz |
| µs | Microseconds |
| cm | Centimeter |
| m | Meter |
| mg | Milligram |
| kg | Kilogram |
| pH | Potential of hydrogen |
| IL | Interleukin |
| TNF-α | Tumor Necrosis Factor-alpha |
| mRNA | Messenger Ribonucleic acid |
| RNA | Ribonucleic acid |
| DNA | Deoxyribonucleic acid |
| cDNA | Complementary Deoxyribonucleic acid |
| qPCR | Quantitative Polymerase Chain Reaction |
| TGF-β | Transforming Growth Factor-beta |

# Chapter I

## *General introduction*

## *General introduction*

### *Tendon injury: terminology*

The terms to describe tendon injury have evolved over the years. Historically, “tendinitis” was the most widely used term to refer to tendon pain, thickening, and dysfunction (Rees et al., 2014). As early as 1917, the term tendinitis was mainly used to describe tendon inflammation (Brickner, 1917; Rees et al., 2014). It usually referred to a clinical syndrome rather than a specific histopathologic entity, since it was used in a clinical context (Maffulli, 1998). It was, however, regarded as a misnomer in the 1970s due to the absence of classic inflammatory cells in the histopathological findings (Khan et al., 2002; Maffulli, 1998; Rees et al., 2014). The term “tendinosis” was therefore introduced to describe degenerative tendon changes with the absence of inflammation (Puddu et al., 1976). Tendinosis was recognized as a distinct entity and not a mere long-term consequence of short-term tendinitis. Nonetheless, both terms were eventually abandoned in favor of “tendinopathy”, as the most appropriate term (Maffulli, 1998; Scott et al., 2020). This term “tendinopathy” has been supported by Maffulli et al. (Maffulli, 1998) as an umbrella descriptor of clinical conditions in and around tendons due to overuse. Tendinopathy became the preferred term of choice, since tendinosis may be a misleading term as it does not refer to a clinical diagnosis and may oversimplify the pathological process involved in this condition (Khan et al., 2002; Maffulli, 1998; Rees et al., 2014).

### *Tendinopathy*

Tendinopathy is a clinical syndrome characterized by persistent tendon pain and loss of function related to mechanical loading (Maffulli, 1998; Scott et al., 2020). This definition, however, does not implicit the existence of a specific pathological or biochemical process which cannot routinely be assessed clinically (Scott et al., 2020). Paratendinopathy of the Achilles tendon may describe pain related to activity in addition to tenderness and swelling around the tendon but with no clinical or imaging evidence of tendinous involvement (Maffulli et al., 2003; Paavola & Järvinen, 2005; Stecco et al., 2014). Crepitation, local areas of hyperemia, and tendon defects or nodules may be found at clinical examination (Paavola & Järvinen, 2005; Van Dijk et al., 2011). The classification of tendinopathy based on chronicity does not follow specific time criteria (Kaux et al., 2011; Paavola et al., 2002). It has been suggested

that if symptoms are present for less than two weeks, it is considered acute tendinopathy (El Hawary et al., 1997), while persistent symptoms for more than 6 weeks may be categorized as chronic (Childress & Beutler, 2013; Clancy, 1983; El Hawary et al., 1997; Fredberg & Stengaard-Pedersen, 2008; Woodley et al., 2007). Nonetheless, others have defined chronic tendinopathy as having symptoms for more than 3 months (Fredberg & Stengaard-Pedersen, 2008; Woodley et al., 2007).

*Risk factors*

It may not be easy to separate the effect of each risk factor from the others, therefore, several factors may predispose to tendinopathy development. A principal risk factor for tendinopathy is aging, which affects the middle-aged population (between 41-60 years) in particular (Clement et al., 1984; de Jonge et al., 2011; Leong et al., 2019; Tuite et al., 1997). Overuse is a common risk factor for tendinopathy, as affected tendons often endure mechanical stress of high magnitude (de Jonge et al., 2011; Heir & Glomsaker, 1996; Neviaser et al., 2012).Tendon degeneration was found to have an association with metabolic-hormonal imbalances (Oliva et al., 2016). A considerably higher risk of tendinopathy was reported in patients with hypercholesterolemia and diabetes mellitus (Abate et al., 2013; Abboud & Kim, 2010; Ranger et al., 2016; Taylor et al., 2017; Tilley et al., 2015). Diabetic patients, moreover, may have an increase of 27% in the risk of rotator cuff calcific tendinopathy (Su et al., 2021). The risk of tendinopathy, tendon tear, and tendon rupture, and complications following tendon surgery may increase with obesity (Franceschi et al., 2014; Gaida et al., 2009; Gaida et al., 2008; Macchi et al., 2020; Owens et al., 2013). Heredity may increase the risk for the development of tendinopathy by fivefold (Gwilym et al., 2009; Harvie et al., 2004; Kraemer et al., 2012; Tashjian et al., 2009).

Drugs and substances may constitute other risk factors for tendinopathy. A higher risk of tendinopathy and tendon tears may be related to alcohol consumption (Owens et al., 2013; Passaretti et al., 2016; Van Der Vlist et al., 2019). Smoking was reported to reduce healing ability and clinical outcomes after tendon repair, and may predispose to rotator cuff tearing (Baumgarten et al., 2010; Carbone et al., 2012; Mallon et al., 2004; Zabrzyński et al., 2021). Fluoroquinolones, long-term corticosteroid use, statins, and aromatase inhibitors may contribute to drug-induced tendinopathy (Alves et al.,

2019; Barge-Caballero et al., 2008; Bidell & Lodise, 2016; Blanco et al., 2005; Eliasson et al., 2019; Kim, 2010; Kirchgesner et al., 2014; Tsai & Yang, 2011). Co-administration of corticosteroids and fluoroquinolones seems to increase the risk of tendinopathy (Alves et al., 2019; Wise et al., 2012). The risk of fluoroquinolones-induced tendinopathy was observed to be significantly higher in patients with renal failure, long-term hemodialysis, end-stage renal disease, and renal transplant (DONCK et al., 1994; Fleisch et al., 2000; Harrell, 1999; Marti et al., 1998; Zabraniecki et al., 1996). Patients with rheumatoid arthritis may present with tendinopathy (McQueen et al., 2005; Shen et al., 2019; Stiskal et al., 1997).

*Pathogenesis theories and their historical progress*

Many hypotheses and theories were developed in attempt to explain the pathogenesis of tendinopathy. Nonetheless, most of these theories agreed that overuse is an important factor associated with the development of tendinopathy. A combination of these factors likely contribute to tendinopathy, as suggested by the failed healing theory (Fu et al., 2010). The theories are overlapping in many aspects, and some are revived or modified from others. Each of these theories focus primarily on the main or initiating event leading to tendinopathy. Nonetheless, many of these theories agree that overuse is an important factor associated with the development of tendinopathy.

*a. Extrinsic*

In 1921, A. W. Meyer proposed that *excessive friction* due to overuse, but with no indication of inflammation, may result in tissue attrition and destruction (MEYER, 1921, 1924). Neer, in 1972, described a characteristic ridge of proliferative spurs on the acromion process, and suggested that damage may occur due to tendon *impingement* by surrounding structures (CHARLES S NEER, 1972).

*b. Intrinsic*

*1. Mechanical theory*

*1.1. Overuse/overload injury*

A vicious cycle of *muscle overload injury* was described by W. B. Kibler in 1990, which identified tissue overload as a principal factor contributing to tissue injury (Kibler, 1990). Archambault et al. in 1995 proposed a relationship between tendon loading, cellular response, and overuse injury; *a loading episode when the tendon is weakened* might lead to tissue injury (Archambault et al., 1995). Arnoczky et al., in 2007, modified Archambault et al.'s theory, and suggested that degenerative changes in tendon-matrix composition and organization may be induced by *repeated strain below the injury threshold* (Arnoczky et al., 2007). Extracellular matrix degeneration may result in temporary weakness of the tissue which may lead to damage if loading is continued (Arnoczky et al., 2007).

The continuum model of tendinopathy, developed by Cook and Purdam in 2009, described the pathology of tendinopathy as *continuum of three stages*; reactive

tendinopathy, tendon dysrepair (failed healing) and degenerative tendinopathy (Cook & Purdam, 2009). According to the continuum model, reactive tendinopathy may occur following acute overload or trauma*;* moreover, *loading is the primary stimulus* that determines the progression or regression of pathology (Cook & Purdam, 2009). The iceberg theory, developed by Abate et al. in 2009, entailed that *pain represents the tip* while the base of the iceberg represents the underlying physiological conditions (Abate et al., 2009). This theory, moreover, defined overuse, localized hyperthermia, and hypoxia as possible factors leading to tendon injury (Abate et al., 2009). In 2016, Cook et al. revised the continuum model, integrating a relationship between tendon structural changes, pain, and dysfunction into the continuum (Cook et al., 2016). Pain may present following acute overload (reactive) or reactive-on-late dysrepair/degenerative tendon pathology (Cook et al., 2016). The revisited model also introduced the concept of “reactive-on-degenerative tendinopathy”, as a hybrid of reactive and degenerative pathologies (Cook et al., 2016).

### *1.2. Degeneration theory*

Several other hypotheses including an *underlying degenerative process* within the tendon leading to tendon rupture were suggested by Codman and Akerson in 1931 (Codman & Akerson, 1931). In 1976, Puddu et al. stated that degenerative lesions of tendons, with or without changes in the peritenon, may precede tendon rupture (Puddu et al., 1976). H. C. Burry proposed in 1978 that *tendon degenerative changes* may be the result of reduced blood flow or over-use (Burry, 1978). However, in 1996, T. E. Cawston stated that *tendon degeneration* may be the result of a common disease mechanism*;* overuse, microtrauma, and hypoxia may lead to alteration in matrix synthesis with deposition of glycosaminoglycan in addition to loss of part of collagen fibrillar network (Cawston et al., 1996).

### *1.3. Inflammation theory*

*The inflammatory model* implying that an inflammatory process in tendons results in pathological changes was widely accepted prior to the 1990s (Rees et al., 2014). This model, however, was later doubted, since acute inflammatory cells were not found in load-bearing regions of tendons (Khan et al., 1999). Fredberg and Stengaard-Pedersen in 2008, suggested that development of tendinopathy and its chronicity may

involve an inflammatory process, although without providing a definitive proof (Fredberg & Stengaard-Pedersen, 2008). Afterwards, it was proposed that both inflammation and degeneration may coexist simultaneously in tendinopathy and may not be mutually exclusive (Abate et al., 2009). In 2011, however, Del Buono et al. proposed that following an acute tendon injury, prolonged systemic, low-grade inflammation and impaired insulin sensitivity may predispose to chronic overuse tendinopathy (Del Buono et al., 2011).

Rees et al. (Rees et al., 2014), subsequently, supported the role of inflammation in tendinopathy, since the evidence for degenerative processes alone was considered insufficient. Then, Dean et al. (Dean et al., 2017; Dean et al., 2015) suggested that the increased number of macrophages and mast cells in tendinopathic tendons compared with healthy controls may warrant further investigation. Furthermore, an inflammatory component to tendinopathy was supported by Mosca et al. (Mosca et al., 2018), based on reviews with more robust definitions of inflammation. In 2018, Tang et al. (Tang et al., 2018) regarded the description of tendinopathy to be a purely degenerative disease an oversimplification, and assumed that inflammation and degeneration may be simultaneously present in tendinopathy. The theory further elaborated that with overuse, microdamage to tendon fibers may occur, which may lead to the secretion of several substances including inflammatory factors (Tang et al., 2018).

*2. Apoptosis theory*

Yuan et al., in 2002, suggested a link between oxidative stress and repetitive loading, which may lead to activation of metalloproteinases and development of degenerative lesions (Yuan et al., 2003; Yuan et al., 2002). Mechanical impingement, moreover, might cause tendon degeneration and tenocyte apoptosis (Tuoheti et al., 2005). Degenerative tendons showed evidence that may suggest apoptosis of fibroblasts or fibroblast-like cells. Reduced number of functional fibroblasts may be a contributing factor to collagen metabolism impairment found in tendon degeneration (Yuan et al., 2002). Ischemia, hypoxia, free radical generation, and nutritional imbalances were proposed as possible factors leading to apoptosis in tendon degeneration, although the exact cause was unidentified (Yuan et al., 2002). Two pathways associated with oxidative stress might be activated for the induction of apoptosis in tendinopathy.

These pathways are c-Jun N-terminal kinase (JNK) and increased cytochrome c-related activation of caspase-3 (Xu & Murrell, 2008).

*3. Vascular theory*

Tendons were suggested to have poor blood supply, with regions of hypovascularity, which may predispose them to injury. Physiologic examinations, nevertheless, may suggest that blood flow is homogenous along the entire Achilles tendon (Rees et al., 2006; Rees et al., 2009). Fibrocartilaginous entheses might be particularly vulnerable to vascular insufficiency, since they are relatively avascular (Rees et al., 2009). However, augmented tendon blood flow and neovascularization were found in Doppler flow examinations and biopsies from patients with degenerative tendinopathy (Merkel et al., 2021; Pufe et al., 2005). It was concluded, nonetheless, that vascularization may indeed affect tensile strength but might not be the only or most influential factor (Theobald et al., 2005). Hypoxia-induced neovessels might be hyperpermeable and might not provide sufficient oxygen and nutrients to tissues. Stabilization of these neovessels might be an effective treatment of tendinopathy (Järvinen, 2020). The role of lymphatic drainage in common tendon disorders might have been ignored in literature. However, it has been found that intact Achilles tendons in rats are void of lymphatic vessels, while they may become present with injury (Tempfer & Traweger, 2015). Neovascularization is a common finding in Achilles tendinopathy, which is usually accompanied by innervation in tendinopathic areas (Tempfer & Traweger, 2015).

*4. Neural theory*

Neurogenic inflammation and increased vascular ingrowth were suggested by Fredberg and Stengaard-Pedersen to mediate adaptive responses to mechanical overload, and tendon weakening and rupture, respectively; collectively designated as the *neurovascular theory* (Fredberg & Stengaard-Pedersen, 2008). Mast cell neurally-mediated degranulation might lead to the release of substance P and calcitonin- gene-related peptide (Rees et al., 2009). Both substance P and calcitonin gene-related peptide might be involved in the initiation of peritendinitis, which might lead to tendon degeneration (Fredberg & Stengaard-Pedersen, 2008). Upregulation of calcitonin gene-related peptide was not found in tendinopathy (Wasker et al., 2023). Additionally,

glutamate, a neurotransmitter, was found within the ultra-dialysate in tendinopathic Achilles tendons (Rees et al., 2006). protein gene product 9.5 (PGP 9.5), N-methyl- D-aspartate Receptors, glutamate, glutamate receptors (mGLUT), neuropeptide Y (NPY) and adrenoreceptors might be upregulated in tendinopathic tendons (Wasker et al., 2023). Central sensitization may explain the lowered pressure pain threshold, both at the site of tendinopathy and at other sites, which were commonly identified in painful tendinopathic tendons at different sites (Plinsinga et al., 2015). Furthermore, extensive ingrowth of sensory nerves, augmented levels of pain mediators, upregulated expression and excitability of pain receptors involved in neuro-immune pathways may be contributing factors to chronic tendon pain (Ackermann et al., 2023). The variability in clinical presentations of morphologically pathologic tendons, nevertheless, may not be fully explained by the neural theory (Rees et al., 2009).

*5. Failed healing response; angiofibroblastic hyperplasia*

In 2010, Fu et al. published a unified theory of tendinopathy pathogenesis; the theory depicted tendinopathy as a three-stage process: injury, failed healing, and clinical presentation (Fu et al., 2010). According to this theory, collagenolytic injuries, without significant pain, may occur in the first stage due to several causes including overuse, trauma, chemicals, and pathogens (Fu et al., 2010). In the failed healing stage, the theory speculated that unfavorable mechanical environment, genetic predisposition, hormonal background, and pharmacological exposures may lead to significant tendon pain and failure to repair the collagenolytic injuries (Fu et al., 2010). Inflammation may be present at the initial stages of repair; however, it may not adequately resolve due to mechanical environment or pharmacological intervention. Increased innervations and vascular in-growth from the paratenon may, moreover, occur during the healing process (Fu et al., 2010). In the clinical presentation stage, spontaneous rupture may occur in asymptomatic patients, whereas, inflammatory pain may be present in symptomatic tendinopathy (Fu et al., 2010).

*Pathophysiology*

The pathophysiology of tendinopathy is multifactorial, however, the exact mechanisms behind its pathology are yet to be understood (Cardoso et al., 2019; Riley, 2004; Xu & Murrell, 2008). The predominant model might suggest that tendon pathology occurs due to a primary response of tenocytes to overload, which leads to tenocyte activation and proliferation, proteoglycans increase, collagen disruption, and neovascularization (Cardoso et al., 2019). Changes in cell activity with age or repetitive strain and microtrauma, and failure to regulate certain matrix metalloproteinase activities as a result of repeated mechanical strain or injury may be implicated in the pathology of tendon injury (Riley, 2004). Tenocytes, before evident tendinopathy, may proliferate, become rounded and apoptotic, and produce more cartilaginous and immature matrix (Xu & Murrell, 2008). Relative stress deprivation may subsequently be induced following disruption of cell-matrix complex leading to metalloproteinase-mediated destruction of the matrix (Xu & Murrell, 2008).

### *Rationale*

*Mechanosensing*

It is well known that tenocytes are able to sense mechanical stimuli (Banes, Tsuzaki, Hu, et al., 1995; Lavagnino & Arnoczky, 2005; Wang, 2006). Electrochemical sensory system(s) may be involved in mechanical load detection through the deformation of the cytoskeleton and/or cell membrane (Adams, 1992; Banes, Tsuzaki, Yamamoto, et al., 1995; Wang et al., 1993; Watson, 1991). Deformation of tendon cells may modify cytoskeletal tension which, through a mechanosensory tensegrity system, and may transmit the signal to the cell nucleus (Arnoczky, Lavagnino, et al., 2002; Ingber, 1997). Many studies have reported alterations in a multitude of expressed genes in response to mechanical stress (Archambault et al., 2002; Arnoczky, Tian, et al., 2002; Banes, Tsuzaki, Hu, et al., 1995; Huisman et al., 2014; Jones et al., 2013; Legerlotz et al., 2013; Maeda et al., 2010; Maeda et al., 2009; Tsuzaki et al., 2003; Wang et al., 2003). Although mechanical stimulation may be required for the maintenance of tendon homeostasis, overstimulation may be involved in the development of tendinopathy (Archambault et al., 2002; Banes, Tsuzaki, Hu, et al., 1995; Tsuzaki et al., 2003; Wang et al., 2003). Excessive stimulation, as well as understimulation, of tenocytes were shown to augment the release of pro-inflammatory cytokines and metalloproteinases (Fu et al., 2002; Lavagnino et al., 2006; Lo et al., 2004; Magra & Maffulli, 2005; Riley et al., 2002).

Several elements and mechanisms may be involved in mechanotransduction of mechanical forces; these may include focal adhesions, the cytoskeleton, the primary cilium, stretching-activated ion channels, changes in second messengers such as intracellular calcium or adenosine triphosphate, integrins, mitogen-activated protein kinases (MAPKs), receptor tyrosine kinases (RTKs), G proteins, Hippo signaling mediated by YAP/TAZ, and extracellular matrix. Focal adhesions may constitute a main mechanism of direct cell-matrix interaction (Stutchbury et al., 2017; Wozniak et al., 2004). The cytoskeleton is proposed to have a role in transduction of mechanical signals (Ingber, 1993; Lavagnino et al., 2015). Changes in cytoskeletal stiffness were reported in response to mechanical stresses to cell surface, which may be mediated by integrins and other transmembrane receptors (Geiger et al., 2001; Ingber, 1991; Katsumi et al., 2004; Wang & Ingber, 1995). Fibroblasts may possess the capacity to

adapt their internal stiffness to match that of their extracellular matrix (Ghosh et al., 2007; Harris et al., 1981; Harris et al., 1980; Solon et al., 2007; Wang, 2000).

Tenocytes can instantly detect and respond to mechanical stimuli by permitting influx of extracellular calcium and releasing intracellular calcium stores (Banes et al., 1994; Banes, Tsuzaki, Hu, et al., 1995; Banes et al., 2003), modifying cytoskeletal filament content and organization, and altering protein expression (A. Banes et al., 1999; Banes et al., 1997; Banes, Tsuzaki, Hu, et al., 1995; Cowan et al., 1998). Moreover, through intercellular signaling pathways, calcium waves may be propagated between interconnected cells via gap junctions (A. J. Banes et al., 1999; Boitano et al., 1992; McNeilly et al., 1996; Sanderson et al., 1994; Waggett et al., 2006), or paracrine signaling mechanisms (Cotrina et al., 1998). Direct passage of calcium ions, inositol (1,4,5)-trisphosphate (IP3), or other signaling molecules through gap junctions may propagate calcium waves and intercellular communication (Wall & Banes, 2005).

Deformation of human tenocytes may lead to an increase in cytosolic calcium and propagation of calcium wave (Kenamond et al., 1997). Transcription factors Scleraxis and Mohawk may stimulate gene expression in response to mechanical stress (Kayama et al., 2016; Maeda et al., 2011). Upregulation of gene expression of collagen I-binding integrins was reported in response to mechanical stimulation of tendon stem/progenitor cells (Popov et al., 2015). An elevated expression of integrins, moreover, was shown to occur as a result of mesenchymal stem cells mechanical stimulation (Subramony et al., 2013).

Levels of TGF-β1 might change in response to mechanical loading, which may suggest a possible role in collagen type-I synthesis (Dideriksen et al., 2013; Fu et al., 2005; Heinemeier et al., 2003; Jiang et al., 2012; Yang et al., 2004). Human tenocytes showed improved cell viability when exposed to TGF-β1 that was blocked with the exposure to TGFβRI/II kinase inhibitor (Fong et al., 2017). Interleukin-6 might be involved in collagen synthesis; an increase of about 5-folds immediately following to 1h treadmill running exercise that may return to basal values within 5h was reported (Andersen et al., 2011). Local administration of insulin-like growth factor-I (IGF-I) might stimulate tendon collagen synthesis (Hansen et al., 2013; Herchenhan et al., 2015).

Nonetheless, no change in IGF-I was detected locally or systemically in response to exercise (Olesen et al., 2007). An elevation of insulin-like growth factor binding protein-1 was found after prolonged acute exercise (Olesen et al., 2007). Prostaglandin E2 (PGE2) might reduce proliferation of human tenocytes and their collagen production (Cilli et al., 2004).

*Mechanosensitive and voltage-gated calcium channels*

Ion channels may be classified to voltage-dependent, ligand-dependent, or mechanosensitive channels (Catterall, 1995; Ghazi et al., 1998). Sodium, chloride, potassium, and calcium channels may have a major role in the mechanical signal transduction (Banes et al., 2001). The potassium, sodium and calcium channels might have similar structure-function relationship, so that they contain conserved transmembrane segments that act as the major voltage sensor (Ertel et al., 2000; Perez-Reyes & Schneider, 1994). Many types of calcium channels were identified and characterized, these may include; T-type (low-voltage activated), L-type (high-voltage activated), N-type, P/Q-type, and R-type (Catterall, 2011; Lipscombe et al., 2004; Perez-Reyes, 2003; Perez-Reyes & Schneider, 1995; Perez-Reyes & Schneider, 1994). Voltage-gated calcium channels may be responsible for initiation of many physiological events through transduction of changes in membrane potential into intracellular calcium transients (Catterall, 2011).

Mechanically activated ion channels may include; DEG/ENaC/ASIC sodium ion channels; transient receptor potential (TRP) polymodal cation channels; mechanosensitive potassium channels: 6/7 transmembrane (TM) domain voltage-gated ($K_v$) and calcium-activated ($K_{ca}$) potassium channels, 2 TM domain inwardly rectifying channels ($K_{ir}$) and 4 TM domain ‘‘two pore domain’’ channels ($K_{2P}$); and PIEZO ion channels (Coste et al., 2010; Ranade et al., 2015). Stretch-activated cation channels that are permeable to potassium and sodium were found in human dermal fibroblasts (Stockbridge & French, 1988). Moreover, cyclic mechanical stretch was reported to induce PIEZO1-mediated calcium influx in human dermal fibroblasts, which promoted proliferation, motility and differentiation (He et al., 2021). Both TRPV4 and PIEZO1 channels may contribute to normal mechanoelectrical transduction, nonetheless, only PIEZO1 channels seem to mediate stretch-activated currents in murine bone marrow stromal cells and chondrocytes (O ‘Neil & Heller, 2005; Rocio Servin-Vences et al., 2017). PIEZO1 channels, furthermore, were reported to be required for stretch-induced influx of calcium in human and murine tenocytes (Nakamichi et al., 2022; Passini et al., 2021). In addition to mechanical stimuli, PIEZO1 channels may be modulated or even gated by voltage (Moroni et al., 2018).

*High-voltage monophasic pulsed current (HVMPC)*

The exact mechanisms governing the interaction between cells and electrical stimulation are yet to be elucidated. Calcium influx was determined to occur following HVMPC stimulation of human dermal fibroblasts, within the first minute, and reaches maximum level after 3 minutes of electrical stimulation (Bourguignon et al., 1989). The parameters of the applied current were 75 V and 100 HZ (Bourguignon et al., 1989). Moreover, increased insulin binding to fibroblasts, due to an increase in insulin receptor number rather than receptor binding affinity, was identified during the second minute of stimulation and reached near maximum level following 3 minutes of exposure (Bourguignon et al., 1989). Further five-fold increase in calcium influx was reported after addition of insulin to HVMPC-treated cells, and this increase was maintained for the subsequent 7 minutes of stimulation (Bourguignon et al., 1989). Similar phenomena were observed following HVMPC stimulation of mouse splenic T-lymphocytes (Bourguignon et al., 1988; Jy et al., 1989).

*Proliferation and migration*

Exposure of human dermal fibroblasts to HVMPC with voltages ranging from 0 to 300 volts, pulse rates of 60-120 HZ, for 20 minutes at room temperature was found to augment DNA synthesis 2 to 24 hours following stimulation (Bourguignon & Bourguignon, 1987). This effect was observed near the cathode and with a maximum increase occurring with 75 V and 100 HZ (Bourguignon & Bourguignon, 1987). The addition of insulin to the HVMPC-treated cells was found to augment DNA synthesis by more than two folds (Bourguignon et al., 1989). Enhanced migration and proliferation of tenocytes were reported at 6 and 7 days, respectively, following cathodal HVMPC stimulation with 50 V and 100 HZ for 30 minutes (Lee et al., 2002). Rat wounds showed a higher dermal fibroblast density following the application of HVMPC stimulation with 50 V and 100 HZ for 30 minutes with alternating polarity (Lee et al., 2003). Accelerated healing and significantly higher number of fibroblasts were reported in response to cathodal HVMPC daily treatment of dermal burn model in pigs for 10 minutes and with 175 V at 60 HZ for 4 weeks (Cruz et al., 1989). The expression of fibroblasts receptors TGF-β1 might have increased in response to HVMPC stimulation (Falanga, 1987). A study stated that keratinocyte proliferation increased following HVMPC stimulation with 30-50 V, 120 HZ and 140 µs for 30 minutes (Eyn-

Young, 2004). Voltages of 300 V or higher were observed to reduce DNA synthesis (Bourguignon & Bourguignon, 1987).

*Collagen synthesis*

Collagen synthesis was found to increase near the cathode at 2 hours post-stimulation with 50 V and 100 HZ for 20 minutes (Bourguignon & Bourguignon, 1987). Another study stated that the addition of insulin to the HVMPC-treated cells, with 75 V and 100 HZ for 3 minutes, increased protein synthesis by more than two folds (Bourguignon et al., 1989). Anodal HVMPC with 170 V and 70 HZ applied for 30 minutes a day for 10 days might have increased collagen synthesis (Kim et al., 1996). Rat skin wounds showed increased collagen deposition in response to HVMPC stimulation with 50 V and 100 HZ for 30 minutes with alternating polarity (Lee et al., 2003). TGF-β1 mRNA expression was reported to increase as a result of HVMPC stimulation of dermal wounds in rats at 6 hours and 7 days post-stimulation (Lee et al., 2010). Another study stated that cathodal-anodal HVMPC enhanced TGF-β1, α-SMA and collagen type I mRNA expression levels in rats, following stimulation with 35-50 V, 100 HZ and 140 μs, for 40 minutes a day, for 7 days (Kim et al., 2014). Higher TGF-β1 and collagen type I expression were found in rat dermal wounds after HVMPC application with visible contraction and alternating polarity with 40 to 85 V for 30 minutes per day for 6 days (Jeon et al., 2015). Moreover, collagen deposition was found to increase at 7 days following cathodal HVMPC stimulation of dermal wounds in rats with 50 V at 100 HZ for 30 minutes (Lee et al., 2010). Full-thickness burn wounds in pigs showed accelerated healing following the application of cathodal HVMPC treatment with 175 V at 60 HZ for 10 minutes daily for 4 weeks (Cruz et al., 1989). Voltages of 300 V or higher were found to inhibit protein synthesis (Bourguignon & Bourguignon, 1987). One study reported that cathodal HVMPC, applied with 30-60 V, pulse width 100 μs and 80 HZ for two hours twice daily for 7 days, might have hampered healing since control group rabbits had better wound closure and tensile-strength (Brown & Gogia, 1987). Six consecutive days of cathodal HVMPC, anodal HVMPC and alternating HVMPC stimulation with sensory threshold and 120 HZ for 30 minutes were ineffective in augmenting collagen synthesis or improving its alignment following Achilles tendon partial rupture in rats (Rampazo et al., 2016).

*Blood flow*

Cathodal HVMPC stimulation with 90 V and pulse rates of 2, 20, 80, 120 HZ for 3 minutes were reported to induce significant increase in rat hindlimb blood flow, with 20 HZ eliciting the greatest increase (T. Mohr et al., 1987). Maximum tolerable voltages, between 115-475 V and pulse frequencies of 2 and 128 HZ, and with negative polarity for 10 minutes resulted in increases blood flow in human calf muscle (Heath & GIBBS, 1992). Negative polarity and higher frequencies of HVMPC stimulation showed the greatest trends to higher blood flow (Hecker et al., 1985). Anodal HVMPC stimulation with pulse rate of 30 HZ and intensities at 10% and 30% of maximal voluntary isometric contraction for plantar flexion did not increase popliteal artery blood flow (Walker et al., 1988). HVMPC stimulation with sensory threshold voltage, 120 HZ, and cathodal, anodal and alternating polarity for 30 minutes were ineffective in improving angiogenesis following Achilles tendon partial rupture in rats (Rampazo et al., 2016). Cutaneous blood flow, microvascular volume and erythrocyte velocity were not influenced by HVMPC stimulation of acupuncture points, with 5 and 120 HZ in asymptomatic subjects (Balogun et al., 1996).

*Antibacterial effect*

Growth inhibition of *Streptococcus A* bacteria *in vitro* was noted around the negative electrode, following a 45-minute application of HVMPC stimulation with 200 V, 120 HZ, 10 second on/off cycle and 2 second ramp time (Kuykendall et al., 2004). High-voltage pulsed electric field treatment for up to 3 days following mouse burn injury model and *Pseudomonas aeruginosa* infection (Wu et al., 2021). The treatment reduced bacterial load and expression of multiple inflammatory-related cytokines; the parameters were as follows: 500 V, 100 µs and 200 HZ, delivered at 3 HZ through two plate electrodes and positioned 1mm apart (Wu et al., 2021). Another study mentioned that HVMPC might inhibit *Staphylococcus aureus* bacterial growth in cell culture, following stimulation with 250 V, 100 HZ and 70 µs interpulse interval for 1 hour (Merriman et al., 2004). Inhibition of *Klebsiella pneumoniae* bacteria was documented after 48 hours of HVMPC stimulation with 250 V, 100 HZ and 70 µs interpulse interval for 120 minutes (Defi, 2014). Different patterns of inhibition were observed at the cathode and anode (Defi, 2014). Bactericidal effects, both direct and indirect, were observed on four different species of bacteria; *Escherichia coli, Klebsiella, Pseudomonas aeruginosa,*

and *Staphylococcus aureus* in response to HVMPC stimulation (Szuminsky et al., 1994). Differences in the zones of inhibition were observed at both the cathode and anode, and the stimulation parameters were 500 V, 120 HZ, 7 µs pulses and 70 µs interpulse interval, for 30 minutes (Szuminsky et al., 1994). HVMPC stimulation, with 32, 64 and 95 V, 100 HZ for 30 and 60 minutes, was shown to inhibit *Staphylococcus aureus, Pseudomonas aeruginosa* and, *Escherichia coli* bacterial proliferation *in vitro* (Gomes et al., 2015). Some degree of growth inhibition of *Staphylococcus aureus, Pseudomonas aeruginosa* and, *Escherichia coli* bacteria was observed in response to HMPVC stimulation with more than 250 V, 120 HZ and 55 µs interpulse interval for at least 2 hours (Kincaid & Lavoie, 1989). Another study reported inhibition of *Escherichia coli* and *Staphylococcus aureus* bacteria *in vitro* following HVMPC stimulation with 400 V, 120 HZ and 70 µs (Kang et al., 1996).

*Post traumatic edema*

A systematic review concluded that cathodal HVMPC, applied with voltages 10% lower than motor threshold and 120 HZ may be effective in reducing edema formation (Snyder et al., 2010). HVMPC, applied with voltages 10% lower than motor threshold and 120 HZ for four 30 minute treatments at 1.5-hour intervals starting 10 minutes after trauma, was reported to be effective in reducing posttraumatic edema formation in frogs (Bettany et al., 1990). A single 30-minute cathodal HVMPC treatment, with voltages 10% less than motor threshold and 120 HZ, via immersion technique, might have significantly reduced posttraumatic edema formation in frog hindlimbs for 4-7.5 hours (Taylor et al., 1992). Nonetheless, the same single 30-minute cathodal HVMPC treatment delivered via surface electrodes, instead of immersion, did not reduce edema formation in frogs (F. Mendel et al., 1992). The discrepancy between these results might be explained by differences in current voltage, treatment insufficiency, and limb dependency (F. Mendel et al., 1992). Limbs treated with four 30-minute cathodal HVMPC treatments, at 10% less than visible motor threshold, 120 HZ, interpulse interval of 75 µs, and with 30-minute rest periods, via immersion technique, showed significantly smaller volumes than that of untreated limbs in rats (F. C. Mendel et al., 1992). Sensory-level (10% less than visible motor threshold) cathodal HVMPC at 120 HZ significantly retarded acute edema in both rats and frogs, at least during and for a short time after treatment, while sensory (10% less than visible motor threshold)

stimulation produced by low-voltage stimulator and anodal HVMPC failed to attenuate edema formation (Mendel & Fish, 1993; F. C. Mendel et al., 1992; Taylor et al., 1992).

HVMPC intervention applied to rat hind limb with 40 V, 80 HZ, 65 to 75 µs, for a period of 20 minutes, did not show a statistically significant difference compared to the control group with regard to paw volume immediately following treatment (T. M. Mohr et al., 1987). Cathodal HVMPC at 10% below visible muscle contraction intensity, 120 HZ, 75 µs, for four 30-minute treatments followed by 30-minute rest each, was reported to reduce edema formation in Zucker-Lean and Brown Norway rats but not in Sprague-Dawley rats (Thornton et al., 1998). Macromolecular leakage might be curbed using cathodal and anodal HVMPC in histamine-treated hamsters; at intensities of 90%, 50% and 10% of visible motor threshold, 120 HZ, and 75 µs (Taylor et al., 1997). Golden hamsters that received 30- or 50-V dose of HVMPC at 120 HZ, and 50 µs, for 5 minutes, showed less post-histamine microvessel leaks compared with control rats (Reed, 1988).

Clinically significant, although not statistically significant, differences were noted in chronic posttraumatic edema reduction, after HVMPC stimulation with minimal observable muscle contraction voltage and 8 HZ, for 30 minutes (Griffin et al., 1990). HVMPC with 100 HZ , 100 µs, for 30 minute stimulation sessions, during 20 visits over 35 days, was reported to be effective in reducing chronic hand edema (Stralka et al., 1998). No improvement in edema was found in patients with acute ankle sprain following the administration of HVMPC stimulation with submotor level voltage, 120 HZ, twin-pulses of 5 and 8 µs and an interpulse interval of 75 µs, for 30 minutes, once a day and either anodal or cathodal polarity (Sandoval et al., 2010). The treatment of acute lateral ankle sprains did not reveal improvement in edema as a result of cathodal HVMPC treatment, in addition to ice, with current intensity to comfort, 28 HZ and 80 HZ, 30 minutes a day for 3 days (Michlovitz et al., 1988).

*Pain*

An increase in pain threshold up to 10 minutes following HVMPC application was reported by a study (Zoghi & Jaberzadeh, 2002). Cathodal HVMPC stimulation for 30 minutes, with voltage above 100 V to motor threshold (visible muscle contraction;

between 100-170 volts), 10 HZ, twin-pulses of 20 μs each and interpulse interval of 100 μs, two to three times a week was found to be effective in reducing pain in female patients with temporomandibular disorder (Gomes et al., 2012). Analgesic effect of anodal HVMPC stimulation for lateral elbow tendinopathy, with 100 V, 100 HZ and 200 μs for 50 minutes, 5 days a week for two weeks (Dolibog et al., 2022). One hour of HVMPC stimulation with voltage at patient's maximum tolerance (mean 294 V), 120 HZ and negative polarity was shown to reduce pain due to levator ani syndrome (Morris & Newton, 1987). Cathodal HVMPC with voltage at patient's maximum tolerance (250-350 V) and 80 HZ, for 1 hour per day, three times over a period of 3 to 10 days was found to be effective for the treatment of levator ani syndrome (Sohn et al., 1982).

Another study stated that HVMPC with voltage at patient's tolerance (100-300 V) and 100 HZ, for 30 minute sessions (gradually increased to 45 minutes) was an effective treatment for levator ani syndrome (Mantilla et al., 2014). Application of HVMPC stimulation following acute ankle sprain, with submotor level voltage, 120 HZ, twin-pulses of 5 and 8 μs and an interpulse interval of 75 μs, for 30 minutes, once a day and either anodal or cathodal polarity revealed no difference in pain status compared with the control group (Sandoval et al., 2010). HVMPC stimulation was ineffective in providing lasting reduction in pain associated with delayed onset muscle soreness (Butterfield et al., 1997).

*Adverse reactions*

Skin irritation due to pH variations as a result of applying HVMPC electrical stimulation with cathodal current and pad electrodes was reported to be negligible (Newton & Karselis, 1983). The treatment characteristics were 100 V, 80-82 HZ, and cathodal current to the active electrodes for 30-minutes (Newton & Karselis, 1983). Nonetheless, one report of a patient who had a persistent red area or burn under the active electrode that resolved within 48 h was found, which was remedied by reducing current intensity (Houghton et al., 2010). Other minor and rare skin reactions that was attributed to contact dermatitis beneath the dispersive electrode were identified (Goldman et al., 2004; Houghton et al., 2010). These skin reactions were treated using a non-adhesive carbon electrode or applying hydrocortisone 2.5% ointment to the affected area for 1-2 weeks (Goldman et al., 2004; Houghton et al., 2010).

Compared to low-voltage neuromuscular stimulation, HVMPC was perceived to be significantly less uncomfortable (Wong, 1986). The treatment protocol evaluated was subject's maximum tolerance for the intensity, 50 HZ, 40 μs, 10 sec on, 10 sec off duty cycle, and ramp of 2 sec, and the total stimulation duration was 6 minutes (Wong, 1986). Other treatment characteristics, however, may lead to different results (Wong, 1986). At least one patient complained of stimulation intensity in another study, which was remedied by reducing it (Akers & Gabrielson, 1984). A complaint of increased pain, moreover, was documented in a patient with severe Raynaud's syndrome after two treatment sessions (Zhou et al., 2016).

Although unlikely that any were related to HVMPC treatment, serious adverse incidents were reported in one study; two patients experienced loss of hemodialysis access necessitating brief hospitalization, and one of these patients developed systemic calciphylaxis and died one week later (Goldman et al., 2004). After the development of excessive exudate, a strong foul odor, and significant redness upon dressing removal, one patient was diagnosed with chronic osteomyelitis (Zhou et al., 2016). No adverse reactions, nevertheless, were reported in a number of studies (Ahmad, 2008; Fitzgerald & Newsome, 1993; Franek et al., 2012; Griffin et al., 1991; Houghton et al., 2003; Polak et al., 2017; Anna Polak et al., 2016; Polak et al., 2018; A Polak et al., 2016).

*Precautions*

Several aspects need to be considered for safe and effective application of electrical stimulation intervention, and HVMPC in particular.

Heterogeneity in cellular phenotypes of tendons and skin, in addition to other tissues, is being discovered (Deng et al., 2021; Guerrero-Juarez et al., 2019; LeBleu & Neilson, 2020; Muhl et al., 2020; Oprescu et al., 2020; Petrany et al., 2020; Philippeos et al., 2018; Solé-Boldo et al., 2020; Vorstandlechner et al., 2020). Moreover, the types and phenotypes of cell present at any given moment, and especially during tissue injury or healing, may not be fully known (Akbar et al., 2021; De Micheli et al., 2020; Eren et al., 2023; Fu et al., 2023; Zhang & Wang, 2010). Consequently, the functional response of each of these phenotypes to electrical stimulation may not be fully known either.

Galvanotaxis, which is the directed polarization and migration of cells in response to electric fields, may depend on the polarity of the used current (Nwogbaga et al., 2023; Zhao et al., 2010). Neutrophils and macrophages may migrate towards the positive electrode (anode), while the negative electrode (cathode) may attract neutrophils, leukocytes, fibroblasts and keratinocytes to the area, if a continuous microamperage direct current is used (Lampe, 1998). Current polarity may be alternated during treatment if healing reached a plateau (Lampe, 1998). Currently, it remains unknown, however, whether all phenotypes within each cell type behave similarly and to what extent, and the circumstances that warrant polarity alternation (Lampe, 1998).

It may be, furthermore, unknown how each of these phenotypes would react to electrical stimulation depending on their phase in the cell cycle (Blackiston et al., 2009; Johnson & Walker, 1999; Kastan & Bartek, 2004; Muhl et al., 2020). Additionally, the phenotype and activity of cells may vary throughout the phases of healing, which adds another layer of complexity to the picture. The severity of the disease may differ between patients, which may even further complicate the choice of the optimal treatment protocol and the appropriate timing thereof. The treatment protocol using HVMPC could be based on results of dermal ulcer studies, however, the parameters of treatment may necessitate further adjustment, particularly that the severity and healing outcomes may vary between these conditions. Further research is therefore

needed to define these parameters, in order to achieve the best results, with the safest approach possible.

The influx of calcium seems to be the basic response to HVMPC exposure, which was observed in human dermal fibroblasts and murine splenic T-lymphocytes. It is yet to be known, nonetheless, whether this response is sustained and to what extent. One of the resultant effects of calcium influx might be cell proliferation, however, the mutagenic effect of HVMPC was not investigated. Nevertheless, despite few reports, the preponderance of evidence suggested that static electric, magnetic, and electromagnetic fields, with the tested types and levels, lacked cytotoxic, transforming, and mutagenic activity (Delgado, 1985; Heynick et al., 2003; Jacobson-Kram et al., 1997; McCann et al., 1998; McCann et al., 1993). Conversely, excessive elevations of cytosolic calcium might be cytotoxic, which may lead to apoptosis (Mattson & Chan, 2003; Nicotera & Orrenius, 1998). Indeed, evidence for increased apoptosis, through different mechanisms, in response to electrical stimulation were found (Chen et al., 2014; Hernández-Bule et al., 2014; Matsuki et al., 2008; Matsuki et al., 2010; Oshima et al., 1998; Oshima et al., 1997; Sebastian et al., 2011; Wang et al., 2012). Electrical stimulation, however, may be contraindicated near unmanaged tumor or malignancy, although without justification (Lampe, 1998; Maltser et al., 2017; Windsor et al., 1993).

Increased pain of patients with peripheral vascular disease in response to electrical stimulation was indeed reported in literature (Lampe, 1998; Levine & Bockstahler, 2014; Sussman & Bates-Jensen, 2001). It was suggested, moreover, that the increase rate of unpleasantness magnitude estimates and pain intensity that correlated to increases in electrical stimulation currents may depend on whether the stimulus caused muscle contraction (Delitto et al., 1992). A significant degree of discomfort was related to electrically induced muscle contraction, which might represent a limiting factor in patients' tolerance to stimulation (Delitto et al., 1992). The only study that reported increased pain with HVMPC, in a patient with severe Raynaud's syndrome, did not mention the current intensity used (Zhou et al., 2016). Nonetheless, some studies on HVMPC intervention used a submotor intensity of stimulation, which might avoid this side effect (Fitzgerald & Newsome, 1993; Goldman et al., 2004; Polak et al., 2017; Anna Polak et al., 2016; A Polak et al., 2016; Santana de Souza et al., 2017).

***Objectives***

The general aims of this thesis were:

a. Identify existing evidence that may support physical therapy interventions for tendinopathy.

b. Determine the efficacy and safety of high-voltage monophasic pulsed current in treating dermal ulcers, and whether dermal ulcers and tendinopathies may share common pathophysiology.

c. Develop an animal model of tendon injury and assess whether the effects of high-voltage monophasic pulsed current on dermal ulcers may be transferred to tendon injuries, as an attempt to translate these effects to tendinopathy.

The outline and objectives of this work were:

**Chapter I**

*General introduction*

Provide an overview on the terminology, risk factors, pathogenesis theories and historical progress and pathophysiology of tendinopathy. Moreover, a rationale for the choice of high-voltage monophasic pulsed current as an intervention, its known effects, adverse reactions and precautions are detailed.

**Chapter II**

*State of the art*

Study I: Review the literature for possible overlap between tendinopathy and pressure ulcers with respect to the pathophysiologic factors leading to their development and failure of healing.

Study II: Review the literature systematically for evidence in the last decade of efficacy of physical therapy interventions for tendinopathy, as a single disease entity, as determined in systematic reviews and/or meta-analyses.

Study III: Review the literature systematically for evidence of physical therapy modalities for the treatment of chronic lateral elbow tendinopathy, as assessed in randomized controlled trials using pain-free grip strength and/or patient-rated tennis elbow evaluation questionnaire as outcome measures.

**Chapter III**

*Original manuscripts*

Study IV: Review the literature systematically to determine and quantify the efficacy of high-voltage monophasic pulsed current in treating stage II-IV pressure ulcers, identify the details of HVMPC intervention parameters and the superior protocol, and ascertain other potential benefits and the safety of HVMPC intervention.

Study V: Review the literature systematically to determine the efficacy of high-voltage monophasic pulsed current in treating diabetic ulcers, assess its effect on skin lesions with each of the pathophysiologic factors potentially contributing to diabetic ulcers, evaluate its safety, and identify treatment parameters.

Study VI: Develop a model of overuse-induced tendon injury in rats and evaluate the possibility of treating it using high-voltage monophasic pulsed current.

**Chapter IV**

*General discussion*

An overall discussion of the results was presented, with emphasis on their possible implications.

**Chapter V**

*Main Conclusions*

The principal conclusions and future perspectives of the work in general were stated.

## References


Abate, M., Schiavone, C., Salini, V., & Andia, I. (2013). Occurrence of tendon pathologies in metabolic disorders. *Rheumatology, 52*(4), 599-608. doi:http://dx.doi.org/10.1093/rheumatology/kes395

Abate, M., Silbernagel, K. G., Siljeholm, C., Di Iorio, A., De Amicis, D., Salini, V., . . . Paganelli, R. (2009). Pathogenesis of tendinopathies: inflammation or degeneration? *Arthritis Research & Therapy, 11*(3), 1-15. doi:http://dx.doi.org/10.1186/ar2723

Abboud, J. A., & Kim, J. S. (2010). The effect of hypercholesterolemia on rotator cuff disease. *Clinical Orthopaedics and Related Research®, 468*(6), 1493-1497. doi:http://dx.doi.org/10.1007/s11999-009-1151-9

Ackermann, P. W., Alim, M. A., Pejler, G., & Peterson, M. (2023). Tendon pain–what are the mechanisms behind it? *Scandinavian Journal of Pain, 23*(1), 14-24. doi:http://dx.doi.org/10.1515/sjpain-2022-0018

Adams, D. S. (1992). Mechanisms of cell shape change: the cytomechanics of cellular response to chemical environment and mechanical loading. *The Journal of cell biology, 117*(1), 83-93. doi:http://dx.doi.org/10.1083/jcb.117.1.83

Ahmad, E. T. (2008). High-voltage pulsed galvanic stimulation: effect of treatment duration on healing of chronic pressure ulcers. *Annals of burns and fire disasters, 21*(3), 124.

Akbar, M., MacDonald, L., Crowe, L. A., Carlberg, K., Kurowska-Stolarska, M., Ståhl, P. L., . . . Millar, N. L. (2021). Single cell and spatial transcriptomics in human tendon disease indicate dysregulated immune homeostasis. *Annals of the Rheumatic Diseases, 80*(11), 1494-1497.

Akers, T., & Gabrielson, A. (1984). The effect of high voltage galvanic stimulation on the rate of healing of decubitus ulcers. *Biomedical sciences instrumentation, 20*, 99-100.

Alves, C., Mendes, D., & Marques, F. B. (2019). Fluoroquinolones and the risk of tendon injury: a systematic review and meta-analysis. *European journal of clinical pharmacology, 75*(10), 1431-1443. doi:http://dx.doi.org/10.1007/s00228-019-02713-1

Andersen, M. B., Pingel, J., Kjær, M., & Langberg, H. (2011). Interleukin-6: a growth factor stimulating collagen synthesis in human tendon. *Journal of applied physiology, 110*(6), 1549-1554. doi:http://dx.doi.org/10.1152/japplphysiol.00037.2010

Archambault, J., Tsuzaki, M., Herzog, W., & Banes, A. J. (2002). Stretch and interleukin-1β induce matrix metalloproteinases in rabbit tendon cells in vitro. *Journal of orthopaedic research, 20*(1), 36-39. doi:http://dx.doi.org/10.1016/S0736-0266(01)00075-4

Archambault, J. M., Wiley, J. P., & Bray, R. C. (1995). Exercise loading of tendons and the development of overuse injuries. *Sports Medicine, 20*(2), 77-89. doi:http://dx.doi.org/10.2165/00007256-199520020-00003

Arnoczky, S. P., Lavagnino, M., & Egerbacher, M. (2007). The mechanobiological aetiopathogenesis of tendinopathy: is it the over-stimulation or the under-stimulation of tendon cells? *International journal of experimental pathology, 88*(4), 217-226. doi:http://dx.doi.org/10.1111/j.1365-2613.2007.00548.x

Arnoczky, S. P., Lavagnino, M., Whallon, J. H., & Hoonjan, A. (2002). In situ cell nucleus deformation in tendons under tensile load; a morphological analysis

using confocal laser microscopy. *Journal of orthopaedic research, 20*(1), 29-35.
Arnoczky, S. P., Tian, T., Lavagnino, M., Gardner, K., Schuler, P., & Morse, P. (2002). Activation of stress-activated protein kinases (SAPK) in tendon cells following cyclic strain: the effects of strain frequency, strain magnitude, and cytosolic calcium. *Journal of orthopaedic research, 20*(5), 947-952. doi:http://dx.doi.org/10.1016/S0736-0266(02)00038-4
Balogun, J., Tang, S., He, Y., Hsieh, J.-M., & Katz, J. (1996). Effects of high-voltage galvanic stimulation of ST36 and ST37 acupuncture points on peripheral blood flow and skin temperature. *Disability and rehabilitation, 18*(10), 523-528. doi:http://dx.doi.org/10.3109/09638289609166039
Banes, A., Horesovsky, G., Tsuzaki, M., Boitano, S., Lawrence, W., Brown, T., . . . Ralphs, J. (1999). The connexin 43 gap junction is a mechanosensitive gene in avian flexor tendon cells. *The Biology of the Synovial Joint, B Caterson, C Archer, Eds., Harwood Academic Publishers, The Netherlands*, 279-299.
Banes, A., Kenamond, C., Yang, X., Horesovsky, G., Miller, L., Boitano, S., & Tsuzaki, M. (1997). *Avian tendon cells express connexin 43 mRNA in response to mechanical stretching.* Paper presented at the Molecular biology of the cell.
Banes, A., Sanderson, M., Boitano, S., Hu, P., Brigman, B., Tsuzaki, M., . . . Lawrence, W. (1994). Mechanical Load±Growth Factors Induce [Ca2+] i Release, Cyclin D1 Expression and DNA Synthesis in Avian Tendon Cells. In *Cell mechanics and cellular engineering* (pp. 210-232): Springer.
Banes, A. J., Lee, G., Graff, R., Otey, C., Archambault, J., Tsuzaki, M., . . . Qi, J. (2001). Mechanical forces and signaling in connective tissue cells: cellular mechanisms of detection, transduction, and responses to mechanical deformation. *Current Opinion in Orthopaedics, 12*(5), 389-396. doi:http://dx.doi.org/10.1097/00001433-200110000-00005
Banes, A. J., Tsuzaki, M., Hu, P., Brigman, B., Brown, T., Almekinders, L., . . . Fischer, T. (1995). PDGF-BB, IGF-I and mechanical load stimulate DNA synthesis in avian tendon fibroblasts in vitro. *Journal of biomechanics, 28*(12), 1505-1513. doi:http://dx.doi.org/10.1016/0021-9290(95)00098-4
Banes, A. J., Tsuzaki, M., Yamamoto, J., Brigman, B., Fischer, T., Brown, T., & Miller, L. (1995). Mechanoreception at the cellular level: the detection, interpretation, and diversity of responses to mechanical signals. *Biochemistry and Cell Biology, 73*(7-8), 349-365. doi:http://dx.doi.org/10.1139/o95-043
Banes, A. J., Wall, M., Garvin, J., & Archambault, J. (2003). Cytomechanics: signaling to mechanical load in connective tissue cells and its role in tissue engineering. In *Functional Tissue Engineering* (pp. 318-334): Springer.
Banes, A. J., Weinhold, P., Yang, X., Tsuzaki, M., Bynum, D., Bottlang, M., & Brown, T. (1999). Gap junctions regulate responses of tendon cells ex vivo to mechanical loading. *Clinical Orthopaedics and Related Research (1976-2007), 367*, S356-S370. doi:http://dx.doi.org/10.1097/00003086-199910001-00034
Barge-Caballero, E., Crespo-Leiro, M. G., Paniagua-Martín, M. J., Muñiz, J., Naya, C., Bouzas-Mosquera, A., . . . Cursack, G. C. (2008). Quinolone-related Achilles tendinopathy in heart transplant patients: incidence and risk factors. *The Journal of heart and lung transplantation, 27*(1), 46-51. doi:http://dx.doi.org/10.1016/j.healun.2007.09.021
Baumgarten, K. M., Gerlach, D., Galatz, L. M., Teefey, S. A., Middleton, W. D., Ditsios, K., & Yamaguchi, K. (2010). Cigarette smoking increases the risk for

rotator cuff tears. *Clinical Orthopaedics and Related Research®, 468*(6), 1534-1541. doi:http://dx.doi.org/10.1007/s11999-009-0781-2
Bettany, J. A., Fish, D. R., & Mendel, F. C. (1990). Influence of high voltage pulsed direct current on edema formation following impact injury. *Physical Therapy, 70*(4), 219-224. doi:http://dx.doi.org/10.1093/ptj/70.4.219
Bidell, M. R., & Lodise, T. P. (2016). Fluoroquinolone-associated tendinopathy: does levofloxacin pose the greatest risk? *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 36*(6), 679-693. doi:http://dx.doi.org/10.1002/phar.1761
Blackiston, D. J., McLaughlin, K. A., & Levin, M. (2009). Bioelectric controls of cell proliferation: ion channels, membrane voltage and the cell cycle. *Cell cycle, 8*(21), 3527-3536. doi:http://dx.doi.org/10.4161/cc.8.21.9888
Blanco, I., Krähenbühl, S., & Schlienger, R. G. (2005). Corticosteroid-associated tendinopathies. *Drug Safety, 28*(7), 633-643. doi:http://dx.doi.org/10.2165/00002018-200528070-00005
Boitano, S., Dirksen, E. R., & Sanderson, M. J. (1992). Intercellular propagation of calcium waves mediated by inositol trisphosphate. *Science, 258*(5080), 292-295. doi:http://dx.doi.org/10.1126/science.1411526
Bourguignon, G. J., & Bourguignon, L. Y. (1987). Electric stimulation of protein and DNA synthesis in human fibroblasts. *The FASEB Journal, 1*(5), 398-402. doi:http://dx.doi.org/10.1096/fasebj.1.5.3678699
Bourguignon, G. J., Jy, W., & Bourguignon, L. Y. (1989). Electric stimulation of human fibroblasts causes an increase in Ca2+ influx and the exposure of additional insulin receptors. *Journal of cellular physiology, 140*(2), 379-385.
Bourguignon, L. Y., Jy, W., Majercik, M. H., & Bourguignon, G. J. (1988). Lymphocyte activation and capping of hormone receptors. *J Cell Biochem, 37*(2), 131-150. doi:http://dx.doi.org/10.1002/jcb.240370202
Brickner, W. M. (1917). PAIN IN THE ARM: SUBDELTOID (SUBACROMIAL) BURSITIS: A FURTHER STUDY OF ITS CLINICAL TYPES, PATHOLOGY AND TREATMENT. *Journal of the American Medical Association, 69*(15), 1237-1243.
Brown, M., & Gogia, P. P. (1987). Effects of high voltage stimulation on cutaneous wound healing in rabbits. *Physical Therapy, 67*(5), 662-667. doi:http://dx.doi.org/10.1093/ptj/67.5.662
Burry, H. (1978). Pathogenesis of some traumatic and degenerative disorders of soft tissue. *Australian and New Zealand journal of medicine, 8*, 163-167. doi:http://dx.doi.org/10.1111/j.1445-5994.1978.tb04816.x
Butterfield, D. L., Draper, D. O., Ricard, M. D., Myrer, J. W., Schulthies, S. S., & Durrant, E. (1997). The effects of high-volt pulsed current electrical stimulation on delayed-onset muscle soreness. *Journal of Athletic Training, 32*(1), 15.
Carbone, S., Gumina, S., Arceri, V., Campagna, V., Fagnani, C., & Postacchini, F. (2012). The impact of preoperative smoking habit on rotator cuff tear: cigarette smoking influences rotator cuff tear sizes. *Journal of shoulder and elbow surgery, 21*(1), 56-60. doi:http://dx.doi.org/10.1016/j.jse.2011.01.039
Cardoso, T. B., Pizzari, T., Kinsella, R., Hope, D., & Cook, J. L. (2019). Current trends in tendinopathy management. *Best Practice & Research Clinical Rheumatology, 33*(1), 122-140. doi:http://dx.doi.org/10.1016/j.berh.2019.02.001

Catterall, W. A. (1995). Structure and function of voltage-gated ion channels. *Annual review of biochemistry, 64*(1), 493-531. doi:http://dx.doi.org/10.1146/annurev.bi.64.070195.002425
Catterall, W. A. (2011). Voltage-gated calcium channels. *Cold Spring Harbor perspectives in biology, 3*(8), a003947. doi:http://dx.doi.org/10.1101/cshperspect.a003947
Cawston, T., Riley, G., & Hazleman, B. (1996). Tendon lesions and soft tissue rheumatism--great outback or great opportunity? *Annals of the Rheumatic Diseases, 55*(1), 1. doi:http://dx.doi.org/10.1136/ard.55.1.1
CHARLES S NEER, I. (1972). Anterior acromioplasty for the chronic impingement syndrome in the shoulder: a preliminary report. *JBJS, 54*(1), 41-50. doi:http://dx.doi.org/10.2106/JBJS.8706.cl
Chen, X., Yin, S., Hu, C., Chen, X., Jiang, K., Ye, S., . . . Zhou, L. (2014). Comparative study of nanosecond electric fields in vitro and in vivo on hepatocellular carcinoma indicate macrophage infiltration contribute to tumor ablation in vivo. *PLoS One, 9*(1), e86421. doi:http://dx.doi.org/10.1371/journal.pone.0086421
Childress, M. A., & Beutler, A. (2013). Management of chronic tendon injuries. *American family physician, 87*(7), 486-490.
Cilli, F., Khan, M., Fu, F., & Wang, J. H.-C. (2004). Prostaglandin E2 affects proliferation and collagen synthesis by human patellar tendon fibroblasts. *Clinical Journal of Sport Medicine, 14*(4), 232-236. doi:http://dx.doi.org/10.1097/00042752-200407000-00006
Clancy, W. G. (1983). Tendinitis and plantar fasciitis in runners. *Orthopedics, 6*(2), 217-233.
Clement, D., Taunton, J., & Smart, G. (1984). Achilles tendinitis and peritendinitis: etiology and treatment. *The American Journal of Sports Medicine, 12*(3), 179-184. doi:http://dx.doi.org/10.1177/036354658401200301
Codman, E. A., & Akerson, I. B. (1931). The pathology associated with rupture of the supraspinatus tendon. *Annals of surgery, 93*(1), 348. doi:http://dx.doi.org/10.1097/00000658-193101000-00043
Cook, J., & Purdam, C. R. (2009). Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. *British journal of sports medicine, 43*(6), 409-416.
Cook, J., Rio, E., Purdam, C., & Docking, S. (2016). Revisiting the continuum model of tendon pathology: what is its merit in clinical practice and research? *British journal of sports medicine, 50*(19), 1187-1191. doi:http://dx.doi.org/10.1136/bjsports-2015-095422
Coste, B., Mathur, J., Schmidt, M., Earley, T. J., Ranade, S., Petrus, M. J., . . . Patapoutian, A. (2010). Piezo1 and Piezo2 are essential components of distinct mechanically activated cation channels. *Science, 330*(6000), 55-60. doi:http://dx.doi.org/10.1126/science.1193270
Cotrina, M. L., Lin, J. H.-C., Alves-Rodrigues, A., Liu, S., Li, J., Azmi-Ghadimi, H., . . . Nedergaard, M. (1998). Connexins regulate calcium signaling by controlling ATP release. *Proceedings of the National Academy of Sciences, 95*(26), 15735-15740. doi:http://dx.doi.org/10.1073/pnas.95.26.15735
Cowan, D. B., Lye, S. J., & Langille, B. L. (1998). Regulation of vascular connexin43 gene expression by mechanical loads. *Circulation research, 82*(7), 786-793. doi:http://dx.doi.org/10.1161/01.RES.82.7.786

Cruz, N. I., Bayrón, F. E., & Suárez, A. J. (1989). Accelerated healing of full-thickness burns by the use of high-voltage pulsed galvanic stimulation in the pig. *Annals of Plastic Surgery, 23*(1), 49-55. doi:http://dx.doi.org/10.1097/00000637-198907000-00009

de Jonge, S., Van den Berg, C., de Vos, R.-J., Van Der Heide, H., Weir, A., Verhaar, J., . . . Tol, J. (2011). Incidence of midportion Achilles tendinopathy in the general population. *British journal of sports medicine, 45*(13), 1026-1028. doi:http://dx.doi.org/10.1136/bjsports-2011-090342

De Micheli, A. J., Swanson, J. B., Disser, N. P., Martinez, L. M., Walker, N. R., Oliver, D. J., . . . Mendias, C. L. (2020). Single-cell transcriptomic analysis identifies extensive heterogeneity in the cellular composition of mouse Achilles tendons. *AMERICAN JOURNAL OF PHYSIOLOGY-CELL PHYSIOLOGY, 319*(5), C885-C894. doi:http://dx.doi.org/10.1152/ajpcell.00372.2020

Dean, B. J., Dakin, S. G., Millar, N. L., & Carr, A. J. (2017). Emerging concepts in the pathogenesis of tendinopathy. *The Surgeon, 15*(6), 349-354. doi:http://dx.doi.org/10.1016/j.surge.2017.05.005

Dean, B. J. F., Gettings, P., Dakin, S. G., & Carr, A. J. (2015). Are inflammatory cells increased in painful human tendinopathy? A systematic review. *British journal of sports medicine*. doi:http://dx.doi.org/10.1136/bjsports-2015-094754

Defi, I. (2014). An in vitro pressure ulcer approach using High Voltage Pulsed Current (HVPC) stimulation. doi:http://dx.doi.org/10.4017/gt.2014.12.3.009.00

Del Buono, A., Battery, L., Denaro, V., Maccauro, G., & Maffulli, N. (2011). Tendinopathy and inflammation: some truths. *International journal of immunopathology and pharmacology, 24*(1_suppl2), 45-50. doi:http://dx.doi.org/10.1177/03946320110241S209

Delgado, J. (1985). Biological effects of extremely low frequency electromagnetic fields. *Journal of Bioelectricity, 4*(1), 75-92. doi:http://dx.doi.org/10.3109/15368378509040362

Delitto, A., Strube, M. J., Shulman, A. D., & Minor, S. D. (1992). A study of discomfort with electrical stimulation. *Physical Therapy, 72*(6), 410-421. doi:http://dx.doi.org/10.1093/ptj/72.6.410

Deng, C.-C., Hu, Y.-F., Zhu, D.-H., Cheng, Q., Gu, J.-J., Feng, Q.-L., . . . Rong, Z. (2021). Single-cell RNA-seq reveals fibroblast heterogeneity and increased mesenchymal fibroblasts in human fibrotic skin diseases. *Nature communications, 12*(1), 3709. doi:http://dx.doi.org/10.1038/s41467-021-24110-y

Dideriksen, K., Sindby, A. K. R., Krogsgaard, M., Schjerling, P., Holm, L., & Langberg, H. (2013). Effect of acute exercise on patella tendon protein synthesis and gene expression. *SpringerPlus, 2*(1), 1-8. doi:http://dx.doi.org/10.1186/2193-1801-2-109

Dolibog, P. T., Porębska, B., Grzegorczyn, S., Chmielewska, D., Ślęzak, A., & Dolibog, P. (2022). Analgesic and Functional Efficiency of High-Voltage Electrical Stimulation in Patients with Lateral Epicondylitis—A Report with a 180-Day Follow-Up. *Journal of Clinical Medicine, 11*(9), 2571. doi:http://dx.doi.org/10.3390/jcm11092571

DONCK, J. B., SEGAERT, M. F., & VANRENTERGHEM, Y. F. (1994). Fluoroquinolones and Achilles tendinopathy in renal transplant recipients. *Transplantation, 58*(6), 736. doi:http://dx.doi.org/10.1086/375078

El Hawary, R., Stanish, W. D., & Curwin, S. L. (1997). Rehabilitation of tendon injuries in sport. *Sports Medicine, 24*(5), 347-358.

Eliasson, P., Dietrich-Zagonel, F., Lundin, A.-C., Aspenberg, P., Wolk, A., & Michaëlsson, K. (2019). Statin treatment increases the clinical risk of tendinopathy through matrix metalloproteinase release–a cohort study design combined with an experimental study. *Scientific Reports, 9*(1), 1-11. doi:http://dx.doi.org/10.1038/s41598-019-53238-7

Eren, A. D., Vermeulen, S., Schmitz, T. C., Foolen, J., & de Boer, J. (2023). The loop of phenotype: Dynamic reciprocity links tenocyte morphology to tendon tissue homeostasis. *Acta Biomaterialia, 163*, 275-286. doi:http://dx.doi.org/10.1016/j.actbio.2022.05.019

Ertel, E. A., Campbell, K. P., Harpold, M. M., Hofmann, F., Mori, Y., Perez-Reyes, E., . . . Birnbaumer, L. (2000). Nomenclature of voltage-gated calcium channels. *Neuron, 25*(3), 533-535. doi:http://dx.doi.org/10.1016/s0896-6273(00)81057-0

Eyn-Young, K. (2004). Keratinocyte proliferation in aged rat skin by high voltage pulsed current stimulation. *Biomedical Science Letters, 10*(4), 361-366.

Falanga, V. (1987). Electrical stimulation increases the expression of fibroblasts receptors for transforming growth factor-beta. *J Invest Dermatol, 88*, A.

Fitzgerald, G. K., & Newsome, D. (1993). Treatment of a large infected thoracic spine wound using high voltage pulsed monophasic current. *Physical Therapy, 73*(6), 355-360.

Fleisch, F., Hartmann, K., & Kuhn, M. (2000). Fluoroquinolone-induced tendinopathy: also occurring with levofloxacin. *Infection, 28*(4), 256. doi:http://dx.doi.org/10.1007/s150100070050

Fong, G., Backman, L. J., Alfredson, H., Scott, A., & Danielson, P. (2017). The effects of substance P and acetylcholine on human tenocyte proliferation converge mechanistically via TGF-β1. *PLoS One, 12*(3), e0174101. doi:http://dx.doi.org/10.1371/journal.pone.0174101

Franceschi, F., Papalia, R., Paciotti, M., Franceschetti, E., Di Martino, A., Maffulli, N., & Denaro, V. (2014). Obesity as a risk factor for tendinopathy: a systematic review. *International Journal of Endocrinology, 2014*. doi:http://dx.doi.org/10.1155/2014/670262

Franek, A., Kostur, R., Polak, A., Taradaj, J., Szlachta, Z., Blaszczak, E., . . . Kucio, C. (2012). Using high-voltage electrical stimulation in the treatment of recalcitrant pressure ulcers: results of a randomized, controlled clinical study. *Ostomy-Wound Management, 58*(3), 30.

Fredberg, U., & Stengaard-Pedersen, K. (2008). Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation. *Scandinavian journal of medicine & science in sports, 18*(1), 3-15.

Fu, S.-C., Rolf, C., Cheuk, Y.-C., Lui, P. P., & Chan, K.-M. (2010). Deciphering the pathogenesis of tendinopathy: a three-stages process. *BMC Sports Science, Medicine and Rehabilitation, 2*(1), 1-12. doi:http://dx.doi.org/10.1186/1758-2555-2-30

Fu, S.-C., Wong, Y.-P., Cheuk, Y.-C., Lee, K.-M., & Chan, K.-M. (2005). TGF-β1 reverses the effects of matrix anchorage on the gene expression of decorin and procollagen type I in tendon fibroblasts. *Clinical Orthopaedics and Related Research®, 431*, 226-232. doi:http://dx.doi.org/10.1097/01.blo.0000145887.48534.6f

Fu, S. C., Chan, B. P., Wang, W., Pau, H. M., Chan, K. M., & Rolf, C. G. (2002). Increased expression of matrix metalloproteinase 1 (MMP1) in 11 patients with

patellar tendinosis. *Acta orthopaedica scandinavica, 73*(6), 658-662. doi:http://dx.doi.org/10.3109/17453670209178031
Fu, W., Yang, R., & Li, J. (2023). Single-cell and spatial transcriptomics reveal changes in cell heterogeneity during progression of human tendinopathy. *BMC biology, 21*(1), 132. doi:http://dx.doi.org/10.1186/s12915-023-01613-2
Gaida, J. E., Ashe, M. C., Bass, S. L., & Cook, J. L. (2009). Is adiposity an under-recognized risk factor for tendinopathy? A systematic review. *Arthritis Care & Research: Official Journal of the American College of Rheumatology, 61*(6), 840-849. doi:http://dx.doi.org/10.1002/art.24518
Gaida, J. E., Cook, J. L., & Bass, S. (2008). Adiposity and tendinopathy. *Disability and rehabilitation, 30*(20-22), 1555-1562. doi:http://dx.doi.org/10.1080/09638280701786864
Geiger, B., Bershadsky, A., Pankov, R., & Yamada, K. M. (2001). Transmembrane crosstalk between the extracellular matrix and the cytoskeleton. *Nature reviews Molecular cell biology, 2*(11), 793-805. doi:http://dx.doi.org/10.1038/35099066
Ghazi, A., Berrier, C., Ajouz, B., & Besnard, M. (1998). Mechanosensitive ion channels and their mode of activation. *Biochimie, 80*(5), 357-362. doi:http://dx.doi.org/10.1016/S0300-9084(00)80003-6
Ghosh, K., Pan, Z., Guan, E., Ge, S., Liu, Y., Nakamura, T., . . . Clark, R. A. (2007). Cell adaptation to a physiologically relevant ECM mimic with different viscoelastic properties. *Biomaterials, 28*(4), 671-679. doi:http://dx.doi.org/10.1016/j.biomaterials.2006.09.038
Goldman, R., Rosen, M., Brewley, B., & Golden, M. (2004). Electrotherapy promotes healing and microcirculation of infrapopliteal ischemic wounds: a prospective pilot study. *Advances in skin & wound care, 17*(6), 284-294.
Gomes, N. C., Berni-Schwarzenbeck, K., Packer, A. C., & Rdrigues-Bigaton, D. (2012). Effect of cathodal high-voltage electrical stimulation on pain in women with TMD. *Brazilian Journal of Physical Therapy, 16*, 10-15. doi:http://dx.doi.org/10.1590/S1413-35552012000100003
Gomes, R. C., Brandino, H. E., Alves de Sousa, N. T., Santos, M. F., Martinez, R., & de Jesus Guirro, R. R. (2015). Polarized currents inhibit in vitro growth of bacteria colonizing cutaneous ulcers. *Wound Repair and Regeneration, 23*(3), 403-411. doi:http://dx.doi.org/10.1111/wrr.12296
Griffin, J. W., Newsome, L. S., Stralka, S. W., & Wright, P. E. (1990). Reduction of chronic posttraumatic hand edema: a comparison of high voltage pulsed current, intermittent pneumatic compression, and placebo treatments. *Physical Therapy, 70*(5), 279-286. doi:http://dx.doi.org/10.1093/ptj/70.5.279
Griffin, J. W., Tooms, R. E., Mendius, R. A., Clifft, J. K., Vander Zwaag, R., & El-Zeky, F. (1991). Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. *Physical Therapy, 71*(6), 433-442. doi:http://dx.doi.org/10.1093/ptj/71.6.433
Guerrero-Juarez, C. F., Dedhia, P. H., Jin, S., Ruiz-Vega, R., Ma, D., Liu, Y., . . . Yang, Z. (2019). Single-cell analysis reveals fibroblast heterogeneity and myeloid-derived adipocyte progenitors in murine skin wounds. *Nature communications, 10*(1), 650. doi:http://dx.doi.org/10.1038/s41467-018-08247-x
Gwilym, S., Watkins, B., Cooper, C., Harvie, P., Auplish, S., Pollard, T., . . . Carr, A. (2009). Genetic influences in the progression of tears of the rotator cuff. *J Bone Joint Surg Br, 91*(7), 915-917. doi:http://dx.doi.org/10.1302/0301-620X.91B7.22353

Hansen, M., Boesen, A., Holm, L., Flyvbjerg, A., Langberg, H., & Kjaer, M. (2013). Local administration of insulin-like growth factor-I (IGF-I) stimulates tendon collagen synthesis in humans. *Scandinavian journal of medicine & science in sports, 23*(5), 614-619. doi:http://dx.doi.org/10.1111/j.1600-0838.2011.01431.x

Harrell, R. M. (1999). Fluoroquinolone-induced tendinopathy: what do we know? *Southern medical journal, 92*(6), 622-625. doi:http://dx.doi.org/10.1097/00007611-199906000-00014

Harris, A. K., Stopak, D., & Wild, P. (1981). Fibroblast traction as a mechanism for collagen morphogenesis. *Nature, 290*(5803), 249-251. doi:http://dx.doi.org/10.1038/290249a0

Harris, A. K., Wild, P., & Stopak, D. (1980). Silicone rubber substrata: a new wrinkle in the study of cell locomotion. *Science, 208*(4440), 177-179. doi:http://dx.doi.org/10.1126/science.6987736

Harvie, P., Ostlere, S., Teh, J., McNally, E., Clipsham, K., Burston, B., . . . Carr, A. (2004). Genetic influences in the aetiology of tears of the rotator cuff: sibling risk of a full-thickness tear. *J Bone Joint Surg Br, 86*(5), 696-700. doi:http://dx.doi.org/10.1302/0301-620X.91B7.22353

He, J., Fang, B., Shan, S., Xie, Y., Wang, C., Zhang, Y., . . . Li, Q. (2021). Mechanical stretch promotes hypertrophic scar formation through mechanically activated cation channel Piezo1. *Cell death & disease, 12*(3), 226. doi:http://dx.doi.org/10.1038/s41419-021-03481-6

Heath, M. E., & GIBBS, S. B. (1992). High-voltage pulsed galvanic stimulation: effects of frequency of current on blood flow in the human calf muscle. *Clinical Science, 82*(6), 607-613. doi:http://dx.doi.org/10.1042/cs0820607

Hecker, B., Carron, H., & Schwartz, D. (1985). Pulsed galvanic stimulation: effects of current frequency and polarity on blood flow in healthy subjects. *Archives of physical medicine and rehabilitation, 66*(6), 369-371.

Heinemeier, K., Langberg, H., Olesen, J. L., & Kjaer, M. (2003). Role of TGF-β1 in relation to exercise-induced type I collagen synthesis in human tendinous tissue. *Journal of applied physiology, 95*(6), 2390-2397. doi:http://dx.doi.org/10.1152/japplphysiol.00403.2003

Heir, T., & Glomsaker, P. (1996). Epidemiology of musculoskeletal injuries among Norwegian conscripts undergoing basic military training. *Scandinavian journal of medicine & science in sports, 6*(3), 186-191. doi:http://dx.doi.org/10.1111/j.1600-0838.1996.tb00088.x

Herchenhan, A., Bayer, M. L., Eliasson, P., Magnusson, S. P., & Kjaer, M. (2015). Insulin-like growth factor I enhances collagen synthesis in engineered human tendon tissue. *Growth Hormone & IGF Research, 25*(1), 13-19. doi:http://dx.doi.org/10.1016/j.ghir.2014.09.001

Hernández-Bule, M. L., Trillo, M. Á., & Úbeda, A. (2014). Molecular mechanisms underlying antiproliferative and differentiating responses of hepatocarcinoma cells to subthermal electric stimulation. *PLoS One, 9*(1), e84636. doi:http://dx.doi.org/10.1371/journal.pone.0084636

Heynick, L. N., Johnston, S. A., & Mason, P. A. (2003). Radio frequency electromagnetic fields: cancer, mutagenesis, and genotoxicity. *Bioelectromagnetics, 24*(S6), S74-S100. doi:http://dx.doi.org/10.1002/bem.10162

Houghton, P. E., Campbell, K. E., Fraser, C. H., Harris, C., Keast, D. H., Potter, P. J., . . . Woodbury, M. G. (2010). Electrical stimulation therapy increases rate of healing of pressure ulcers in community-dwelling people with spinal cord

injury. *Archives of physical medicine and rehabilitation, 91*(5), 669-678. doi:http://dx.doi.org/10.1016/j.apmr.2009.12.026
Houghton, P. E., Kincaid, C. B., Lovell, M., Campbell, K. E., Keast, D. H., Woodbury, M. G., & Harris, K. A. (2003). Effect of electrical stimulation on chronic leg ulcer size and appearance. *Physical Therapy, 83*(1), 17-28. doi:http://dx.doi.org/10.1093/ptj/83.1.17
Huisman, E., Lu, A., McCormack, R. G., & Scott, A. (2014). Enhanced collagen type I synthesis by human tenocytes subjected to periodic in vitro mechanical stimulation. *BMC musculoskeletal disorders, 15*, 1-8. doi:http://dx.doi.org/10.1186/1471-2474-15-386
Ingber, D. (1991). Integrins as mechanochemical transducers. *Current opinion in cell biology, 3*(5), 841-848. doi:http://dx.doi.org/10.1016/0955-0674(91)90058-7
Ingber, D. E. (1993). Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. *Journal of Cell Science, 104*(3), 613-627. doi:http://dx.doi.org/10.1242/jcs.104.3.613
Ingber, D. E. (1997). Tensegrity: the architectural basis of cellular mechanotransduction. *Annual review of physiology, 59*(1), 575-599. doi:http://dx.doi.org/10.1146/annurev.physiol.59.1.575
Jacobson-Kram, D., Tepper, J., Kuo, P., San, R. H., Curry, P. T., Wagner, V. O., & Putman, D. L. (1997). Evaluation of potential genotoxicity of pulsed electric and electromagnetic fields used for bone growth stimulation. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 388*(1), 45-57. doi:http://dx.doi.org/10.1016/S1383-5718(96)00134-9
Järvinen, T. A. (2020). Neovascularisation in tendinopathy: from eradication to stabilisation? In (Vol. 54, pp. 1-2): BMJ Publishing Group Ltd and British Association of Sport and Exercise Medicine.
Jeon, J.-K., Park, S.-K., & Lee, J.-H. (2015). Effects of high voltage pulsed current stimulation with a visible contraction intensity on expression of TGF-β1 and synthesis of type I collagen in wound-induced white rats. *Journal of physical therapy science, 27*(5), 1485-1490. doi:http://dx.doi.org/10.1589/jpts.27.1485
Jiang, C., Shao, L., Wang, Q., & Dong, Y. (2012). Repetitive mechanical stretching modulates transforming growth factor-β induced collagen synthesis and apoptosis in human patellar tendon fibroblasts. *Biochemistry and Cell Biology, 90*(5), 667-674. doi:http://dx.doi.org/10.1139/o2012-024
Johnson, D. G., & Walker, C. L. (1999). Cyclins and cell cycle checkpoints. *Annual review of pharmacology and toxicology, 39*(1), 295-312. doi:http://dx.doi.org/10.1146/annurev.pharmtox.39.1.295
Jones, E. R., Jones, G. C., Legerlotz, K., & Riley, G. P. (2013). Cyclical strain modulates metalloprotease and matrix gene expression in human tenocytes via activation of TGFβ. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1833*(12), 2596-2607. doi:http://dx.doi.org/10.1016/j.bbamcr.2013.06.019
Jy, W., Fregien, N., Bourguignon, G., & Bourguignon, L. Y. (1989). Role of Ca2+ in the regulation of hormone receptor exposure during lymphocyte activation. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 983*(2), 153-160. doi:http://dx.doi.org/10.1016/0005-2736(89)90228-9
Kang, E.-J., Roh, J.-S., Lee, J.-S., Yi, C.-H., & Kim, T.-H. (1996). A comparison of the inhibitive effect of high voltage pulsed current stimulation and microcurrent electrical neuromuscular stimulation on bacterial growth. *Physical Therapy Korea, 3*(1), 12-23.

Kastan, M. B., & Bartek, J. (2004). Cell-cycle checkpoints and cancer. *Nature, 432*(7015), 316-323. doi:http://dx.doi.org/10.1038/nature03097
Katsumi, A., Orr, A. W., Tzima, E., & Schwartz, M. A. (2004). Integrins in mechanotransduction. *Journal of Biological Chemistry, 279*(13), 12001-12004. doi:http://dx.doi.org/10.1074/jbc.R300038200
Kaux, J.-F., Forthomme, B., Le Goff, C., Crielaard, J.-M., & Croisier, J.-L. (2011). Current opinions on tendinopathy. *Journal of sports science & medicine, 10*(2), 238.
Kayama, T., Mori, M., Ito, Y., Matsushima, T., Nakamichi, R., Suzuki, H., . . . Asahara, H. (2016). Gtf2ird1-dependent mohawk expression regulates mechanosensing properties of the tendon. *Molecular and cellular biology, 36*(8), 1297-1309. doi:http://dx.doi.org/10.1128/MCB.00950-15
Kenamond, C., Weinhold, P., Bynum, D., Tsuzaki, M., Benjamin, M., Ralphs, J., . . . Banes, A. (1997). Human tendon cells express connexin-43 and propagate a calcium wave in response to mechanical stimulation. *Trans Orthop Res Soc, 22*(179-180), 116.
Khan, K. M., Cook, J. L., Bonar, F., Harcourt, P., & Åstrom, M. (1999). Histopathology of common tendinopathies. *Sports Medicine, 27*(6), 393-408. doi:http://dx.doi.org/10.2165/00007256-199927060-00004
Khan, K. M., Cook, J. L., Kannus, P., Maffulli, N., & Bonar, S. (2002). Time to abandon the “tendinitis” myth: painful, overuse tendon conditions have a non-inflammatory pathology. In: British Medical Journal Publishing Group.
Kibler, W. (1990). Clinical aspects of muscle injury. *Med Sci Sports Exerc, 22*(4), 450-452.
Kim, G. K. (2010). The risk of fluoroquinolone-induced tendinopathy and tendon rupture: what does the clinician need to know? *The Journal of clinical and aesthetic dermatology, 3*(4), 49.
Kim, S.-H., Park, R.-J., & Kwon, H.-C. (1996). Effect of High Voltage Pulsed Galvanic Current on Wound Healing in Rabbits. *Physical Therapy Korea, 3*(3), 67-81.
Kim, T. H., Cho, H.-y., & Lee, S. M. (2014). High-voltage pulsed current stimulation enhances wound healing in diabetic rats by restoring the expression of collagen, α-smooth muscle actin, and TGF-β1. *The Tohoku journal of experimental medicine, 234*(1), 1-6. doi:http://dx.doi.org/10.1620/tjem.234.1
Kincaid, C. B., & Lavoie, K. H. (1989). Inhibition of bacterial growth in vitro following stimulation with high voltage, monophasic, pulsed current. *Physical Therapy, 69*(8), 651-655. doi:http://dx.doi.org/10.1093/ptj/69.8.651
Kirchgesner, T., Larbi, A., Omoumi, P., Malghem, J., Zamali, N., Manelfe, J., . . . Dallaudière, B. (2014). Drug-induced tendinopathy: from physiology to clinical applications. *Joint Bone Spine, 81*(6), 485-492. doi:http://dx.doi.org/10.1016/j.jbspin.2014.03.022
Kraemer, R., Wuerfel, W., Lorenzen, J., Busche, M., Vogt, P. M., & Knobloch, K. (2012). Analysis of hereditary and medical risk factors in Achilles tendinopathy and Achilles tendon ruptures: a matched pair analysis. *Archives of Orthopaedic and Trauma Surgery, 132*(6), 847-853. doi:http://dx.doi.org/10.1007/s00402-012-1476-9
Kuykendall, C. A., Hutchinson, T. L., & Bloedaum, A. P. (2004). 112 effects of high voltage pulsed current on bacterial viability: an in vitro study. *Wound Repair and Regeneration, 12*(2), A29-A29. doi:http://dx.doi.org/10.1111/j.1067-1927.2004.0abstractdf.x

Lampe, K. E. (1998). Electrotherapy in tissue repair. *Journal of Hand Therapy, 11*(2), 131-139. doi:http://dx.doi.org/10.1016/S0894-1130(98)80011-2
Lavagnino, M., Arnoczky, S., Caballero, O., Robertson, E., & Nashi, S. (2006). In vitro stress-deprivation alters the mechanostat set point of tendon cells. *Trans Orthop Res Soc, 31*, 329.
Lavagnino, M., & Arnoczky, S. P. (2005). In vitro alterations in cytoskeletal tensional homeostasis control gene expression in tendon cells. *Journal of orthopaedic research, 23*(5), 1211-1218. doi:http://dx.doi.org/10.1016/j.orthres.2005.04.001
Lavagnino, M., Wall, M. E., Little, D., Banes, A. J., Guilak, F., & Arnoczky, S. P. (2015). Tendon mechanobiology: Current knowledge and future research opportunities. *Journal of orthopaedic research, 33*(6), 813-822. doi:http://dx.doi.org/10.1002/jor.22871
LeBleu, V. S., & Neilson, E. G. (2020). Origin and functional heterogeneity of fibroblasts. *The FASEB Journal, 34*(3), 3519-3536. doi:http://dx.doi.org/10.1096/fj.201903188R
Lee, J.-H., Jekal, S.-J., & Park, R.-J. (2002). Enhance of migration and proliferation of cells from tendon biopsies by high voltage pulsed current stimulation. *Journal of Korean Physical Therapy, 14*(2), 162-171.
Lee, J.-H., Park, C.-E., & Park, R.-J. (2010). Electrical stimulation induces the collagen deposition and TGF-β1 mRNA expression in skin wound of rat. *Journal of Korean Physical Therapy, 22*(3), 87-92.
Lee, J.-h., Song, I.-y., & Jong-Gyu, K. (2003). Acceleration of wound healing and collagen deposition in rat skin by high voltage pulsed current stimulation. *Journal of Korean Physical Therapy, 15*(4), 1-12.
Legerlotz, K., Jones, G., Screen, H., & Riley, G. (2013). Cyclic loading of tendon fascicles using a novel fatigue loading system increases interleukin-6 expression by tenocytes. *Scandinavian journal of medicine & science in sports, 23*(1), 31-37. doi:http://dx.doi.org/10.1111/j.1600-0838.2011.01410.x
Leong, H. T., Chuen Fu, S., He, X., Han Oh, J., Yamamoto, N., & Yung, S. H. P. (2019). Risk factors for rotator cuff tendinopathy: a systematic review and meta-analysis. *Journal of Rehabilitation Medicine, 51*(9), 627-637. doi:http://dx.doi.org/10.2340/16501977-2598
Levine, D., & Bockstahler, B. (2014). Electrical stimulation. *Canine rehabilitation and physical therapy, 2*, 342-358.
Lipscombe, D., Helton, T. D., & Xu, W. (2004). L-type calcium channels: the low down. *Journal of Neurophysiology, 92*(5), 2633-2641. doi:http://dx.doi.org/10.1152/jn.00486.2004
Lo, I. K., Marchuk, L. L., Hollinshead, R., Hart, D. A., & Frank, C. B. (2004). Matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase mRNA levels are specifically altered in torn rotator cuff tendons. *The American Journal of Sports Medicine, 32*(5), 1223-1229. doi:http://dx.doi.org/10.1177/0363546503262200
Macchi, M., Spezia, M., Elli, S., Schiaffini, G., & Chisari, E. (2020). Obesity increases the risk of tendinopathy, tendon tear and rupture, and postoperative complications: a systematic review of clinical studies. *Clinical Orthopaedics and Related Research®, 478*(8), 1839-1847. doi:http://dx.doi.org/10.1097/CORR.0000000000001261
Maeda, E., Fleischmann, C., Mein, C. A., Shelton, J. C., Bader, D. L., & Lee, D. A. (2010). Functional analysis of tenocytes gene expression in tendon fascicles

subjected to cyclic tensile strain. *Connective tissue research, 51*(6), 434-444. doi:http://dx.doi.org/10.3109/03008201003597056
Maeda, E., Shelton, J. C., Bader, D. L., & Lee, D. A. (2009). Differential regulation of gene expression in isolated tendon fascicles exposed to cyclic tensile strain in vitro. *Journal of applied physiology, 106*(2), 506-512. doi:http://dx.doi.org/10.1152/japplphysiol.90981.2008
Maeda, T., Sakabe, T., Sunaga, A., Sakai, K., Rivera, A. L., Keene, D. R., . . . Schweitzer, R. (2011). Conversion of mechanical force into TGF-β-mediated biochemical signals. *Current Biology, 21*(11), 933-941. doi:http://dx.doi.org/10.1016/j.cub.2011.04.007
Maffulli, N. (1998). Overuse tendon conditions: time to change a confusing terminology. *Arthroscopy, 14*(8), 840-843.
Maffulli, N., Wong, J., & Almekinders, L. C. (2003). Types and epidemiology of tendinopathy. *Clin Sports Med, 22*(4), 675-692. doi:http://dx.doi.org/10.1016/S0278-5919(03)00004-8
Magra, M., & Maffulli, N. (2005). Matrix metalloproteases: a role in overuse tendinopathies. *British journal of sports medicine, 39*(11), 789-791. doi:http://dx.doi.org/10.1136/bjsm.2005.017855
Mallon, W. J., Misamore, G., Snead, D. S., & Denton, P. (2004). The impact of preoperative smoking habits on the results of rotator cuff repair. *Journal of shoulder and elbow surgery, 13*(2), 129-132. doi:http://dx.doi.org/10.1016/j.jse.2003.11.002
Maltser, S., Cristian, A., Silver, J. K., Morris, G. S., & Stout, N. L. (2017). A focused review of safety considerations in cancer rehabilitation. *PM&R, 9*(9), S415-S428. doi:http://dx.doi.org/10.1016/j.pmrj.2017.08.403
Mantilla, N., Paris, B., Abcarian, H., Cintron, J., Zavala, A., & Singer, M. (2014). Efficacy of electrogalvanic stimulation in treatment of levator ani syndrome revisited. *J Surg, 10*, 2. doi:http://dx.doi.org/10.7438/1584-9341-10-2-12
Marti, H., Stoller, R., & Frey, F. (1998). Fluoroquinolones as a cause of tendon disorders in patients with renal failure/renal transplants. *British journal of rheumatology, 37*(3), 343-344. doi:http://dx.doi.org/10.1093/rheumatology/37.3.343
Matsuki, N., Ishikawa, T., Imai, Y., & Yamaguchi, T. (2008). Low voltage pulses can induce apoptosis. *Cancer letters, 269*(1), 93-100. doi:http://dx.doi.org/10.1016/j.canlet.2008.04.019
Matsuki, N., Takeda, M., Ishikawa, T., Kinjo, A., Hayasaka, T., Imai, Y., & Yamaguchi, T. (2010). Activation of caspases and apoptosis in response to low-voltage electric pulses. *Oncology reports, 23*(5), 1425-1433. doi:http://dx.doi.org/10.3892/or_00000780
Mattson, M. P., & Chan, S. L. (2003). Calcium orchestrates apoptosis. *Nature cell biology, 5*(12), 1041-1043. doi:http://dx.doi.org/10.1038/ncb1203-1041
McCann, J., Dietrich, F., & Rafferty, C. (1998). The genotoxic potential of electric and magnetic fields: an update. *Mutation Research/Reviews in Mutation Research, 411*(1), 45-86. doi:http://dx.doi.org/10.1016/S1383-5742(98)00006-4
McCann, J., Dietrich, F., Rafferty, C., & Martin, A. O. (1993). A critical review of the genotoxic potential of electric and magnetic fields. *Mutation Research/Reviews in Genetic Toxicology, 297*(1), 61-95. doi:http://dx.doi.org/10.1016/0165-1110(93)90008-B
McNeilly, C., Banes, A., Benjamin, M., & Ralphs, J. (1996). Tendon cells in vivo form a three dimensional network of cell processes linked by gap junctions. *Journal*

*of anatomy, 189*(Pt 3), 593. doi:http://dx.doi.org/10.1046/j.1469-7580.1997.19030477.x
McQueen, F., Beckley, V., Crabbe, J., Robinson, E., Yeoman, S., & Stewart, N. (2005). Magnetic resonance imaging evidence of tendinopathy in early rheumatoid arthritis predicts tendon rupture at six years. *Arthritis & Rheumatism, 52*(3), 744-751. doi:http://dx.doi.org/10.1002/art.20947
Mendel, F., Caputi, C., Karnes, J., & Fish, D. (1992). High voltage pulsed current using surface electrodes: effect on acute edema formation after hyperflexion injury in frogs. *Journal of Orthopaedic & Sports Physical Therapy, 16*(3), 140-144. doi:http://dx.doi.org/10.2519/jospt.1992.16.3.140
Mendel, F. C., & Fish, D. R. (1993). New perspectives in edema control via electrical stimulation. *Journal of Athletic Training, 28*(1), 63.
Mendel, F. C., Wylegala, J. A., & Fish, D. R. (1992). Influence of high voltage pulsed current on edema formation following impact injury in rats. *Physical Therapy, 72*(9), 668-673. doi:http://dx.doi.org/10.1093/ptj/72.9.668
Merkel, M. F. R., Hellsten, Y., Magnusson, S. P., & Kjaer, M. (2021). Tendon blood flow, angiogenesis, and tendinopathy pathogenesis. *Translational sports medicine, 4*(6), 756-771. doi:http://dx.doi.org/10.1002/tsm2.280
Merriman, H. L., Hegyi, C. A., Albright-Overton, C. R., Carlos Jr, J., Putnam, R. W., & Mulcare, J. A. (2004). A comparison of four electrical stimulation types on Staphylococcus aureus growth in vitro. *Journal of rehabilitation research and development, 41*(2), 139. doi:http://dx.doi.org/10.1682/JRRD.2004.02.0139
MEYER, A. W. (1921). Unrecognized occupational destruction of the tendon of the long head of the biceps brachii. *Archives of Surgery, 2*(1), 130-144. doi:http://dx.doi.org/10.1001/archsurg.1921.01110040139007
Meyer, A. W. (1924). Further evidences of attrition in the human body. *American Journal of Anatomy, 34*(1), 241-267. doi:http://dx.doi.org/10.1002/aja.1000340108
Michlovitz, S., Smith, W., & Watkins, M. (1988). Ice and high voltage pulsed stimulation in treatment of acute lateral ankle sprains. *Journal of Orthopaedic & Sports Physical Therapy, 9*(9), 301-304. doi:http://dx.doi.org/10.2519/jospt.1988.9.9.301
Mohr, T., Akers, T. K., & Wessman, H. C. (1987). Effect of high voltage stimulation on blood flow in the rat hind limb. *Physical Therapy, 67*(4), 526-533. doi:http://dx.doi.org/10.1093/ptj/67.4.526
Mohr, T. M., Akers, T. K., & Landry, R. G. (1987). Effect of high voltage stimulation on edema reduction in the rat hind limb. *Physical Therapy, 67*(11), 1703-1707. doi:http://dx.doi.org/10.1093/ptj/67.11.1703
Moroni, M., Servin-Vences, M. R., Fleischer, R., Sánchez-Carranza, O., & Lewin, G. R. (2018). Voltage gating of mechanosensitive PIEZO channels. *Nature communications, 9*(1), 1096. doi:http://dx.doi.org/10.1038/s41467-018-03502-7
Morris, L., & Newton, R. A. (1987). Use of high voltage pulsed galvanic stimulation for patients with levator ani syndrome. *Physical Therapy, 67*(10), 1522-1525. doi:http://dx.doi.org/10.1093/ptj/67.10.1522
Mosca, M. J., Rashid, M. S., Snelling, S. J., Kirtley, S., Carr, A. J., & Dakin, S. G. (2018). Trends in the theory that inflammation plays a causal role in tendinopathy: a systematic review and quantitative analysis of published reviews. *BMJ Open Sport—Exercise Medicine, 4*(1).

Muhl, L., Genové, G., Leptidis, S., Liu, J., He, L., Mocci, G., . . . Chivukula, I. V. (2020). Single-cell analysis uncovers fibroblast heterogeneity and criteria for fibroblast and mural cell identification and discrimination. *Nature communications, 11*(1), 3953. doi:http://dx.doi.org/10.1038/s41467-020-17740-1

Nakamichi, R., Ma, S., Nonoyama, T., Chiba, T., Kurimoto, R., Ohzono, H., . . . Wang, G. (2022). The mechanosensitive ion channel PIEZO1 is expressed in tendons and regulates physical performance. *Science translational medicine, 14*(647), eabj5557. doi:http://dx.doi.org/10.1126/scitranslmed.abj5557

Neviaser, A., Andarawis-Puri, N., & Flatow, E. (2012). Basic mechanisms of tendon fatigue damage. *Journal of shoulder and elbow surgery, 21*(2), 158-163. doi:http://dx.doi.org/10.1016/j.jse.2011.11.014

Newton, R. A., & Karselis, T. C. (1983). Skin pH following high voltage pulsed galvanic stimulation. *Physical Therapy, 63*(10), 1593-1596.

Nicotera, P., & Orrenius, S. (1998). The role of calcium in apoptosis. *Cell calcium, 23*(2-3), 173-180. doi:http://dx.doi.org/10.1016/S0143-4160(98)90116-6

Nwogbaga, I., Kim, A. H., & Camley, B. A. (2023). Physical limits on galvanotaxis. *Physical Review E, 108*(6), 064411. doi:http://dx.doi.org/10.1103/PhysRevE.108.064411

O 'Neil, R. G., & Heller, S. (2005). The mechanosensitive nature of TRPV channels. *Pflügers Archiv, 451*, 193-203. doi:http://dx.doi.org/10.1007/s00424-005-1424-4

Olesen, J. L., Heinemeier, K. M., Gemmer, C., Kjær, M., Flyvbjerg, A., & Langberg, H. (2007). Exercise-dependent IGF-I, IGFBPs, and type I collagen changes in human peritendinous connective tissue determined by microdialysis. *Journal of applied physiology, 102*(1), 214-220. doi:http://dx.doi.org/10.1152/japplphysiol.01205.2005

Oliva, F., Piccirilli, E., Berardi, A. C., Frizziero, A., Tarantino, U., & Maffulli, N. (2016). Hormones and tendinopathies: the current evidence. *British medical bulletin, 117*(1), 39-58. doi:http://dx.doi.org/10.1093/bmb/ldv054

Oprescu, S. N., Yue, F., Qiu, J., Brito, L. F., & Kuang, S. (2020). Temporal dynamics and heterogeneity of cell populations during skeletal muscle regeneration. *IScience, 23*(4). doi:http://dx.doi.org/10.1016/j.isci.2020.100993

Oshima, Y., Sakamoto, T., Kawano, Y.-I., Hata, Y., Yoshikawa, H., Sonoda, K.-H., . . . Inomata, H. (1998). Synergistic effect of electric pulses and bleomycin on cultured rabbit subconjunctival fibroblasts. *Graefe's archive for clinical and experimental ophthalmology, 236*, 52-60. doi:http://dx.doi.org/10.1007/s004170050042

Oshima, Y., Sakamoto, T., Sonoda, K.-h., Yoshida, H., Ishibashi, T., & Inomata, H. (1997). Effect of electric pulses and antiproliferative drugs on cultured bovine retinal pigment epithelial cells. *Current eye research, 16*(1), 64-70. doi:http://dx.doi.org/10.1076/ceyr.16.1.64.5112

Owens, B. D., Wolf, J. M., Seelig, A. D., Jacobson, I. G., Boyko, E. J., Smith, B., . . . Team, M. C. S. (2013). Risk factors for lower extremity tendinopathies in military personnel. *Orthopaedic Journal of Sports Medicine, 1*(1), 2325967113492707. doi:http://dx.doi.org/10.1177/2325967113492707

Paavola, M., & Järvinen, T. A. (2005). Paratendinopathy. *Foot Ankle Clin, 10*(2), 279-292. doi:http://dx.doi.org/10.1016/j.fcl.2005.01.008

Paavola, M., Kannus, P., Järvinen, T. A., Khan, K., Józsa, L., & Järvinen, M. (2002). Achilles tendinopathy. *JBJS, 84*(11), 2062-2076.

Passaretti, D., Candela, V., Venditto, T., Giannicola, G., & Gumina, S. (2016). Association between alcohol consumption and rotator cuff tear. *Acta orthopaedica, 87*(2), 165-168. doi:http://dx.doi.org/10.3109/17453674.2015.1119599
Passini, F. S., Jaeger, P. K., Saab, A. S., Hanlon, S., Chittim, N. A., Arlt, M. J., . . . Bollhalder, M. (2021). Shear-stress sensing by PIEZO1 regulates tendon stiffness in rodents and influences jumping performance in humans. *Nature biomedical engineering, 5*(12), 1457-1471. doi:http://dx.doi.org/10.1038/s41551-021-00716-x
Perez-Reyes, E. (2003). Molecular physiology of low-voltage-activated t-type calcium channels. *Physiological reviews, 83*(1), 117-161. doi:http://dx.doi.org/10.1152/physrev.00018.2002
Perez-Reyes, E., & Schneider, T. (1995). Molecular biology of calcium channels. *Kidney international, 48*(4), 1111-1124. doi:http://dx.doi.org/10.1038/ki.1995.395
Perez-Reyes, E., & Schneider, T. (1994). Calcium channels: structure, function, and classification. *Drug development research, 33*(3), 295-318. doi:http://dx.doi.org/10.1002/ddr.430330311
Petrany, M. J., Swoboda, C. O., Sun, C., Chetal, K., Chen, X., Weirauch, M. T., . . . Millay, D. P. (2020). Single-nucleus RNA-seq identifies transcriptional heterogeneity in multinucleated skeletal myofibers. *Nature communications, 11*(1), 6374. doi:http://dx.doi.org/10.1038/s41467-020-20063-w
Philippeos, C., Telerman, S. B., Oulès, B., Pisco, A. O., Shaw, T. J., Elgueta, R., . . . Lynch, M. D. (2018). Spatial and single-cell transcriptional profiling identifies functionally distinct human dermal fibroblast subpopulations. *Journal of Investigative Dermatology, 138*(4), 811-825. doi:http://dx.doi.org/10.1016/j.jid.2018.01.016
Plinsinga, M. L., Brink, M. S., Vicenzino, B., & Van Wilgen, C. P. (2015). Evidence of nervous system sensitization in commonly presenting and persistent painful tendinopathies: a systematic review. *Journal of Orthopaedic & Sports Physical Therapy, 45*(11), 864-875. doi:http://dx.doi.org/10.2519/jospt.2015.5895
Polak, A., Kloth, L. C., Blaszczak, E., Taradaj, J., Nawrat-Szoltysik, A., Ickowicz, T., . . . Kucio, C. (2017). The efficacy of pressure ulcer treatment with cathodal and cathodal-anodal high-voltage monophasic pulsed current: a prospective, randomized, controlled clinical trial. *Physical Therapy, 97*(8), 777-789.
Polak, A., Kloth, L. C., Blaszczak, E., Taradaj, J., Nawrat-Szoltysik, A., Walczak, A., . . . Kucio, C. (2016). Evaluation of the healing progress of pressure ulcers treated with cathodal high-voltage monophasic pulsed current: results of a prospective, double-blind, randomized clinical trial. *Advances in skin & wound care, 29*(10), 447-459. doi:http://dx.doi.org/10.1097/01.ASW.0000493164.75337.de
Polak, A., Kucio, C., Kloth, L. C., Paczula, M., Hordynska, E., Ickowicz, T., . . . Ficek, K. (2018). A randomized, controlled clinical study to assess the effect of anodal and cathodal electrical stimulation on periwound skin blood flow and pressure ulcer size reduction in persons with neurological injuries. *OSTOMY WOUND MANAGEMENT*. doi:http://dx.doi.org/10.25270/owm.2018.2.1029
Polak, A., Taradaj, J., Nawrat-Szoltysik, A., Stania, M., Dolibog, P., Blaszczak, E., . . . Kucio, C. (2016). Reduction of pressure ulcer size with high-voltage pulsed current and high-frequency ultrasound: a randomised trial. *Journal of Wound Care, 25*(12), 742-754. doi:http://dx.doi.org/10.12968/jowc.2016.25.12.742

Popov, C., Burggraf, M., Kreja, L., Ignatius, A., Schieker, M., & Docheva, D. (2015). Mechanical stimulation of human tendon stem/progenitor cells results in upregulation of matrix proteins, integrins and MMPs, and activation of p38 and ERK1/2 kinases. *BMC Molecular Biology, 16*(1), 1-11. doi:http://dx.doi.org/10.1186/s12867-015-0036-6

Puddu, G., Ippolito, E., & Postacchini, F. (1976). A classification of Achilles tendon disease. *The American Journal of Sports Medicine, 4*(4), 145-150. doi:http://dx.doi.org/10.1177/036354657600400404

Pufe, T., Petersen, W., Mentlein, R., & Tillmann, B. (2005). The role of vasculature and angiogenesis for the pathogenesis of degenerative tendons disease. *Scandinavian journal of medicine & science in sports, 15*(4), 211-222.

Rampazo, É. P., Liebano, R. E., Pinfildi, C. E., Folha, R. A., & Ferreira, L. M. (2016). High voltage pulsed current in collagen realignment, synthesis, and angiogenesis after Achilles tendon partial rupture. *Brazilian Journal of Physical Therapy, 20*, 312-319. doi:http://dx.doi.org/10.1590/bjpt-rbf.2014.0167

Ranade, S. S., Syeda, R., & Patapoutian, A. (2015). Mechanically activated ion channels. *Neuron, 87*(6), 1162-1179. doi:http://dx.doi.org/10.1016/j.neuron.2015.08.032

Ranger, T. A., Wong, A. M., Cook, J. L., & Gaida, J. E. (2016). Is there an association between tendinopathy and diabetes mellitus? A systematic review with meta-analysis. *British journal of sports medicine, 50*(16), 982-989. doi:http://dx.doi.org/10.1136/bjsports-2015-094735

Reed, B. V. (1988). Effect of high voltage pulsed electrical stimulation on microvascular permeability to plasma proteins: a possible mechanism in minimizing edema. *Physical Therapy, 68*(4), 491-495. doi:http://dx.doi.org/10.1093/ptj/68.4.491

Rees, J., Wilson, A., & Wolman, R. (2006). Current concepts in the management of tendon disorders. *Rheumatology, 45*(5), 508-521. doi:http://dx.doi.org/10.1093/rheumatology/kel046

Rees, J. D., Maffulli, N., & Cook, J. (2009). Management of tendinopathy. *The American Journal of Sports Medicine, 37*(9), 1855-1867. doi:http://dx.doi.org/10.1177/0363546508324283

Rees, J. D., Stride, M., & Scott, A. (2014). Tendons–time to revisit inflammation. *British journal of sports medicine, 48*(21), 1553-1557. doi:http://dx.doi.org/10.1136/bjsports-2012-091957

Riley, G. (2004). The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology, 43*(2), 131-142. doi:http://dx.doi.org/10.1093/rheumatology/keg448

Riley, G. P., Curry, V., DeGroot, J., van El, B., Verzijl, N., Hazleman, B. L., & Bank, R. A. (2002). Matrix metalloproteinase activities and their relationship with collagen remodelling in tendon pathology. *Matrix Biology, 21*(2), 185-195. doi:http://dx.doi.org/10.1016/S0945-053X(01)00196-2

Rocio Servin-Vences, M., Moroni, M., Lewin, G. R., & Poole, K. (2017). Direct measurement of TRPV4 and PIEZO1 activity reveals multiple mechanotransduction pathways in chondrocytes. *Elife, 6*, e21074.

Sanderson, M. J., Charles, A. C., Boitano, S., & Dirksen, E. R. (1994). Mechanisms and function of intercellular calcium signaling. *Molecular and cellular endocrinology, 98*(2), 173-187. doi:http://dx.doi.org/10.1016/0303-7207(94)90136-8

Sandoval, M. C., Ramirez, C., Camargo, D. M., & Salvini, T. F. (2010). Effect of high-voltage pulsed current plus conventional treatment on acute ankle sprain. *Brazilian Journal of Physical Therapy, 14*, 193-199. doi:http://dx.doi.org/10.1590/S1413-35552010000300012

Santana de Souza, A. C., Lomasso Costa, M. M., Vieira Costa, P. H., & Souza da Silva, F. (2017). Efeito da estimulação elétrica de alta voltagem para o tratamento de úlceras por pressão: um estudo experimental de caso único. *Fisioterapia Brasil, 18*(6).

Scott, A., Squier, K., Alfredson, H., Bahr, R., Cook, J. L., Coombes, B., . . . Lewis, J. S. (2020). Icon 2019: international scientific tendinopathy symposium consensus: clinical terminology. *British journal of sports medicine, 54*(5), 260-262. doi:http://dx.doi.org/10.1136/bjsports-2019-100885

Sebastian, A., Allan, E., Allan, D., Colthurst, J., & Bayat, A. (2011). Addition of novel degenerate electrical waveform stimulation with photodynamic therapy significantly enhances its cytotoxic effect in keloid fibroblasts: first report of a potential combination therapy. *Journal of Dermatological Science, 64*(3), 174-184. doi:http://dx.doi.org/10.1016/j.jdermsci.2011.08.012

Shen, P.-C., Chang, P.-C., Jou, I.-M., Chen, C.-H., Lee, F.-H., & Hsieh, J.-L. (2019). Hand tendinopathy risk factors in Taiwan: A population-based cohort study. *Medicine, 98*(1). doi:http://dx.doi.org/10.1097/MD.0000000000013795

Snyder, A. R., Perotti, A. L., Lam, K. C., & Bay, R. C. (2010). The influence of high-voltage electrical stimulation on edema formation after acute injury: a systematic review. *Journal of sport rehabilitation, 19*(4), 436-451. doi:http://dx.doi.org/10.1123/jsr.19.4.436

Sohn, N., Weinstein, M. A., & Robbins, R. D. (1982). The levator syndrome and its treatment with high-voltage electrogalvanic stimulation. *The American Journal of Surgery, 144*(5), 580-582. doi:http://dx.doi.org/10.1016/0002-9610(82)90586-4

Solé-Boldo, L., Raddatz, G., Schütz, S., Mallm, J.-P., Rippe, K., Lonsdorf, A. S., . . . Lyko, F. (2020). Single-cell transcriptomes of the human skin reveal age-related loss of fibroblast priming. *Communications biology, 3*(1), 188. doi:http://dx.doi.org/10.1038/s42003-020-0922-4

Solon, J., Levental, I., Sengupta, K., Georges, P. C., & Janmey, P. A. (2007). Fibroblast adaptation and stiffness matching to soft elastic substrates. *Biophysical journal, 93*(12), 4453-4461. doi:http://dx.doi.org/10.1529/biophysj.106.101386

Stecco, C., Cappellari, A., Macchi, V., Porzionato, A., Morra, A., Berizzi, A., & De Caro, R. (2014). The paratendineous tissues: an anatomical study of their role in the pathogenesis of tendinopathy. *Surgical and Radiologic Anatomy, 36*, 561-572. doi:http://dx.doi.org/10.1007/s00276-013-1244-8

Stiskal, M., Szolar, D. H., Stenzel, I., Steiner, E., MESARIC, P., Czembirek, H., & Preidler, K. W. (1997). Magnetic resonance imaging of Achilles tendon in patients with rheumatoid arthritis. *Investigative radiology, 32*(10), 602-608. doi:http://dx.doi.org/10.1097/00004424-199710000-00004

Stockbridge, L. L., & French, A. S. (1988). Stretch-activated cation channels in human fibroblasts. *Biophysical journal, 54*(1), 187-190. doi:http://dx.doi.org/10.1016/S0006-3495(88)82944-8

Stralka, S. W., Jackson, J. A., & Lewis, A. R. (1998). Treatment of hand and wrist pain: A randomized clinical trial of high voltage pulsed, direct current built into

a wrist splint. *AAOHN Journal, 46*(5), 233-236. doi:http://dx.doi.org/10.1177/216507999804600502
Stutchbury, B., Atherton, P., Tsang, R., Wang, D.-Y., & Ballestrem, C. (2017). Distinct focal adhesion protein modules control different aspects of mechanotransduction. *Journal of Cell Science, 130*(9), 1612-1624. doi:http://dx.doi.org/10.1242/jcs.195362
Su, Y. C., Chung, C. H., Ke, M. J., Chen, L. C., Chien, W. C., & Wu, Y. T. (2021). Increased risk of shoulder calcific tendinopathy in diabetes mellitus: a nationwide, population-based, matched cohort study. *International Journal of Clinical Practice*, e14549. doi:http://dx.doi.org/10.1111/ijcp.14549
Subramony, S. D., Dargis, B. R., Castillo, M., Azeloglu, E. U., Tracey, M. S., Su, A., & Lu, H. H. (2013). The guidance of stem cell differentiation by substrate alignment and mechanical stimulation. *Biomaterials, 34*(8), 1942-1953. doi:http://dx.doi.org/10.1016/j.biomaterials.2012.11.012
Sussman, C., & Bates-Jensen, B. M. (2001). Wound care: a collaborative practice manual for physical therapists and nurses. *(No Title)*.
Szuminsky, N. J., Albers, A. C., Unger, P., & Eddy, J. G. (1994). Effect of narrow, pulsed high voltages on bacterial viability. *Physical Therapy, 74*(7), 660-667. doi:http://dx.doi.org/10.1093/ptj/74.7.660
Tang, C., Chen, Y., Huang, J., Zhao, K., Chen, X., Yin, Z., . . . Shen, W. (2018). The roles of inflammatory mediators and immunocytes in tendinopathy. *Journal of Orthopaedic Translation, 14*, 23-33. doi:http://dx.doi.org/10.1016/j.jot.2018.03.003
Tashjian, R. Z., Farnham, J. M., Albright, F. S., Teerlink, C. C., & Cannon-Albright, L. A. (2009). Evidence for an inherited predisposition contributing to the risk for rotator cuff disease. *The Journal of Bone and Joint Surgery. American volume., 91*(5), 1136. doi:http://dx.doi.org/10.2106/JBJS.H.00831
Taylor, B., Cheema, A., & Soslowsky, L. (2017). Tendon pathology in hypercholesterolemia and familial hypercholesterolemia. *Current Rheumatology Reports, 19*(12), 1-6. doi:http://dx.doi.org/10.1007/s11926-017-0704-2
Taylor, K., Fish, D. R., Mendel, F. C., & Burton, H. W. (1992). Effect of a single 30-minute treatment of high voltage pulsed current on edema formation in frog hind limbs. *Physical Therapy, 72*(1), 63-68. doi:http://dx.doi.org/10.1093/ptj/72.1.63
Taylor, K., Mendel, F. C., Fish, D. R., Hard, R., & Burton, H. W. (1997). Effect of high-voltage pulsed current and alternating current on macromolecular leakage in hamster cheek pouch microcirculation. *Physical Therapy, 77*(12), 1729-1740. doi:http://dx.doi.org/10.1093/ptj/77.12.1729
Tempfer, H., & Traweger, A. (2015). Tendon vasculature in health and disease. *Frontiers in Physiology, 6*, 330. doi:http://dx.doi.org/10.3389/fphys.2015.00330
Theobald, P., Benjamin, M., Nokes, L., & Pugh, N. (2005). Review of the vascularisation of the human Achilles tendon. *Injury, 36*(11), 1267-1272. doi:http://dx.doi.org/10.1016/j.injury.2005.02.012
Thornton, R. M., Mendel, F. C., & Fish, D. R. (1998). Effects of electrical stimulation on edema formation in different strains of rats. *Physical Therapy, 78*(4), 386-394. doi:http://dx.doi.org/10.1093/ptj/78.4.386
Tilley, B. J., Cook, J. L., Docking, S. I., & Gaida, J. E. (2015). Is higher serum cholesterol associated with altered tendon structure or tendon pain? A

systematic review. *British journal of sports medicine, 49*(23), 1504-1509. doi:http://dx.doi.org/10.1136/bjsports-2015-095100
Tsai, W.-C., & Yang, Y.-M. (2011). Fluoroquinolone-associated tendinopathy. *Chang Gung Med J, 34*(5), 461-467.
Tsuzaki, M., Bynum, D., Almekinders, L., Yang, X., Faber, J., & Banes, A. (2003). ATP modulates load-inducible IL-1β, COX 2, and MMP-3 gene expression in human tendon cells. *J Cell Biochem, 89*(3), 556-562. doi:http://dx.doi.org/10.1002/jcb.10534
Tuite, D., Renström, P., & O'brien, M. (1997). The aging tendon. *Scandinavian journal of medicine & science in sports, 7*(2), 72-77. doi:http://dx.doi.org/10.1111/j.1600-0838.1997.tb00122.x
Tuoheti, Y., Itoi, E., Pradhan, R. L., Wakabayashi, I., Takahashi, S., Minagawa, H., . . . Shimada, Y. (2005). Apoptosis in the supraspinatus tendon with stage II subacromial impingement. *Journal of shoulder and elbow surgery, 14*(5), 535-541. doi:http://dx.doi.org/10.1016/j.jse.2005.01.001
Van Der Vlist, A. C., Breda, S. J., Oei, E. H., Verhaar, J. A., & de Vos, R.-J. (2019). Clinical risk factors for Achilles tendinopathy: a systematic review. *British journal of sports medicine, 53*(21), 1352-1361. doi:http://dx.doi.org/10.1136/bjsports-2018-099991
Van Dijk, C., Van Sterkenburg, M., Wiegerinck, J., Karlsson, J., & Maffulli, N. (2011). Terminology for Achilles tendon related disorders. *Knee Surgery, Sports Traumatology, Arthroscopy, 19*, 835-841. doi:http://dx.doi.org/10.1007/s00167-010-1374-z
Vorstandlechner, V., Laggner, M., Kalinina, P., Haslik, W., Radtke, C., Shaw, L., . . . Mildner, M. (2020). Deciphering the functional heterogeneity of skin fibroblasts using single-cell RNA sequencing. *The FASEB Journal, 34*(3), 3677-3692. doi:http://dx.doi.org/10.1096/fj.201902001RR
Waggett, A. D., Benjamin, M., & Ralphs, J. R. (2006). Connexin 32 and 43 gap junctions differentially modulate tenocyte response to cyclic mechanical load. *European journal of cell biology, 85*(11), 1145-1154. doi:http://dx.doi.org/10.1016/j.ejcb.2006.06.002
Walker, D. C., Currier, D. P., & Threlkeld, A. J. (1988). Effects of high voltage pulsed electrical stimulation on blood flow. *Physical Therapy, 68*(4), 481-485. doi:http://dx.doi.org/10.1093/ptj/68.4.481
Wall, M., & Banes, A. (2005). Early responses to mechanical load in tendon: role for calcium signaling, gap junctions and intercellular communication. *J Musculoskelet Neuronal Interact, 5*(1), 70-84.
Wang, J., Guo, J., Wu, S., Feng, H., Sun, S., Pan, J., . . . Beebe, S. J. (2012). Synergistic effects of nanosecond pulsed electric fields combined with low concentration of gemcitabine on human oral squamous cell carcinoma in vitro. doi:http://dx.doi.org/10.1371/journal.pone.0043213
Wang, J. H.-C. (2000). Substrate deformation determines actin cytoskeleton reorganization: a mathematical modeling and experimental study. *Journal of theoretical biology, 202*(1), 33-41. doi:http://dx.doi.org/10.1006/jtbi.1999.1035
Wang, J. H.-C. (2006). Mechanobiology of tendon. *Journal of biomechanics, 39*(9), 1563-1582. doi:http://dx.doi.org/10.1016/j.jbiomech.2005.05.011
Wang, J. H.-C., Jia, F., Yang, G., Yang, S., Campbell, B. H., Stone, D., & Woo, S. L.-Y. (2003). Cyclic mechanical stretching of human tendon fibroblasts increases the production of prostaglandin E 2 and levels of cyclooxygenase expression:

a novel in vitro model study. *Connective tissue research, 44*(3-4), 128-133. doi:http://dx.doi.org/10.1080/03008200390223909
Wang, N., Butler, J. P., & Ingber, D. E. (1993). Mechanotransduction across the cell surface and through the cytoskeleton. *Science, 260*(5111), 1124-1127. doi:http://dx.doi.org/10.1007/s00249-009-0542-x
Wang, N., & Ingber, D. E. (1995). Probing transmembrane mechanical coupling and cytomechanics using magnetic twisting cytometry. *Biochemistry and Cell Biology, 73*(7-8), 327-335. doi:http://dx.doi.org/10.1139/o95-041
Wasker, S. V. Z., Challoumas, D., Weng, W., Murrell, G. A., & Millar, N. L. (2023). Is neurogenic inflammation involved in tendinopathy? A systematic review. *BMJ open sport & exercise medicine, 9*(1), e001494. doi:http://dx.doi.org/10.1136/bmjsem-2022-001494
Watson, P. (1991). Function follows form: generation of intracellular signals by cell deformation. *The FASEB Journal, 5*(7), 2013-2019. doi:http://dx.doi.org/10.1096/fasebj.5.7.1707019
Windsor, R. E., Lester, J. P., & Herring, S. A. (1993). Electrical stimulation in clinical practice. *The Physician and sportsmedicine, 21*(2), 85-93. doi:http://dx.doi.org/10.1080/00913847.1993.11947556
Wise, B. L., Peloquin, C., Choi, H., Lane, N. E., & Zhang, Y. (2012). Impact of age, sex, obesity, and steroid use on quinolone-associated tendon disorders. *The American journal of medicine, 125*(12), 1228. e1223-1228. e1228. doi:http://dx.doi.org/10.1016/j.amjmed.2012.05.027
Wong, R. A. (1986). High voltage versus low voltage electrical stimulation: force of induced muscle contraction and perceived discomfort in healthy subjects. *Physical Therapy, 66*(8), 1209-1214. doi:http://dx.doi.org/10.1093/ptj/66.8.1209
Woodley, B. L., Newsham-West, R. J., & Baxter, G. D. (2007). Chronic tendinopathy: effectiveness of eccentric exercise. *British journal of sports medicine, 41*(4), 188-198. doi:http://dx.doi.org/10.1136/bjsm.2006.029769
Wozniak, M. A., Modzelewska, K., Kwong, L., & Keely, P. J. (2004). Focal adhesion regulation of cell behavior. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1692*(2-3), 103-119. doi:http://dx.doi.org/10.1016/j.bbamcr.2004.04.007
Wu, M., Rubin, A. E., Dai, T., Schloss, R., Usta, O. B., Golberg, A., & Yarmush, M. (2021). High-voltage, pulsed electric fields eliminate Pseudomonas aeruginosa stable infection in a mouse burn model. *Advances in Wound Care, 10*(9), 477-489. doi:http://dx.doi.org/10.1089/wound.2019.1147
Xu, Y., & Murrell, G. A. (2008). The basic science of tendinopathy. *Clin Orthop Relat Res, 466*(7), 1528-1538. doi:http://dx.doi.org/10.1007/s11999-008-0286-4
Yang, G., Crawford, R. C., & Wang, J. H. (2004). Proliferation and collagen production of human patellar tendon fibroblasts in response to cyclic uniaxial stretching in serum-free conditions. *Journal of biomechanics, 37*(10), 1543-1550. doi:http://dx.doi.org/10.1016/j.jbiomech.2004.01.005
Yuan, J., Murrell, G. A., Trickett, A., & Wang, M.-X. (2003). Involvement of cytochrome c release and caspase-3 activation in the oxidative stress-induced apoptosis in human tendon fibroblasts. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1641*(1), 35-41. doi:http://dx.doi.org/10.1016/S0167-4889(03)00047-8

Yuan, J., Murrell, G. A., Wei, A. Q., & Wang, M. X. (2002). Apoptosis in rotator cuff tendonopathy. *Journal of orthopaedic research, 20*(6), 1372-1379. doi:http://dx.doi.org/10.1016/S0736-0266(02)00075-X

Zabraniecki, L., Negrier, I., Vergne, P., Arnaud, M., Bonnet, C., Bertin, P., & Treves, R. (1996). Fluoroquinolone induced tendinopathy: report of 6 cases. *Journal of Rheumatology, 23*(3), 516-520.

Zabrzyński, J., Huri, G., Gagat, M., Łapaj, Ł., Yataganbaba, A., Szwedowski, D., . . . Paczesny, Ł. (2021). The impact of smoking on clinical results following the rotator cuff and biceps tendon complex arthroscopic surgery. *Journal of Clinical Medicine, 10*(4), 599. doi:http://dx.doi.org/10.3390/jcm10040599

Zhang, J., & Wang, J. H. (2010). Characterization of differential properties of rabbit tendon stem cells and tenocytes. *BMC musculoskeletal disorders, 11*(1), 1-11. doi:http://dx.doi.org/10.1186/1471-2474-11-10

Zhao, M., Penninger, J., & Isseroff, R. R. (2010). Electrical activation of wound-healing pathways. *Adv Skin Wound Care, 1*(1), 567-573. doi:http://dx.doi.org/10.1089/9781934854013.567

Zhou, K., Krug, K., Stachura, J., Niewczyk, P., Ross, M., Tutuska, J., & Ford, G. (2016). Silver-Collagen Dressing and High-voltage, Pulsed-current Therapy for the Treatment of Chronic Full-thickness Wounds: A Case Series. *Ostomy/wound management, 62*(3), 36-44.

Zoghi, M., & Jaberzadeh, S. (2002). Effects of high voltage electro-auriculotherapy on experimental pain threshold. *Physiotherapy, 88*(11), 658-666. doi:http://dx.doi.org/10.1016/S0031-9406(05)60108-3

# Dissertation articles

**Chapter II**

**State of the art**

Study I. Failed healing response: are pressure ulcers and tendinopathies two faces of the same coin? (submitted)

Study II. Physical therapy for tendinopathy: An umbrella review of systematic reviews and meta-analyses

Study III. Efficacy of physical therapy interventions for chronic lateral elbow tendinopathy: a systematic review

**Chapter III**

**Original manuscripts**

Study IV. High Voltage Monophasic Pulsed Current (HVMPC) for stage II-IV pressure ulcer healing. A systematic review and meta-analysis

Study V. The effect of high-voltage monophasic pulsed current on diabetic ulcers and their potential pathophysiologic factors: A systematic review and meta-analysis

Study VI. Induction and potential treatment of overuse tendon injury (in preparation)

# Chapter II

## *State of the art*

I

**Failed healing response: are pressure ulcers and tendinopathies two faces of the same coin?**


## Abstract

Striking similarities despite obvious differences may characterize the pathophysiology of pressure ulcers and tendinopathies. Mesenchymal stem cells are common precursors to both dermal fibroblasts and tenocytes. Dedifferentiation of dermal fibroblasts and subsequent redifferentiation to various connective tissue cells, which include tenocytes, has been found to occur during regeneration. Moreover, induced pluripotency of human dermal fibroblasts to form all lineages, including tenocytes, was successfully achieved. Mechanical overload, aging, and sustained inflammatory state may contribute to failed healing response in both conditions.


## Connective tissue structure

Despite the obvious differences between skin dermis and tendon tissues, both structures may be considered examples of fibrous connective tissues, due to their composition of resident fibroblasts, and extracellular matrix (Culav et al., 1999; Dunn & Silver, 1983; Oxlund, 1986). Their extracellular matrices are predominantly composed of collagen, elastin, microfibrils, proteoglycans, and glycoproteins, although in varying proportions (Pfisterer et al., 2021; Screen et al., 2015). Type I collagen is the main collagen of ligaments, tendons, skin, and many interstitial connective tissues (Henriksen & Karsdal, 2016). Collagen type I typically represents more than 95% of total collagen content of normal tendons, assuming that hydroxyproline content from non-collagenous proteins is negligible (Riley et al., 1994b). Small amounts of type III collagen, among others, may also be present, in macroscopically normal tendons (on average less than 5% of total collagen content) (Riley et al., 1994b; von der Mark, 1981). Type III collagen may be also found in the endotenon and epitenon of normal tendons (Becker et al., 1976; Duance et al., 1977). Both types, however, are typically present in skin dermis of adults, with type I collagen being much more abundant than type III collagen (Cheng et al., 2011; Epstein Jr & Munderloh, 1978; Lovell et al., 1987).

The ratio of type I: III collagens is determined to range from 81-90%: 10-19% at different depths of the skin dermis (Epstein Jr & Munderloh, 1978).

It is well known that fibroblasts are diverse mesenchymal cells that are responsible for the synthesis of collagens, proteoglycans, elastin, fibronectin, microfibrillar proteins and laminins (Plikus et al., 2021). Moreover, fibroblasts are involved in modifying the microstructure of extracellular matrix via covalent crosslinking, protein glycosylation, and controlled proteolysis through the release of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) (Plikus et al., 2021). Human tendon fibroblasts were shown to produce collagens (Chard et al., 1987; Xia et al., 2006; Yang et al., 2004), MMPs (Corps et al., 2004; Dalton et al., 1995; Tsuzaki, Bynum, et al., 2003; Tsuzaki, Guyton, et al., 2003), and TIMPs (Dalton et al., 1995; Vater et al., 1979). Human dermal fibroblasts, likewise, were found to express collagens (Booth et al., 1980; Fleischmajer et al., 1981; Uitto et al., 1980), MMPs (Bauer et al., 1975; Scharffetter-Kochanek et al., 1993; Welgus et al., 1985; Wilhelm et al., 1986), and TIMPs (Stricklin & Welgus, 1983; Welgus et al., 1985; Welgus & Stricklin, 1983). Fibroblasts are therefore capable of remodeling the matrix in response to chemical and mechanical signals in their microenvironment milieu (Hynes, 2009; Lu et al., 2011). Fibroblasts, moreover, secrete a multitude of cytokines, growth factors, and adipokines, which may influence their own function, and the function of nearby cells (Plikus et al., 2021; Zhang & An, 2007). An array of signaling pathways regulate fibroblast function, proliferation, and differentiation (Plikus et al., 2021).

Nevertheless, heterogeneity in fibroblast phenotypes may exist within and across tissues (Plikus et al., 2021). At least ten major subtypes (clusters) of fibroblast populations were identified in human dermis (Ascensión et al., 2021; Ganier et al., 2022). Moreover, at least six distinct tenocyte populations were found in healthy human tendon (Gomez-Collignon et al., 2022; Kendal et al., 2020). Differences in contraction pattern, within, and between skin dermis and tendon fibroblasts may be present (Eastwood et al., 1996). Skin fibroblasts may have a higher growth rate and protein synthesis than tendon cells (Evans & Trail, 1998). Tendon cells may furthermore differ from skin fibroblasts in their ability to form three-dimensional cylindrical structures in vitro (Evans & Trail, 1998). However, the application of a static strain to human dermal fibroblasts was shown to generate neo-tendon tissue indistinguishable from that

engineered with human tenocytes (Deng et al., 2009; Nakanishi et al., 2019). Mesenchymal and induced pluripotent stem cells, moreover, were shown, under certain conditions, to differentiate into tenocyte-like cells (Komura et al., 2020; Kuo & Tuan, 2008). A few clinical studies already reported the possibility of using autologous dermal fibroblasts for the treatment of tendon disease (Clarke et al., 2011; Connell et al., 2009; Obaid et al., 2012).

## Tissue injury and adaptation

### Adaptation

The mechanical properties of connective tissues may depend on the geometric orientation of collagen and elastin fibers in the matrix (Comninou & Yannas, 1976). Albeit variability between tissues in their constituents may be found, most connective tissues with high tensile strength (e.g. skin and tendon) are composed of high collagen and low elastin content (Nimni & Harkness, 2018). A stress-strain curve represents the relationship between tensile force and extension, which may demonstrate the mechanical behavior of connective tissues (Nimni & Harkness, 2018). It may be divided into three phases for most tissues: low stress (toe), linear, and high stress (failure) phases (Nimni & Harkness, 2018). Although physiologic loading of skin and tendons may operate up to the first half of the second phase (BROWN, 1973; Maganaris & Paul, 1999), sustained or repeated physiologic loading may be sufficient to induce mechanical failure of these tissues (Goldstein et al., 1987; Stekelenburg et al., 2008).

Acute mechanical loading of tendons may result in an immediate reduction followed by augmented collagen protein synthesis in tendons and peritendinous tissues, which peaks around 24h and remains increased for up to 72h after exercise (Christensen et al., 2011; Heinemeier et al., 2003; H. Langberg, D. Skovgaard, L. J. Petersen, et al., 1999; Miller et al., 2005; Moerch et al., 2013). Similarly, immediately after exercise, a reduction in collagen degradation may occur, which returns to pre-exercise values within 72h (Heinemeier et al., 2003; H. Langberg, D. Skovgaard, L. J. Petersen, et al., 1999). Nonetheless, mechanical strain might provide resistance against collagen degradation at the tissue and fibril level, while individual collagen molecules might show a biphasic response such that higher loads predispose collagen to cleavage, but lower loads provide resistance against degradation (Kjaer, 2004; Saini et al., 2020).

Skin response to mechanical stress may be classified depending on loading configuration to pressure, shear, friction and tension. Exposure to low or moderate durations of pressure may be acceptable for intact skin, although reversible damage might occur. Reparative mechanisms may be overcome by catabolic processes if a certain force or time is exceeded. Deeper tissues may be more vulnerable to injury than superficial tissues; with pathological changes progressing from the muscles towards the skin. Friction at a low frequency and high force may be sufficient to cause a blister within the epidermis. Below a specific threshold, adaptive thickening of skin, rather than injury, may occur in response to friction. Increases in the thickness of stratum corneum may reduce the risk of failure. Animal studies showed that tension may lead to redistribution of collagen matrix so that the matrix is thickened, with exception to the dermis, and skin surface area is increased. Collagen fibril diameter, collagen crosslinking, and sulfated proteoglycans may increase in response to higher tensile forces (Ogawa et al., 2012; Sanders et al., 1995; Verhaegen et al., 2012).

**Connective tissue injury**

Excessive mechanical load, among other causes, may result in connective tissue injury (Bouten et al., 2003; Kellett, 1986; Stekelenburg et al., 2008). Intense and/or prolonged pressure or pressure in combination with shear may contribute to the development of pressure injury and/or deep tissue injury (Coleman et al., 2014; Edsberg et al., 2016; Stekelenburg et al., 2008). Mechanical overload, moreover, may result in acute, chronic, or acute on chronic soft tissue injuries (Dutton et al., 2004; Kellett, 1986). Repeated overload, friction, and/or excessive compression seem to be important factors in the onset of overuse tendinopathy (Cook & Purdam, 2009; Kannus, 1997; Kellett, 1986; Renström & Johnson, 1985). Nonetheless, it is yet to be determined whether mechanical load is directly responsible for these injuries, as several intrinsic and extrinsic factors may play a role in their development (Fontanez et al., 2022).

**Theories of aetiology and pathophysiology**

Neither the pathophysiology of tendinopathy nor pressure ulcers is fully understood (Cardoso et al., 2019; Kottner et al., 2009). Biomechanical forces, inflammasome activation, ischemia, reperfusion injury, impaired lymphatic function, aging and sustained cell deformation were suggested to contribute to pressure ulcer development

(Bouten et al., 2003; Coleman et al., 2014; Kottner et al., 2009; Mustoe et al., 2006; Stojadinovic et al., 2013; Wywialowski, 1999). Alternatively, initial cell death and tissue damage due to sustained deformation of soft tissues, followed by secondary inflammatory-oedema related damage and ischemic damage were postulated as possible factors leading to pressure ulcers (Gefen et al., 2022).

Multiple hypotheses, theories and models were developed in attempt to explain the pathophysiology of tendinopathy. These may include; excessive friction due to overuse (MEYER, 1921, 1924), impingement by surrounding structures (CHARLES S NEER, 1972), muscle overload injury (Kibler, 1990), a loading episode when the tendon is weakened (Archambault et al., 1995), repeated strain below the injury threshold (Arnoczky et al., 2007), the continuum model of tendinopathy (Cook & Purdam, 2009), the revised continuum model (Cook et al., 2016), the iceberg theory (Abate et al., 2009), an underlying degenerative process (Burry, 1978; Cawston et al., 1996; Codman & Akerson, 1931), the inflammatory model (Rees et al., 2014), the apoptosis theory (Yuan et al., 2003; Yuan et al., 2002), the vascular theory (Ahmed et al., 1998; Pufe et al., 2005), the neural theory (Hart, 1995; Plinsinga et al., 2015) and failed healing theory (Fu et al., 2010a). A combination of these factors likely contributes to tendinopathy, as suggested by the failed healing theory, although the exact pathogenesis is yet to be determined.

**Risk factors**

Several risk factors may predispose to the development of tendinopathy; however, it may be difficult to study the effect of each factor in isolation from the others. Aging is a main risk factor for tendinopathy, which most commonly affects the middle-aged population (between 41-60 years) in particular (Clement et al., 1984; de Jonge et al., 2011; Leong et al., 2019; Tuite et al., 1997). A common risk factor for tendinopathy is overuse, since affected tendons often sustain high levels of mechanical stress (de Jonge et al., 2011; Heir & Glomsaker, 1996; Neviaser et al., 2012). Metabolic-hormonal imbalances were demonstrated to have an association with tendon degeneration (Oliva et al., 2016). Patients with diabetes mellitus and hypercholesterolemia may have a significantly higher risk of developing tendinopathy (Abate et al., 2013; Abboud & Kim, 2010; Ranger et al., 2016; Taylor et al., 2017; Tilley et al., 2015). The risk of calcific tendinopathy of rotator cuff muscles may increase by 27% in diabetic patients

(Su et al., 2021). Obesity, moreover, may increase the risk of tendinopathy, tendon tear, and tendon rupture, and may also augment complications following tendon surgery (Franceschi et al., 2014; Gaida et al., 2009; Gaida et al., 2008; Macchi et al., 2020; Owens et al., 2013). Heredity may be a significant risk factor for the development of tendinopathy, increasing the risk by fivefold (Gwilym et al., 2009; Harvie et al., 2004; Kraemer et al., 2012; Tashjian et al., 2009).

Other risk factors for tendinopathy may include exposure to substances and drugs. Alcohol consumption may be associated with an increased risk for tendinopathy and tendon tears (Owens et al., 2013; Passaretti et al., 2016; Van Der Vlist et al., 2019). Smoking may predispose for rotator cuff tearing, and was shown to reduce healing ability and clinical outcomes after repair (Baumgarten et al., 2010; Carbone et al., 2012; Mallon et al., 2004; Zabrzyński et al., 2021). Drug-induced tendinopathy may occur due to the administration of fluoroquinolones, long-term corticosteroid use, statins, and aromatase inhibitors (Alves et al., 2019; Barge-Caballero et al., 2008; Bidell & Lodise, 2016; Blanco et al., 2005; Eliasson et al., 2019; Kim, 2010; Kirchgesner et al., 2014; Tsai & Yang, 2011). Concomitant administration of fluoroquinolones and corticosteroids seems to augment the risk of tendinopathy (Alves et al., 2019; Wise et al., 2012). A significant increase in the risk of fluoroquinolones-induced tendinopathy was observed in patients with renal failure, long-term hemodialysis, end-stage renal disease, and renal transplant (DONCK et al., 1994; Fleisch et al., 2000; Harrell, 1999; Marti et al., 1998; Zabraniecki et al., 1996). Tendinopathy may also be present in patients with rheumatoid arthritis (McQueen et al., 2005; Shen et al., 2019; Stiskal et al., 1997) (Figure 1).

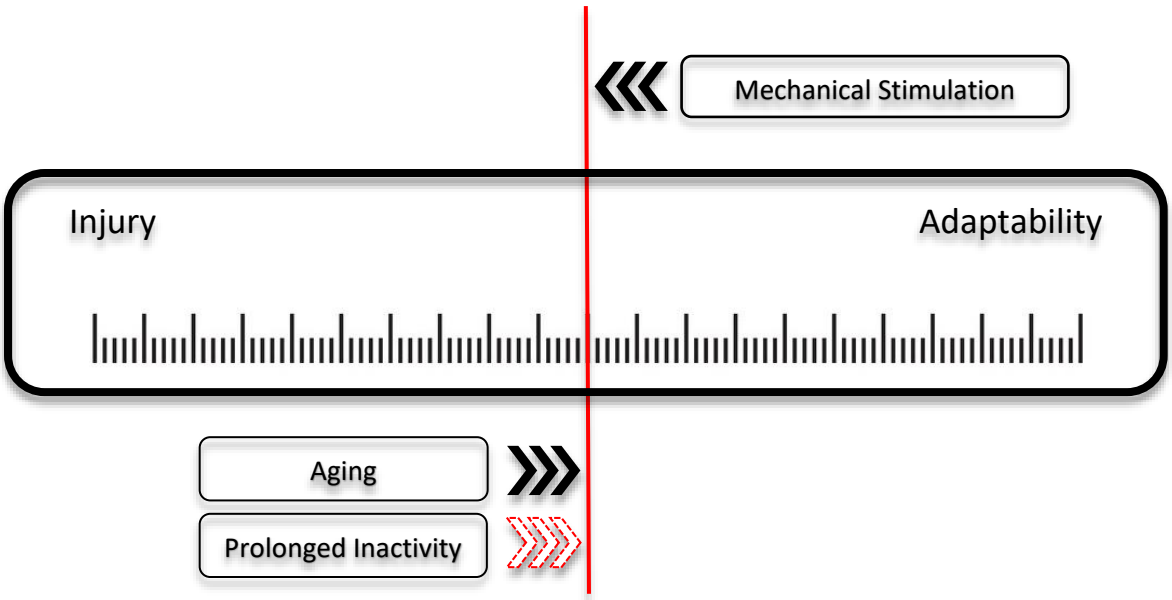



Figure 1. An illustration of the relationship between basic factors that may influence the threshold of adaptability and injury; while adequate mechanical stimulation may enhance adaptability, excessive mechanical stimulation might lead to injury. Aging and prolonged inactivity, however, may have opposing effects which may reduce adaptability and facilitate injury.

Mobility related variable may include frequency and magnitude of movement, whereas, activity variables are related to larger-scale measures such as bedfast, chairfast and ambulation. Lower activity/mobility levels may increase pressure-ulcer risk, with mobility measures being more able to differentiate whether pressure ulcers might occur (Brandeis et al., 1994; Coleman et al., 2013; Lindgren et al., 2004). Stage I pressure ulcer may be strongly associated with increased subsequent stage II pressure ulcer odds (Coleman et al., 2013). Moreover, the role of diabetes in increasing the probability of pressure-ulcer incidence may be supported by strong evidence (Coleman et al., 2013; Keller et al., 2002). Anemia and hypoalbuminemia may constitute risk factors for the development of pressure ulcers (Coleman et al., 2013; Keller et al., 2002). Moisture, furthermore, might be a contributing factor to the development of pressure ulcers (Brandeis et al., 1994; Coleman et al., 2013; Keller et al., 2002). Significant predictors of pressure ulcer occurrence may be friction and shear (Coleman et al., 2013; Wann-Hansson et al., 2008).

A potential contributor to pressure ulcer risk may be medication, although supported by limited evidence (Coleman et al., 2013; Kaitani et al., 2010). Hospitalized patients

with limited activity, nevertheless, may be at significant and independent risk of developing pressure ulcers due to the following factors: nonblanchable erythema, immobility, lymphocytopenia, dry skin and reduced body weight (Allman et al., 1995; Børsting et al., 2018). A higher pressure-ulcer risk in intensive care unit (ICU) patients may be associated with these factors: history of vascular disease, Dopamine® or Dobutamine® administration, continuous veno-venous hemofiltration, intermittent hemodialysis and mechanical ventilation, while, sedative use, chair sitting and body temperature above 38 °C were reported to reduce pressure ulcer risk (Nijs et al., 2009; Serrano et al., 2017). It is yet to be confirmed, nonetheless, whether increased body temperature may contribute to pressure-ulcer development (Coleman et al., 2013). Age, length of ICU stay, duration of mean blood pressure <60-70 mmHg, diabetes, spinal cord injury and renal insufficiency emerged as other risk factors for pressure-ulcer development in ICUs and nursing homes (Alderden et al., 2017; Bours et al., 2001; Brandeis et al., 1994; Frankel et al., 2007; Keller et al., 2002; Serrano et al., 2017; Slowikowski & Funk, 2010). The severity of pressure ulcers may not be affected by the number of medications administered (Arai et al., 2023). However, corticosteroid use and total caloric intake may impact the healing of pressure ulcers (Arai et al., 2020).

**Tissue and cell injury**

Physical agents including mechanical trauma are known causes of cellular injury (Cobb et al., 1996; D'lima et al., 2001). Other causes of cell injury may include ischemia and aging (Jennings et al., 1990; Trump et al., 1976; Warner, 1997). The main mechanisms of cell injury may include ATP depletion, cell membrane permeabilization, intracellular pathways disruption, and DNA damage (Miller & Zachary, 2017; Trump et al., 1976; Trump et al., 1974). Cell repair, adaptation, or degeneration may occur in response to reversible injury (Miller & Zachary, 2017). Irreversible injury and/or irreparable DNA damage, however, may lead to cell senescence, apoptosis, dysplasia, or necrosis (Galluzzi et al., 2008; Hetts, 1998; Miller & Zachary, 2017).

**Threshold of injury**

While there are attempts to identify a mechanical threshold for tissue injury in literature, it seems that such value may be both patient-specific and tissue-type specific (Gefen, 2018; Oomens et al., 2010). A single damage threshold, therefore, could not be defined

due in part to this specificity (Gefen, 2018; Oomens et al., 2010). The threshold of injury may depend on magnitude of mechanical load and exposure time (load/time combination) (Gefen, 2009; Oomens et al., 2010). Several intrinsic factors, moreover, may influence the threshold for injury; including tissue composition, tissue stiffness, individual internal anatomy, and thickness of soft tissue layers (Gefen, 2018). Similarly, there seems to be no agreement on a threshold of microdamage in tendon tissue matrix, since a range from 4% up to 7.5% strain was reported as injurious (Lee et al., 2017; Stauber et al., 2020; Zitnay et al., 2017).

Sustained soft tissue deformations may cause cell damage within a few minutes, however, clinically visible damage may only become apparent following hours of sustained load (Gefen et al., 2022). Ischemia from capillary closure, ischemia-reperfusion injury, and tissue deformation have been proposed, among others, as etiological pathways for pressure injuries (Gefen et al., 2022). The same mechanisms were suggested to be involved in tendinopathy; ischemia and ischemia-reperfusion injuries could lead to tendinopathy (Alfredson, 2005; Archambault et al., 1995; Kannus, 1997), moreover, nuclear deformation of tendon cells may represent a potential cellular mechanism in tendon overuse injuries (Arnoczky, Lavagnino, et al., 2002).

**Acute healing:**

a. Skin

Hemostasis is accomplished, immediately after injury, through vasoconstriction and platelet aggregation to form a vascular plug. Platelets, moreover, influence leukocyte chemotaxis, release proteolytic enzymes and growth factors. Growth factors stimulate proliferation of fibroblast and smooth muscle cells, and increase collagen synthesis. Neutrophils are the first cells to appear in the wound site to initiate ingestion of bacteria. Macrophages, subsequently, secrete growth factors that lead to continued mitogenesis later in wound healing, since platelets cease to exist in the tissue. Injured cells release thromboplastin which activates a cascade leading to fibrin formation. The formed fibrin network serves as a scaffold over which fibroblasts and epithelial cells migrate and provides temporary stability to the defect. Macrophages are observed in the wound during the first 5 days, while lymphocytes reach their maximum number at about day 6 of healing. Macrophages release proteinases, such as elastases, collagenases and plasminogen activators, which contribute to wound debridement. Macrophages,

furthermore, may influence collagen synthesis and neovascularization (Kanzler et al., 1986; Rodrigues et al., 2019; Stadelmann et al., 1998).

Epidermal cells in wound margin begin to migrate into the wound within 24 hours. Hypoxic conditions in the central dead space of the wound might augment the release of growth factors, which may stimulate angiogenesis. Low oxygen tension potentiates fibroblast proliferation. Migration of fibroblasts into the wound commences at 48-72 hours, and a “lag phase” of two days may exist between fibroblast migration and active collagen synthesis (Howes et al., 1929). Collagen type III is formed early in the wound and its synthesis reaches maximum levels between 5 and 7 days. Proteoglycans and glycosaminoglycans are additionally formed along with collagen and reach maximum synthesis levels about 2 weeks after wounding. Type III collagen is subsequently replaced by type I collagen. Myofibroblasts, which are cells with both fibroblast and smooth muscle cell features, are responsible for wound contraction (Kanzler et al., 1986; Rodrigues et al., 2019; Stadelmann et al., 1998). Heterogeneity in stromal and immune cell phenotypes is noteworthy and understanding its contribution to wound healing is of growing interest (Rodrigues et al., 2019).

b. Tendon

Almost immediately after tendon injury, hematoma formation is followed by platelet aggregation, which causes the recruitment of leucocytes and the expression of pro-inflammatory cytokines. Neutrophils are the first responders to injury which subsequently attract macrophages. Growth factors released by macrophages may stimulate fibroblast proliferation and extracellular matrix formation. Capillary formation is promoted by angiogenic factors, which is crucial for collagen synthesis. Epitenon cells near the repair site proliferate and migrate to the superficial areas of the repair surface. Neovascularization occurs with blood vessels formation into the repair site. The endotenon shows moderate increase in cellularity, however, the epitenon demonstrates the main proliferative response. The paratenon might have a vital role in tendon healing, since it is a source of tendon stem/progenitor cells and might contribute to tendon blood supply (Ahmed et al., 1998; Edwards, 1946; Mayer, 1916; Stein et al., 2000; Zantop et al., 2003). Additionally, tenocytes migrate and proliferate at the injury site. Sustained elevation in the production of type III collagen is observed throughout the healing process. Non-collagenous proteins and proteoglycans are synthesized at

high rates at about 10 days after repair. Tendons may show an initial “lag period” which is followed by a progressive increase in tensile strength (Mason & Allen, 1941). Greater tensile strength is provided through the replacement of type III collagen fibers with type I collagen. A gradual decline in tendon vascularity, cellularity and water content are accompanied by increased elongation of previously rounded tenocytes (Hope & Saxby, 2007; Leong et al., 2020).

**Chronic healing:**

a. Pressure ulcer

The characteristic changes observed in the blanchable erythema stage may be mild mainly lymphocytic perivascular infiltrate, with mild to moderate edema in the papillary dermis. Red blood cell engorgement of blood vessels in the papillary dermis may be found in the non-blanchable erythema stage. Diffuse eosinophilia with erosions and crust formation, focal eosinophilia and necrosis with or without subepidermal separation, epidermal atrophy with subepidermal blister, and a subepidermal bulla with a relatively normal-appearing epidermis may constitute epidermal responses and characterize the dermatitis phase. Necrotic changes in the epidermis and fat with dermal infiltration of inflammatory cells may be found in the early ulcer stage. A healing ulcer may show fibroblast proliferation, formation of blood vessels and edema. A chronic ulcer, nonetheless, may be covered by a layer of red blood cells and acute inflammatory cells. Biopsies from stage III-IV chronic pressure ulcers were found to have high levels of neutrophils, which may be responsible for matrix degradation. Furthermore, macrophages, lymphocytes and mast cells were found. Vacuolated or degenerated fibroblasts, and numerous fat droplets may be identified in pressure ulcers (Berg & Rudolph, 1995; Diegelmann, 2003; Vande Berg et al., 1998; Witkowski & Parish, 1982).

b. Tendinopathy

Histopathological changes in chronic tendon disease may include paratenonitis (tenosynovitis, tenovaginitis, peritendinitis), paratenonitis with tendinosis, tendinosis, tendinitis and calcifying tendinopathy. Acute paratenonitis may be characterized by edema, swelling, tenosynovium hyperemia, adipocyte necrosis, and leucocytic and lymphocytic infiltration. Subsequently, crepitation during motion may occur due to fibrinous exudation deposition in tendon sheath. Chronic paratenonitis, nonetheless,

may show fibroblast proliferation, blood vessel formation, moderate perivascular lymphocytic infiltration and thickening of peritendinous tissues. Adipocyte necrosis and extracellular fat droplets may be frequently found initially in chronic paratenonitis, but are later replaced by fibrous tissues. Edema, collagen fiber misalignment, and focal cell necrosis may be found in tendinosis. Accumulation of extracellular lipids and glycosaminoglycans, formation of blood vessels, and calcification may be observed as the condition progresses (Järvinen et al., 1997; Jozsa et al., 1980; Jozsa & Kannus, 1997; Kannus & Jozsa, 1991).

Mucoid and lipid (tendolipomatosis) degeneration, fibrinoid degeneration and necrosis, and capillary formation are common findings in tendinosis and chronic tendinitis. Degenerated, necrotic, and activated tenocytes were identified in hypoxic degenerative tendinopathy. Fibroblast proliferation, angiogenesis, and infiltration of lymphocytes and macrophages are sometimes found in degenerated tendon and may indicate healing. Inflammatory cellular response may often be identified in partial tendon tear. It is unknown, however, whether this inflammatory response precedes or follows partial tendon rupture. Spontaneous tendon rupture may be preceded with degenerative tendinopathy, mucoid degeneration, tendolipomatosis, and calcifying tendinopathy, either alone or combined (Järvinen et al., 1997; Jozsa et al., 1980; Jozsa & Kannus, 1997; Kannus & Jozsa, 1991).

**Failed healing response:**

Aging, inflammation and mechanical stress were commonly proposed as the factors leading to degenerative tendon changes and pressure ulcers (Järvinen et al., 1997; Stojadinovic et al., 2013). Despite attempts, failed healing seems to occur in tendinopathy possibly due to continuous overloading which may increase inflammatory cytokines (D'Addona et al., 2017; Dean et al., 2017; Del Buono et al., 2011; Fu et al., 2010b; Rees et al., 2014). Upregulated and/or prolonged expression of inflammatory cytokines and disrupted collagen matrix may contribute to delayed pressure ulcer healing (Diegelmann, 2003; Jiang et al., 2014). Failed healing may occur in more than 40% of stage III and IV pressure ulcers following treatment, which is associated with increased risk of mortality (Allman, 1997).

**Tissue and cell deformation**

The response of fibroblast cells to mechanical stress may depend on tissue origin (Mackley et al., 2006). Sustained and/or excessive tissue and cell deformation may contribute to pressure ulcer development. Deformation-induced cell death events and deformation associated with inflammatory edema, which may elevate interstitial pressure and augment cell-scale mechanical loading even further, were described by Lustig et al. (Lustig et al., 2021) and other cell-death threshold models (Linder-Ganz et al., 2006; Oomens et al., 2015; Stekelenburg et al., 2008). Cyclic axial stretching of human dermal fibroblasts was shown to reduce apoptosis (Huang et al., 2013). Interestingly, release of mechanical tension was found to induce apoptosis of human dermal fibroblasts (Grinnell et al., 1999).

High strain mechanical loading was reported to induce apoptosis of tenocytes in an *in vitro* and an *ex vivo* models (C. Jiang et al., 2012; Scott et al., 2005). Apoptosis of tendon cells may occur due to activation of stress-activated protein kinases (SAPKs) in response to repetitive loading (Arnoczky, Tian, et al., 2002). Overloading of tenocytes may lead to upregulation of proinflammatory cytokines and metalloproteinases, which may degrade the matrix (Tsuzaki, Bynum, et al., 2003; Tsuzaki, Guyton, et al., 2003). Cyclic strain of human tenocytes was shown to induce IL-1β, cyclooxygenase 2 (COX-2) and prostaglandin E2 (PGE2) gene expression (Li et al., 2004; Tsuzaki, Bynum, et al., 2003; Yang et al., 2005). IL-1β, subsequently, may induce the release of COX-2 and PGE2 in human tendon cells (Tsuzaki, Guyton, et al., 2003; Yang et al., 2005). Release of tensile strain on human tendon construct might reduce the expression of tenocyte markers such as tenomodulin, scleraxis, Col1 and Col3 mRNA (Bayer et al., 2014).

**Tissue and cell hypoxia**

As tension increases during passive stretch and isometric contraction, a progressive decline in tendon and paratenon blood flow may be observed, with values significantly reduced near calcaneal insertion (Åström, 2000; Åstroöm & Westlin, 1994; Kubo et al., 2017; Schatzker & Brånemark, 1969). Blood flow and volume might, however, be higher than resting levels during the recovery period (Kubo et al., 2008; Kubo et al., 2017). Tendinopathic tendons might demonstrate a flattened post-occlusive reactive

hyperemia compared with a high peak response in intact tendons (Arverud et al., 2016).

Peritendinous blood flow may increase during intermittent static contractions, whereas dynamic loading might augment metabolic activity and the expression of prostaglandin E2 (PGE2), thromboxane B2 (TXB2), bradykinin and adenosine (Bojsen-Møller et al., 2006; Boushel et al., 2000; Kalliokoski et al., 2005; Kjaer et al., 2000; LANGBERG et al., 1998, 1999; Langberg et al., 2002; Langberg et al., 2001; H. Langberg, D. Skovgaard, M. Karamouzis, et al., 1999; H. Langberg, D. Skovgaard, L. J. Petersen, et al., 1999). COX-2 might mediate prostaglandin release during intermittent isometric exercise and following resistance exercise, which might regulate peritendinous blood flow (Langberg et al., 2003). Higher lactate concentrations were identified in tendinopathic tendons compared with normal tendons (Alfredson et al., 2002). Inconsistent pattern of blood flow might be observed in response to prolonged pressure in elderly patients (Frantz et al., 1993; Hagisawa et al., 1991). Pressure ulcers might develop postoperatively in patients with impaired blood flow during surgery (Sanada et al., 1997). Shear combined with pressure, moreover, was reported to decrease transcutaneous oxygen and blood flow (Manorama et al., 2013; Manorama et al., 2010). Low resting systolic blood pressure might correlate with the incidence of pressure ulcers (Schubert, 1991). Non-blanching erythema might be associated with subsequent development of pressure ulcers (Nixon et al., 2007; Schubert et al., 1994). Post-occlusive reactive hyperemia might be impaired in elderly and spinal cord injury patients (Hagisawa et al., 1991; Jan et al., 2013; Schubert, 1991). Pressure combined with shear, long and repeated periods of ischemia without allowing sufficient recovery might increase reactive hyperemia (de Wert et al., 2015; Thorfinn et al., 2007).

An oxygen-dependent HIF-1α (hypoxia-inducible factor-1α) or HIF-2α subunit and a HIF-1β subunit form hypoxia-inducible factors, which are one of the response regulators to hypoxia (Hashimoto & Shibasaki, 2015; Semenza, 2000). Cultured human tenocytes might be less sensitive to hypoxia than dermal fibroblasts (Webster & Burry, 1982). Both cell types, however, might show reduction in collagen and total protein synthesis in response to hypoxia (Webster & Burry, 1982). Hypoxia of young, aged and tendinopathic human tenocytes might lead to changes towards a fibrochondrocyte phenotype in young and tendinopathic tenocytes, and a mineralized

fibrochondrocyte phenotype in aged tenocytes (McBeath et al., 2019). Furthermore, areas of fibrochondrocyte and mineralized fibrochondrocyte phenotypes correlated with a co-localized HIF-1α expression (McBeath et al., 2019). Hypoxia of human tenocytes, moreover, was shown to upregulate the expression of HIF-1α and VEGF, and several proapoptotic proteins that may mediate hypoxia-induced apoptosis (Liang et al., 2012; Millar et al., 2012).

HIF-1α levels may increase in response to peroxide-treatment of primary human tenocytes in both high and low glucose media (Poulsen et al., 2014). Under low-glucose conditions, HIF-1α might upregulate the expression of sox9 and scleraxis (Poulsen et al., 2014). In addition, HIF-1α inhibition might reduce the migration and proliferation of tenocytes (Jiao et al., 2022). The expression of HIF-1α was found to increase in tendinopathy, mild rotator cuff tendon impingement and tendon tears (Benson et al., 2010; Jiao et al., 2022; Lakemeier, Reichelt, Patzer, et al., 2010; Millar et al., 2012; Mosca et al., 2017). Signs of hypoxia were identified in tenocytes in degenerated tendons, with calcification occurring in areas with fibrocartilaginous phenotype (Jozsa et al., 1980; Jozsa et al., 1982). Hypoxia and HIF-1α might, moreover, promote retention of chondrogenic phenotype, inhibit the expression of fibroblast-like markers and encourage redifferentiation of human chondrocytes (Duval et al., 2009; Mennan et al., 2019).

Acute hypoxia was found to upregulate TGF-β1 expression and collagen synthesis by human dermal fibroblasts, which might be mediated by HIF-1α (Falanga et al., 1993; Falanga et al., 1991; Falanga et al., 2002; Kang et al., 2020; Kim et al., 2019; Mingyuan et al., 2018; Siddiqui et al., 1996; Yamanaka & Ishikawa, 2000). Chronic exposure to hypoxia (more than six cell-culture passages), nonetheless, might result in reduction of TGF-β1 and collagen expression (Siddiqui et al., 1996; Yamanaka & Ishikawa, 2000). Aged human dermal fibroblasts might have reduced migration and TGF-β1 receptor expression when exposed to hypoxia (Mogford et al., 2002). Acute hypoxia might increase the proliferation rate of cultured human dermal fibroblasts, whereas chronic hypoxia might reduce it (Falanga & Kirsner, 1993; Siddiqui et al., 1996; Storch & Talley, 1988). Cultured pressure ulcer fibroblasts might produce elevated levels of TGF-β1 when senescent (Brunner et al., 2021; Vande Berg et al., 2005). However,

TGF-β1 mRNA expression was not detected in biopsies from chronic pressure ulcers (Schmid et al., 1993).

A subset of patients with tendinopathic and ruptured tendons may show vascular endothelial growth factor-A (VEGF) and VEGF receptors-1 and -2 expression, while control tendons might not (Alfredson et al., 2003; Lakemeier, Reichelt, Patzer, et al., 2010; Lakemeier, Reichelt, Timmesfeld, et al., 2010; Legerlotz et al., 2012; Petersen et al., 2004; Pufe et al., 2001; Savitskaya et al., 2011; Scott, Lian, Bahr, Hart, & Duronio, 2008). Tendinopathic tendons, moreover, might demonstrate elevated angiogenesis and blood flow (Merkel et al., 2021; Petersen et al., 2004; Scott, Lian, Bahr, Hart, & Duronio, 2008). Human dermal fibroblasts might upregulate VEGF mRNA in response to hypoxia (Scheid et al., 2000; Steinbrech et al., 1999). Higher or lower levels of VEGF, particularly in granulation tissue surrounding the central necrosis and wound exudate, were reported in chronic pressure ulcers (Jiang et al., 2014; Pufe et al., 2003; Takayama et al., 2010).

**Aging**

The expression of p53 protein might increase in intrinsically aged human dermal fibroblasts, which might suggest an incipient cellular senescence (Lupa et al., 2015). An age dependent increase in senescence-associated β-galactosidase in both human dermal fibroblasts and keratinocytes was identified in dermal samples from donors of different age (Dimri et al., 1995). This might indicate, therefore, that number of senescent cells may increase with age (Dimri et al., 1995). Gene expression levels in human dermal fibroblasts might be altered in three in-vitro aging models, with cell growth rate being reduced with aging (Lago & Puzzi, 2019). An age-related change, moreover, in mitochondrial biogenesis process might lead to a significant reduction in oxidative phosphorylation efficiency of human dermal fibroblasts (Bowman & Birch-Machin, 2016; Greco et al., 2003). COX-2 and PTGES1 (prostaglandin E synthase) might progressively increase with aging, which might result in increased PGE2 levels in elderly skin (Li et al., 2015). Skin biopsies from old donors were found to have reduced dermal fibroblast proliferation and impaired extracellular matrix homeostasis compared with young subjects (Dumas et al., 1994; Fisher et al., 2009; Khorramizadeh et al., 1999; Li et al., 2015; Phillips et al., 1994; Qin et al., 2017; Rorteau et al., 2022;

Schneider, 1979; Solé-Boldo et al., 2020). Replicative senescence of human dermal fibroblasts might be correlated with overexpression of collagenase (West et al., 1989).

The integrity of tendon structure, in addition to, gene and protein expression involved in collagen turnover pathways might be preserved with aging, while tenocytes might tend to decrease in number and show impaired migration with age (Asai et al., 2022; Gagliano et al., 2018; Hubbard & Soutas-Little, 1984). A significant reduction with age, however, in total glycosaminoglycan, chondroitin sulphate and dermatan sulphate was found in another study (Riley et al., 1994a). The tenogenic differentiation and growth capacities of tendon stem progenitor cells (TSPCs) might be significantly reduced with aging (Han et al., 2017; Klatte-Schulz et al., 2012). Moreover, TSPCs derived from aged human tendons might have lower cell density with a fragile phenotype, characterized by significantly higher values of senescence markers and apoptosis compared with young TSPCs (Yan et al., 2020).

**Sustained inflammatory state**

Prolonged low-grade inflammation may predispose to chronic tendinopathies (Crowe et al., 2019; D'Addona et al., 2017; Dakin et al., 2018; Del Buono et al., 2011; Fredberg & Stengaard-Pedersen, 2008; Mosca et al., 2017; Mosca et al., 2018). Similarly, chronic pressure ulcers might show high-grades of inflammation and collagen matrix disruption (Bronneberg et al., 2007; Edsberg et al., 2012; Jiang et al., 2014). Senescent fibroblasts might express SASPs, which might contribute to persistent inflammation and wound chronicity (Freund et al., 2010; Harding et al., 2005; Sanada et al., 2018). Persistent senescent cells might augment the proapoptotic and proinflammatory qualities of SASP in response to extracellular signals, such as damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) (Chaib et al., 2022). A threshold beyond which the accumulation of senescent fibroblasts might impede healing might be around 15% (Harding et al., 2005). Tenocytes activation markers might remain elevated following exposure to IL-1β, leading to persistent expression of IL-6 and IL-8 mRNA levels in chronic tendinopathic tendons compared with healthy controls (Dakin et al., 2017). Tenocytes from tendinopathic tendons, moreover, might express higher NF-κB and Interferon target genes following treatment with IFNγ compared with healthy tendons (Dakin et al., 2015).

Tendinopathic and torn tendons might demonstrate infiltration of mast cells and macrophages (Dakin et al., 2018; Gotoh et al., 1997; Kragsnaes et al., 2014; Matthews et al., 2006; Millar et al., 2010; Scott, Lian, Bahr, Hart, Duronio, et al., 2008). Exposure of tenocytes to TNFα might significantly reduce their viability and induce apoptosis (Backman et al., 2014). Moreover, TNFα might lead to reduced collagen type I deposition and upregulated MMP-1, TNFα and IL-1β expression by human tenocytes (John et al., 2010). The prevalence of mast cells might be higher in tendinopathic tendons compared with normal controls (Scott, Lian, Bahr, Hart, Duronio, et al., 2008). Mast cells might induce the production of COX-2 and PGE2 by tenocytes, which might lead to reduced COL1 mRNA expression and type I procollagen protein production (Behzad et al., 2013). The release of COX-2 and PGE2 by tenocytes might be mediated through transforming growth factor beta 1 (TGF-β1) (Behzad et al., 2013).

An increased number of mast cells, macrophages and $CD8^+$ T was identified in intrinsically aged human skin compared with young skin (Pilkington et al., 2019). A two-step clearance mechanism might be necessary for phagocytosis; macrophages may release TNFα to induce apoptosis in senescent human dermal fibroblasts, and subsequently dead cells are phagocytosed by macrophages (Ogata et al., 2021). Senescence-associated secretory phenotype (SASP) might suppress both steps of the macrophage clearance mechanism leading to accumulation of senescent cells (Ogata et al., 2021). SASP-related pro-inflammatory cytokines might induce the expression of the non-classical MHC molecule HLA-E (Pereira et al., 2019). An increased expression of HLA-E in senescent human dermal fibroblasts might contribute to their accumulation in sections from older individuals, possibly due to inhibited natural killer (NK) and $CD8^+$ T cells response against senescent cells (Pereira et al., 2019). Furthermore, mechanical stimulation of mast cells and macrophages might lead to degranulation and phenotype polarization, respectively (Ballotta et al., 2014; Fujishiro et al., 2004; Wang et al., 2010; Yang et al., 2000; Zhang et al., 2012).

**Cell senescence and apoptosis**

Senescent cells fail to divide although they typically remain metabolically active (Campisi & d'Adda di Fagagna, 2007; Hayflick & Moorhead, 1961). The senescence growth arrest is inherently permanent since physiological stimuli cannot stimulate senescent cells to proliferate (Campisi & d'Adda di Fagagna, 2007). Oxidative stress

may lead to loss in proliferative capacity and induction of senescence of human fibroblasts, which might be mediated through p16, p21 and p53 (Chen et al., 2004; Frippiat et al., 2001; Kang et al., 2011; Lee et al., 1999; Ogata et al., 2021; Stöckl et al., 2006). Human tenocytes exposed to peroxide-treatment might undergo apoptosis through caspase-3 and -7 activation (Gallorini et al., 2022). Cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) expression might be upregulated during fibroblast senescence (Zdanov et al., 2007).

Upregulation of senescence-associated β-galactosidase (SA-β-Gal), p16, p21 and p53, and senescence-associated secretory phenotype markers IL-1*α* and IL-6, with reduction in the expression of scleraxis, tenomodulin, biglycan, decorin, Col1, and Col3 were reported in human TSPCs isolated from tendinopathic tendons (Cai et al., 2022; Han et al., 2017; Kohler et al., 2013; Wang et al., 2022). The severity of tendinopathy might be positively correlated with p16, p21, p53 and senescence-associated β-galactosidase expression levels, and might be higher in human tendinopathic tenocytes compared with normal controls (Chen et al., 2022). Moreover, human tenocytes isolated from tendon tears might express increased values of senescence-associated secretory phenotype mediators (IL-6 and IL-8), and cellular senescence markers (p16 and p53) (Hawthorne et al., 2023; Lundgreen et al., 2011). A higher expression of type III collagen might occur in human tenocyte cultures from tendinopathic and ruptured tendons compared with normal tendons (Maffulli et al., 2000).

Aging might facilitate the senescence of human dermal fibroblasts when exposed to hydrogen peroxide, while TGF-β1 seems to prevent it (Gurjala et al., 2005). An increased expression of p21 and p53 was reported in human dermal wounds when compared with intact skin; with earliest p53 increase within 3 days of onset, and considerable increases at 8 days and between 3 and 11 weeks of onset (Chia et al., 2021; Hausmann et al., 1998; Jiang et al., 2020). Human dermal fibroblasts cultured from pressure ulcers might become prematurely senescent, with more cells staining positively for SA-β-Gal compared with normal skin (Vande Berg et al., 2005; Vande Berg et al., 1998). Pressure ulcer sections might stain positively for p21 cell cycle marker indicating senescence of fibroblasts (Vande Berg et al., 2005; Vande Berg et al., 2001).

Apoptosis is programmed destruction of cellular constituents, which might occur, like cellular senescence in response to extreme cellular stress (Campisi & d'Adda di Fagagna, 2007; Ellis et al., 1991). Caspases-3, -7 and -9 may have unique roles during the apoptosis process, with caspases-3 essential for efficient execution of apoptosis (Brentnall et al., 2013). Senescent human fibroblasts might resist apoptosis by downregulating caspase-3 (Marcotte et al., 2004). Tendinopathic tendons might demonstrate increased cellularity, however, apoptotic cell death mediated through caspase-3 was additionally identified (Chen et al., 2010; Gaida et al., 2012; Lian et al., 2007; Pearce et al., 2009). Apoptosis was also detected in degenerative tendons in patients with tendinopathy, rotator cuff impingement and tendon tears (Benson et al., 2010; Chen et al., 2010; Lundgreen et al., 2013; Tuoheti et al., 2005; Wu et al., 2011; Yuan et al., 2002). Chronic pressure ulcers, likewise, were found to express higher levels of caspase-3, and compared with acute wound and normal skin, higher TNFα and IL-1β mRNAs but lower expression of VEGF and bFGF and their receptors (Jiang et al., 2014; L. Jiang et al., 2012) (Figure 2).

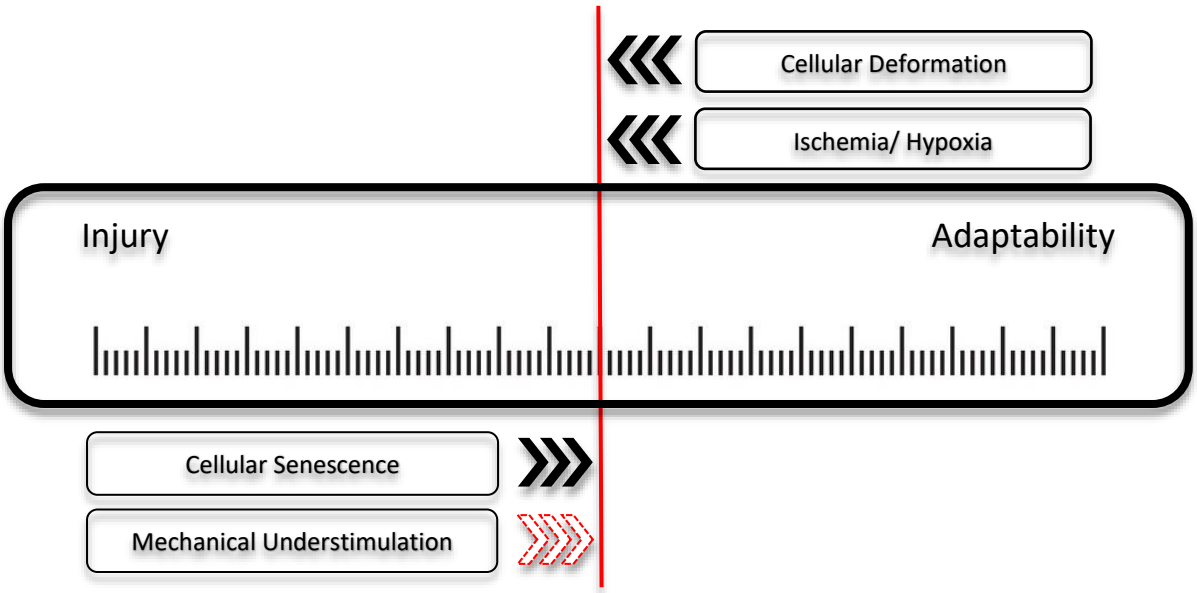



Figure 2. An illustration of the relationship between physiologic (or pathophysiologic) factors that may influence the threshold of adaptability and injury; mechanical stimulation may be translated as cellular deformation and ischemia/hypoxia, whereas, aging and prolonged inactivity may be translated as cellular senescence and mechanical understimulation, respectively.

**Excessive matrix degradation**

Stretching of human dermal fibroblasts was associated with an increase in MMP-1 and MMP-3 mRNA expression, although without change in collagen synthesis (Huang et al., 2013). Conversely, cyclic uniaxial mechanical strain was reported to increase synthesis of procollagen type I and TIMP-1, but reduce synthesis of MMP-1 (Binda et al., 2014). Another study, nonetheless, found little effect on MMP activity in response to cyclic mechanical strain (Zhang et al., 2014). Cyclic loading, furthermore, increased MMP-9 and reduced MMP-3 expression, while higher levels of MMP-2 were produced under static loading (Prajapati, Chavally-Mis, et al., 2000). Mechanical loading of human dermal fibroblasts was reported to augment the mRNA expression of MMP-1,-2,-3, and TIMP-1,-2 (Mudera et al., 2000). Ramp loading and unloading at a slow rate may cause a 5-fold increase in MMP-9 release (Prajapati, Eastwood, et al., 2000). More than 10-fold and 25-fold elevations of MMP-2 and MMP-9, respectively, were found in pressure ulcers exudate compared with healing wounds (Yager et al., 1996). Moreover, pro-MMP-2 and pro-MMP-9 were regarded as the main active enzymes in pressure ulcer biopsies (Rogers et al., 1995). Chronic ulcers, including pressure ulcers, were characterized by significant elevations in MMP-1 and MMP-8, and reduction in TIMP-1 (Nwomeh et al., 1999). Increased ratio of MMP-9/TIMP-1 levels was reported to negatively correlate with pressure ulcer healing (Ladwig et al., 2002).

Gene expression of IL-1β, MMP-1, and MMP-3 was shown to be induced by cyclic strain of human tenocytes (Tsuzaki, Bynum, et al., 2003; Yang et al., 2005). Moreover, the release of MMP-1, -3, -13 and aggrecanase-1 in human tendon cells may be induced by IL-1β (Tsuzaki, Guyton, et al., 2003; Yang et al., 2005). Physical exercise was shown to augment pro-MMP-9 release following exercise, which remained high for 3 days, while pro-MMP-2 level dropped immediately after exercise for a day and was slightly elevated at day 3 post-exercise (Koskinen et al., 2004). MMP-2 mRNA expression levels might be downregulated following a bout of resistance exercise (Sullivan et al., 2009). Painful tendons showed lower MMP-3,-10 and TIMP-3, and higher levels of ADAM-12 and MMP-23 mRNA expression levels compared with normal tendons (Jones et al., 2006). Ruptured tendons showed a higher gene expression of proteoglycan core protein versican and decorin, collagen type I, MMP-2 and -9, TIMP-1 and -2 (Karousou et al., 2008). Reduced activity of MMP-2, however, following tendon rupture was reported by another study (Riley et al., 2002).

Furthermore, ruptured tendons expressed higher levels of ADAMs-8,-12, MMP-1,-9,-19,-25, and TIMP-1, and lower levels of MMP-3,-7, and TIMP-2,-3,-4 (Jones et al., 2006). Increased activity of MMP-1 and reduced activity of MMP-3 were documented after tendon rupture (Riley et al., 2002). MMP-13 mRNA levels were shown to increase, whereas a reduction in MMP-3, TIMP-2, -3 and -4 mRNA levels were found in torn rotator cuff tendons (Lo et al., 2004). Degenerated long head biceps tendon, in combination with rotator cuff tears, showed increased MMP-1 and -9 expression and decreased MMP-3 expression (Lakemeier, Schwuchow, et al., 2010). A significant increase in MMP-9 and TIMP-1 expression was reported in patients with patellar tendinopathy (Parkinson et al., 2010). MMP-3 and TIMP-2 were suggested to have significant roles in the pathophysiology of chronic Achilles tendinopathy (Nie et al., 2019). Lower levels of MMP-3 mRNA, but significantly higher levels of type I and type III collagen mRNAs were expressed in tendinopathic Achilles tendons compared with healthy controls (Ireland et al., 2001) (Figure 3).

**Pain**

Sustained mechanical stimulation was found to augment pain intensity at tendon and tendon-bone junctions (Gibson et al., 2006). A consensus has been recently reached to refer to persistent tendon pain related to mechanical loading as tendinopathy (Scott et al., 2020). The mechanisms behind tendon pain in tendinopathy, nonetheless, remain not fully understood (Rio et al., 2014). Tendon pain with loading, tenderness and impaired function are some clinical features of tendinopathy (Kountouris & Cook, 2007; Ramos et al., 2009). An association between persistent painful tendinopathies and nervous system sensitization seems likely (Plinsinga et al., 2015; Tompra et al., 2015). Substantial sensory nerve ingrowth into tendon proper, up-regulated pain receptor excitability and expression, increased levels of pain mediators and involvement of neuro-immune pathways in pain regulation may be observed in chronic painful tendons (Ackermann, 2013; Ackermann et al., 2023; Ackermann et al., 2016). Pain is a common problem in patients with pressure ulcers, and the nature of pain experience is complex in these patients (Gorecki et al., 2011; Hopkins et al., 2006). Pain was severe and constant for some patients and was even reported at dressing change (Briggs et al., 2013; McGinnis et al., 2014; Szor & Bourguignon, 1999).

## Edema

Tendinopathic patellar tendon tissue sections showed an increase in water content compared with healthy control tendons (Tom et al., 2009). Significantly higher water content was found in torn supraspinatus and subscapularis tendons (Riley et al., 1994b). Tendon tissue edema, moreover, may characterize hypoxic degenerative tendinopathy (Jozsa & Kannus, 1997). Accumulation of proteoglycans in tendinopathic tendons might explain the observed increase in water content, since large proteoglycan are known to bind water (Chard et al., 1994; Parkinson et al., 2010; Riley et al., 1994a; Tom et al., 2009; Yanagishita, 1993).

Patients at risk of developing pressure ulcers showed signs of dermal and subdermal edema upon high-resolution ultrasound examination (Quintavalle et al., 2006). Moreover, increased subepidermal moisture (increased edema and inflammation) was reported in patients with stage I pressure injuries (Bates-Jensen et al., 2017; Bates-Jensen et al., 2008; Kim et al., 2018). Edema might predispose to ulcer formation and may delay wound healing once an ulcer is established (Perdue & Wilson, 1989). Areas of dissociated edema were reported in biopsies of early stage and chronic pressure ulcers (Berg & Rudolph, 1995; Diegelmann, 2003; Witkowski & Parish, 1982).

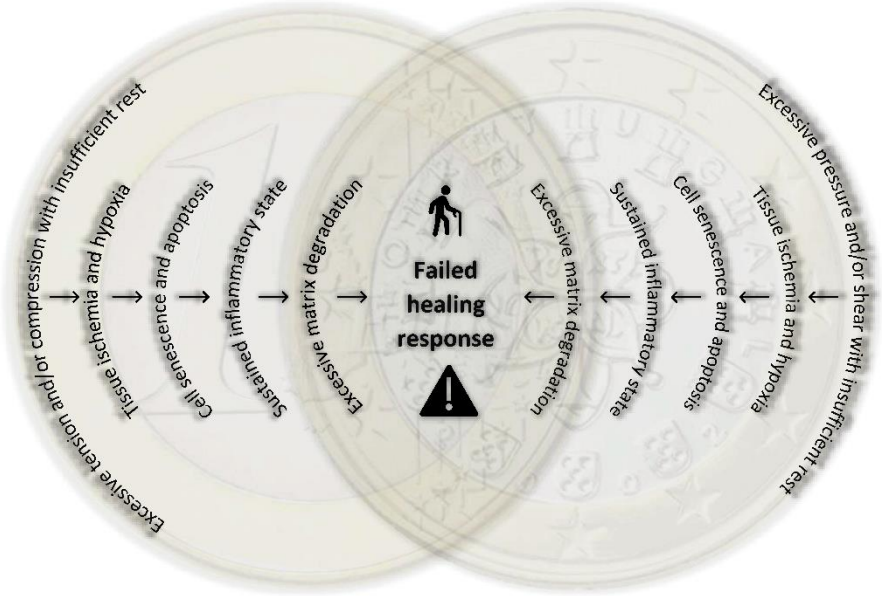



Figure 3. An illustration of the possible pathophysiologic mechanisms of tendinopathy and pressure ulcers leading to failed healing response; the old man walking with a cane signifies aging.

## Limitations

Tendinopathy may encompass a heterogenous clinical and histological spectrum compared with pressure ulcers (Jomaa et al., 2020). Tendinopathy, moreover, might not be identified as an inflammatory condition due to the absence of neutrophils. A consensus, nonetheless, on the definition of inflammatory cells might not have been established (Dean et al., 2016). However, the presence of macrophages and T-lymphocytes in tendinopathic tendons may contradict this conclusion (Dean et al., 2016). Neutrophils are commonly found in the granulation tissue of pressure ulcers (Diegelmann, 2003).

## References


Abate, M., Schiavone, C., Salini, V., & Andia, I. (2013). Occurrence of tendon pathologies in metabolic disorders. *Rheumatology, 52*(4), 599-608. doi:http://dx.doi.org/10.1093/rheumatology/kes395

Abate, M., Silbernagel, K. G., Siljeholm, C., Di Iorio, A., De Amicis, D., Salini, V., . . . Paganelli, R. (2009). Pathogenesis of tendinopathies: inflammation or degeneration? *Arthritis Research & Therapy, 11*(3), 1-15. doi:http://dx.doi.org/10.1186/ar2723

Abboud, J. A., & Kim, J. S. (2010). The effect of hypercholesterolemia on rotator cuff disease. *Clinical Orthopaedics and Related Research®, 468*(6), 1493-1497. doi:http://dx.doi.org/10.1007/s11999-009-1151-9

Ackermann, P. W. (2013). Neuronal regulation of tendon homoeostasis. *International journal of experimental pathology, 94*(4), 271-286. doi:http://dx.doi.org/10.1111/iep.12028

Ackermann, P. W., Alim, M. A., Pejler, G., & Peterson, M. (2023). Tendon pain–what are the mechanisms behind it? *Scandinavian Journal of Pain, 23*(1), 14-24. doi:http://dx.doi.org/10.1515/sjpain-2022-0018

Ackermann, P. W., Salo, P., & Hart, D. A. (2016). Tendon innervation. *Metabolic influences on risk for tendon disorders*, 35-51. doi:http://dx.doi.org/10.1007/978-3-319-33943-6_4

Ahmed, I., Lagopoulos, M., McConnell, P., Soames, R., & Sefton, G. (1998). Blood supply of the Achilles tendon. *Journal of orthopaedic research, 16*(5), 591-596. doi:http://dx.doi.org/10.1002/jor.1100160511

Alderden, J., Rondinelli, J., Pepper, G., Cummins, M., & Whitney, J. (2017). Risk factors for pressure injuries among critical care patients: a systematic review. *International journal of nursing studies, 71*, 97-114. doi:http://dx.doi.org/10.1016/j.ijnurstu.2017.03.012

Alfredson, H. (2005). The chronic painful Achilles and patellar tendon: research on basic biology and treatment. *Scandinavian journal of medicine & science in sports, 15*(4), 252-259. doi:http://dx.doi.org/10.1111/j.1600-0838.2005.00466.x

Alfredson, H., Bjur, D., Thorsen, K., Lorentzon, R., & Sandström, P. (2002). High intratendinous lactate levels in painful chronic Achilles tendinosis. An investigation using microdialysis technique. *Journal of orthopaedic research, 20*(5), 934-938. doi:http://dx.doi.org/10.1016/S0736-0266(02)00021-9

Alfredson, H., Lorentzon, M., Bäckman, S., Bäckman, A., & Lerner, U. H. (2003). cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic Achilles tendinosis. *Journal of orthopaedic research, 21*(6), 970-975. doi:http://dx.doi.org/10.1016/S0736-0266(03)00107-4

Allman, R. M. (1997). Pressure ulcer prevalence, incidence, risk factors, and impact. *Clinics in geriatric medicine, 13*(3), 421-436. doi:http://dx.doi.org/10.1016/S0749-0690(18)30152-6

Allman, R. M., Goode, P. S., Patrick, M. M., Burst, N., & Bartolucci, A. A. (1995). Pressure ulcer risk factors among hospitalized patients with activity limitation. *JAMA, 273*(11), 865-870. doi:http://dx.doi.org/10.1001/jama.1995.03520350047027

Alves, C., Mendes, D., & Marques, F. B. (2019). Fluoroquinolones and the risk of tendon injury: a systematic review and meta-analysis. *European journal of clinical pharmacology, 75*(10), 1431-1443. doi:http://dx.doi.org/10.1007/s00228-019-02713-1

Arai, K., Yamamoto, K., Suzuki, T., Shiko, Y., Kawasaki, Y., Mitsukawa, N., & Ishii, I. (2023). Factors affecting severity of pressure ulcers: Impact of number of medications. *Wound Repair and Regeneration, 31*(5), 671-678. doi:http://dx.doi.org/10.1111/wrr.13113

Arai, K., Yamamoto, K. h., Suzuki, T., Mitsukawa, N., & Ishii, I. (2020). Risk factors affecting pressure ulcer healing: Impact of prescription medications. *Wound Repair and Regeneration, 28*(3), 409-415. doi:http://dx.doi.org/10.1111/wrr.12791

Archambault, J. M., Wiley, J. P., & Bray, R. C. (1995). Exercise loading of tendons and the development of overuse injuries. *Sports Medicine, 20*(2), 77-89. doi:http://dx.doi.org/10.2165/00007256-199520020-00003

Arnoczky, S. P., Lavagnino, M., & Egerbacher, M. (2007). The mechanobiological aetiopathogenesis of tendinopathy: is it the over-stimulation or the under-stimulation of tendon cells? *International journal of experimental pathology, 88*(4), 217-226. doi:http://dx.doi.org/10.1111/j.1365-2613.2007.00548.x

Arnoczky, S. P., Lavagnino, M., Whallon, J. H., & Hoonjan, A. (2002). In situ cell nucleus deformation in tendons under tensile load; a morphological analysis using confocal laser microscopy. *Journal of orthopaedic research, 20*(1), 29-35. doi:http://dx.doi.org/10.1016/S0736-0266(01)00080-8

Arnoczky, S. P., Tian, T., Lavagnino, M., Gardner, K., Schuler, P., & Morse, P. (2002). Activation of stress-activated protein kinases (SAPK) in tendon cells following cyclic strain: the effects of strain frequency, strain magnitude, and cytosolic calcium. *Journal of orthopaedic research, 20*(5), 947-952. doi:http://dx.doi.org/10.1016/S0736-0266(02)00038-4

Arverud, E. D., Persson-Lindell, O., Sundquist, F., Labruto, F., Edman, G., & Ackermann, P. W. (2016). Microcirculation in healing and healthy Achilles tendon assessed with invasive laser doppler flowmetry. *Muscles, ligaments and tendons journal, 6*(1), 90. doi:http://dx.doi.org/10.11138/mltj/2016.6.1.090

Asai, K., Nakase, J., Ishikawa, T., Yoshimizu, R., Kimura, M., Ozaki, N., & Tsuchiya, H. (2022). Differences in cellular and microstructural properties of the semitendinosus muscle tendon between young and adult patients. *Journal of orthopaedic science, 27*(2), 478-485. doi:http://dx.doi.org/10.1016/j.jos.2021.01.012

Ascensión, A. M., Fuertes-Alvarez, S., Ibanez-Sole, O., Izeta, A., & Araúzo-Bravo, M. J. (2021). Human dermal fibroblast subpopulations are conserved across single-cell RNA sequencing studies. *Journal of Investigative Dermatology, 141*(7), 1735-1744. e1735. doi:http://dx.doi.org/10.1016/j.jid.2020.11.028

Åström, M. (2000). Laser Doppler flowmetry in the assessment of tendon blood flow. *Scandinavian journal of medicine & science in sports, 10*(6), 365-367. doi:http://dx.doi.org/10.1034/j.1600-0838.2000.010006365.x

Åstroöm, M., & Westlin, N. (1994). Blood flow in the human Achilles tendon assessed by laser Doppler flowmetry. *Journal of orthopaedic research, 12*(2), 246-252. doi:http://dx.doi.org/10.1002/jor.1100120214

Backman, L. J., Eriksson, D. E., & Danielson, P. (2014). Substance P reduces TNF-α-induced apoptosis in human tenocytes through NK-1 receptor stimulation. *British journal of sports medicine, 48*(19), 1414-1420. doi:http://dx.doi.org/10.1136/bjsports-2014-094099

Ballotta, V., Driessen-Mol, A., Bouten, C. V., & Baaijens, F. P. (2014). Strain-dependent modulation of macrophage polarization within scaffolds.

*Biomaterials, 35*(18), 4919-4928. doi:http://dx.doi.org/10.1016/j.biomaterials.2014.03.002

Barge-Caballero, E., Crespo-Leiro, M. G., Paniagua-Martín, M. J., Muñiz, J., Naya, C., Bouzas-Mosquera, A., . . . Cursack, G. C. (2008). Quinolone-related Achilles tendinopathy in heart transplant patients: incidence and risk factors. *The Journal of heart and lung transplantation, 27*(1), 46-51. doi:http://dx.doi.org/10.1016/j.healun.2007.09.021

Bates-Jensen, B. M., McCreath, H. E., & Patlan, A. (2017). Subepidermal moisture detection of pressure induced tissue damage on the trunk: the pressure ulcer detection study outcomes. *Wound Repair and Regeneration, 25*(3), 502-511. doi:http://dx.doi.org/10.1111/wrr.12548

Bates-Jensen, B. M., McCreath, H. E., Pongquan, V., & Apeles, N. C. R. (2008). Subepidermal moisture differentiates erythema and stage I pressure ulcers in nursing home residents. *Wound Repair and Regeneration, 16*(2), 189-197. doi:http://dx.doi.org/10.1111/j.1524-475X.2008.00359.x

Bauer, E. A., Stricklin, G. P., Jeffrey, J. J., & Eisen, A. Z. (1975). Collagenase production by human skin fibroblasts. *Biochemical and biophysical research communications, 64*(1), 232-240. doi:http://dx.doi.org/10.1016/0006-291X(75)90243-0

Baumgarten, K. M., Gerlach, D., Galatz, L. M., Teefey, S. A., Middleton, W. D., Ditsios, K., & Yamaguchi, K. (2010). Cigarette smoking increases the risk for rotator cuff tears. *Clinical Orthopaedics and Related Research®, 468*(6), 1534-1541. doi:http://dx.doi.org/10.1007/s11999-009-0781-2

Bayer, M. L., Schjerling, P., Herchenhan, A., Zeltz, C., Heinemeier, K. M., Christensen, L., . . . Kjaer, M. (2014). Release of tensile strain on engineered human tendon tissue disturbs cell adhesions, changes matrix architecture, and induces an inflammatory phenotype. *PLoS One, 9*(1), e86078. doi:http://dx.doi.org/10.1371/journal.pone.0086078

Becker, U., Nowack, H., Gay, S., & Timpl, R. (1976). Production and specificity of antibodies against the aminoterminal region in type III collagen. *Immunology, 31*(1), 57.

Behzad, H., Sharma, A., Mousavizadeh, R., Lu, A., & Scott, A. (2013). Mast cells exert pro-inflammatory effects of relevance to the pathophyisology of tendinopathy. *Arthritis Research & Therapy, 15*(6), 1-11. doi:http://dx.doi.org/10.1186/ar4374

Benson, R., McDonnell, S., Knowles, H., Rees, J., Carr, A., & Hulley, P. (2010). Tendinopathy and tears of the rotator cuff are associated with hypoxia and apoptosis. *The Journal of Bone & Joint Surgery British Volume, 92*(3), 448-453. doi:http://dx.doi.org/10.1302/0301-620X.92B3.23074

Berg, J. V., & Rudolph, R. (1995). Pressure (decubitus) ulcer: variation in histopathology—a light and electron microscope study. *Human pathology, 26*(2), 195-200. doi:http://dx.doi.org/10.1016/0046-8177(95)90037-3

Bidell, M. R., & Lodise, T. P. (2016). Fluoroquinolone-associated tendinopathy: does levofloxacin pose the greatest risk? *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 36*(6), 679-693. doi:http://dx.doi.org/10.1002/phar.1761

Binda, D., Tissot, M., Viennet, C., Saas, P., Muret, P., & Humbert, P. (2014). In vitro study of the impact of mechanical tension on the dermal fibroblast phenotype in the context of skin wound healing. *Journal of biomechanics, 47*(14), 3555-3561. doi:http://dx.doi.org/10.1016/j.jbiomech.2014.07.015

Blanco, I., Krähenbühl, S., & Schlienger, R. G. (2005). Corticosteroid-associated tendinopathies. *Drug Safety, 28*(7), 633-643. doi:http://dx.doi.org/10.2165/00002018-200528070-00005

Bojsen-Møller, J., Kalliokoski, K. K., Seppanen, M., Kjaer, M., & Magnusson, S. (2006). Low-intensity tensile loading increases intratendinous glucose uptake in the Achilles tendon. *Journal of applied physiology, 101*(1), 196-201. doi:http://dx.doi.org/10.1152/japplphysiol.00004.2006

Booth, B. A., Polak, K. L., & Uitto, J. (1980). Collagen biosynthesis by human skin fibroblasts. I. Optimization of the culture conditions for synthesis of type I and type III procollagens. *Biochimica et Biophysica Acta (BBA)-Nucleic Acids and Protein Synthesis, 607*(1), 145-160. doi:http://dx.doi.org/10.1016/0005-2787(80)90228-2

Børsting, T. E., Tvedt, C. R., Skogestad, I. J., Granheim, T. I., Gay, C. L., & Lerdal, A. (2018). Prevalence of pressure ulcer and associated risk factors in middle-and older-aged medical inpatients in Norway. *Journal of clinical nursing, 27*(3-4), e535-e543. doi:http://dx.doi.org/10.1111/jocn.14088

Bours, G., Laat, d. E., Halfens, R., & Lubbers, M. (2001). Prevalence, risk factors and prevention of pressure ulcers in Dutch intensive care units: results of a cross-sectional survey. *Intensive Care Medicine, 27*, 1599-1605. doi:http://dx.doi.org/10.1007/s001340101061

Boushel, R., Langberg, H., Green, S., Skovgaard, D., Bülow, J., & Kjær, M. (2000). Blood flow and oxygenation in peritendinous tissue and calf muscle during dynamic exercise in humans. *The Journal of physiology, 524*(1), 305-313. doi:http://dx.doi.org/10.1111/j.1469-7793.2000.t01-2-00305.x

Bouten, C. V., Oomens, C. W., Baaijens, F. P., & Bader, D. L. (2003). The etiology of pressure ulcers: skin deep or muscle bound? *Archives of physical medicine and rehabilitation, 84*(4), 616-619. doi:http://dx.doi.org/10.1053/apmr.2003.50038

Bowman, A., & Birch-Machin, M. A. (2016). Age-dependent decrease of mitochondrial complex II activity in human skin fibroblasts. *Journal of Investigative Dermatology, 136*(5), 912-919. doi:http://dx.doi.org/10.1016/j.jid.2016.01.017

Brandeis, G. H., Ooi, W. L., Hossain, M., Morris, J. N., & Lipsitz, L. A. (1994). A longitudinal study of risk factors associated with the formation of pressure ulcers in nursing homes. *Journal of the American Geriatrics Society, 42*(4), 388-393. doi:http://dx.doi.org/10.1111/j.1532-5415.1994.tb07486.x

Brentnall, M., Rodriguez-Menocal, L., De Guevara, R. L., Cepero, E., & Boise, L. H. (2013). Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis. *BMC cell biology, 14*, 1-9. doi:http://dx.doi.org/10.1186/1471-2121-14-32

Briggs, M., Collinson, M., Wilson, L., Rivers, C., McGinnis, E., Dealey, C., . . . Stevenson, R. (2013). The prevalence of pain at pressure areas and pressure ulcers in hospitalised patients. *BMC nursing, 12*(1), 1-6. doi:http://dx.doi.org/10.1186/1472-6955-12-19

Bronneberg, D., Spiekstra, S. W., Cornelissen, L. H., Oomens, C. W., Gibbs, S., Baaijens, F. P., & Bouten, C. V. (2007). Cytokine and chemokine release upon prolonged mechanical loading of the epidermis. *EXPERIMENTAL DERMATOLOGY, 16*(7), 567-573. doi:http://dx.doi.org/10.1111/j.1600-0625.2007.00566.x

BROWN, I. A. (1973). A scanning electron microscope study of the effects of uniaxial tension on human skin. *British Journal of Dermatology, 89*(4), 383-393. doi:http://dx.doi.org/10.1111/j.1365-2133.1973.tb02993.x

Brunner, G., Roux, M., Böhm, V., & Meiners, T. (2021). Cellular and molecular changes that predispose skin in chronic spinal cord injury to pressure ulcer formation. *International wound journal, 18*(5), 728-737. doi:http://dx.doi.org/10.1111/iwj.13575

Burry, H. (1978). Pathogenesis of some traumatic and degenerative disorders of soft tissue. *Australian and New Zealand journal of medicine, 8*, 163-167. doi:http://dx.doi.org/10.1111/j.1445-5994.1978.tb04816.x

Cai, Z., Zhang, Y., Liu, S., & Liu, X. (2022). Celecoxib, beyond anti-inflammation, alleviates tendon-derived stem cell senescence in degenerative rotator cuff tendinopathy. *The American Journal of Sports Medicine, 50*(9), 2488-2496. doi:http://dx.doi.org/10.1177/03635465221098133

Campisi, J., & d'Adda di Fagagna, F. (2007). Cellular senescence: when bad things happen to good cells. *Nature reviews Molecular cell biology, 8*(9), 729-740. doi:http://dx.doi.org/10.1038/nrm2233

Carbone, S., Gumina, S., Arceri, V., Campagna, V., Fagnani, C., & Postacchini, F. (2012). The impact of preoperative smoking habit on rotator cuff tear: cigarette smoking influences rotator cuff tear sizes. *Journal of shoulder and elbow surgery, 21*(1), 56-60. doi:http://dx.doi.org/10.1016/j.jse.2011.01.039

Cardoso, T. B., Pizzari, T., Kinsella, R., Hope, D., & Cook, J. L. (2019). Current trends in tendinopathy management. *Best Practice & Research Clinical Rheumatology, 33*(1), 122-140. doi:http://dx.doi.org/10.1016/j.berh.2019.02.001

Cawston, T., Riley, G., & Hazleman, B. (1996). Tendon lesions and soft tissue rheumatism--great outback or great opportunity? *Annals of the Rheumatic Diseases, 55*(1), 1. doi:http://dx.doi.org/10.1136/ard.55.1.1

Chaib, S., Tchkonia, T., & Kirkland, J. L. (2022). Cellular senescence and senolytics: the path to the clinic. *Nature medicine, 28*(8), 1556-1568. doi:http://dx.doi.org/10.1038/s41591-022-01923-y

Chard, M., Cawston, T., Riley, G., Gresham, G., & Hazleman, B. (1994). Rotator cuff degeneration and lateral epicondylitis: a comparative histological study. *Annals of the Rheumatic Diseases, 53*(1), 30-34. doi:http://dx.doi.org/10.1136/ard.53.1.30

Chard, M., Wright, J., & Hazleman, B. (1987). Isolation and growth characteristics of adult human tendon fibroblasts. *Annals of the Rheumatic Diseases, 46*(5), 385-390. doi:http://dx.doi.org/10.1136/ard.46.5.385

CHARLES S NEER, I. (1972). Anterior acromioplasty for the chronic impingement syndrome in the shoulder: a preliminary report. *JBJS, 54*(1), 41-50. doi:http://dx.doi.org/10.2106/JBJS.8706.cl

Chen, J.-H., Stoeber, K., Kingsbury, S., Ozanne, S. E., Williams, G. H., & Hales, C. N. (2004). Loss of proliferative capacity and induction of senescence in oxidatively stressed human fibroblasts. *Journal of Biological Chemistry, 279*(47), 49439-49446. doi:http://dx.doi.org/10.1074/jbc.M409153200

Chen, J., Wang, A., Xu, J., & Zheng, M. (2010). In chronic lateral epicondylitis, apoptosis and autophagic cell death occur in the extensor carpi radialis brevis tendon. *Journal of shoulder and elbow surgery, 19*(3), 355-362. doi:http://dx.doi.org/10.1016/j.jse.2009.07.064

Chen, S.-Y., Jou, I.-M., Ko, P.-Y., Hsu, K.-L., Su, W.-R., Kuo, L.-C., . . . Wu, P.-T. (2022). Amelioration of experimental tendinopathy by lentiviral CD44 gene therapy targeting senescence-associated secretory phenotypes. *Molecular Therapy-Methods & Clinical Development, 26*, 157-168. doi:http://dx.doi.org/10.1016/j.omtm.2022.06.006

Cheng, W., Yan-hua, R., Fang-gang, N., & Guo-an, Z. (2011). The content and ratio of type I and III collagen in skin differ with age and injury. *African Journal of Biotechnology, 10*(13), 2524-2529. doi:http://dx.doi.org/10.5897/AJB10.1999

Chia, C. W., Sherman-Baust, C. A., Larson, S. A., Pandey, R., Withers, R., Karikkineth, A. C., . . . Sen, R. (2021). Age-associated expression of p21and p53 during human wound healing. *Aging cell, 20*(5), e13354. doi:http://dx.doi.org/10.1111/acel.13354

Christensen, B., Dandanell, S., Kjaer, M., & Langberg, H. (2011). Effect of anti-inflammatory medication on the running-induced rise in patella tendon collagen synthesis in humans. *Journal of applied physiology, 110*(1), 137-141. doi:http://dx.doi.org/10.1152/japplphysiol.00942.2010

Clarke, A. W., Alyas, F., Morris, T., Robertson, C. J., Bell, J., & Connell, D. A. (2011). Skin-derived tenocyte-like cells for the treatment of patellar tendinopathy. *The American Journal of Sports Medicine, 39*(3), 614-623. doi:http://dx.doi.org/10.1177/0363546510387095

Clement, D., Taunton, J., & Smart, G. (1984). Achilles tendinitis and peritendinitis: etiology and treatment. *The American Journal of Sports Medicine, 12*(3), 179-184. doi:http://dx.doi.org/10.1177/036354658401200301

Cobb, J., Hotchkiss, R., Karl, I., & Buchman, T. (1996). Mechanisms of cell injury and death. *British Journal of Anaesthesia, 77*(1), 3-10. doi:http://dx.doi.org/10.1093/bja/77.1.3

Codman, E. A., & Akerson, I. B. (1931). The pathology associated with rupture of the supraspinatus tendon. *Annals of surgery, 93*(1), 348. doi:http://dx.doi.org/10.1097/00000658-193101000-00043

Coleman, S., Gorecki, C., Nelson, E. A., Closs, S. J., Defloor, T., Halfens, R., . . . Nixon, J. (2013). Patient risk factors for pressure ulcer development: systematic review. *International journal of nursing studies, 50*(7), 974-1003. doi:http://dx.doi.org/10.1016/j.ijnurstu.2012.11.019

Coleman, S., Nixon, J., Keen, J., Wilson, L., McGinnis, E., Dealey, C., . . . Schols, J. M. (2014). A new pressure ulcer conceptual framework. *Journal of advanced nursing, 70*(10), 2222-2234. doi:http://dx.doi.org/10.1111/jan.12405

Comninou, M., & Yannas, I. V. (1976). Dependence of stress-strain nonlinearity of connective tissues on the geometry of collagen fibres. *Journal of biomechanics, 9*(7), 427-433. doi:http://dx.doi.org/10.1016/0021-9290(76)90084-1

Connell, D., Datir, A., Alyas, F., & Curtis, M. (2009). Treatment of lateral epicondylitis using skin-derived tenocyte-like cells. *British journal of sports medicine, 43*(4), 293-298. doi:http://dx.doi.org/10.1136/bjsm.2008.056457

Cook, J., & Purdam, C. R. (2009). Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. *British journal of sports medicine, 43*(6), 409-416.

Cook, J., Rio, E., Purdam, C., & Docking, S. (2016). Revisiting the continuum model of tendon pathology: what is its merit in clinical practice and research? *British journal of sports medicine, 50*(19), 1187-1191. doi:http://dx.doi.org/10.1136/bjsports-2015-095422

Corps, A. N., Curry, V. A., Buttle, D. J., Hazleman, B. L., & Riley, G. P. (2004). Inhibition of interleukin-1β-stimulated collagenase and stromelysin expression in human tendon fibroblasts by epigallocatechin gallate ester. *Matrix Biology, 23*(3), 163-169. doi:http://dx.doi.org/10.1016/j.matbio.2004.05.001

Crowe, L. A., McLean, M., Kitson, S. M., Melchor, E. G., Patommel, K., Cao, H. M., . . . Spencer, S. J. (2019). S100A8 & S100A9: Alarmin mediated inflammation in tendinopathy. *Scientific Reports, 9*(1), 1463. doi:http://dx.doi.org/10.1038/s41598-018-37684-3

Culav, E. M., Clark, C. H., & Merrilees, M. J. (1999). Connective tissues: matrix composition and its relevance to physical therapy. *Physical Therapy, 79*(3), 308-319. doi:http://dx.doi.org/10.1093/ptj/79.3.308

D'Addona, A., Maffulli, N., Formisano, S., & Rosa, D. (2017). Inflammation in tendinopathy. *The Surgeon, 15*(5), 297-302. doi:http://dx.doi.org/10.1016/j.surge.2017.04.004

D'lima, D. D., Hashimoto, S., Chen, P. C., Colwell Jr, C. W., & Lotz, M. K. (2001). Impact of mechanical trauma on matrix and cells. *Clinical Orthopaedics and Related Research (1976-2007), 391*, S90-S99. doi:http://dx.doi.org/10.1097/00003086-200110001-00009

Dakin, S. G., Buckley, C. D., Al-Mossawi, M. H., Hedley, R., Martinez, F. O., Wheway, K., . . . Carr, A. J. (2017). Persistent stromal fibroblast activation is present in chronic tendinopathy. *Arthritis Research & Therapy, 19*, 1-11. doi:http://dx.doi.org/10.1186/s13075-016-1218-4

Dakin, S. G., Martinez, F. O., Yapp, C., Wells, G., Oppermann, U., Dean, B. J., . . . Roche, L. (2015). Inflammation activation and resolution in human tendon disease. *Science translational medicine, 7*(311), 311ra173-311ra173. doi:http://dx.doi.org/10.1126/scitranslmed.aac4269

Dakin, S. G., Newton, J., Martinez, F. O., Hedley, R., Gwilym, S., Jones, N., . . . Appleton, L. (2018). Chronic inflammation is a feature of Achilles tendinopathy and rupture. *British journal of sports medicine, 52*(6), 359-367. doi:http://dx.doi.org/10.1136/bjsports-2017-098161

Dalton, S., Cawston, T., Riley, G., Bayley, I., & Hazleman, B. (1995). Human shoulder tendon biopsy samples in organ culture produce procollagenase and tissue inhibitor of metalloproteinases. *Annals of the Rheumatic Diseases, 54*(7), 571-577. doi:http://dx.doi.org/10.1136/ard.54.7.571

de Jonge, S., Van den Berg, C., de Vos, R.-J., Van Der Heide, H., Weir, A., Verhaar, J., . . . Tol, J. (2011). Incidence of midportion Achilles tendinopathy in the general population. *British journal of sports medicine, 45*(13), 1026-1028. doi:http://dx.doi.org/10.1136/bjsports-2011-090342

de Wert, L. A., Bader, D. L., Oomens, C. W., Schoonhoven, L., Poeze, M., & Bouvy, N. D. (2015). A new method to evaluate the effects of shear on the skin. *Wound Repair and Regeneration, 23*(6), 885-890. doi:http://dx.doi.org/10.1111/wrr.12368

Dean, B. J., Dakin, S. G., Millar, N. L., & Carr, A. J. (2017). Emerging concepts in the pathogenesis of tendinopathy. *The Surgeon, 15*(6), 349-354. doi:http://dx.doi.org/10.1016/j.surge.2017.05.005

Dean, B. J. F., Gettings, P., Dakin, S. G., & Carr, A. J. (2016). Are inflammatory cells increased in painful human tendinopathy? A systematic review. *British journal of sports medicine, 50*(4), 216-220. doi:http://dx.doi.org/10.1136/bjsports-2015-094754

Del Buono, A., Battery, L., Denaro, V., Maccauro, G., & Maffulli, N. (2011). Tendinopathy and inflammation: some truths. *International journal of immunopathology and pharmacology, 24*(1_suppl2), 45-50. doi:http://dx.doi.org/10.1177/03946320110241S209

Deng, D., Liu, W., Xu, F., Yang, Y., Zhou, G., Zhang, W. J., . . . Cao, Y. (2009). Engineering human neo-tendon tissue in vitro with human dermal fibroblasts under static mechanical strain. *Biomaterials, 30*(35), 6724-6730. doi:http://dx.doi.org/10.1016/j.biomaterials.2009.08.054

Diegelmann, R. F. (2003). Excessive neutrophils characterize chronic pressure ulcers. *Wound Repair and Regeneration, 11*(6), 490-495. doi:http://dx.doi.org/10.1046/j.1524-475X.2003.11617.x

Dimri, G. P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., . . . Pereira-Smith, O. (1995). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proceedings of the National Academy of Sciences, 92*(20), 9363-9367. doi:http://dx.doi.org/10.1073/pnas.92.20.9363

DONCK, J. B., SEGAERT, M. F., & VANRENTERGHEM, Y. F. (1994). Fluoroquinolones and Achilles tendinopathy in renal transplant recipients. *Transplantation, 58*(6), 736. doi:http://dx.doi.org/10.1086/375078

Duance, V., Restall, D., Beard, H., Bourne, F., & Bailey, A. (1977). The location of three collagen types in skeletal muscle. *FEBS letters, 79*(2), 248-252. doi:http://dx.doi.org/10.1016/0014-5793(77)80797-7

Dumas, M., Chaudagne, C., Bonté, F., & Meybeck, A. (1994). In vitro biosynthesis of type I and III collagens by human dermal fibroblasts from donors of increasing age. *Mechanisms of ageing and development, 73*(3), 179-187. doi:http://dx.doi.org/10.1016/0047-6374(94)90050-7

Dunn, M. G., & Silver, F. H. (1983). Viscoelastic behavior of human connective tissues: relative contribution of viscous and elastic components. *Connective tissue research, 12*(1), 59-70. doi:http://dx.doi.org/10.3109/03008208309005612

Dutton, M., Magee, D., Hengeveld, E., Banks, K., Atkinson, K., Coutts, F., & Hassenkamp, A. (2004). *Orthopaedic examination, evaluation, and intervention* (Vol. 1): McGraw-Hill Medical.

Duval, E., Leclercq, S., Elissalde, J. M., Demoor, M., Galéra, P., & Boumédiene, K. (2009). Hypoxia-inducible factor 1α inhibits the fibroblast-like markers type I and type III collagen during hypoxia-induced chondrocyte redifferentiation: Hypoxia not only induces type II collagen and aggrecan, but it also inhibits type I and type III collagen in the hypoxia-inducible factor 1α–dependent redifferentiation of chondrocytes. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology, 60*(10), 3038-3048. doi:http://dx.doi.org/10.1002/art.24851

Eastwood, M., Porter, R., Khan, U., McGrouther, G., & Brown, R. (1996). Quantitative analysis of collagen gel contractile forces generated by dermal fibroblasts and the relationship to cell morphology. *Journal of cellular physiology, 166*(1), 33-42. doi:http://dx.doi.org/10.1002/(SICI)1097-4652(199601)166:1<33::AID-JCP4>3.0.CO;2-H

Edsberg, L. E., Black, J. M., Goldberg, M., McNichol, L., Moore, L., & Sieggreen, M. (2016). Revised national pressure ulcer advisory panel pressure injury staging system: revised pressure injury staging system. *Journal of Wound, Ostomy, and Continence Nursing, 43*(6), 585. doi:http://dx.doi.org/10.1097/WON.0000000000000281

Edsberg, L. E., Wyffels, J. T., Brogan, M. S., & Fries, K. M. (2012). Analysis of the proteomic profile of chronic pressure ulcers. *Wound Repair and Regeneration, 20*(3), 378-401. doi:http://dx.doi.org/10.1111/j.1524-475X.2012.00791.x
Edwards, D. (1946). The blood supply and lymphatic drainage of tendons. *Journal of anatomy, 80*(Pt 3), 147.
Eliasson, P., Dietrich-Zagonel, F., Lundin, A.-C., Aspenberg, P., Wolk, A., & Michaëlsson, K. (2019). Statin treatment increases the clinical risk of tendinopathy through matrix metalloproteinase release–a cohort study design combined with an experimental study. *Scientific Reports, 9*(1), 1-11. doi:http://dx.doi.org/10.1038/s41598-019-53238-7
Ellis, R. E., Yuan, J., & Horvitz, H. R. (1991). Mechanisms and functions of cell death. *Annual review of cell biology, 7*(1), 663-698. doi:http://dx.doi.org/10.1146/annurev.cb.07.110191.003311
Epstein Jr, E., & Munderloh, N. (1978). Human skin collagen. Presence of type I and type III at all levels of the dermis. *Journal of Biological Chemistry, 253*(5), 1336-1337. doi:http://dx.doi.org/10.1016/S0021-9258(17)34870-6
Evans, C., & Trail, I. (1998). Fibroblast-like cells from tendons differ from skin fibroblasts in their: Ability to form three-dimensional structures in vitro. *The Journal of Hand Surgery: British & European Volume, 23*(5), 633-641. doi:http://dx.doi.org/10.1016/S0266-7681(98)80018-1
Falanga, V., & Kirsner, R. S. (1993). Low oxygen stimulates proliferation of fibroblasts seeded as single cells. *Journal of cellular physiology, 154*(3), 506-510. doi:http://dx.doi.org/10.1002/jcp.1041540308
Falanga, V., Martin, T. A., Takagi, H., Kirsner, R. S., Helfman, T., Pardes, J., & Ochoa, M. S. (1993). Low oxygen tension increases mRNA levels of alpha 1 (I) procollagen in human dermal fibroblasts. *Journal of cellular physiology, 157*(2), 408-412. doi:http://dx.doi.org/10.1002/jcp.1041570225
Falanga, V., Qian, V. S. W., Danielpour, D., Katz, M. H., Roberts, A. B., & Sporn, M. B. (1991). Hypoxia upregulates the synthesis of TGF-β1 by human dermal fibroblasts. *Journal of Investigative Dermatology, 97*(4), 634-637. doi:http://dx.doi.org/10.1111/1523-1747.ep12483126
Falanga, V., Zhou, L., & Yufit, T. (2002). Low oxygen tension stimulates collagen synthesis and COL1A1 transcription through the action of TGF-β1. *Journal of cellular physiology, 191*(1), 42-50. doi:http://dx.doi.org/10.1002/jcp.10065
Fisher, G. J., Quan, T., Purohit, T., Shao, Y., Cho, M. K., He, T., . . . Voorhees, J. J. (2009). Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin. *The American journal of pathology, 174*(1), 101-114. doi:http://dx.doi.org/10.2353/ajpath.2009.080599
Fleisch, F., Hartmann, K., & Kuhn, M. (2000). Fluoroquinolone-induced tendinopathy: also occurring with levofloxacin. *Infection, 28*(4), 256. doi:http://dx.doi.org/10.1007/s150100070050
Fleischmajer, R., Timpl, R., Tuderman, L., Raisher, L., Wiestner, M., Perlish, J. S., & Graves, P. N. (1981). Ultrastructural identification of extension aminopropeptides of type I and III collagens in human skin. *Proceedings of the National Academy of Sciences, 78*(12), 7360-7364. doi:http://dx.doi.org/10.1073/pnas.78.12.7360
Fontanez, R., De Jesus, K., Sepulveda, F., & Micheo, W. (2022). Tendinopathy. In *Clinical Guide to Musculoskeletal Medicine* (pp. 645-650): Springer.
Franceschi, F., Papalia, R., Paciotti, M., Franceschetti, E., Di Martino, A., Maffulli, N., & Denaro, V. (2014). Obesity as a risk factor for tendinopathy: a systematic

review. *International Journal of Endocrinology, 2014*. doi:http://dx.doi.org/10.1155/2014/670262
Frankel, H., Sperry, J., & Kaplan, L. (2007). Risk factors for pressure ulcer development in a best practice surgical intensive care unit. *The American Surgeon, 73*(12), 1215-1217. doi:http://dx.doi.org/10.1177/000313480707301203
Frantz, R., Xakellis, G. C., & Arteaga, M. (1993). The effects of prolonged pressure on skin blood flow in elderly patients at risk for pressure ulcers. *Decubitus, 6*(6), 16-20.
Fredberg, U., & Stengaard-Pedersen, K. (2008). Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation. *Scandinavian journal of medicine & science in sports, 18*(1), 3-15. doi:http://dx.doi.org/10.1111/j.1600-0838.2007.00746.x
Freund, A., Orjalo, A. V., Desprez, P.-Y., & Campisi, J. (2010). Inflammatory networks during cellular senescence: causes and consequences. *Trends in molecular medicine, 16*(5), 238-246. doi:http://dx.doi.org/10.1016/j.molmed.2010.03.003
Frippiat, C., Chen, Q. M., Zdanov, S., Magalhaes, J.-P., Remacle, J., & Toussaint, O. (2001). Subcytotoxic H2O2 stress triggers a release of transforming growth factor-β1, which induces biomarkers of cellular senescence of human diploid fibroblasts. *Journal of Biological Chemistry, 276*(4), 2531-2537. doi:http://dx.doi.org/10.1074/jbc.M006809200
Fu, S.-C., Rolf, C., Cheuk, Y.-C., Lui, P. P., & Chan, K.-M. (2010a). Deciphering the pathogenesis of tendinopathy: a three-stages process. *BMC Sports Science, Medicine and Rehabilitation, 2*(1), 1-12. doi:http://dx.doi.org/10.1186/1758-2555-2-30
Fu, S.-C., Rolf, C., Cheuk, Y.-C., Lui, P. P., & Chan, K.-M. (2010b). Deciphering the pathogenesis of tendinopathy: a three-stages process. *BMC Sports Science, Medicine and Rehabilitation, 2*, 1-12.
Fujishiro, T., Nishikawa, T., Shibanuma, N., Akisue, T., Takikawa, S., Yamamoto, T., . . . Kurosaka, M. (2004). Effect of cyclic mechanical stretch and titanium particles on prostaglandin E2 production by human macrophages in vitro. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 68*(3), 531-536. doi:http://dx.doi.org/10.1002/jbm.a.20098
Gagliano, N., Menon, A., Cabitza, F., Compagnoni, R., & Randelli, P. (2018). Morphological and molecular characterization of human hamstrings shows that tendon features are not influenced by donor age. *Knee Surgery, Sports Traumatology, Arthroscopy, 26*, 343-352. doi:http://dx.doi.org/10.1007/s00167-017-4661-0
Gaida, J. E., Ashe, M. C., Bass, S. L., & Cook, J. L. (2009). Is adiposity an under-recognized risk factor for tendinopathy? A systematic review. *Arthritis Care & Research: Official Journal of the American College of Rheumatology, 61*(6), 840-849. doi:http://dx.doi.org/10.1002/art.24518
Gaida, J. E., Bagge, J., Purdam, C., Cook, J., Alfredson, H., & Forsgren, S. (2012). Evidence of the TNF-α system in the human Achilles tendon: expression of TNF-α and TNF receptor at both protein and mRNA levels in the tenocytes. *Cells Tissues Organs, 196*(4), 339-352. doi:http://dx.doi.org/10.1159/000335475

Gaida, J. E., Cook, J. L., & Bass, S. (2008). Adiposity and tendinopathy. *Disability and rehabilitation, 30*(20-22), 1555-1562. doi:http://dx.doi.org/10.1080/09638280701786864
Gallorini, M., Antonetti Lamorgese Passeri, C., Cataldi, A., Berardi, A. C., & Osti, L. (2022). Hyaluronic Acid Alleviates Oxidative Stress and Apoptosis in Human Tenocytes via Caspase 3 and 7. *International journal of molecular sciences, 23*(15), 8817. doi:http://dx.doi.org/10.3390/ijms23158817
Galluzzi, L., Vicencio, J. M., Kepp, O., Tasdemir, E., Maiuri, M. C., & Kroemer, G. (2008). To die or not to die: that is the autophagic question. *CURRENT MOLECULAR MEDICINE-NETHERLANDS THEN UAE-, 8*(2), 78. doi:http://dx.doi.org/10.2174/156652408783769616
Ganier, C., Rognoni, E., Goss, G., Lynch, M., & Watt, F. M. (2022). Fibroblast Heterogeneity in Healthy and Wounded Skin. *Cold Spring Harbor perspectives in biology*, a041238. doi:http://dx.doi.org/10.1101/cshperspect.a041238
Gefen, A. (2009). Reswick and Rogers pressure-time curve for pressure ulcer risk. Part 1. *Nursing Standard (through 2013), 23*(45), 64. doi:http://dx.doi.org/10.7748/ns2009.07.23.45.64.c7115
Gefen, A. (2018). The future of pressure ulcer prevention is here: detecting and targeting inflammation early. *EWMA J, 19*(2), 7-13.
Gefen, A., Brienza, D. M., Cuddigan, J., Haesler, E., & Kottner, J. (2022). Our contemporary understanding of the aetiology of pressure ulcers/pressure injuries. *International wound journal, 19*(3), 692-704. doi:http://dx.doi.org/10.1111/iwj.13667
Gibson, W., Arendt-Nielsen, L., & Graven-Nielsen, T. (2006). Referred pain and hyperalgesia in human tendon and muscle belly tissue. *Pain, 120*(1-2), 113-123. doi:http://dx.doi.org/10.1016/j.pain.2005.10.023
Goldstein, S. A., Armstrong, T. J., Chaffin, D. B., & Matthews, L. S. (1987). Analysis of cumulative strain in tendons and tendon sheaths. *Journal of biomechanics, 20*(1), 1-6. doi:http://dx.doi.org/10.1016/0021-9290(87)90261-2
Gomez-Collignon, A., Brown, R., Carr, A., Dakin, S., Lach, A., Loizou, C., . . . Kendal, A. (2022). Single cell multi-omics characterise discrete human tendon cells populations that persist in vitro and on fibrous scaffolds. *European Cells & Materials, 43*, 1-20. doi:http://dx.doi.org/10.22203/eCM.v044a01
Gorecki, C., Closs, S. J., Nixon, J., & Briggs, M. (2011). Patient-reported pressure ulcer pain: a mixed-methods systematic review. *Journal of Pain and Symptom Management, 42*(3), 443-459. doi:http://dx.doi.org/10.1016/j.jpainsymman.2010.11.016
Gotoh, M., Hamada, K., Yamakawa, H., Tomonaga, A., Inoue, A., & Fukuda, H. (1997). Significance of granulation tissue in torn supraspinatus insertions: An immunohistochemical study with antibodies against interleukin-1β, cathepsin D, and matrix metalloprotease-1. *Journal of orthopaedic research, 15*(1), 33-39. doi:http://dx.doi.org/10.1002/jor.1100150106
Greco, M., Villani, G., Mazzucchelli, F., Bresolin, N., Papa, S., & Attardi, G. (2003). Marked aging-related decline in efficiency of oxidative phosphorylation in human skin fibroblasts. *The FASEB Journal, 17*(12), 1706-1708. doi:http://dx.doi.org/10.1096/fj.02-1009fje
Grinnell, F., Zhu, M., Carlson, M. A., & Abrams, J. M. (1999). Release of mechanical tension triggers apoptosis of human fibroblasts in a model of regressing granulation tissue. *Experimental cell research, 248*(2), 608-619. doi:http://dx.doi.org/10.1006/excr.1999.4440

Gurjala, A. N., Liu, W. R., Mogford, J. E., Procaccini, P. S., & Mustoe, T. A. (2005). Age-dependent response of primary human dermal fibroblasts to oxidative stress: cell survival, pro-survival kinases, and entrance into cellular senescence. *Wound Repair and Regeneration, 13*(6), 565-575. doi:http://dx.doi.org/10.1111/j.1524-475X.2005.00079.x

Gwilym, S., Watkins, B., Cooper, C., Harvie, P., Auplish, S., Pollard, T., . . . Carr, A. (2009). Genetic influences in the progression of tears of the rotator cuff. *J Bone Joint Surg Br, 91*(7), 915-917. doi:http://dx.doi.org/10.1302/0301-620X.91B7.22353

Hagisawa, S., Barbenel, J., & Kenedi, R. (1991). Influence of age on postischaemic reactive hyperaemia. *Clinical Physics and Physiological Measurement, 12*(3), 227. doi:http://dx.doi.org/10.1088/0143-0815/12/3/003

Han, W., Wang, B., Liu, J., & Chen, L. (2017). The p16/miR-217/EGR1 pathway modulates age-related tenogenic differentiation in tendon stem/progenitor cells. *Acta biochimica et biophysica Sinica, 49*(11), 1015-1021. doi:http://dx.doi.org/10.1093/abbs/gmx104

Harding, K. G., Moore, K., & Phillips, T. J. (2005). Wound chronicity and fibroblast senescence–implications for treatment. *International wound journal, 2*(4), 364-368. doi:http://dx.doi.org/10.1111/j.1742-4801.2005.00149.x

Harrell, R. M. (1999). Fluoroquinolone-induced tendinopathy: what do we know? *Southern medical journal, 92*(6), 622-625. doi:http://dx.doi.org/10.1097/00007611-199906000-00014

Hart, D. (1995). Inflammatory processes in repetitive motion and over-use syndromes: potential role of neurogenic mechanisms in tendons and ligaments. *Repetitive motion disorders of the upper extremity*, 247-262.

Harvie, P., Ostlere, S., Teh, J., McNally, E., Clipsham, K., Burston, B., . . . Carr, A. (2004). Genetic influences in the aetiology of tears of the rotator cuff: sibling risk of a full-thickness tear. *J Bone Joint Surg Br, 86*(5), 696-700. doi:http://dx.doi.org/10.1302/0301-620X.91B7.22353

Hashimoto, T., & Shibasaki, F. (2015). Hypoxia-inducible factor as an angiogenic master switch. *Frontiers in pediatrics, 3*, 33. doi:http://dx.doi.org/10.3389/fped.2015.00033

Hausmann, R., Nerlich, A., & Betz, P. (1998). The time-related expression of p53 protein in human skin wounds–a quantitative immunohistochemical analysis. *International journal of legal medicine, 111*, 169-172. doi:http://dx.doi.org/10.1007/s004140050142

Hawthorne, B. C., Wellington, I. J., Sabitsky, J. T., Murphy, K. V., Karsmarski, O. P., Thomas, R. O., . . . Gulati, S. (2023). Human rotator cuff tears demonstrate an age-dependent increase in markers of cellular senescence and selective removal of senescent cells with dasatinib+ quercetin increases genetic expression of COL1A1 in vitro. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*. doi:http://dx.doi.org/10.1016/j.arthro.2023.05.036

Hayflick, L., & Moorhead, P. S. (1961). The serial cultivation of human diploid cell strains. *Experimental cell research, 25*(3), 585-621. doi:http://dx.doi.org/10.1016/0014-4827(61)90192-6

Heinemeier, K., Langberg, H., Olesen, J. L., & Kjaer, M. (2003). Role of TGF-β1 in relation to exercise-induced type I collagen synthesis in human tendinous tissue. *Journal of applied physiology, 95*(6), 2390-2397. doi:http://dx.doi.org/10.1152/japplphysiol.00403.2003

Heir, T., & Glomsaker, P. (1996). Epidemiology of musculoskeletal injuries among Norwegian conscripts undergoing basic military training. *Scandinavian journal of medicine & science in sports, 6*(3), 186-191. doi:http://dx.doi.org/10.1111/j.1600-0838.1996.tb00088.x
Henriksen, K., & Karsdal, M. (2016). Type I collagen. In *Biochemistry of collagens, laminins and elastin* (pp. 1-11): Elsevier.
Hetts, S. W. (1998). To die or not to die: an overview of apoptosis and its role in disease. *JAMA, 279*(4), 300-307. doi:http://dx.doi.org/10.1001/jama.279.4.300
Hope, M., & Saxby, T. S. (2007). Tendon healing. *Foot Ankle Clin, 12*(4), 553-567. doi:http://dx.doi.org/10.1016/j.fcl.2007.07.003
Hopkins, A., Dealey, C., Bale, S., Defloor, T., & Worboys, F. (2006). Patient stories of living with a pressure ulcer. *Journal of advanced nursing, 56*(4), 345-353. doi:http://dx.doi.org/10.1111/j.1365-2648.2006.04007.x
Howes, E. L., Sooy, J. W., & Harvey, S. C. (1929). The healing of wounds as determined by their tensile strength. *Journal of the American Medical Association, 92*(1), 42-45. doi:http://dx.doi.org/10.1001/jama.1929.02700270046011
Huang, C., Miyazaki, K., Akaishi, S., Watanabe, A., Hyakusoku, H., & Ogawa, R. (2013). Biological effects of cellular stretch on human dermal fibroblasts. *Journal of Plastic, Reconstructive & Aesthetic Surgery, 66*(12), e351-e361. doi:http://dx.doi.org/10.1016/j.bjps.2013.08.002
Hubbard, R., & Soutas-Little, R. (1984). Mechanical properties of human tendon and their age dependence. doi:http://dx.doi.org/10.1115/1.3138471
Hynes, R. O. (2009). The extracellular matrix: not just pretty fibrils. *Science, 326*(5957), 1216-1219. doi:http://dx.doi.org/10.1126/science.1176009
Ireland, D., Harrall, R., Curry, V., Holloway, G., Hackney, R., Hazleman, B., & Riley, G. (2001). Multiple changes in gene expression in chronic human Achilles tendinopathy. *Matrix Biology, 20*(3), 159-169. doi:http://dx.doi.org/10.1016/S0945-053X(01)00128-7
Jan, Y.-K., Liao, F., Rice, L. A., & Woods, J. A. (2013). Using reactive hyperemia to assess the efficacy of local cooling on reducing sacral skin ischemia under surface pressure in people with spinal cord injury: a preliminary report. *Archives of physical medicine and rehabilitation, 94*(10), 1982-1989. doi:http://dx.doi.org/10.1016/j.apmr.2013.03.022
Järvinen, M., Jozsa, L., Kannus, P., Järvinen, T., Kvist, M., & Leadbetter, W. (1997). Histopathological findings in chronic tendon disorders. *Scandinavian journal of medicine & science in sports, 7*(2), 86-95.
Jennings, R., Murry, C. E., Steenbergen Jr, C., & Reimer, K. (1990). Development of cell injury in sustained acute ischemia. *CIRCULATION, 82*(3 Suppl), II2-12.
Jiang, C., Shao, L., Wang, Q., & Dong, Y. (2012). Repetitive mechanical stretching modulates transforming growth factor-β induced collagen synthesis and apoptosis in human patellar tendon fibroblasts. *Biochemistry and Cell Biology, 90*(5), 667-674. doi:http://dx.doi.org/10.1139/o2012-024
Jiang, D., de Vries, J. C., Muschhammer, J., Schatz, S., Ye, H., Hein, T., . . . Scharffetter-Kochanek, K. (2020). Local and transient inhibition of p21 expression ameliorates age-related delayed wound healing. *Wound Repair and Regeneration, 28*(1), 49-60. doi:http://dx.doi.org/10.1111/wrr.12763
Jiang, L., Dai, Y., Cui, F., Pan, Y., Zhang, H., Xiao, J., & Xiaobing, F. (2014). Expression of cytokines, growth factors and apoptosis-related signal

molecules in chronic pressure ulcer wounds healing. *Spinal Cord, 52*(2), 145-151. doi:http://dx.doi.org/10.1038/sc.2013.132
Jiang, L., Zhang, E., Yang, Y., Zhang, C., Fu, X., & Xiao, J. (2012). Effectiveness of apoptotic factors expressed on the wounds of patients with stage III pressure ulcers. *Journal of Wound Ostomy & Continence Nursing, 39*(4), 391-396. doi:http://dx.doi.org/10.1097/WON.0b013e318259c47e
Jiao, X., Zhang, Y., Li, W., Zhou, X., Chu, W., Li, Y., . . . Gan, Y. (2022). HIF-1α inhibition attenuates severity of Achilles tendinopathy by blocking NF-κB and MAPK pathways. *International Immunopharmacology, 106*, 108543. doi:http://dx.doi.org/10.1016/j.intimp.2022.108543
John, T., Lodka, D., Kohl, B., Ertel, W., Jammrath, J., Conrad, C., . . . Schulze-Tanzil, G. (2010). Effect of pro-inflammatory and immunoregulatory cytokines on human tenocytes. *Journal of orthopaedic research, 28*(8), 1071-1077. doi:http://dx.doi.org/10.1002/jor.21079
Jomaa, G., Kwan, C.-K., Fu, S.-C., Ling, S. K.-K., Chan, K.-M., Yung, P. S.-H., & Rolf, C. (2020). A systematic review of inflammatory cells and markers in human tendinopathy. *BMC musculoskeletal disorders, 21*, 1-13. doi:http://dx.doi.org/10.1186/s12891-020-3094-y
Jones, G. C., Corps, A. N., Pennington, C. J., Clark, I. M., Edwards, D. R., Bradley, M. M., . . . Riley, G. P. (2006). Expression profiling of metalloproteinases and tissue inhibitors of metalloproteinases in normal and degenerate human achilles tendon. *Arthritis & Rheumatism, 54*(3), 832-842. doi:http://dx.doi.org/10.1002/art.21672
Jozsa, L., Balint, B., & Reffy, A. (1980). Calcifying tendinopathy. *Archives of orthopaedic and traumatic surgery, 97*, 305-307. doi:http://dx.doi.org/10.1007/BF00380713
Jozsa, L., Balint, B., Reffy, A., & Demel, Z. (1982). Hypoxic alterations of tenocytes in degenerative tendinopathy. *Archives of orthopaedic and traumatic surgery, 99*, 243-246. doi:http://dx.doi.org/10.1007/BF00381401
Jozsa, L., & Kannus, P. (1997). Histopathological findings in spontaneous tendon ruptures. *Scandinavian journal of medicine & science in sports, 7*(2), 113-118.
Kaitani, T., Tokunaga, K., Matsui, N., & Sanada, H. (2010). Risk factors related to the development of pressure ulcers in the critical care setting. *Journal of clinical nursing, 19*(3-4), 414-421. doi:http://dx.doi.org/10.1111/j.1365-2702.2009.03047.x
Kalliokoski, K. K., Langberg, H., Ryberg, A. K., Scheede-Bergdahl, C., Doessing, S., Kjaer, A., . . . Kjaer, M. (2005). The effect of dynamic knee-extension exercise on patellar tendon and quadriceps femoris muscle glucose uptake in humans studied by positron emission tomography. *Journal of applied physiology, 99*(3), 1189-1192. doi:http://dx.doi.org/10.1152/japplphysiol.00283.2005
Kang, H. T., Lee, K. B., Kim, S. Y., Choi, H. R., & Park, S. C. (2011). Autophagy impairment induces premature senescence in primary human fibroblasts. *PLoS One, 6*(8), e23367. doi:http://dx.doi.org/10.1371/journal.pone.0023367
Kang, Y., Roh, M. R., Rajadurai, S., Rajadurai, A., Kumar, R., Njauw, C.-N., . . . Tsao, H. (2020). Hypoxia and HIF-1α regulate collagen production in keloids. *Journal of Investigative Dermatology, 140*(11), 2157-2165. doi:http://dx.doi.org/10.1016/j.jid.2020.01.036
Kannus, P. (1997). Etiology and pathophysiology of chronic tendon disorders in sports. *Scandinavian journal of medicine & science in sports, 7*(2), 78-85. doi:http://dx.doi.org/10.1111/j.1600-0838.1997.tb00123.x

Kannus, P., & Jozsa, L. (1991). Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. *JBJS, 73*(10), 1507-1525.
Kanzler, M. H., Gorsulowsky, D. C., & Swanson, N. A. (1986). Basic mechanisms in the healing cutaneous wound. *The Journal of dermatologic surgery and oncology, 12*(11), 1156-1164. doi:http://dx.doi.org/10.1111/j.1524-4725.1986.tb02099.x
Karousou, E., Ronga, M., Vigetti, D., Passi, A., & Maffulli, N. (2008). Collagens, proteoglycans, MMP-2, MMP-9 and TIMPs in human achilles tendon rupture. *Clin Orthop Relat Res, 466*, 1577-1582. doi:http://dx.doi.org/10.1007/s11999-008-0255-y
Keller, P. B., Wille, J., van Ramshorst, B., & van der Werken, C. (2002). Pressure ulcers in intensive care patients: a review of risks and prevention. *Intensive Care Medicine, 28*, 1379-1388. doi:http://dx.doi.org/10.1007/s00134-002-1487-z
Kellett, J. (1986). Acute soft tissue injuries--a review of the literature. *Med Sci Sports Exerc, 18*(5), 489-500.
Kendal, A. R., Layton, T., Al-Mossawi, H., Appleton, L., Dakin, S., Brown, R., . . . Carr, A. (2020). Multi-omic single cell analysis resolves novel stromal cell populations in healthy and diseased human tendon. *Scientific Reports, 10*(1), 1-14.
Khorramizadeh, M., Tredget, E., Telasky, C., Shen, Q., & Ghahary, A. (1999). Aging differentially modulates the expression of collagen and collagenase in dermal fibroblasts. *Molecular and Cellular Biochemistry, 194*, 99-108. doi:http://dx.doi.org/10.1023/A:1006909021352
Kibler, W. (1990). Clinical aspects of muscle injury. *Med Sci Sports Exerc, 22*(4), 450-452.
Kim, C.-G., Park, S., Ko, J. W., & Jo, S. (2018). The relationship of subepidermal moisture and early stage pressure injury by visual skin assessment. *Journal of tissue viability, 27*(3), 130-134. doi:http://dx.doi.org/10.1016/j.jtv.2018.05.002
Kim, G. K. (2010). The risk of fluoroquinolone-induced tendinopathy and tendon rupture: what does the clinician need to know? *The Journal of clinical and aesthetic dermatology, 3*(4), 49.
Kim, J., Kim, B., Kim, S. M., Yang, C. E., Song, S. Y., Lee, W. J., & Lee, J. H. (2019). Hypoxia-induced epithelial-to-mesenchymal transition mediates fibroblast abnormalities via EKR activation in cutaneous wound healing. *International journal of molecular sciences, 20*(10), 2546. doi:http://dx.doi.org/10.3390/ijms20102546
Kirchgesner, T., Larbi, A., Omoumi, P., Malghem, J., Zamali, N., Manelfe, J., . . . Dallaudière, B. (2014). Drug-induced tendinopathy: from physiology to clinical applications. *Joint Bone Spine, 81*(6), 485-492. doi:http://dx.doi.org/10.1016/j.jbspin.2014.03.022
Kjaer, M. (2004). Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. *Physiological reviews, 84*(2), 649-698. doi:http://dx.doi.org/10.1152/physrev.00031.2003
Kjaer, M., Langberg, H., Skovgaard, D., Olesen, J., Bülow, J., Krogsgaard, M., & Boushel, R. (2000). In vivo studies of peritendinous tissue in exercise. *Scandinavian journal of medicine & science in sports, 10*(6), 326-331. doi:http://dx.doi.org/10.1034/j.1600-0838.2000.010006326.x

Klatte-Schulz, F., Pauly, S., Scheibel, M., Greiner, S., Gerhardt, C., Schmidmaier, G., & Wildemann, B. (2012). Influence of age on the cell biological characteristics and the stimulation potential of male human tenocyte-like cells. *Eur Cell Mater, 24*, 74-89. doi:http://dx.doi.org/10.22203/ecm.v024a06

Kohler, J., Popov, C., Klotz, B., Alberton, P., Prall, W. C., Haasters, F., . . . Jakob, F. (2013). Uncovering the cellular and molecular changes in tendon stem/progenitor cells attributed to tendon aging and degeneration. *Aging cell, 12*(6), 988-999. doi:http://dx.doi.org/10.1111/acel.12124

Komura, S., Satake, T., Goto, A., Aoki, H., Shibata, H., Ito, K., . . . Akiyama, H. (2020). Induced pluripotent stem cell-derived tenocyte-like cells promote the regeneration of injured tendons in mice. *Scientific Reports, 10*(1), 3992. doi:http://dx.doi.org/10.1038/s41598-020-61063-6

Koskinen, S. O., Heinemeier, K. M., Olesen, J. L., Langberg, H., & Kjaer, M. (2004). Physical exercise can influence local levels of matrix metalloproteinases and their inhibitors in tendon-related connective tissue. *Journal of applied physiology, 96*(3), 861-864. doi:http://dx.doi.org/10.1152/japplphysiol.00489.2003

Kottner, J., Balzer, K., Dassen, T., & Heinze, S. (2009). Pressure ulcers: a critical review of definitions and classifications. *Ostomy Wound Manage, 55*(9), 22-29.

Kountouris, A., & Cook, J. (2007). Rehabilitation of Achilles and patellar tendinopathies. *Best Practice & Research Clinical Rheumatology, 21*(2), 295-316. doi:http://dx.doi.org/10.1016/j.berh.2006.12.003

Kraemer, R., Wuerfel, W., Lorenzen, J., Busche, M., Vogt, P. M., & Knobloch, K. (2012). Analysis of hereditary and medical risk factors in Achilles tendinopathy and Achilles tendon ruptures: a matched pair analysis. *Archives of Orthopaedic and Trauma Surgery, 132*(6), 847-853. doi:http://dx.doi.org/10.1007/s00402-012-1476-9

Kragsnaes, M. S., Fredberg, U., Stribolt, K., Kjaer, S. G., Bendix, K., & Ellingsen, T. (2014). Stereological quantification of immune-competent cells in baseline biopsy specimens from achilles tendons: results from patients with chronic tendinopathy followed for more than 4 years. *The American Journal of Sports Medicine, 42*(10), 2435-2445. doi:http://dx.doi.org/10.1177/0363546514542329

Kubo, K., Ikebukuro, T., Tsunoda, N., & Kanehisa, H. (2008). Noninvasive measures of blood volume and oxygen saturation of human Achilles tendon by red laser lights. *Acta Physiologica, 193*(3), 257-264. doi:http://dx.doi.org/10.1111/j.1748-1716.2008.01841.x

Kubo, K., Ishigaki, T., & Ikebukuro, T. (2017). Measurement of blood flow in the human Achilles tendon in vivo. *The Journal of Physical Fitness and Sports Medicine, 6*(4), 251-256. doi:http://dx.doi.org/10.7600/jpfsm.6.251

Kuo, C. K., & Tuan, R. S. (2008). Mechanoactive tenogenic differentiation of human mesenchymal stem cells. *Tissue Engineering Part A, 14*(10), 1615-1627. doi:http://dx.doi.org/10.1089/ten.tea.2006.0415

Ladwig, G. P., Robson, M. C., Liu, R., Kuhn, M. A., Muir, D. F., & Schultz, G. S. (2002). Ratios of activated matrix metalloproteinase-9 to tissue inhibitor of matrix metalloproteinase-1 in wound fluids are inversely correlated with healing of pressure ulcers. *Wound Repair and Regeneration, 10*(1), 26-37. doi:http://dx.doi.org/10.1046/j.1524-475X.2002.10903.x

Lago, J. C., & Puzzi, M. B. (2019). The effect of aging in primary human dermal fibroblasts. *PLoS One, 14*(7), e0219165. doi:http://dx.doi.org/10.1371/journal.pone.0219165

Lakemeier, S., Reichelt, J. J., Patzer, T., Fuchs-Winkelmann, S., Paletta, J. R., & Schofer, M. D. (2010). The association between retraction of the torn rotator cuff and increasing expression of hypoxia inducible factor 1α and vascular endothelial growth factor expression: an immunohistological study. *BMC musculoskeletal disorders, 11*, 1-7. doi:http://dx.doi.org/10.1186/1471-2474-11-230

Lakemeier, S., Reichelt, J. J., Timmesfeld, N., Fuchs-Winkelmann, S., Paletta, J. R., & Schofer, M. D. (2010). The relevance of long head biceps degeneration in the presence of rotator cuff tears. *BMC musculoskeletal disorders, 11*, 1-7. doi:http://dx.doi.org/10.1186/1471-2474-11-191

Lakemeier, S., Schwuchow, S. A., Peterlein, C. D., Foelsch, C., Fuchs-Winkelmann, S., Archontidou-Aprin, E., . . . Schofer, M. D. (2010). Expression of matrix metalloproteinases 1, 3, and 9 in degenerated long head biceps tendon in the presence of rotator cuff tears: an immunohistological study. *BMC musculoskeletal disorders, 11*(1), 1-7. doi:http://dx.doi.org/10.1186/1471-2474-11-271

LANGBERG, BÜLOW, & KJÆR. (1998). Blood flow in the peritendinous space of the human Achilles tendon during exercise. *Acta Physiologica Scandinavica, 163*(2), 149-153. doi:http://dx.doi.org/10.1046/j.1365-201X.1998.00361.x

Langberg, Bülow, & Kjær. (1999). Standardized intermittent static exercise increases peritendinous blood flow in human leg. *Clinical Physiology, 19*(1), 89-93. doi:http://dx.doi.org/10.1046/j.1365-2281.1999.00148.x

Langberg, H., Bjørn, C., Boushel, R., Hellsten, Y., & Kjaer, M. (2002). Exercise-induced increase in interstitial bradykinin and adenosine concentrations in skeletal muscle and peritendinous tissue in humans. *The Journal of physiology, 542*(3), 977-983. doi:http://dx.doi.org/10.1113/jphysiol.2002.018077

Langberg, H., Boushel, R., Skovgaard, D., Risum, N., & Kjaer, M. (2003). Cyclo-oxygenase-2 mediated prostaglandin release regulates blood flow in connective tissue during mechanical loading in humans. *The Journal of physiology, 551*(2), 683-689. doi:http://dx.doi.org/10.1113/jphysiol.2003.046094

Langberg, H., Olesen, J., Skovgaard, D., & Kjær, M. (2001). Age related blood flow around the Achilles tendon during exercise in humans. *European Journal of Applied Physiology, 84*, 246-248. doi:http://dx.doi.org/10.1007/s004210170013

Langberg, H., Skovgaard, D., Karamouzis, M., Bülow, J., & Kjær, M. (1999). Metabolism and inflammatory mediators in the peritendinous space measured by microdialysis during intermittent isometric exercise in humans. *The Journal of physiology, 515*(3), 919-927. doi:http://dx.doi.org/10.1111/j.1469-7793.1999.919ab.x

Langberg, H., Skovgaard, D., Petersen, L. J., Bülow, J., & Kjær, M. (1999). Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. *The Journal of physiology, 521*(1), 299-306. doi:http://dx.doi.org/10.1111/j.1469-7793.1999.00299.x

Lee, A. C., Fenster, B. E., Ito, H., Takeda, K., Bae, N. S., Hirai, T., . . . Finkel, T. (1999). Ras proteins induce senescence by altering the intracellular levels of

reactive oxygen species. *Journal of Biological Chemistry, 274*(12), 7936-7940. doi:http://dx.doi.org/10.1074/jbc.274.12.7936
Lee, A. H., Szczesny, S. E., Santare, M. H., & Elliott, D. M. (2017). Investigating mechanisms of tendon damage by measuring multi-scale recovery following tensile loading. *Acta Biomaterialia, 57*, 363-372. doi:http://dx.doi.org/10.1016/j.actbio.2017.04.011
Legerlotz, K., Jones, E. R., Screen, H. R., & Riley, G. P. (2012). Increased expression of IL-6 family members in tendon pathology. *Rheumatology, 51*(7), 1161-1165. doi:http://dx.doi.org/10.1093/rheumatology/kes002
Leong, H. T., Chuen Fu, S., He, X., Han Oh, J., Yamamoto, N., & Yung, S. H. P. (2019). Risk factors for rotator cuff tendinopathy: a systematic review and meta-analysis. *Journal of Rehabilitation Medicine, 51*(9), 627-637. doi:http://dx.doi.org/10.2340/16501977-2598
Leong, N. L., Kator, J. L., Clemens, T. L., James, A., Enamoto-Iwamoto, M., & Jiang, J. (2020). Tendon and ligament healing and current approaches to tendon and ligament regeneration. *Journal of Orthopaedic Research®, 38*(1), 7-12. doi:http://dx.doi.org/10.1002/jor.24475
Li, Y., Lei, D., Swindell, W. R., Xia, W., Weng, S., Fu, J., . . . Gudjonsson, J. E. (2015). Age-associated increase in skin fibroblast–derived prostaglandin E2 contributes to reduced collagen levels in elderly human skin. *Journal of Investigative Dermatology, 135*(9), 2181-2188. doi:http://dx.doi.org/10.1038/jid.2015.157
Li, Z., Yang, G., Khan, M., Stone, D., Woo, S. L., & Wang, J. H. (2004). Inflammatory response of human tendon fibroblasts to cyclic mechanical stretching. *The American Journal of Sports Medicine, 32*(2), 435-440. doi:http://dx.doi.org/10.1177/0095399703258680
Lian, Ø., Scott, A., Engebretsen, L., Bahr, R., Duronio, V., & Khan, K. (2007). Excessive apoptosis in patellar tendinopathy in athletes. *The American Journal of Sports Medicine, 35*(4), 605-611. doi:http://dx.doi.org/10.1177/0363546506295702
Liang, M., Cornell, H. R., Zargar Baboldashti, N., Thompson, M. S., Carr, A. J., & Hulley, P. A. (2012). Regulation of hypoxia-induced cell death in human tenocytes. *Advances in orthopedics, 2012*. doi:http://dx.doi.org/10.1155/2012/984950
Linder-Ganz, E., Engelberg, S., Scheinowitz, M., & Gefen, A. (2006). Pressure–time cell death threshold for albino rat skeletal muscles as related to pressure sore biomechanics. *Journal of biomechanics, 39*(14), 2725-2732. doi:http://dx.doi.org/10.1016/j.jbiomech.2005.08.010
Lindgren, M., Unosson, M., Fredrikson, M., & Ek, A. C. (2004). Immobility–a major risk factor for development of pressure ulcers among adult hospitalized patients: a prospective study. *Scandinavian journal of caring sciences, 18*(1), 57-64. doi:http://dx.doi.org/10.1046/j.0283-9318.2003.00250.x
Lo, I. K., Marchuk, L. L., Hollinshead, R., Hart, D. A., & Frank, C. B. (2004). Matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase mRNA levels are specifically altered in torn rotator cuff tendons. *The American Journal of Sports Medicine, 32*(5), 1223-1229. doi:http://dx.doi.org/10.1177/0363546503262200
Lovell, C., Smolenski, K., Duance, V., Light, N., Young, S., & Dyson, M. (1987). Type I and III collagen content and fibre distribution in normal human skin during

ageing. *British Journal of Dermatology, 117*(4), 419-428. doi:http://dx.doi.org/10.1111/j.1365-2133.1987.tb04921.x
Lu, P., Takai, K., Weaver, V. M., & Werb, Z. (2011). Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harbor perspectives in biology, 3*(12), a005058. doi:http://dx.doi.org/10.1101/cshperspect.a005058
Lundgreen, K., Lian, Ø., Scott, A., & Engebretsen, L. (2013). Increased levels of apoptosis and p53 in partial-thickness supraspinatus tendon tears. *Knee Surgery, Sports Traumatology, Arthroscopy, 21*, 1636-1641. doi:http://dx.doi.org/10.1007/s00167-012-2226-9
Lundgreen, K., Lian, Ø. B., Engebretsen, L., & Scott, A. (2011). Tenocyte apoptosis in the torn rotator cuff: a primary or secondary pathological event? *British journal of sports medicine, 45*(13), 1035-1039. doi:http://dx.doi.org/10.1136/bjsm.2010.083188
Lupa, D. M. W., Kalfalah, F., Safferling, K., Boukamp, P., Poschmann, G., Volpi, E., . . . Huebenthal, U. (2015). Characterization of skin aging–associated secreted proteins (SAASP) produced by dermal fibroblasts isolated from intrinsically aged human skin. *Journal of Investigative Dermatology, 135*(8), 1954-1968. doi:http://dx.doi.org/10.1038/jid.2015.120
Lustig, A., Margi, R., Orlov, A., Orlova, D., Azaria, L., & Gefen, A. (2021). The mechanobiology theory of the development of medical device-related pressure ulcers revealed through a cell-scale computational modeling framework. *Biomechanics and Modeling in Mechanobiology, 20*(3), 851-860. doi:http://dx.doi.org/10.1007/s10237-021-01432-w
Macchi, M., Spezia, M., Elli, S., Schiaffini, G., & Chisari, E. (2020). Obesity increases the risk of tendinopathy, tendon tear and rupture, and postoperative complications: a systematic review of clinical studies. *Clinical Orthopaedics and Related Research®, 478*(8), 1839-1847. doi:http://dx.doi.org/10.1097/CORR.0000000000001261
Mackley, J. R., Ando, J., Herzyk, P., & Winder, S. J. (2006). Phenotypic responses to mechanical stress in fibroblasts from tendon, cornea and skin. *Biochemical Journal, 396*(2), 307-316. doi:http://dx.doi.org/10.1042/BJ20060057
Maffulli, N., Ewen, S. W., Waterston, S. W., Reaper, J., & Barrass, V. (2000). Tenocytes from ruptured and tendinopathic achilles tendons produce greater quantities of type III collagen than tenocytes from normal achilles tendons: an in vitro model of human tendon healing. *The American Journal of Sports Medicine, 28*(4), 499-505. doi:http://dx.doi.org/10.1177/03635465000280040901
Maganaris, C. N., & Paul, J. P. (1999). In vivo human tendon mechanical properties. In: Wiley Online Library.
Mallon, W. J., Misamore, G., Snead, D. S., & Denton, P. (2004). The impact of preoperative smoking habits on the results of rotator cuff repair. *Journal of shoulder and elbow surgery, 13*(2), 129-132. doi:http://dx.doi.org/10.1016/j.jse.2003.11.002
Manorama, A., Meyer, R., Wiseman, R., & Bush, T. R. (2013). Quantifying the effects of external shear loads on arterial and venous blood flow: implications for pressure ulcer development. *Clinical Biomechanics, 28*(5), 574-578. doi:http://dx.doi.org/10.1016/j.clinbiomech.2013.04.001
Manorama, A. A., Baek, S., Vorro, J., Sikorskii, A., & Bush, T. R. (2010). Blood perfusion and transcutaneous oxygen level characterizations in human skin with changes in normal and shear loads—implications for pressure ulcer

formation. *Clinical Biomechanics, 25*(8), 823-828. doi:http://dx.doi.org/10.1016/j.clinbiomech.2010.06.003
Marcotte, R., Lacelle, C., & Wang, E. (2004). Senescent fibroblasts resist apoptosis by downregulating caspase-3. *Mechanisms of ageing and development, 125*(10-11), 777-783. doi:http://dx.doi.org/10.1016/j.mad.2004.07.007
Marti, H., Stoller, R., & Frey, F. (1998). Fluoroquinolones as a cause of tendon disorders in patients with renal failure/renal transplants. *British journal of rheumatology, 37*(3), 343-344. doi:http://dx.doi.org/10.1093/rheumatology/37.3.343
Mason, M. L., & Allen, H. S. (1941). The rate of healing of tendons: an experimental study of tensile strength. *Annals of surgery, 113*(3), 424. doi:http://dx.doi.org/10.1097/00000658-194103000-00009
Matthews, T., Hand, G., Rees, J., Athanasou, N., & Carr, A. (2006). Pathology of the torn rotator cuff tendon: reduction in potential for repair as tear size increases. *The Journal of Bone & Joint Surgery British Volume, 88*(4), 489-495. doi:http://dx.doi.org/10.1302/0301-620X.88B4.16845
Mayer, L. (1916). The physiological method of tendon transplantation. I. Historical; anatomy and physiology of tendons. *Sdurg Gynecol Obstet, 22*, 182-192.
McBeath, R., Edwards, R. W., O'Hara, B. J., Maltenfort, M. G., Parks, S. M., Steplewski, A., . . . Shapiro, I. M. (2019). Tendinosis develops from age-and oxygen tension-dependent modulation of Rac1 activity. *Aging cell, 18*(3), e12934. doi:http://dx.doi.org/10.1111/acel.12934
McGinnis, E., Briggs, M., Collinson, M., Wilson, L., Dealey, C., Brown, J., . . . Nelson, E. A. (2014). Pressure ulcer related pain in community populations: a prevalence survey. *BMC nursing, 13*(1), 1-10. doi:http://dx.doi.org/10.1186/1472-6955-13-16
McQueen, F., Beckley, V., Crabbe, J., Robinson, E., Yeoman, S., & Stewart, N. (2005). Magnetic resonance imaging evidence of tendinopathy in early rheumatoid arthritis predicts tendon rupture at six years. *Arthritis & Rheumatism, 52*(3), 744-751. doi:http://dx.doi.org/10.1002/art.20947
Mennan, C., Garcia, J., McCarthy, H., Owen, S., Perry, J., Wright, K., . . . Roberts, S. (2019). Human articular chondrocytes retain their phenotype in sustained hypoxia while normoxia promotes their immunomodulatory potential. *Cartilage, 10*(4), 467-479. doi:http://dx.doi.org/10.1177/1947603518769714
Merkel, M. F. R., Hellsten, Y., Magnusson, S. P., & Kjaer, M. (2021). Tendon blood flow, angiogenesis, and tendinopathy pathogenesis. *Translational sports medicine, 4*(6), 756-771. doi:http://dx.doi.org/10.1002/tsm2.280
MEYER, A. W. (1921). Unrecognized occupational destruction of the tendon of the long head of the biceps brachii. *Archives of Surgery, 2*(1), 130-144. doi:http://dx.doi.org/10.1001/archsurg.1921.01110040139007
Meyer, A. W. (1924). Further evidences of attrition in the human body. *American Journal of Anatomy, 34*(1), 241-267. doi:http://dx.doi.org/10.1002/aja.1000340108
Millar, N. L., Hueber, A. J., Reilly, J. H., Xu, Y., Fazzi, U. G., Murrell, G. A., & McInnes, I. B. (2010). Inflammation is present in early human tendinopathy. *The American Journal of Sports Medicine, 38*(10), 2085-2091. doi:http://dx.doi.org/10.1177/0363546510372613
Millar, N. L., Reilly, J. H., Kerr, S. C., Campbell, A. L., Little, K. J., Leach, W. J., . . . McInnes, I. B. (2012). Hypoxia: a critical regulator of early human

tendinopathy. *Annals of the Rheumatic Diseases, 71*(2), 302-310. doi:http://dx.doi.org/10.1136/ard.2011.154229
Miller, B. F., Olesen, J. L., Hansen, M., Døssing, S., Crameri, R. M., Welling, R. J., . . . Babraj, J. A. (2005). Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. *The Journal of physiology, 567*(3), 1021-1033. doi:http://dx.doi.org/10.1113/jphysiol.2005.093690
Miller, M. A., & Zachary, J. F. (2017). Mechanisms and morphology of cellular injury, adaptation, and death. *Pathologic basis of veterinary disease*, 2. doi:http://dx.doi.org/10.1016/B978-0-323-35775-3.00001-1
Mingyuan, X., Qianqian, P., Shengquan, X., Chenyi, Y., Rui, L., Yichen, S., & Jinghong, X. (2018). Hypoxia-inducible factor-1α activates transforming growth factor-β1/Smad signaling and increases collagen deposition in dermal fibroblasts. *Oncotarget, 9*(3), 3188. doi:http://dx.doi.org/10.18632/oncotarget.23225
Moerch, L., Pingel, J., Boesen, M., Kjaer, M., & Langberg, H. (2013). The effect of acute exercise on collagen turnover in human tendons: influence of prior immobilization period. *European Journal of Applied Physiology, 113*, 449-455. doi:http://dx.doi.org/10.1007/s00421-012-2450-5
Mogford, J. E., Tawil, N., Chen, A., Gies, D., Xia, Y., & Mustoe, T. A. (2002). Effect of age and hypoxia on TGFβ1 receptor expression and signal transduction in human dermal fibroblasts: impact on cell migration. *Journal of cellular physiology, 190*(2), 259-265. doi:http://dx.doi.org/10.1002/jcp.10060
Mosca, M. J., Carr, A. J., Snelling, S. J., Wheway, K., Watkins, B., & Dakin, S. G. (2017). Differential expression of alarmins—S100A9, IL-33, HMGB1 and HIF-1α in supraspinatus tendinopathy before and after treatment. *BMJ open sport & exercise medicine, 3*(1), e000225. doi:http://dx.doi.org/10.1136/bmjsem-2017-000225
Mosca, M. J., Rashid, M. S., Snelling, S. J., Kirtley, S., Carr, A. J., & Dakin, S. G. (2018). Trends in the theory that inflammation plays a causal role in tendinopathy: a systematic review and quantitative analysis of published reviews. *BMJ open sport & exercise medicine, 4*(1), e000332. doi:http://dx.doi.org/10.1136/bmjsem-2017-000332
Mudera, V. C., Pleass, R., Eastwood, M., Tarnuzzer, R., Schultz, G., Khaw, P., . . . Brown, R. (2000). Molecular responses of human dermal fibroblasts to dual cues: contact guidance and mechanical load. *Cell motility and the cytoskeleton, 45*(1), 1-9. doi:http://dx.doi.org/10.1002/(SICI)1097-0169(200001)45:1<1::AID-CM1>3.0.CO;2-J
Mustoe, T. A., O'shaughnessy, K., & Kloeters, O. (2006). Chronic wound pathogenesis and current treatment strategies: a unifying hypothesis. *Plastic and reconstructive surgery, 117*(7S), 35S-41S. doi:http://dx.doi.org/10.1097/01.prs.0000225431.63010.1b
Nakanishi, Y., Okada, T., Takeuchi, N., Kozono, N., Senju, T., Nakayama, K., & Nakashima, Y. (2019). Histological evaluation of tendon formation using a scaffold-free three-dimensional-bioprinted construct of human dermal fibroblasts under in vitro static tensile culture. *Regenerative therapy, 11*, 47-55. doi:http://dx.doi.org/10.1016/j.reth.2019.02.002
Neviaser, A., Andarawis-Puri, N., & Flatow, E. (2012). Basic mechanisms of tendon fatigue damage. *Journal of shoulder and elbow surgery, 21*(2), 158-163. doi:http://dx.doi.org/10.1016/j.jse.2011.11.014

Nie, G., Wen, X., Liang, X., Zhao, H., Li, Y., & Lu, J. (2019). Additional evidence supports association of common genetic variants in MMP3 and TIMP2 with increased risk of chronic Achilles tendinopathy susceptibility. *Journal of science and medicine in sport, 22*(10), 1074-1078. doi:http://dx.doi.org/10.1016/j.jsams.2019.05.021

Nijs, N., Toppets, A., Defloor, T., Bernaerts, K., Milisen, K., & Van Den Berghe, G. (2009). Incidence and risk factors for pressure ulcers in the intensive care unit. *Journal of clinical nursing, 18*(9), 1258-1266. doi:http://dx.doi.org/10.1111/j.1365-2702.2008.02554.x

Nimni, M. E., & Harkness, R. D. (2018). Molecular structure and functions of collagen. In *Collagen* (pp. 1-19): CRC Press.

Nixon, J., Cranny, G., & Bond, S. (2007). Skin alterations of intact skin and risk factors associated with pressure ulcer development in surgical patients: a cohort study. *International journal of nursing studies, 44*(5), 655-663. doi:http://dx.doi.org/10.1016/j.ijnurstu.2006.02.010

Nwomeh, B. C., Liang, H.-X., Cohen, I. K., & Yager, D. R. (1999). MMP-8 is the predominant collagenase in healing wounds and nonhealing ulcers. *Journal of Surgical Research, 81*(2), 189-195. doi:http://dx.doi.org/10.1006/jsre.1998.5495

Obaid, H., Clarke, A., Rosenfeld, P., Leach, C., & Connell, D. (2012). Skin-derived fibroblasts for the treatment of refractory Achilles tendinosis: preliminary short-term results. *JBJS, 94*(3), 193-200. doi:http://dx.doi.org/10.2106/JBJS.J.00781

Ogata, Y., Yamada, T., Hasegawa, S., Sanada, A., Iwata, Y., Arima, M., . . . Akamatsu, H. (2021). SASP-induced macrophage dysfunction may contribute to accelerated senescent fibroblast accumulation in the dermis. *EXPERIMENTAL DERMATOLOGY, 30*(1), 84-91. doi:http://dx.doi.org/10.1111/exd.14205

Ogawa, R., Okai, K., Tokumura, F., Mori, K., Ohmori, Y., Huang, C., . . . Akaishi, S. (2012). The relationship between skin stretching/contraction and pathologic scarring: the important role of mechanical forces in keloid generation. *Wound Repair and Regeneration, 20*(2), 149-157. doi:http://dx.doi.org/10.1111/j.1524-475X.2012.00766.x

Oliva, F., Piccirilli, E., Berardi, A. C., Frizziero, A., Tarantino, U., & Maffulli, N. (2016). Hormones and tendinopathies: the current evidence. *British medical bulletin, 117*(1), 39-58. doi:http://dx.doi.org/10.1093/bmb/ldv054

Oomens, C., Loerakker, S., & Bader, D. (2010). The importance of internal strain as opposed to interface pressure in the prevention of pressure related deep tissue injury. *Journal of tissue viability, 19*(2), 35-42. doi:http://dx.doi.org/10.1016/j.jtv.2009.11.002

Oomens, C. W., Bader, D. L., Loerakker, S., & Baaijens, F. (2015). Pressure induced deep tissue injury explained. *Annals of biomedical engineering, 43*(2), 297-305. doi:http://dx.doi.org/10.1007/s10439-014-1202-6

Owens, B. D., Wolf, J. M., Seelig, A. D., Jacobson, I. G., Boyko, E. J., Smith, B., . . . Team, M. C. S. (2013). Risk factors for lower extremity tendinopathies in military personnel. *Orthopaedic Journal of Sports Medicine, 1*(1), 2325967113492707. doi:http://dx.doi.org/10.1177/2325967113492707

Oxlund, H. (1986). Relationships between the biomechanical properties, composition and molecular structure of connective tissues. *Connective tissue research, 15*(1-2), 65-72. doi:http://dx.doi.org/10.3109/03008208609001974

Parkinson, J., Samiric, T., Ilic, M. Z., Cook, J., Feller, J. A., & Handley, C. J. (2010). Change in proteoglycan metabolism is a characteristic of human patellar tendinopathy. *Arthritis & Rheumatism, 62*(10), 3028-3035. doi:http://dx.doi.org/10.1002/art.27587
Passaretti, D., Candela, V., Venditto, T., Giannicola, G., & Gumina, S. (2016). Association between alcohol consumption and rotator cuff tear. *Acta orthopaedica, 87*(2), 165-168. doi:http://dx.doi.org/10.3109/17453674.2015.1119599
Pearce, C. J., Ismail, M., & Calder, J. D. (2009). Is apoptosis the cause of noninsertional achilles tendinopathy? *The American Journal of Sports Medicine, 37*(12), 2440-2444. doi:http://dx.doi.org/10.1177/0363546509340264
Perdue, R. W., & Wilson, J. L. (1989). Decubitus ulcers. *The Journal of the American Board of Family Practice, 2*(1), 43-48. doi:http://dx.doi.org/10.3122/jabfm.2.1.43
Pereira, B. I., Devine, O. P., Vukmanovic-Stejic, M., Chambers, E. S., Subramanian, P., Patel, N., . . . Valdovinos, A. (2019). Senescent cells evade immune clearance via HLA-E-mediated NK and CD8+ T cell inhibition. *Nature communications, 10*(1), 2387. doi:http://dx.doi.org/10.1038/s41467-019-10335-5
Petersen, W., Pufe, T., Zantop, T., Tillmann, B., Tsokos, M., & Mentlein, R. (2004). Expression of VEGFR-1 and VEGFR-2 in degenerative Achilles tendons. *Clinical Orthopaedics and Related Research®, 420*, 286-291. doi:http://dx.doi.org/10.1097/00003086-200403000-00040
Pfisterer, K., Shaw, L. E., Symmank, D., & Weninger, W. (2021). The extracellular matrix in skin inflammation and infection. *Frontiers in Cell and Developmental Biology*, 1578. doi:http://dx.doi.org/10.3389/fcell.2021.682414
Phillips, C. L., Combs, S. B., & Pinnell, S. R. (1994). Effects of ascorbic acid on proliferation and collagen synthesis in relation to the donor age of human dermal fibroblasts. *Journal of Investigative Dermatology, 103*(2), 228-232. doi:http://dx.doi.org/10.1111/1523-1747.ep12393187
Pilkington, S., Barron, M., Watson, R., Griffiths, C., & Bulfone-Paus, S. (2019). Aged human skin accumulates mast cells with altered functionality that localize to macrophages and vasoactive intestinal peptide-positive nerve fibres. *British Journal of Dermatology, 180*(4), 849-858. doi:http://dx.doi.org/10.1111/bjd.17268
Plikus, M. V., Wang, X., Sinha, S., Forte, E., Thompson, S. M., Herzog, E. L., . . . Horsley, V. (2021). Fibroblasts: Origins, definitions, and functions in health and disease. *Cell, 184*(15), 3852-3872. doi:http://dx.doi.org/10.1016/j.cell.2021.06.024
Plinsinga, M. L., Brink, M. S., Vicenzino, B., & Van Wilgen, C. P. (2015). Evidence of nervous system sensitization in commonly presenting and persistent painful tendinopathies: a systematic review. *Journal of Orthopaedic & Sports Physical Therapy, 45*(11), 864-875. doi:http://dx.doi.org/10.2519/jospt.2015.5895
Poulsen, R., Knowles, H., Carr, A., & Hulley, P. (2014). Cell differentiation versus cell death: extracellular glucose is a key determinant of cell fate following oxidative stress exposure. *Cell death & disease, 5*(2), e1074-e1074. doi:http://dx.doi.org/10.1038/cddis.2014.52
Prajapati, R. T., Chavally-Mis, B., Herbage, D., Eastwood, M., & Brown, R. A. (2000). Mechanical loading regulates protease production by fibroblasts in three-

dimensional collagen substrates. *Wound Repair and Regeneration, 8*(3), 226-237. doi:http://dx.doi.org/10.1046/j.1524-475x.2000.00226.x
Prajapati, R. T., Eastwood, M., & Brown, R. A. (2000). Duration and orientation of mechanical loads determine fibroblast cyto-mechanical activation: monitored by protease release. *Wound Repair and Regeneration, 8*(3), 238-246. doi:http://dx.doi.org/10.1046/j.1524-475x.2000.00238.x
Pufe, T., Paulsen, F., Petersen, W., Mentlein, R., & Tsokos, M. (2003). The angiogenic peptide vascular endothelial growth factor (VEGF) is expressed in chronic sacral pressure ulcers. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 200*(1), 130-136. doi:http://dx.doi.org/10.1007/s004280100422
Pufe, T., Petersen, W., Mentlein, R., & Tillmann, B. (2005). The role of vasculature and angiogenesis for the pathogenesis of degenerative tendons disease. *Scandinavian journal of medicine & science in sports, 15*(4), 211-222. doi:http://dx.doi.org/10.1111/j.1600-0838.2005.00465.x
Pufe, T., Petersen, W., Tillmann, B., & Mentlein, R. (2001). The angiogenic peptide vascular endothelial growth factor is expressed in foetal and ruptured tendons. *Virchows Archiv, 439*, 579-585. doi:http://dx.doi.org/10.1007/s004280100422
Qin, Z., Balimunkwe, R., & Quan, T. (2017). Age-related reduction of dermal fibroblast size upregulates multiple matrix metalloproteinases as observed in aged human skin in vivo. *British Journal of Dermatology, 177*(5), 1337-1348. doi:http://dx.doi.org/10.1111/bjd.15379
Quintavalle, P. R., Lyder, C. H., Mertz, P. J., Phillips-Jones, C., & Dyson, M. (2006). Use of high-resolution, high-frequency diagnostic ultrasound to investigate the pathogenesis of pressure ulcer development. *Advances in skin & wound care, 19*(9), 498-505. doi:http://dx.doi.org/10.1097/00129334-200611000-00010
Ramos, L. A., Carvalho, R. T. d., Garms, E., Navarro, M. S., Abdalla, R. J., & Cohen, M. (2009). Prevalence of pain on palpation of the inferior pole of the patella among patients with complaints of knee pain. *Clinics, 64*, 199-202. doi:http://dx.doi.org/10.1590/S1807-59322009000300009
Ranger, T. A., Wong, A. M., Cook, J. L., & Gaida, J. E. (2016). Is there an association between tendinopathy and diabetes mellitus? A systematic review with meta-analysis. *British journal of sports medicine, 50*(16), 982-989. doi:http://dx.doi.org/10.1136/bjsports-2015-094735
Rees, J. D., Stride, M., & Scott, A. (2014). Tendons–time to revisit inflammation. *British journal of sports medicine, 48*(21), 1553-1557. doi:http://dx.doi.org/10.1136/bjsports-2012-091957
Renström, P., & Johnson, R. J. (1985). Overuse injuries in sports. *Sports Medicine, 2*(5), 316-333. doi:http://dx.doi.org/10.2165/00007256-198502050-00002
Riley, G., Harrall, R., Constant, C., Chard, M., Cawston, T., & Hazleman, B. (1994a). Glycosaminoglycans of human rotator cuff tendons: changes with age and in chronic rotator cuff tendinitis. *Annals of the Rheumatic Diseases, 53*(6), 367-376. doi:http://dx.doi.org/10.1136/ard.53.6.367
Riley, G., Harrall, R., Constant, C., Chard, M., Cawston, T., & Hazleman, B. (1994b). Tendon degeneration and chronic shoulder pain: changes in the collagen composition of the human rotator cuff tendons in rotator cuff tendinitis. *Annals of the Rheumatic Diseases, 53*(6), 359-366. doi:http://dx.doi.org/10.1136/ard.53.6.359
Riley, G. P., Curry, V., DeGroot, J., van El, B., Verzijl, N., Hazleman, B. L., & Bank, R. A. (2002). Matrix metalloproteinase activities and their relationship with

collagen remodelling in tendon pathology. *Matrix Biology, 21*(2), 185-195. doi:http://dx.doi.org/10.1016/S0945-053X(01)00196-2
Rio, E., Moseley, L., Purdam, C., Samiric, T., Kidgell, D., Pearce, A. J., . . . Cook, J. (2014). The pain of tendinopathy: physiological or pathophysiological? *Sports Medicine, 44*, 9-23. doi:http://dx.doi.org/10.1007/s40279-013-0096-z
Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. (2019). Wound healing: a cellular perspective. *Physiological reviews, 99*(1), 665-706. doi:http://dx.doi.org/10.1152/physrev.00067.2017
Rogers, A. A., Burnett, S., Moore, J. C., Shakespeare, P. G., & John Chen, W. (1995). Involvement of proteolytic enzymes—plasminogen activators and matrix metalloproteinases—in the pathophysiology of pressure ulcers. *Wound Repair and Regeneration, 3*(3), 273-283. doi:http://dx.doi.org/10.1046/j.1524-475X.1995.30307.x
Rorteau, J., Chevalier, F. P., Bonnet, S., Barthélemy, T., Lopez-Gaydon, A., Martin, L. S., . . . Lamartine, J. (2022). Maintenance of chronological aging features in culture of normal human dermal fibroblasts from old donors. *Cells, 11*(5), 858. doi:http://dx.doi.org/10.3390/cells11050858
Saini, K., Cho, S., Dooling, L. J., & Discher, D. E. (2020). Tension in fibrils suppresses their enzymatic degradation–A molecular mechanism for 'use it or lose it'. *Matrix Biology, 85*, 34-46. doi:http://dx.doi.org/10.1016/j.matbio.2019.06.001
Sanada, F., Taniyama, Y., Muratsu, J., Otsu, R., Shimizu, H., Rakugi, H., & Morishita, R. (2018). Source of chronic inflammation in aging. *Frontiers in cardiovascular medicine, 5*, 12. doi:http://dx.doi.org/10.3389/fcvm.2018.00012
Sanada, H., Nagakawa, T., Yamamoto, M., Higashidani, K., Tsuru, H., & Sugama, J. (1997). The role of skin blood flow in pressure ulcer development during surgery. *Advances in wound care: the journal for prevention and healing, 10*(6), 29-34.
Sanders, J. E., Goldstein, B. S., & Leotta, D. F. (1995). Skin response to mechanical stress: adaptation rather than breakdown-a review of the literature. *Journal of rehabilitation research and development, 32*, 214-214.
Savitskaya, Y. A., Izaguirre, A., Sierra, L., Perez, F., Cruz, F., Villalobos, E., . . . Ibarra, C. (2011). Effect of angiogenesis-related cytokines on rotator cuff disease: the search for sensitive biomarkers of early tendon degeneration. *Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders, 4*, CMAMD. S7071. doi:http://dx.doi.org/10.4137/CMAMD.S7071
Scharffetter-Kochanek, K., Wlaschek, M., Briviba, K., & Sies, H. (1993). Singlet oxygen induces collagenase expression in human skin fibroblasts. *FEBS letters, 331*(3), 304-306. doi:http://dx.doi.org/10.1016/0014-5793(93)80357-Z
Schatzker, J., & Brånemark, P.-I. (1969). Intravital observation on the microvascular anatomy and microcirculation of the tendon. *Acta orthopaedica scandinavica, 40*(sup126), 1-42. doi:http://dx.doi.org/10.3109/ort.1969.40.suppl-126.01
Scheid, A., Wenger, R., Christina, H., Camenisch, I., Ferenc, A., Stauffer, U., . . . Meuli, M. (2000). Hypoxia-regulated gene expression in fetal wound regeneration and adult wound repair. *Pediatric surgery international, 16*, 232-236. doi:http://dx.doi.org/10.1007/s003830050735
Schmid, P., Cox, D., Bilbe, G., McMaster, G., Morrison, C., Stähelin, H., . . . Seiler, W. (1993). TGF-βs and TGF-β type II receptor in human epidermis: differential expression in acute and chronic skin wounds. *The Journal of pathology, 171*(3), 191-197. doi:http://dx.doi.org/10.1002/path.1711710307

Schneider, E. L. (1979). Aging and cultured human skin in fibroblasts. *Journal of Investigative Dermatology, 73*(1), 15-18. doi:http://dx.doi.org/10.1111/1523-1747.ep12532753
Schubert, V. (1991). Hypotension as a risk factor for the development of pressure sores in elderly subjects. *Age and ageing, 20*(4), 255-261. doi:http://dx.doi.org/10.1093/ageing/20.4.255
Schubert, V., Perbeck, L., & Schubert, P. Å. (1994). Skin microcirculatory and thermal changes in elderly subjects with early stage of pressure sores. *Clinical Physiology, 14*(1), 1-13. doi:http://dx.doi.org/10.1111/j.1475-097X.1994.tb00484.x
Scott, A., Khan, K., Heer, J., Cook, J., Lian, O., & Duronio, V. (2005). High strain mechanical loading rapidly induces tendon apoptosis: an ex vivo rat tibialis anterior model. *British journal of sports medicine, 39*(5), e25-e25. doi:http://dx.doi.org/10.1136/bjsm.2004.015164
Scott, A., Lian, Ø., Bahr, R., Hart, D. A., & Duronio, V. (2008). VEGF expression in patellar tendinopathy: a preliminary study. *Clin Orthop Relat Res, 466*, 1598-1604. doi:http://dx.doi.org/10.1007/s11999-008-0272-x
Scott, A., Lian, Ø., Bahr, R., Hart, D. A., Duronio, V., & Khan, K. M. (2008). Increased mast cell numbers in human patellar tendinosis: correlation with symptom duration and vascular hyperplasia. *British journal of sports medicine, 42*(9), 753-757. doi:http://dx.doi.org/10.1136/bjsm.2007.040212
Scott, A., Squier, K., Alfredson, H., Bahr, R., Cook, J. L., Coombes, B., . . . Lewis, J. S. (2020). Icon 2019: international scientific tendinopathy symposium consensus: clinical terminology. *British journal of sports medicine, 54*(5), 260-262. doi:http://dx.doi.org/10.1136/bjsports-2019-100885
Screen, H. R., Berk, D. E., Kadler, K. E., Ramirez, F., & Young, M. F. (2015). Tendon functional extracellular matrix. *Journal of orthopaedic research, 33*(6), 793-799. doi:http://dx.doi.org/10.1002/jor.22818
Semenza, G. L. (2000). HIF-1: mediator of physiological and pathophysiological responses to hypoxia. *Journal of applied physiology, 88*(4), 1474-1480. doi:http://dx.doi.org/10.1152/jappl.2000.88.4.1474
Serrano, M. L., Méndez, M. G., Cebollero, F. C., & Rodríguez, J. L. (2017). Risk factors for pressure ulcer development in Intensive Care Units: A systematic review. *Medicina Intensiva (English Edition), 41*(6), 339-346. doi:http://dx.doi.org/10.1016/j.medine.2017.04.006
Shen, P.-C., Chang, P.-C., Jou, I.-M., Chen, C.-H., Lee, F.-H., & Hsieh, J.-L. (2019). Hand tendinopathy risk factors in Taiwan: A population-based cohort study. *Medicine, 98*(1). doi:http://dx.doi.org/10.1097/MD.0000000000013795
Siddiqui, A., Galiano, R. D., Connors, D., Gruskin, E., Wu, L., & Mustoe, T. A. (1996). Differential effects of oxygen on human dermal fibroblasts: acute versus chronic hypoxia. *Wound Repair and Regeneration, 4*(2), 211-218. doi:http://dx.doi.org/10.1046/j.1524-475X.1996.40207.x
Slowikowski, G. C., & Funk, M. (2010). Factors associated with pressure ulcers in patients in a surgical intensive care unit. *Journal of Wound Ostomy & Continence Nursing, 37*(6), 619-626. doi:http://dx.doi.org/10.1097/WON.0b013e3181f90a34
Solé-Boldo, L., Raddatz, G., Schütz, S., Mallm, J.-P., Rippe, K., Lonsdorf, A. S., . . . Lyko, F. (2020). Single-cell transcriptomes of the human skin reveal age-related loss of fibroblast priming. *Communications biology, 3*(1), 188. doi:http://dx.doi.org/10.1038/s42003-020-0922-4

Stadelmann, W. K., Digenis, A. G., & Tobin, G. R. (1998). Physiology and healing dynamics of chronic cutaneous wounds. *The American Journal of Surgery, 176*(2), 26S-38S. doi:http://dx.doi.org/10.1016/S0002-9610(98)00183-4
Stauber, T., Blache, U., & Snedeker, J. G. (2020). Tendon tissue microdamage and the limits of intrinsic repair. *Matrix Biology, 85*, 68-79. doi:http://dx.doi.org/10.1016/j.matbio.2019.07.008
Stein, V., Laprell, H., Tinnemeyer, S., & Petersen, W. (2000). Quantitative assessment of intravascular volume of the human Achilles tendon. *Acta orthopaedica scandinavica, 71*(1), 60-63. doi:http://dx.doi.org/10.1080/00016470052943919
Steinbrech, D. S., Longaker, M. T., Mehrara, B. J., Saadeh, P. B., Chin, G. S., Gerrets, R. P., . . . Gittes, G. K. (1999). Fibroblast response to hypoxia: the relationship between angiogenesis and matrix regulation. *Journal of Surgical Research, 84*(2), 127-133. doi:http://dx.doi.org/10.1006/jsre.1999.5627
Stekelenburg, A., Gawlitta, D., Bader, D. L., & Oomens, C. W. (2008). Deep tissue injury: how deep is our understanding? *Archives of physical medicine and rehabilitation, 89*(7), 1410-1413. doi:http://dx.doi.org/10.1016/j.apmr.2008.01.012
Stiskal, M., Szolar, D. H., Stenzel, I., Steiner, E., MESARIC, P., Czembirek, H., & Preidler, K. W. (1997). Magnetic resonance imaging of Achilles tendon in patients with rheumatoid arthritis. *Investigative radiology, 32*(10), 602-608. doi:http://dx.doi.org/10.1097/00004424-199710000-00004
Stöckl, P., Hütter, E., Zwerschke, W., & Jansen-Dürr, P. (2006). Sustained inhibition of oxidative phosphorylation impairs cell proliferation and induces premature senescence in human fibroblasts. *Experimental Gerontology, 41*(7), 674-682. doi:http://dx.doi.org/10.1016/j.exger.2006.04.009
Stojadinovic, O., Minkiewicz, J., Sawaya, A., Bourne, J. W., Torzilli, P., de Rivero Vaccari, J. P., . . . Tomic-Canic, M. (2013). Deep tissue injury in development of pressure ulcers: a decrease of inflammasome activation and changes in human skin morphology in response to aging and mechanical load. *PLoS One, 8*(8), e69223. doi:http://dx.doi.org/10.1371/journal.pone.0069223
Storch, T. G., & Talley, G. D. (1988). Oxygen concentration regulates the proliferative response of human fibroblasts to serum and growth factors. *Experimental cell research, 175*(2), 317-325. doi:http://dx.doi.org/10.1016/0014-4827(88)90195-4
Stricklin, G. P., & Welgus, H. (1983). Human skin fibroblast collagenase inhibitor. Purification and biochemical characterization. *Journal of Biological Chemistry, 258*(20), 12252-12258. doi:http://dx.doi.org/10.1016/S0021-9258(17)44166-4
Su, Y. C., Chung, C. H., Ke, M. J., Chen, L. C., Chien, W. C., & Wu, Y. T. (2021). Increased risk of shoulder calcific tendinopathy in diabetes mellitus: a nationwide, population-based, matched cohort study. *International Journal of Clinical Practice*, e14549. doi:http://dx.doi.org/10.1111/ijcp.14549
Sullivan, B. E., Carroll, C. C., Jemiolo, B., Trappe, S. W., Magnusson, S. P., Døssing, S., . . . Trappe, T. A. (2009). Effect of acute resistance exercise and sex on human patellar tendon structural and regulatory mRNA expression. *Journal of applied physiology, 106*(2), 468-475. doi:http://dx.doi.org/10.1152/japplphysiol.91341.2008
Szor, J. K., & Bourguignon, C. (1999). Description of pressure ulcer pain at rest and at dressing change. *Journal of WOCN, 26*(3), 115-120. doi:http://dx.doi.org/10.1016/S1071-5754(99)90028-1

Takayama, M., Kuramoto, Y., Okuyama, R., Yamasaki, K., & Aiba, S. (2010). The exudate of pressure ulcers contains a substantial amount of vascular endothelial growth factor. *The Tohoku journal of experimental medicine, 221*(4), 315-319. doi:http://dx.doi.org/10.1620/tjem.221.315

Tashjian, R. Z., Farnham, J. M., Albright, F. S., Teerlink, C. C., & Cannon-Albright, L. A. (2009). Evidence for an inherited predisposition contributing to the risk for rotator cuff disease. *The Journal of Bone and Joint Surgery. American volume., 91*(5), 1136. doi:http://dx.doi.org/10.2106/JBJS.H.00831

Taylor, B., Cheema, A., & Soslowsky, L. (2017). Tendon pathology in hypercholesterolemia and familial hypercholesterolemia. *Current Rheumatology Reports, 19*(12), 1-6. doi:http://dx.doi.org/10.1007/s11926-017-0704-2

Thorfinn, J., Sjöberg, F., & Lidman, D. (2007). Perfusion of buttock skin in healthy volunteers after long and short repetitive loading evaluated by laser Doppler perfusion imager. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery, 41*(6), 297-302. doi:http://dx.doi.org/10.1080/02844310701633249

Tilley, B. J., Cook, J. L., Docking, S. I., & Gaida, J. E. (2015). Is higher serum cholesterol associated with altered tendon structure or tendon pain? A systematic review. *British journal of sports medicine, 49*(23), 1504-1509. doi:http://dx.doi.org/10.1136/bjsports-2015-095100

Tom, S., Parkinson, J., Ilic, M. Z., Cook, J., Feller, J. A., & Handley, C. J. (2009). Changes in the composition of the extracellular matrix in patellar tendinopathy. *Matrix Biology, 28*(4), 230-236. doi:http://dx.doi.org/10.1016/j.matbio.2009.04.001

Tompra, N., van Dieën, J. H., & Coppieters, M. W. (2015). Central pain processing is altered in people with Achilles tendinopathy. *British journal of sports medicine*. doi:http://dx.doi.org/10.1136/bjsports-2015-095476

Trump, B., Berezesky, I., Collan, Y., Kahng, M., & Mergner, W. (1976). Recent studies on the pathophysiology of ischemic cell injury. *Beiträge zur Pathologie, 158*(4), 363-388. doi:http://dx.doi.org/10.1016/S0005-8165(76)80135-7

Trump, B. F., Laiho, K. A., Mergner, W. J., & Arstila, A. U. (1974). Studies on the subcellular pathophysiology of acute lethal cell injury. *Beiträge zur Pathologie, 152*(3), 243-271. doi:http://dx.doi.org/10.1016/S0005-8165(74)80177-0

Tsai, W.-C., & Yang, Y.-M. (2011). Fluoroquinolone-associated tendinopathy. *Chang Gung Med J, 34*(5), 461-467.

Tsuzaki, M., Bynum, D., Almekinders, L., Yang, X., Faber, J., & Banes, A. (2003). ATP modulates load-inducible IL-1β, COX 2, and MMP-3 gene expression in human tendon cells. *J Cell Biochem, 89*(3), 556-562. doi:http://dx.doi.org/10.1002/jcb.10534

Tsuzaki, M., Guyton, G., Garrett, W., Archambault, J., Herzog, W., Almekinders, L., . . . Banes, A. (2003). IL-1β induces COX2, MMP-1,-3 and-13, ADAMTS-4, IL-1β and IL-6 in human tendon cells. *Journal of orthopaedic research, 21*(2), 256-264. doi:http://dx.doi.org/10.1016/S0736-0266(02)00141-9

Tuite, D., Renström, P., & O'brien, M. (1997). The aging tendon. *Scandinavian journal of medicine & science in sports, 7*(2), 72-77. doi:http://dx.doi.org/10.1111/j.1600-0838.1997.tb00122.x

Tuoheti, Y., Itoi, E., Pradhan, R. L., Wakabayashi, I., Takahashi, S., Minagawa, H., . . . Shimada, Y. (2005). Apoptosis in the supraspinatus tendon with stage II

subacromial impingement. *Journal of shoulder and elbow surgery, 14*(5), 535-541. doi:http://dx.doi.org/10.1016/j.jse.2005.01.001

Uitto, J., Booth, B. A., & Polak, K. L. (1980). Collagen biosynthesis by human skin fibroblasts II. Isolation and further characterization of type I and type III procollagens synthesized in culture. *Biochimica et Biophysica Acta (BBA)-Protein Structure, 624*(2), 545-561. doi:http://dx.doi.org/10.1016/0005-2795(80)90095-1

Van Der Vlist, A. C., Breda, S. J., Oei, E. H., Verhaar, J. A., & de Vos, R.-J. (2019). Clinical risk factors for Achilles tendinopathy: a systematic review. *British journal of sports medicine, 53*(21), 1352-1361. doi:http://dx.doi.org/10.1136/bjsports-2018-099991

Vande Berg, J. S., Rose, M. A., Haywood-Reid, P. L., Rudolph, R., Payne, W. G., & Robson, M. C. (2005). Cultured pressure ulcer fibroblasts show replicative senescence with elevated production of plasmin, plasminogen activator inhibitor-1, and transforming growth factor-β1. *Wound Repair and Regeneration, 13*(1), 76-83. doi:http://dx.doi.org/10.1111/j.1067-1927.2005.130110.x

Vande Berg, J. S., Rudolph, R., Hollan, C., & Haywood-Reid, P. L. (1998). Fibroblast senescence in pressure ulcers. *Wound Repair and Regeneration, 6*(1), 38-49. doi:http://dx.doi.org/10.1046/j.1524-475X.1998.60107.x

Vande Berg, J. S., Smith, P. D., Haywood-Reid, P. L., Munson, A. B., Soules, K. A., & Robson, M. C. (2001). Dynamic forces in the cell cycle affecting fibroblasts in pressure ulcers. *Wound Repair and Regeneration, 9*(1), 19-27. doi:http://dx.doi.org/10.1046/j.1524-475x.2001.00019.x

Vater, C. A., Mainardi, C. L., & Harris Jr, E. D. (1979). Inhibitor of human collagenase from cultures of human tendon. *Journal of Biological Chemistry, 254*(8), 3045-3053. doi:http://dx.doi.org/10.1016/S0021-9258(17)30180-1

Verhaegen, P. D., Schouten, H. J., Tigchelaar-Gutter, W., van Marle, J., van Noorden, C. J., Middelkoop, E., & van Zuijlen, P. P. (2012). Adaptation of the dermal collagen structure of human skin and scar tissue in response to stretch: an experimental study. *Wound Repair and Regeneration, 20*(5), 658-666. doi:http://dx.doi.org/10.1111/j.1524-475X.2012.00827.x

von der Mark, K. (1981). Localization of Collagen Types in Tissues. In D. A. Hall & D. S. Jackson (Eds.), *International Review of Connective Tissue Research* (Vol. 9, pp. 265-324): Elsevier.

Wang, C., Zhou, Z., Song, W., Cai, Z., Ding, Z., Chen, D., . . . He, Y. (2022). Inhibition of IKKβ/NF-κB signaling facilitates tendinopathy healing by rejuvenating inflamm-aging induced tendon-derived stem/progenitor cell senescence. *Molecular Therapy-Nucleic Acids, 27*, 562-576. doi:http://dx.doi.org/10.1016/j.omtn.2021.12.026

Wang, L., Ding, G., Gu, Q., & Schwarz, W. (2010). Single-channel properties of a stretch-sensitive chloride channel in the human mast cell line HMC-1. *European Biophysics Journal, 39*, 757-767. doi:http://dx.doi.org/10.1007/s00249-009-0542-x

Wann-Hansson, C., Hagell, P., & Willman, A. (2008). Risk factors and prevention among patients with hospital-acquired and pre-existing pressure ulcers in an acute care hospital. *Journal of clinical nursing, 17*(13), 1718-1727. doi:http://dx.doi.org/10.1111/j.1365-2702.2008.02286.x

Warner, H. R. (1997). Aging and regulation of apoptosis. *Current topics in cellular regulation, 35*, 107-121. doi:http://dx.doi.org/10.1016/S0070-2137(97)80004-0

Webster, D., & Burry, H. (1982). The effects of hypoxia on human skin, lung and tendon cells in vitro. *British journal of experimental pathology, 63*(1), 50.
Welgus, H. G., Jeffrey, J. J., Eisen, A. Z., Roswit, W. T., & Stricklin, G. P. (1985). Human skin fibroblast collagenase: interaction with substrate and inhibitor. *Collagen and related research, 5*(2), 167-179. doi:http://dx.doi.org/10.1016/S0174-173X(85)80038-8
Welgus, H. G., & Stricklin, G. (1983). Human skin fibroblast collagenase inhibitor. Comparative studies in human connective tissues, serum, and amniotic fluid. *Journal of Biological Chemistry, 258*(20), 12259-12264. doi:http://dx.doi.org/10.1016/S0021-9258(17)44167-6
West, M. D., Pereira-Smith, O. M., & Smith, J. R. (1989). Replicative senescence of human skin fibroblasts correlates with a loss of regulation and overexpression of collagenase activity. *Experimental cell research, 184*(1), 138-147. doi:http://dx.doi.org/10.1016/0014-4827(89)90372-8
Wilhelm, S. M., Eisen, A. Z., Teter, M., Clark, S. D., Kronberger, A., & Goldberg, G. (1986). Human fibroblast collagenase: glycosylation and tissue-specific levels of enzyme synthesis. *Proceedings of the National Academy of Sciences, 83*(11), 3756-3760. doi:http://dx.doi.org/10.1073/pnas.83.11.3756
Wise, B. L., Peloquin, C., Choi, H., Lane, N. E., & Zhang, Y. (2012). Impact of age, sex, obesity, and steroid use on quinolone-associated tendon disorders. *The American journal of medicine, 125*(12), 1228. e1223-1228. e1228. doi:http://dx.doi.org/10.1016/j.amjmed.2012.05.027
Witkowski, J. A., & Parish, L. C. (1982). Histopathology of the decubitus ulcer. *Journal of the American Academy of Dermatology, 6*(6), 1014-1021. doi:http://dx.doi.org/10.1016/S0190-9622(82)70085-4
Wu, B., Chen, J., Rosa, T. D., Yu, Q., Wang, A., Xu, J., & Zheng, M.-H. (2011). Cellular response and extracellular matrix breakdown in rotator cuff tendon rupture. *Archives of Orthopaedic and Trauma Surgery, 131*, 405-411. doi:http://dx.doi.org/10.1007/s00402-010-1157-5
Wywialowski, E. F. (1999). Tissue perfusion as a key underlying concept of pressure ulcer development and treatment. *Journal of Vascular Nursing, 17*(1), 12-16. doi:http://dx.doi.org/10.1016/S1062-0303(99)90003-1
Xia, W., Szomor, Z., Wang, Y., & Murrell, G. A. (2006). Nitric oxide enhances collagen synthesis in cultured human tendon cells. *Journal of orthopaedic research, 24*(2), 159-172. doi:http://dx.doi.org/10.1002/jor.20060
Yager, D. R., Zhang, L.-Y., Liang, H.-X., Diegelmann, R. F., & Cohen, I. K. (1996). Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. *Journal of Investigative Dermatology, 107*(5), 743-748. doi:http://dx.doi.org/10.1111/1523-1747.ep12365637
Yamanaka, M., & Ishikawa, O. (2000). Hypoxic conditions decrease the mRNA expression of proα1 (I) and (III) collagens and increase matrix metalloproteinases-1 of dermal fibroblasts in three-dimensional cultures. *Journal of Dermatological Science, 24*(2), 99-104. doi:http://dx.doi.org/10.1016/S0923-1811(00)00086-4
Yan, Z., Yin, H., Brochhausen, C., Pfeifer, C. G., Alt, V., & Docheva, D. (2020). Aged tendon stem/progenitor cells are less competent to form 3D tendon organoids due to cell autonomous and matrix production deficits. *Frontiers in bioengineering and biotechnology, 8*, 406. doi:http://dx.doi.org/10.3389/fbioe.2020.00406

Yanagishita, M. (1993). Function of proteoglycans in the extracellular matrix. *Pathology International, 43*(6), 283-293. doi:http://dx.doi.org/10.1111/j.1440-1827.1993.tb02569.x

Yang, G., Crawford, R. C., & Wang, J. H. (2004). Proliferation and collagen production of human patellar tendon fibroblasts in response to cyclic uniaxial stretching in serum-free conditions. *Journal of biomechanics, 37*(10), 1543-1550. doi:http://dx.doi.org/10.1016/j.jbiomech.2004.01.005

Yang, G., Im, H.-J., & Wang, J. H.-C. (2005). Repetitive mechanical stretching modulates IL-1β induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. *Gene, 363*, 166-172. doi:http://dx.doi.org/10.1016/j.gene.2005.08.006

Yang, J.-H., Sakamoto, H., Xu, E. C., & Lee, R. T. (2000). Biomechanical regulation of human monocyte/macrophage molecular function. *The American journal of pathology, 156*(5), 1797-1804. doi:http://dx.doi.org/10.1016/S0002-9440(10)65051-1

Yuan, J., Murrell, G. A., Trickett, A., & Wang, M.-X. (2003). Involvement of cytochrome c release and caspase-3 activation in the oxidative stress-induced apoptosis in human tendon fibroblasts. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1641*(1), 35-41. doi:http://dx.doi.org/10.1016/S0167-4889(03)00047-8

Yuan, J., Murrell, G. A., Wei, A. Q., & Wang, M. X. (2002). Apoptosis in rotator cuff tendonopathy. *Journal of orthopaedic research, 20*(6), 1372-1379. doi:http://dx.doi.org/10.1016/S0736-0266(02)00075-X

Zabraniecki, L., Negrier, I., Vergne, P., Arnaud, M., Bonnet, C., Bertin, P., & Treves, R. (1996). Fluoroquinolone induced tendinopathy: report of 6 cases. *Journal of Rheumatology, 23*(3), 516-520.

Zabrzyński, J., Huri, G., Gagat, M., Łapaj, Ł., Yataganbaba, A., Szwedowski, D., . . . Paczesny, Ł. (2021). The impact of smoking on clinical results following the rotator cuff and biceps tendon complex arthroscopic surgery. *Journal of Clinical Medicine, 10*(4), 599. doi:http://dx.doi.org/10.3390/jcm10040599

Zantop, T., Tillmann, B., & Petersen, W. (2003). Quantitative assessment of blood vessels of the human Achilles tendon: an immunohistochemical cadaver study. *Archives of Orthopaedic and Trauma Surgery, 123*, 501-504. doi:http://dx.doi.org/10.1007/s00402-003-0491-2

Zdanov, S., Bernard, D., Debacq-Chainiaux, F., Martien, S., Gosselin, K., Vercamer, C., . . . Abbadie, C. (2007). Normal or stress-induced fibroblast senescence involves COX-2 activity. *Experimental cell research, 313*(14), 3046-3056. doi:http://dx.doi.org/10.1016/j.yexcr.2007.04.033

Zhang, D., Spielmann, A., Wang, L., Ding, G., Huang, F., Gu, Q., & Schwarz, W. (2012). Mast-cell degranulation induced by physical stimuli involves the activation of transient-receptor-potential channel TRPV2. *Physiological research, 61*(1), 113. doi:http://dx.doi.org/10.33549/physiolres.932053

Zhang, J.-M., & An, J. (2007). Cytokines, inflammation and pain. *International anesthesiology clinics, 45*(2), 27. doi:http://dx.doi.org/10.1097/AIA.0b013e318034194e

Zhang, Y., Lin, Z., Foolen, J., Schoen, I., Santoro, A., Zenobi-Wong, M., & Vogel, V. (2014). Disentangling the multifactorial contributions of fibronectin, collagen and cyclic strain on MMP expression and extracellular matrix remodeling by fibroblasts. *Matrix Biology, 40*, 62-72. doi:http://dx.doi.org/10.1016/j.matbio.2014.09.001

Zitnay, J. L., Li, Y., Qin, Z., San, B. H., Depalle, B., Reese, S. P., . . . Weiss, J. A. (2017). Molecular level detection and localization of mechanical damage in collagen enabled by collagen hybridizing peptides. *Nature communications, 8*(1), 14913. doi:http://dx.doi.org/10.1038/ncomms14913

II

Physical Therapy in Sport 46 (2020) 30–46

Contents lists available at ScienceDirect

Physical Therapy in Sport

journal homepage: www.elsevier.com/ptsp

Review Article

# Physical therapy for tendinopathy: An umbrella review of systematic reviews and meta-analyses

Beshoy Girgis*, José Alberto Duarte

*CIAFEL, Faculty of Sport, University of Porto, R. Dr. Plácido Costa, 91, 4200-450, Porto, Portugal*

ARTICLE INFO

*Article history:*
Received 30 April 2020
Received in revised form
1 August 2020
Accepted 4 August 2020

*Keywords:*
Tendon injuries
Tendinopathy
Conservative treatment
Review

ABSTRACT

*Objective:* To summarize evidence in the last decade regarding the efficacy of physical therapy interventions to treat tendinopathy, as a single disease entity, as determined in systematic reviews (SRs) and/or meta-analyses (MAs).

*Methods:* Electronic search of PubMed, PEDro, and Scopus database was performed from year 2010 to January 2020. The methodological quality of the identified studies was assessed using the AMSTAR 2 tool. Studies scoring 9 points or higher were further analyzed using GRADE principles.

*Results:* 40 SRs and/or MAs were included in qualitative synthesis, whereas only 5 MAs were included in quantitative synthesis. Low-level laser therapy (LLLT) intervention showed a pooled improvement in pain reduction of 1.53 cm; 95% CI, [1.14, 1.91] ($I^2 = 1.9\%$, $p = 0.361$) on visual analogue scale, and grip strength of 9.59 kg; 95% CI, [5.90, 13.27].

*Conclusions:* Moderate-quality evidence may support these following interventions: LLLT revealed a statistically and potentially clinically significant improvement in pain and function on the short-term. Extracorporeal shockwave therapy showed a statistically significant enhancement in pain and function at all follow-up durations; however, its clinical significance was undetermined. Eccentric exercise was supported by qualitative evidence only. Caution is advised when interpreting results due to possible pathological differences in tendinopathy at each region.

© 2020 Elsevier Ltd. All rights reserved.

## 1. Introduction

Tendinopathy is a clinical syndrome characterized by pain, swelling, and reduced performance (Maffulli, 1998; Scott et al., 2020). It may involve any tendon, however, it commonly involves wrist flexors and extensors at the elbow, Rotator cuff, and Patellar and Achilles tendons (Maffulli, Wong, & Almekinders, 2003; Xu & Murrell, 2008). The incidence of tendinopathy in sport is rising due to increased training frequency and participation in sports (Ackermann & Renström, 2012; Mead, Gumucio, Awan, Mendias, & Sugg, 2018; Renstrom & Woo, 2008). The pathophysiology of tendinopathy is not fully understood but recent evidence has indicated a potential role of inflammation (Dean, Gettings, Dakin, & Carr, 2016; Mosca et al., 2018). Several reviews, however, have supported the histopathological similarity in tendinopathy at various anatomical sites (Abate et al., 2009; Järvinen et al., 1997; Maffulli et al., 2004; Xu & Murrell, 2008). Conservative treatment is considered the mainstay in the treatment of tendinopathy (Andres & Murrell, 2008; Mead et al., 2018). Ultrasound therapy (US), manual therapy (MT), low-level laser therapy (LLLT), extracorporeal shockwave therapy (ESWT), and exercise therapy (ET) are common interventions for tendinopathy, among others. Nevertheless, most of the available therapeutic modalities are supported by weak evidence (Cardoso, Pizzari, Kinsella, Hope, & Cook, 2019; Maffulli, Longo, Loppini, & Denaro, 2010). ET is regarded as an essential part of the treatment of tendinopathy (Dilger & Chimenti, 2019; Littlewood, May, & Walters, 2013; Mead et al., 2018). The efficacy of eccentric exercise (EE), in particular, is supported by several studies (Andres & Murrell, 2008; Jayaseelan, Mischke, & Strazzulla, 2019; Magnussen, Dunn, & Thomson, 2009; Rutland et al., 2010). The optimal treatment protocol, nonetheless, is yet undefined (Couppé, Svensson, Silbernagel, Langberg, & Magnusson, 2015; Dilger & Chimenti, 2019; Dimitrios & Malliaras, 2016; Littlewood, Malliaras, & Chance-Larsen, 2015; Meyer, Tumilty, & Baxter, 2009). Since there are many reviews on the topic in literature, summarizing existing evidence may facilitate the intervention

* Corresponding author.
*E-mail addresses:* begirgis@gmail.com, up201301273@fade.up.pt, begirgis@gmail.com, up201301273@fade.up.pt (B. Girgis), jarduarte@fade.up.pt (J.A. Duarte).

https://doi.org/10.1016/j.ptsp.2020.08.002
1466-853X/© 2020 Elsevier Ltd. All rights reserved.

decision-making process. Furthermore, nearly all of these reviews are modality-oriented rather than disease-oriented reviews. The objective of the present review was to summarize evidence in the last decade regarding the efficacy of physical therapy interventions to treat tendinopathy, as a single disease entity, as determined in systematic reviews and/or meta-analyses.

## 2. Methods

### 2.1. Eligibility criteria

Inclusion was limited to systematic reviews and/or meta-analyses with the objective to determine the efficacy of physical therapy intervention(s) in treating tendinopathy (without restriction to a specific anatomical site). Moreover, studies had to involve adequate description of methodology to be included; defined as: a systematic review and/or a meta-analysis involving an explicitly stated search strategy and search terms, explicitly described eligibility criteria, and explicitly defined methods of statistical synthesis (if applicable). Only studies published in peer reviewed journals, since year 2010, and written in English language were included. Studies including invasive, pharmacologic, and/or surgical interventions(s), non-efficacy studies involving physical therapy intervention(s), and protocols of studies were excluded (Table 1).

### 2.2. Information sources and search strategy

Electronic search of the National Institutes of Health (NIH) PubMed, the Physiotherapy Evidence Database PEDro, and Scopus database was performed from year 2010 to January 2020. Inspection of bibliographic references complemented the electronic search. The terms used in the electronic search were: *systematic review, meta-analysis, tendinitis, tendinosis, and tendinopathy*. The search strategy of PubMed database was: ((“Tendinopathy/rehabilitation"[Mesh] OR “Tendinopathy/therapy"[Mesh]) AND ((systematic[sb] OR Meta-Analysis[ptyp]) AND English[lang])) AND (“2010/01/19"[PDat]: “2020/01/16"[PDat]). The search strategy of Scopus database was: TITLE-ABS (tendinopathy) OR TITLE-ABS (tendinitis) OR TITLE-ABS (tendinosis) AND PUBYEAR > 2010 AND (LIMIT-TO ( DOCTYPE, “re")).

### 2.3. Study selection process

A standard data sheet was used for data collection and management. Duplicates were identified and removed electronically using EndNote X9.2 software (Bld13018). Following the removal of duplicates and non-matching studies, full-text articles were reviewed for possible inclusion. Full-text articles, moreover, were checked for eligibility in cases of abstracts. None of the authors were contacted for additional data.

### 2.4. Data items

The data extracted from each study were study title, search methodology, number and type of included studies, patient population, intervention(s) and parameters of intervention(s); if applicable, outcome(s) and/or outcome measure(s); if applicable, follow-up period(s), results, limitations, and conclusion(s). Pooled estimates of effect size(s), if applicable, were also extracted.

### 2.5. Methodological quality assessment

The methodological quality of the identified systematic reviews and/or meta-analyses were assessed using the Assessment of Multiple Systematic Reviews tool (AMSTAR 2) (a measurement tool to assess systematic reviews) (Shea et al., 2017). The AMSTAR instrument has been developed and validated to assess the quality of systematic reviews of randomized and non-randomized studies of healthcare interventions (Shea et al., 2007a, 2007b, 2009). The AMSTAR 2 guidance document was used to aid the evaluation process. The AMSTAR 2 instrument has a total of 16 items and includes more simple response categories than the original AMSTAR. All items on the AMSTAR 2 tool were weighted equally. The assessment results were arbitrarily divided into four categories, similar to other studies using the AMSTAR tool (Flodgren et al., 2011; Ryan et al., 2014). The rating of overall confidence (OC) was categorized, depending on fulfilled criteria, as follows; critically low (1-4), low (5-8), moderate (9-12), and high (13-16). Studies scoring 9 points or higher were further analyzed using Grading of Recommendations Assessment, Development, and Evaluation (GRADE) principles (Group, 2004) to determine the quality of evidence.

### 2.6. Outcome measures

Since pain with movement is a characteristic feature of tendinopathy, pain is often measured in the context of a physical task that induces pain (MacDermid & Silbernagel, 2015). Moreover, pain commonly reduces patient's activity levels, impairs range of motion, strength and function (MacDermid & Silbernagel, 2015). The outcomes were pain and function. Pain, as determined through; patient-reported pain scores using the visual analogue scale (VAS) or the numeric pain rating scale (NPRS) (Carlsson, 1983; Dixon & Bird, 1981; Hawker, Mian, Kendzerska, & French, 2011; Johnson, 2005).

Function, as determined through; *lateral elbow tendinopathy (LET)*: grip strength (pain-free grip strength) (Blanchette & Normand, 2011; Hamilton, Balnave, & Adams, 1994; Innes, 1999; Lim, 2013; Mathiowetz, Weber, Volland, & Kashman, 1984; Smidt et al., 2002; Stratford, 1987; Stratford et al., 1989, 1993; Stratford & Levy, 1994) and patient-rated tennis elbow evaluation (PRTEE) questionnaire (Evans et al., 2019; MacDermid, 2005; Newcomer, Martinez-Silvestrini, Schaefer, Gay, & Arendt, 2005; Overend, Wuori-Fearn, Kramer, & MacDermid, 1999; Rompe, Overend, & MacDermid, 2007; Vincent & MacDermid, 2014); *Achilles*

**Table 1**
Summary of eligibility criteria.

| Inclusion criteria | Exclusion criteria |
|---|---|
| • Systematic review and/or meta-analysis assessing the efficacy of physical therapy intervention(s) for tendinopathy | • Including invasive, pharmacologic, and/or surgical intervention(s) |
| • Systematic review and/or meta-analysis with adequate description of methodology | • Non-efficacy systematic review and/or meta-analysis involving physical therapy intervention(s) |
| • Published in peer reviewed journal | • Protocol of a systematic review and/or a meta-analysis |
| • Published since year 2010 | |
| • Written in English language | |

*tendinopathy (AT)*: the heel-rise test (Lunsford & Perry, 1995; Ross & Fontenot, 2000), the hopping and drop counter movement jump tests (MacDermid & Silbernagel, 2015), and the Victorian Institute of Sport Assessment-Achilles (VISA-A) questionnaire (Iversen, Bartels, & Langberg, 2012; Robinson et al., 2001); *Patellar tendinopathy (PT)*: the Victorian Institute of Sport Assessment-Patella (VISA-P) questionnaire (Dauber, Ball, Lewis, & Neidermeyer, 2018); *hip-region tendinopathies (HT)*: the Victorian Institute of Sport Assessment-Proximal Hamstring Tendons (VISA-H) (Cacchio, De Paulis, & Maffulli, 2014), the Victorian Institute for Sport Assessment for Gluteal Tendinopathy (VISA-G) (Fearon et al., 2015), and the Copenhagen Hip and Groin Outcome Score (HA-GOS) (Thorborg, Hölmich, Christensen, Petersen, & Roos, 2011); *Rotator cuff tendinopathy (RT)*: the Functional Impairment Test-Head and Neck/Shoulder/Arm (FIT-HaNSA) (Evans, Adkins, Clarke, & Cox, 2018), disabilities of the arm, shoulder and hand (DASH) questionnaire and the QuickDASH questionnaire (Gummesson, Ward, & Atroshi, 2006; Jester, Harth, Wind, Germann, & Sauerbier, 2005), the Rotator Cuff Quality-of-Life measure (RC-QoL) (Eubank, Mohtadi, Lafave, Wiley, & Emery, 2017; Razmjou, Bean, van Osnabrugge, MacDermid, & Holtby, 2006), Constant-Murley score (CMS) (Rocourt et al., 2008), and strength and range of motion (ROM); and for calcific tendinopathy, calcific deposit size.

### 2.7. Synthesis of results

The results were classified by intervention modality and therapy for practical reasons and simplification. Only MAs with moderate rating of OC or higher were included in the statistical synthesis in order to reach accurate and reliable results. Meta-analysis of results was performed using comprehensive meta-analysis software V3.3.070 (CMA; Biostat, NJ) to calculate a weighted mean difference (WMD) and 95% confidence interval (CI). Mean differences (MD) and 95% CI, or standardized mean differences (SMD) and 95% CI were utilized as reported in each study. Variability, between studies, in units of measurement were resolved through converting results to a standardized unit. Random-effects model was used due to the small number of included studies. Confidence intervals, in contrast to p-value, reflect the direction and the strength of the studied effect (du Prel, Hommel, Röhrig, & Blettner, 2009; Shakespeare, Gebski, Veness, & Simes, 2001). Heterogeneity was calculated using $I^2$ and p-value; $I^2$ values of 25%, 50%, 75% correspond to low, moderate, high heterogeneity, respectively (Higgins, Thompson, Deeks, & Altman, 2003).

## 3. Results

### 3.1. Results of the systematic review

A total of 886 citations, including title and abstract, were identified using electronic search of databases; PubMed, n = 166; PEDro, n = 55; Scopus, n = 634; and bibliography, n = 31. 769 citations were assessed for eligibility following the elimination of duplicates, n = 117. 67 full-text articles were further examined for eligibility after non-matching records were excluded, n = 702, and subsequently 27 full-text articles (out of 67) were excluded, with reasons; non-efficacy studies, n = 7; protocols of studies, n = 3; studies including invasive, pharmacologic, surgical interventions, n = 12; and written in languages other than English, n = 5. A list of excluded studies is provided in supplementary data. Forty systematic reviews (SRs) and/or meta-analyses (MAs) matched the eligibility criteria and were included in qualitative synthesis (Tumilty et al., 2010; Chang, Wu, Yang, & Jiang, 2010; Huisstede, Gebremariam, van der Sande, Hay, & Koes, 2011; Hutchison, Beard, Pallister, Topliss, & Williams, 2011; Lee, Cheng, & Grimmer-Somers, 2011; Joseph, Taft, Moskwa, & Denegar, 2012; Littlewood, Ashton, Chance-Larsen, May, & Sturrock, 2012; Rowe et al., 2012; Sussmilch-Leitch, Collins, Bialocerkowski, Warden, & Crossley, 2012; Al-Abbad & Simon, 2013; Cullinane, Boocock, & Trevelyan, 2014; Dimitrios, Konstantinos, & Antonis, 2013; Hoogvliet, Randsdorp, Dingemanse, Koes, & Huisstede, 2013; Ioppolo et al., 2013; Bannuru, Flavin, Vaysbrot, Harvey, & McAlindon, 2014; Dingemanse, Randsdorp, Koes, & Huisstede, 2014; Haslerud, Magnussen, Joensen, Lopes-Martins, & Bjordal, 2015; Loew et al., 2014; Mani-Babu, Morrissey, Waugh, Screen, & Barton, 2015; Desjardins-Charbonneau, Roy, Dionne, & Desmeules, 2015; Desjardins-Charbonneau et al., 2015; Desmeules et al., 2015; Krey, Borchers, & McCamey, 2015; Nogueira Júnior & Júnior, 2015; Scott, Munteanu, & Menz, 2014; Weber, Thai, Neuheuser, Groover, & Christ, 2015; 1017Boudreault, Desmeules, Roy, Dionne, & Frémont, ; Desmeules et al., 2016a; Desmeules et al., 2016b; Ortega-Castillo & Medina-Porqueres, 2016; McKivigan, Yamashita, & Smith, 2017; Punnoose, Norrish, & Pak, 2017; Liao et al., 2018a; Liao et al., 2018b; Lim & Wong, 2018; Lucado, Dale, Vincent, & Day, 2018; Wilson et al., 2018; Hickey, Walker, Lee, & Vitato, 2019; Murphy et al., 2019; Yan et al., 2019), whereas only 5 MAs (out of 19) were included in quantitative synthesis. The flow of the review is presented according to preferred reporting items for systematic reviews and meta-analyses (PRISMA) in Fig. 1.

### 3.2. Methodological quality assessment results

The AMSTAR 2 tool was used to appraise the included SRs; the rating of overall confidence (OC) in the results ranged from critically low to high (Table 2). One study had a high rating of OC (Murphy et al., 2019), Sixteen studies had a moderate rating of OC (Tumilty et al., 2010; Lee et al., 2011; Al-Abbad & Simon, 2013; Bannuru et al., 2014; Haslerud et al., 2015; Loew et al., 2014; Desjardins-Charbonneau et al., 2015; Desmeules et al., 2015; Scott et al., 2014; Boudreault et al., 1017; Desmeules et al., 2016b; Ortega-Castillo & Medina-Porqueres, 2016; Liao et al., 2018a; Liao et al., 2018b; Lim & Wong, 2018; Wilson et al., 2018), twenty one studies had a low rating of OC (Chang et al., 2010; Cullinane et al., 2014; Desjardins-Charbonneau et al., 2015; Desmeules et al., 2016a; Dingemanse et al., 2014; Hickey et al., 2019; Hoogvliet et al., 2013; Huisstede et al., 2011; Hutchison et al., 2011; Ioppolo et al., 2013; Joseph et al., 2012; Krey et al., 2015; Littlewood et al., 2012; Lucado et al., 2018; Mani-Babu et al., 2015; McKivigan et al., 2017; Punnoose et al., 2017; Rowe et al., 2012; Sussmilch-Leitch et al., 2012; Weber et al., 2015; Yan et al., 2019), and two studies had a critically low rating of OC (Dimitrios et al., 2013; Nogueira Júnior & Júnior, 2015). Few studies included an explicit statement that the review methods were established prior to the conduct of the review; five studies included a published protocol (Liao et al., 2018a; Lim & Wong, 2018; Murphy et al., 2019; Ortega-Castillo & Medina-Porqueres, 2016; Wilson et al., 2018), while three studies partially fulfilled this criterion (criterion 2) (Hutchison et al., 2011; Krey et al., 2015; Littlewood et al., 2012). Three studies explained their selection of the study designs in the inclusion criteria (criterion 3) (Al-Abbad & Simon, 2013; Chang et al., 2010; Lee et al., 2011). Five studies provided a list of excluded studies and justified exclusion (criterion 7) (Haslerud et al., 2015; Loew et al., 2014; Desmeules et al., 2015; Boudreault et al., 1017; Desmeules et al., 2016b). Seven studies did not describe the included studies in adequate detail (criterion 8) (Chang et al., 2010; Dimitrios et al., 2013; Dingemanse et al., 2014; Hoogvliet et al., 2013; Lucado et al., 2018; Nogueira Júnior & Júnior, 2015; Wilson et al., 2018). Two studies reported on the sources of funding for the included studies (criterion 10) (Liao et al., 2018b; Loew et al., 2014).

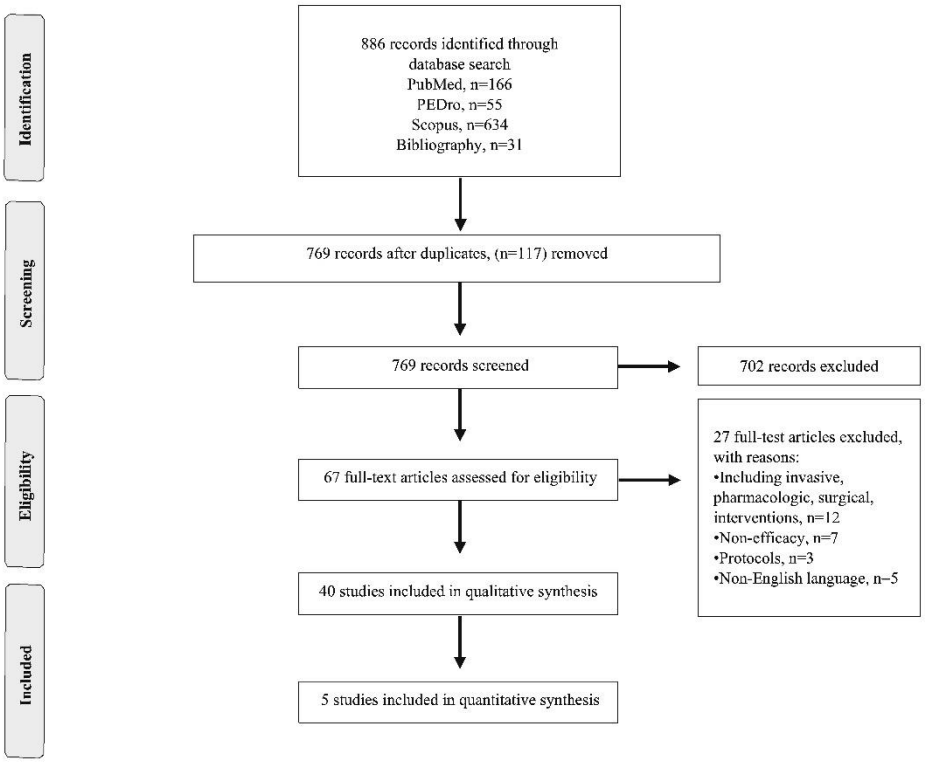



**Fig. 1.** Study flow diagram according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

### 3.3. Findings of included studies

#### 3.3.1. Low level laser therapy

Three studies with moderate rating of OC (Tumilty et al., 2010; Haslerud et al., 2015; Boudreault et al., 1017), two studies with low rating of OC (Rowe et al., 2012; Sussmilch-Leitch et al., 2012), and one study with critically low OC (Nogueira Júnior & Júnior, 2015) supported the application of LLLT, both as monotherapy and in conjunction with other physical therapy interventions, for pain relief in patients with RT and AT. Tumilty et al. (Tumilty et al., 2010) reported a reduction in pain scores of −13.64 mm; 95% CI, [-26.17, 1.11] on VAS and improvement in grip strength of 9.59 kg; 95% CI, [5.90, 13.27]. Haslerud et al. (Haslerud et al., 2015) mentioned significant pain relief of 20.41 mm; 95% CI, [12.38 to 28.44] of LLLT as monotherapy, and 16.00 mm; 95% CI, [11.88, 20.12] of LLLT in addition to ET on VAS. Boudreault et al., (Boudreault et al., 1017) described an improvement in overall pain of 1.38 cm; 95% CI, [0.91, 1.85] on VAS. One study with low rating of OC (Chang et al., 2010) reported that applying LLLT on myofascial trigger points improved pain, grip strength and ROM ($p < 0.05$) in patients with LET. Nonetheless, one study with low rating of OC (Dingemanse et al., 2014) concluded that evidence regarding the efficacy of LLLT vs placebo, in patients with LET, was conflicting. One study with moderate rating of OC (Boudreault et al., 1017) indicated that existing evidence did not support the effects of LLLT on function in patients with RT. A study with moderate rating of OC (Desmeules et al., 2015) indicated that LLLT was found to be more effective than US in reducing pain in patients with RT.

#### 3.3.2. Extracorporeal shockwave therapy

Three studies with moderate rating of OC (Al-Abbad & Simon, 2013; Liao et al., 2018a, 2018b), and one with low rating of OC (Rowe et al., 2012) concluded that ESWT was effective in reducing pain and improving function in patients with AT and PT. Liao and Tsauo et al. (Liao et al., 2018a) reported a significant reduction in pain (SMD) of −1.41; 95% CI, [-2.01, −0.82], and improvement in function (SMD) of 2.59; 95% CI, [1.54, 3.64] at all follow-up periods. Liao and Xie et al. (Liao et al., 2018b) concluded that ESWT had significant effect on pain reduction (SMD) of −1.49; 95% CI, [-2.11, −0.87] and range of motion restoration (SMD) of 1.76; 95% CI, [1.43, 2.09]. Two studies with moderate rating of OC (Lee et al., 2011; Bannuru et al., 2014) supported the application of ESWT for calcific tendinitis of the shoulder. Ioppolo et al. (Ioppolo et al., 2013) reported a total resorption ratio of 27.19; 95% CI, [7.20, 102.67] and a partial resorption ratio of 16.22; 95% CI, [3.33, 79.01]. Moreover, two studies with low rating of OC (Huisstede et al., 2011; Ioppolo et al., 2013) also supported the use of ESWT for shoulder calcific tendinitis. Two studies with low rating of OC (Mani-Babu et al., 2015; Punnoose et al., 2017) showed conflicting results regarding the efficacy of ESWT for AT and PT. Punnoose et al. (Punnoose et al., 2017) indicated no significant differences between intervention and control groups on VISA-A scores 5.74; 95% CI, [-15.02, 26.51] and VISA-P scores 10.01; 95% CI, [-7.86, 27.87], for AT and PT, respectively. Yan et al. (Yan et al., 2019) reported lower pain (VAS score) in ESWT group at 1 month of −1.42; 95% CI, [-2.14, −0.70], at 3 months of −1.65; 95% CI, [-1.81, −1.49], and at 6 months of −0.73; 95% CI, [-1.06, −0.40], and higher grip strength scores at 3 months of 1.53; 95% CI, [1.17, 1.88] after intervention compared to US.

#### 3.3.3. Exercise therapy

Three studies with moderate rating of OC (Ortega-Castillo & Medina-Porqueres, 2016; Lim & Wong, 2018; Wilson et al., 2018) and three with low rating of OC (Littlewood et al., 2012; Hoogvliet et al., 2013; Desmeules et al., 2016a) concluded that ET reduced pain and improved function in patients with LET, AT, PT, and RT. One study with high rating of OC (Murphy et al., 2019), three studies with moderate rating of OC (Ortega-Castillo & Medina-Porqueres, 2016; Lim & Wong, 2018; Wilson et al., 2018), and three studies

**Table 2**
Summary of methodological quality assessment of studies. 1. Did the research questions and inclusion criteria for the review include the components of PICO? 2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol? 3. Did the review authors explain their selection of the study designs for inclusion in the review? 4. Did the review authors use a comprehensive literature search strategy? 5. Did the review authors perform study selection in duplicate? 6. Did the review authors perform data extraction in duplicate? 7. Did the review authors provide a list of excluded studies and justify the exclusions? 8. Did the review authors describe the included studies in adequate detail? 9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review? 10. Did the review authors report on the sources of funding for the studies included in the review? 11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results? 12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis? 13. Did the review authors account for RoB in primary studies when interpreting/discussing the results of the review? 14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review? 15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review? 16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review? The rating of overall confidence (OC) was categorized, depending on fulfilled criteria, as follows; critically low (1-4), low (5-8), moderate (9-12), and high (13-16); 1, met; 0, unmet; P, partially met; N/A, inapplicable.

| Studies | AMSTAR 2 items | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | OC |
| Tumilty et al. (Tumilty et al., 2010) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | Moderate |
| Chang et al. (Chang et al., 2010) | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | Low |
| Huisstede et al. (Huisstede et al., 2011) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 0 | Low |
| Hutchison et al. (Hutchison et al., 2011) | 1 | P | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 0 | 1 | N/A | 0 | Low |
| Lee et al. (Lee et al., 2011) | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 0 | 1 | N/A | 1 | Moderate |
| Joseph et al. (Joseph et al., 2012) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 0 | 1 | N/A | 0 | Low |
| Littlewood et al. (Littlewood et al., 2012) | 1 | P | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 0 | Low |
| Rowe et al. (Rowe et al., 2012) | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 1 | Low |
| Sussmilch-Leitch et al. (Sussmilch-Leitch et al., 2012) | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | Low |
| Al-Abbad et al. (Al-Abbad & Simon, 2013) | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 1 | Moderate |
| Cullinane et al. (Cullinane et al., 2014) | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 1 | Low |
| Dimitrios et al. (Dimitrios et al., 2013) | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | N/A | N/A | 0 | 1 | N/A | 0 | C. Low |
| Hoogvliet et al. (Hoogvliet et al., 2013) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 1 | Low |
| Ioppolo et al. (Ioppolo et al., 2013) | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Low |
| Bannuru et al. (Bannuru et al., 2014) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 1 | Moderate |
| Dingemanse et al. (Dingemanse et al., 2014) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 1 | Low |
| Haslerud et al. (Haslerud et al., 2015) | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | Moderate |
| Loew et al. (Loew et al., 2014) | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | N/A | N/A | 1 | 1 | N/A | 1 | Moderate |
| Mani-Babu et al. (Mani-Babu et al., 2015) | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | N/A | N/A | 0 | 1 | N/A | 1 | Low |
| D-Charbonneau et al. (Desjardins-Charbonneau et al., 2015) | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | Moderate |
| D-Charbonneau et al. (Desjardins-Charbonneau et al., 2015) | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | Low |
| Desmeules et al. (Desmeules et al., 2015) | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | Moderate |
| Krey et al. (Krey et al., 2015) | 0 | P | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | N/A | N/A | 0 | 1 | N/A | 1 | Low |
| Nogueira Júnior et al. (Nogueira Júnior & Júnior, 2015) | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | N/A | N/A | 0 | 1 | N/A | 0 | C. Low |
| Scott et al. (Scott et al., 2014) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | Moderate |
| Weber et al. (Weber et al., 2015) | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | Low |
| Boudreault et al., (Boudreault et al., 1017) | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | Moderate |
| Desmeules et al. (Desmeules et al., 2016a) | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 0 | Low |
| Desmeules et al. (Desmeules et al., 2016b) | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 1 | Moderate |
| Ortega-Castillo et al. (Ortega-Castillo & Medina-Porqueres, 2016) | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 0 | 1 | N/A | 1 | Moderate |
| McKivigan et al. (McKivigan et al., 2017) | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 0 | 0 | N/A | 0 | Low |
| Punnoose et al. (Punnoose et al., 2017) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | Low |
| Liao et al. (Liao et al., 2018a) | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | Moderate |
| Liao et al. (Liao et al., 2018b) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | Moderate |
| Lim et al. (Lim & Wong, 2018) | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | N/A | N/A | 1 | 1 | N/A | 1 | Moderate |
| Lucado et al. (Lucado et al., 2018) | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | Low |
| Wilson et al. (Wilson et al., 2018) | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | Moderate |
| Hickey et al. (Hickey et al., 2019) | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | Low |
| Murphy et al. (Murphy et al., 2019) | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | High |
| Yan et al. (Yan et al., 2019) | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | Low |

with low rating of OC (Rowe et al., 2012; Cullinane et al., 2014; Hickey et al., 2019) supported EE for treating LET, AT and PT. Murphy et al. (Murphy et al., 2019) suggested that heavy eccentric calf training may be superior to both natural history by VISA-A: 20.6; 95% CI, [11.7, 29.5] and traditional physiotherapy by VISA-A: 17.70; 95% CI, [3.75, 31.66], but may be inferior to other exercise interventions (very-low to low quality of evidence). A meta-analysis with low rating of OC (Sussmilch-Leitch et al., 2012) identified positive effects of EE in addition to LLLT at 12 weeks in patients with AT (pain VAS: −0.59, 95% CI, [−1.11, −0.07]). A systematic review with low rating of OC (Hutchison et al., 2011) indicated that beneficial effects of EE in combination with LLLT or ESWT was supported by some evidence. Nevertheless, the latter studies (Hutchison et al., 2011; Sussmilch-Leitch et al., 2012) stated that EE was not superior to shockwave therapy at 16 weeks (VISA-A:−0.55; 95% CI, [−2.21, 1.11]), or other physical therapy modalities.

### 3.3.4. Manual therapy

One study with moderate rating of OC (Loew et al., 2014) and another with low rating of OC (Joseph et al., 2012) have concluded that existing evidence is insufficient to determine the efficacy of deep friction massage in patients with tendinopathy. Two studies with low rating of OC (Hoogvliet et al., 2013; Lucado et al., 2018) supported joint mobilization and manipulation techniques for LET. Lucado et al. (Lucado et al., 2018) reported positive effects of mobilization with movement on pain reduction (VAS) of 0.43; 95% CI, [0.15, 0.71] and grip strength improvement of 0.31; 95% CI, [0.11, 0.51]. A study with low rating of OC (Desjardins-Charbonneau et al., 2015) indicated that MT may reduce pain in patients with RT, however, whether it can improve function is unclear. Desjardins-

Charbonneau et al. (Desjardins-Charbonneau et al., 2015) reported a small but statistically significant reduction in pain of 1.1; 95% CI, [0.6, 1.6] on VAS. A study with low rating of OC (Krey et al., 2015) stated that current evidence suggest that tendon dry needling improved patient-reported outcome measures in patients with tendinopathy.

#### 3.3.5. Ultrasound therapy

A study with moderate rating of OC (Desmeules et al., 2015) concluded that US therapy was not superior to placebo, LLLT, or ET. Desmeules et al. (Desmeules et al., 2015) reported that US in conjunction with ET was not superior to ET alone (Constant-Murley score: −0.26; 95% CI, [-3.84, 3.32]). One study with low rating of OC (Dingemanse et al., 2014) stated that US therapy was potentially effective for the management of LET. A study with low rating of OC (Yan et al., 2019) compared US therapy to ESWT and indicated that US therapy was less effective in reducing pain and improving function in patients with LET. Inconclusive evidence regarding the efficacy of US for the treatment of AT was reported in one study with low rating of OC (Rowe et al., 2012).

#### 3.3.6. Orthotic therapy

A study with moderate rating of OC (Desjardins-Charbonneau et al., 2015) stated that there was insufficient evidence to determine the efficacy of kinesiology taping or non-elastic taping used alone or in addition to other interventions for the management of RT. Desjardins-Charbonneau et al. (Desjardins-Charbonneau et al., 2015) reported that kinesiology taping alone improved pain-free flexion by 8.7; 95% CI, [8.0, 9.5] and pain-free abduction by 10.3; 95% CI, [9.1, 11.4]. Another study with moderate rating of OC (Scott et al., 2014) concluded that foot orthoses were equivalent to physical therapy or no treatment (weak evidence), and that the use of adhesive taping alone or combined with foot orthoses was supported by very weak evidence. Conflicting evidence regarding the efficacy of AirHeel™ brace was reported (Rowe et al., 2012; Scott et al., 2014), whereas the addition of night splints to EE was opposed by one study with moderate rating of OC (Wilson et al., 2018) and two studies with low rating of OC (Rowe et al., 2012; Sussmilch-Leitch et al., 2012). In-shoe foot orthoses was supported by limited evidence (Rowe et al., 2012).

#### 3.3.7. Transcutaneous electrical nerve stimulation

One study with moderate rating of OC (Desmeules et al., 2016b) and one study with low rating of OC (Dingemanse et al., 2014) indicated that existing evidence was inadequate to support the effectiveness of TENS to treat RT or LET.

#### 3.3.8. Iontophoresis

One study with low rating of OC (McKivigan et al., 2017) and one with critically low rating of OC (Dimitrios et al., 2013) concluded that evidence to determine the efficacy of iontophoresis in treating patients with LET was inconclusive. A Summary of characteristics of studies is provided in Table 3.

### 3.4. Results of the meta-analysis

Only 5 MAs with moderate rating of OC (Tumilty et al., 2010; Haslerud et al., 2015; Boudreault et al., 1017; Liao et al., 2018a; Liao et al., 2018b) showed sufficient quality and homogeneity for the meta-analytic procedures to be plausible and meaningful. Sensitivity analysis was not performed due to the small number of studies. LLLT intervention, either alone versus placebo or combined with exercise versus exercise plus placebo, showed an improvement (MD) in pain reduction of 1.53 cm; 95% CI, [1.14, 1.91] ($I^2$ = 1.9%, p = 0.361) on VAS. The follow-up period for LLLT intervention ranged between (2–12 weeks) (Fig. 2).

ESWT intervention, either alone or combined with other interventions versus placebo or other interventions, revealed an improvement (SMD) in pain reduction of 1.27; 95% CI, [0.89, 1.65] ($I^2$ = 0.000%, p = 0.560) at ≤1 month; 0.93; 95% CI, [0.55, 1.31] ($I^2$ = 0.000%, p = 0.688) at > 1 month and ≤3 months; 1.91; 95% CI, [1.17, 2.66] ($I^2$ = 0.000%, p = 0.913) at > 3 months and ≤6 months; and 1.99; 95% CI, [0.84, 3.15] ($I^2$ = 26.152%, p = 0.245) at ≥ 12 months (Fig. 3). Moreover, ESWT intervention, either alone or combined with other interventions versus placebo or other interventions, demonstrated positive effects (SMD) on function of 2.41; 95% CI, [1.66, 3.17] ($I^2$ = 0.000%, p = 0.649) at ≤1 month, 1.41; 95% CI, [0.83, 1.99] ($I^2$ = 0.000%, p = 0.763) at > 1 month and ≤3 months, 2.38; 95% CI, [1.41, 3.35] ($I^2$ = 0.000%, p = 0.907) at > 3 months and ≤6 months, and 3.44; 95% CI, [1.45, 5.44] ($I^2$ = 43.51%, p = 0.183) at ≥ 12 months (Fig. 4).

## 4. Discussion

The identified reviews assessed the efficacy of a variety of physical therapy interventions for tendinopathy. Nonetheless, the identified evidence was insufficient to discuss all of these interventions. The GRADE principles (Group, 2004) were subsequently used to appraise results of the present review in order to determine the quality of evidence. Moderate-quality evidence that may support the efficacy of low-level laser therapy, extracorporeal shockwave therapy, and exercise therapy was found. The discussion section, therefore, will cover these interventions primarily.

Exercise therapy (ET) demonstrated positive effects in reducing pain and improving function (Lim & Wong, 2018; Ortega-Castillo & Medina-Porqueres, 2016; Wilson et al., 2018). The efficacy of eccentric exercise (EE), in particular, was supported by several SRs (Lim & Wong, 2018; Murphy et al., 2019; Ortega-Castillo & Medina-Porqueres, 2016; Wilson et al., 2018). EE seems to be more suitable for long-term pain reduction and improvement in function, while isometric exercises appear to more effective for short-term pain relief (Lim & Wong, 2018). Moderate-quality evidence, moreover, recommended EE over concentric exercise for reducing pain (Wilson et al., 2018). No significant difference was found between EE and heavy slow resistance exercise in reducing pain or improving function (Wilson et al., 2018). Nonetheless, a statistically-significant difference was found between high-dose and low-dose eccentric training in pain reduction of 6.3 mm; 95% CI, [−4.45, 17.04] (Wilson et al., 2018) on VAS. This difference, however, may not be clinically significant since the minimal clinically important difference (MCID) in pain reduction was reported to be 1.4 cm (Tashjian, Deloach, Porucznik, & Powell, 2009).

The results of the current review regarding EE are consistent with literature (Carcia et al., 2010; Burcal, Rosen, Taylor, & Nicola, 2019). Even-though ET, EE in particular, seems to be the most supported intervention in literature, it was merely supported by qualitative evidence. In the current review, likewise, no quantitative evidence for the efficacy of ET was found. Several potential mechanisms have been proposed to explain the efficacy of EE, including neuromuscular alterations (O'Neill, Watson, & Barry, 2015), increased collagen synthesis, and pain habituation (Murtaugh & Ihm, 2013). The optimal protocol and dosage of exercise, however, remain unknown (Couppé et al., 2015; Littlewood et al., 2015; Meyer et al., 2009; Raman, MacDermid, & Grewal, 2012; Young, Rhon, de Zoete, Cleland, & Snodgrass, 2018). Notably, since the pathogenesis of tendinopathy may include overuse and the optimal protocol is currently unknown, the "appropriate" dosage of exercise may be difficult to establish, which may hinder the applicability and safety of ET.

Extracorporeal shockwave therapy (ESWT) showed a

**Table 3**
Summary of characteristics of studies. LLLT: Low-level laser therapy; LE, Lateral epicondylitis; MTrPs, Myofascial trigger points; RCTs, Randomized controlled trials; ESWT, Extracorporeal shockwave therapy; RC, Rotator cuff; DFM, Deep friction massage; AT, Achilles tendinopathy; VAS, Visual analogue scale; CONSORT; Consolidated Standards of Reporting Trials; LET, Lateral elbow tendinopathy; KT, Kinesiology taping; NET, Non-elastic taping; MT, Manual therapy; GTPS, Greater trochanteric pain syndrome; PT, Patellar tendinopathy; MD, Mean difference; RR, Risk ratio; US, Ultrasound therapy; TENS, Transcutaneous electrical nerve stimulation; VISA-P, the Victorian Institute of Sport Assessment-Patella questionnaire; VISA-A, the Victorian Institute of Sport Assessment—Achilles Questionnaire; TSR, Treatment success rate; ROM, Range of motion; KSTDs, knee soft tissue disorders; CI, Confidence interval; HSR, Heavy slow resistance; MWM, Mobilization with movement; HECT, Heavy eccentric calf training; The rating of overall confidence (OC) was categorized, depending on fulfilled criteria, as follows; critically low (1-4), low (5-8), moderate (9-12), and high (13-16).

| Studies | Title | Search strategy | Results | Conclusion | OC |
|---|---|---|---|---|---|
| Tumilty et al. (Tumilty et al., 2010) | Low Level Laser Treatment of Tendinopathy: A Systematic Review with Meta-analysis | The following databases were searched from inception to August 1, 2008: MEDLINE, PubMed, CINAHL, AMED, EMBASE, All EBM reviews, PEDro (Physiotherapy Evidence Database), SCOPUS. Controlled clinical trials evaluating LLLT as a primary intervention for any tendinopathy were included in the review. | Twenty-five controlled clinical trials met the inclusion criteria. There were conflicting findings from multiple trials: 12 showed positive effects and 13 were inconclusive or showed no effect. In two instances where pooling of data was possible, LLLT showed a positive effect size. | LLLT can potentially be effective in treating tendinopathy when recommended dosages are used. The 12 positive studies provide strong evidence that positive outcomes are associated with the use of current dosage recommendations for the treatment of tendinopathy. | Moderate |
| Chang et al. (Chang et al., 2010) | Therapeutic Effects of Low-Level Laser on Lateral Epicondylitis from Differential Interventions of Chinese-Western Medicine: Systematic Review | We searched several electronic databases, including Medline, PubMed, and CINAHL, and explored studies that were randomized controlled trials on the therapeutic effects of LLLT on LE from 1990 to February 2009. | We selected ten articles, and in seven of them the irradiation was conducted on tender points or MTrPs in the experimental groups. The results revealed that applying LLLT on tender points or MTrPs is an effective means to improve the effect size (ES) of pain release after treatment (pooled ES: 0.71, 95% CI: 0.82 −0.60) and follow-up (pooled ES: 1.05, 95% CI: 1.16−0.94). | We suggest that using LLLT on tender points or MTrPs of LE could effectively improve therapeutic effects. | Low |
| Huisstede et al. (Huisstede et al., 2011) | Evidence for effectiveness of Extracorporal Shock-Wave Therapy (ESWT) to treat calcific and non-calcific rotator cuff tendinosis e A systematic review | The Cochrane Library, PubMed, Embase, Pedro, and Cinahl were searched for relevant systematic reviews and RCTs. | Seventeen RCTs (11 calcific, 6 non-calcific) were included. For calcific RC-tendinosis, strong evidence was found for effectiveness in favour of high-ESWT versus low-ESWT in short-term. Moderate evidence was found in favour of high-ESWT versus placebo in short-, mid- and long-term and versus low-ESWT in mid- and long-term. | This review shows that only high-ESWT is effective for treating calcific RC-tendinosis. No evidence was found for the effectiveness of ESWT to treat non-calcific RC-tendinosis. | Low |
| Hutchison et al. (Hutchison et al., 2011) | Is physiotherapy effective for patients with a chronic mid-body Achilles tendinopathy? A systematic review of non-surgical and non-pharmacological interventions | A search of the following electronic databases was conducted: Medline, CINAHL, EMBASE, AMED, sports discuss, web of knowledge, rehabilitation and sports medicine sources, cochrane library, and PEDro (physiotherapy evidence database) (January 1999 to January 2011). | Nine publications met the review inclusion criteria. Methodological quality was adequate for all nine studies; however, blinding was a limitation for most. Interventions investigated were; exercises (n = 2), low-level laser therapy (n = 1), low-energy shockwave treatment (SWT) (n = 3), air cast brace (n = 2), and insoles (n = 1). | There is an insufficient evidence to determine which method of physiotherapy is most appropriate for a chronic mid-body Achilles tendinopathy. | Low |
| Lee et al. (Lee et al., 2011) | The midterm effectiveness of extracorporeal shockwave therapy in the management of chronic calcific shoulder tendinitis | Articles were electronically searched from the Cochrane Controlled Trials Register, MEDLINE, CINAHL, PUBMED, EMBASE, SPORTSDiscus and PEDro using a comprehensive search strategy. Studies were included if they were randomized controlled trials testing the midterm effectiveness of ESWT for chronic calcific tendonitis. | Six of the nine included studies scored 7 or more for methodologic quality. All studies had follow-up periods of at least 6 months. Common methodologic flaws were insufficient blinding of clinicians and assessors. There was consistent evidence of midterm effectiveness of ESWT in reducing pain and improving shoulder function for patients with chronic calcified tendinitis. | Our review suggests that ESWT is moderately effective in reducing pain and improving function in individuals with chronic shoulder calcific tendinitis, for up to a year after its application (Level of Evidence B).There is no clear evidence regarding the optimal dosage in energy level, intervals between sessions, and number of sessions required for optimal recovery from the condition. | Moderate |
| Joseph et al. (Joseph et al., 2012) | Deep Friction Massage to Treat Tendinopathy: A Systematic Review of a Classic Treatment in the Face of a New Paradigm of Understanding | The authors surveyed research articles in all languages by searching PubMed, Scopus, Pedro, CINAHL, PsycINFO, and the Cochrane Library. | Nine studies met the inclusion criteria. Evidence Synthesis: The heterogeneity of dependent measures did not allow for meta-analysis. | The varied locations, study designs, etiopathogenesis, and outcome tools used to examine the efficacy of DFM make a unified conclusion tenuous. | Low |
| Littlewood et al. (Littlewood et al., 2012) | Exercise for rotator cuff tendinopathy: a systematic review | An electronic search of AMED, CiNAHL, Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, PEDro and SPORTDiscus was undertaken from their inception to November 2010 and supplemented by hand | Five articles detailing four studies were included, all of which were regarded as presenting a low risk of bias. Overall, the literature was supportive of the use of exercise in terms of pain and functional disability. | The available literature is supportive of the use of exercise but due to the paucity of research and associated limitations further study is indicated. | Low |

**Table 3** (*continued*)

| Studies | Title | Search strategy | Results | Conclusion | OC |
|---|---|---|---|---|---|
| | | searching related articles and contact with topic experts. | | | |
| Rowe et al. (Rowe et al., 2012) | Conservative management of mid-portion Achilles tendinopathy: a mixed methods study, integrating systematic review and clinical reasoning | Searches of the electronic databases, PubMed, ISI Web of Science, PEDro, CINAHL, Embase, and Google Scholar were conducted for all papers published from inception to November 2011. | 47 studies fulfilled the inclusion criteria. Evidence was strong for eccentric loading exercises and extracorporeal shockwave therapy; moderate for, splinting/bracing, active rest, low-level laser therapy and concentric exercises (i.e. inferior to eccentric exercise). In-shoe foot orthoses and therapeutic ultrasound had limited evidence. | Empirical research indicates strong evidence for eccentric loading exercises and extra-corporeal shockwave therapy and moderate evidence for low-level laser therapy. Additionally, the physiotherapists interviewed reported anecdotal evidence for concentric exercises, soft-tissue mobilizations and foot orthoses. | Low |
| Sussmilch-Leitch et al. (Sussmilch-Leitch et al., 2012) | Physical therapies for Achilles tendinopathy: systematic review and meta-analysis | Reference lists of eligible papers were hand-searched. Randomized controlled trials (RCTs) were included if they evaluated at least one non-pharmacological, non-surgical intervention for AT using at least one outcome of pain and/or function. | Four studies were excluded due to high risk of bias, leaving 19 studies, the majority of which evaluated midportion AT. Effect sizes from individual RCTs support the use of eccentric exercise. Meta-analyses identified significant effects favouring the addition of laser therapy to eccentric exercise at 12 weeks (pain VAS: standardized mean difference −0.59, 95% confidence interval −1.11 to −0.07). | Practitioners can consider eccentric exercise as an initial intervention for AT, with the addition of laser therapy as appropriate. Shock wave therapy may represent an effective alternative. High-quality RCTs following CONSORT guidelines are required to further evaluate the efficacy of physical therapies and determine optimal clinical pathways for AT. | Low |
| Al-Abbad et al. (Al-Abbad & Simon, 2013) | The Effectiveness of Extracorporeal Shock Wave Therapy on Chronic Achilles Tendinopathy: A Systematic Review | Articles were electronically searched from the Cochrane Controlled Trials Register, MEDLINE, CINAHL, EMBASE, and SPORTDiscus using a comprehensive search strategy. | Four of the included studies were randomized controlled trials, and 2 were pre-post study designs. Common methodological deficiencies included not blinding the clinician and participants. There was consistent evidence from 4 reviewed studies on the effectiveness of ESWT in the management of patients with chronic Achilles tendinopathies at a minimum 3 months' follow-up. | Overall, our review showed satisfactory evidence for the effectiveness of low-energy ESWT in the treatment of chronic insertional and noninsertional Achilles tendinopathies at a minimum 3 months' follow-up before considering surgery if other conservative management fails. | Moderate |
| Cullinane et al. (Cullinane et al., 2014) | Is eccentric exercise an effective treatment for lateral epicondylitis? A systematic review | Six electronic databases were searched (ProQuest, Medline via EBSCO, AMED, Scopus, Web of Science, and CINAHL). A systematic review was undertaken to identify randomized and controlled clinical trials incorporating eccentric exercise as a treatment for patients diagnosed with lateral epicondylitis. | Twelve studies met the inclusion criteria. Following treatment, all groups inclusive of eccentric exercise reported decreased pain and improved function and grip strength from baseline. Seven studies reported improvements in pain, function, and/or grip strength for therapy treatments inclusive of eccentric exercise when compared with those excluding eccentric exercise. | The majority of consistent findings support the inclusion of eccentric exercise as part of a multimodal therapy programme for improved outcomes in patients with lateral epicondylitis. | Low |
| Dimitrios et al. (Dimitrios et al., 2013) | Effectiveness of Iontophoresis for Lateral Elbow Tendinopathy | Randomized controlled trials (RCTs) identified by a search strategy in six databases were used in combination with reference checking. RCTs that included iontophoresis, patients with LET, and at least one of the clinically relevant outcome measures were selected. A qualitative analysis of the selected studies was conducted using the Chalmers' technique. | Four RCTs fulfilled the criteria and were included in the review. Although these studies had satisfactory methodology, shortcomings were not absent; conflicting results were revealed as to the effectiveness of iontophoresis for LET management. | Iontophoresis need not be ruled out for LET as it is a dose-response modality, and the optimal treatment dose has obviously not yet have been discovered. Further research with well-designed RCTs is needed to establish the absolute and relative effectiveness of this intervention for LET. | C. Low |
| Hoogvliet et al. (Hoogvliet et al., 2013) | Does effectiveness of exercise therapy and mobilization techniques offer guidance for the treatment of lateral and medial epicondylitis? A systematic review | Searches in PubMed, Embase, Cinahl and Pedro were performed to identify relevant randomized clinical trials (RCTs) and systematic reviews. | One review and 12 RCTs, all studying lateral epicondylitis, were included. Different therapeutic regimes were evaluated: stretching, strengthening, concentric/eccentric exercises and manipulation of the cervical or thoracic spine, elbow or wrist. No statistical pooling of the results could be performed owing to | Although not yet conclusive, these results support the belief that strength training decreases symptoms in tendinosis. The short-term analgesic effect of manipulation techniques may allow more vigorous stretching and strengthening exercises. | Low |

(*continued on next page*)

**Table 3** (*continued*)

| Studies | Title | Search strategy | Results | Conclusion | OC |
|---|---|---|---|---|---|
| | | | heterogeneity of the included studies. | | |
| Ioppolo et al. (Ioppolo et al., 2013) | Clinical Improvement and Resorption of Calcifications in Calcific Tendinitis of the Shoulder After Shock Wave Therapy at 6 Months' Follow-Up: A Systematic Review and Meta-Analysis | Articles were searched from the Cochrane Library, MEDLINE, Embase, CINAHL, and Ovid database. | 6 studies were included for review. We found a clinical improvement with a pooled total resorption ratio of 27.19 (95% confidence interval [CI], 7.20e102.67) and a pooled partial resorption ratio of 16.22 (95% CI, 3.33e79.01). | SWT increases shoulder function, reduces pain, and is effective in dissolving calcifications. These results were maintained over the following 6 months. | Low |
| Bannuru et al. (Bannuru et al., 2014) | High-Energy Extracorporeal Shock-Wave Therapy for Treating Chronic Calcific Tendinitis of the Shoulder | MEDLINE, Cochrane Central Register of Controlled Trials, EMBASE, Web of Science, and Google Scholar were searched up to November 1, 2013. | Twenty-eight RCTs met the inclusion criteria. Studies were heterogeneous. Twenty RCTs compared ESWT energy levels and placebo and consistently showed that high-energy ESWT was significantly better than placebo in decreasing pain and improving function and resorption of calcifications in calcific tendinitis. | High-energy ESWT is effective for improving pain and shoulder function in chronic calcific shoulder tendinitis and can result in complete resolution of calcifications | Moderate |
| Dingemanse et al. (Dingemanse et al., 2014) | Evidence for the effectiveness of electrophysical modalities for treatment of medial and lateral epicondylitis: a systematic review | Searches in PubMed, EMBASE, CINAHL and Pedro were performed to identify relevant randomized clinical trials (RCTs) and systematic reviews. | A total of 2 reviews and 20 RCTs were included, all of which concerned LE. Moderate evidence was found for the effectiveness of ultrasound versus placebo on mid-term follow-up. Ultrasound plus friction massage showed moderate evidence of effectiveness versus laser therapy on short-term follow-up. | Potential effectiveness of ultrasound and laser for the management of LE was found. To draw more definite conclusions high-quality RCTs examining different intensities are needed as well as studies focusing on long-term follow-up results. | Low |
| Haslerud et al. (Haslerud et al., 2015) | The Efficacy of Low-Level Laser Therapy for Shoulder Tendinopathy: A Systematic Review and Meta-Analysis of Randomized Controlled Trials | A literature search for randomized controlled clinical trials was performed on Medline, PubMed, Embase, CINAHL, PEDro and the Cochrane Controlled Trial Register databases. | Seventeen randomized controlled trials met the inclusion criteria. Significant and clinically important pain relief was found with weighted mean differences (WMD) over placebo, for LLLT as monotherapy at 20.41 mm (95% CI: 12.38 to 28.44) and as adjunct to exercise therapy at 16.00 mm (95% CI: 11.88 to 20.12) pain reduction on VAS. | This review shows that optimal LLLT can offer clinically relevant pain relief and initiate a more rapid course of improvement, both alone and in combination with physiotherapy interventions. | Moderate |
| Loew et al. (Loew et al., 2014) | Deep transverse friction massage for treating lateral elbow or lateral knee tendinitis (Review) | We searched the following electronic databases: the specialized central registry of the Cochrane Field of Physical and Related Therapies, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Clinicaltrials.gov, and the Physiotherapy Evidence Database (PEDro), up until July 2014. | Two RCTs met the inclusion criteria. No statistically significant differences were reported within five weeks for mean change in pain (VAS) (MD -6.60, 95%CI -28.60 to 15.40; 7% absolute improvement), grip strength (MD 0.10, 95% CI -0.16 to 0.36) and function (MD -1.80, 95% CI -0.18.64 to 15.04; 2% improvement), pain-free function index (MD 1.10, 95% CI -1.00 to 3.20) and functional status (RR 3.3, 95% CI 0.4 to 24.3). | We do not have sufficient evidence to determine the effects of deep transverse friction on pain, improvement in grip strength, and functional status for patients with lateral elbow tendinitis or knee tendinitis, as no evidence of clinically important benefits was found. | Moderate |
| Mani-Babu et al. (Mani-Babu et al., 2015) | The Effectiveness of Extracorporeal Shock Wave Therapy in Lower Limb Tendinopathy. A Systematic Review | PubMed (Medline), Embase, Web of Knowledge, Cochrane, and CINAHL were searched from inception to February 2013 for studies of any design investigating the effectiveness of ESWT in GTPS, PT, and AT. | A total of 20 studies were identified. Moderate evidence indicates that ESWT is more effective than eccentric loading for insertional AT and equal to eccentric loading for midportion AT in the short term. Additionally, there is moderate evidence that combining ESWT and eccentric loading in midportion AT may produce superior outcomes to eccentric loading alone. | Extracorporeal shock wave therapy is an effective intervention and should be considered for GTPS, PT, and AT particularly when other nonoperative treatments have failed. | Low |
| Desjardins-Charbonneau et al. (Desjardins-Charbonneau et al., 2015) | THE EFFICACY OF TAPING FOR ROTATOR CUFF TENDINOPATHY: A SYSTEMATIC REVIEW AND META-ANALYSIS | An electronic bibliographical search in MEDLINE, EMBASE, the Physiotherapy Evidence Database (PEDro) and the Cumulative Index to Nursing & Allied Health Literature (CINAHL) was conducted in order to identify | Ten RCTs were included in the present review. Based on pooled results of two studies (n = 72), KT used alone resulted in significant gain in pain free flexion (MD: 8.7° 95%CI 8.0°–9.5°) and in pain free | Although KT significantly improved pain free range of motion, there is insufficient evidence to formally conclude on the efficacy of KT or NET used alone or in conjunction with | Moderate |

**Table 3** (*continued*)

| Studies | Title | Search strategy | Results | Conclusion | OC |
|---|---|---|---|---|---|
| | | citations indexed until October 2014. | abduction (MD: 10.3° 95%CI 9.1° −11.4°). | other interventions in patients with RC tendinopathy. | |
| Desjardins-charbonneau et al. (Desjardins-Charbonneau et al., 2015) | The Efficacy of Manual Therapy for Rotator Cuff Tendinopathy: A Systematic Review and Meta-analysis | An electronic bibliographical search was conducted in MEDLINE, Embase, PEDro, and CINAHL from their dates of inception to June 2014. | Twenty-one studies were included. A small but statistically significant overall effect for pain reduction of MT compared with a placebo or in addition to another intervention was observed (n = 406), which may or may not be clinically important. | For patients with RC tendinopathy, based on low- to moderate-quality evidence, MT may decrease pain; however, it is unclear whether it can improve function. | Low |
| Desmeules et al. (Desmeules et al., 2015) | The efficacy of therapeutic ultrasound for rotator cuff tendinopathy: A systematic review and meta-analysis | We searched four bibliographical databases: PubMed, PeDRO, CINAHL, EMBASE, from their date of inception to December 2013. Reference lists of retrieved studies and previous systematic reviews were also searched to identify additional relevant publications | Eleven RCTs were included. Therapeutic US did not provide greater benefits than a placebo intervention or advice in terms of pain reduction and functional improvement. | Based on low to moderate level evidence, therapeutic US does not provide any benefit compared to a placebo or advice, to laser therapy or when combined to exercise | Moderate |
| Krey et al. (Krey et al., 2015) | Tendon needling for treatment of tendinopathy: A systematic review | A literature review was completed using Medline and Cochrane Database searches up to November 2013 to identify all English-language and translated clinical papers that evaluated the use of tendon needling for the treatment of tendinosis. | In 2 studies evaluating tendon needling in lateral epicondylosis, one showed an improvement in a subjective visual analogue scale score of 34% (significant change >25%) from baseline at 6 months. The other showed an improvement of 56.1% in a visual analogue scale score from baseline. | The evidence suggests that tendon needling improves patient-reported outcome measures in patients with tendinopathy. There is a trend that shows that the addition of autologous blood products may further improve theses outcomes. | Low |
| Nogueira Júnior et al. (Nogueira Júnior & Júnior, 2015) | THE EFFECTS OF LASER TREATMENT IN TENDINOPATHY: A SYSTEMATIC REVIEW | A search was performed in the electronic databases Pub-Med, Medline, Capes Journals Database and Latin American and Caribbean Health Sciences Literature (LILACS), without restrictions regarding the period of publication. | Three studies were included. Conclusions about the effectiveness of low-intensity laser therapy cannot be made due to the low statistical power of the study. The low intensity laser in this dosage can reduce inflammation in activated Achilles tendinitis. There was an acceleration of the recovery process when laser was associated with an eccentric exercise program. | After the analysis, we selected three articles that showed that the use of low-level laser therapy, compared to placebo, is effective in treatment of tendinopathy. | C. Low |
| Scott et al. (Scott et al., 2014) | Effectiveness of Orthotic Devices in the Treatment of Achilles Tendinopathy: A Systematic Review | Electronic bibliographic databases (MEDLINE, EMBASE, Current Contents, CINAHL and SPORTDiscus) were searched in May 2014. | Twelve studies satisfied the inclusion criteria; nine studies investigated mid-portion AT, whilst three studies did not distinguish between mid-portion and insertional pathology. Weak evidence showed that foot orthoses were equivalent to physical therapy, and equivalent to no treatment. | The findings of this study indicate that foot orthoses, the AirHeel brace, and ankle joint dorsiflexion night splints are equivalent to calf muscle eccentric exercises in the management of mid-portion AT. | Moderate |
| Weber et al. (Weber et al., 2015) | Efficacy of physical therapy for the treatment of lateral epicondylitis: a meta-analysis | We searched PUBMED, EMBASE and the Cochrane Database until April 2012 using medical subject headings related to epicondylitis when possible. | 16 studies met inclusion criteria. Analyses were conducted for overall pain relief, pain relief during maximum handgrip strength tests, and maximum handgrip strength. There were not enough studies to conduct an analysis of physical function or other outcome variables. | Differences between treatment and control groups were larger than differences between treatments. Control group gains were 50–66% as high as treatment group gains. | Low |
| Boudreault et al., (Boudreault et al., 1017) | The Efficacy of Laser Therapy for Rotator Cuff Tendinopathy: A Systematic Review and Meta-Analysis | A literature search was conducted in Pubmed, CINAHL, Embase and PEDro databases for randomized controlled trials (RCTs) published until May 2014, comparing the efficacy of LT to any other intervention. | Thirteen RCTs were included. It was concluded that LT may provide short-term pain relief of minimally significant clinical importance compared to placebo, ultrasound therapy, or clinical recommendations alone. | Low to moderate grade evidence supports that LT may reduce pain in the short term in adults with RC tendinopathy, while its effects on function and ROM are not supported. | Moderate |
| Desmeules et al. (Desmeules et al., 2016a) | Efficacy of exercise therapy in workers with rotator cuff tendinopathy: a systematic review | A literature search in four bibliographical databases (Pubmed, CINAHL, EMBASE, and PEDro) was conducted from inception up to February 2015. | Ten studies were included. Three RCTs of moderate methodological quality concluded that exercises were superior to a placebo or no intervention in terms of function and return-to-work outcomes. | There is low to moderate-grade evidence that therapeutic exercises provided in a clinical setting are an effective modality to treat workers suffering from RC | Low |

(*continued on next page*)

**Table 3** (*continued*)

| Studies | Title | Search strategy | Results | Conclusion | OC |
|---|---|---|---|---|---|
| | | | | tendinopathy and to promote return-to-work. | |
| Desmeules et al. (Desmeules et al., 2016b) | Efficacy of transcutaneous electrical nerve stimulation for rotator cuff tendinopathy: a systematic review | A literature search was conducted in four databases (CINAHL, Embase, Pubmed and PeDRO) for randomized controlled trials published from date of inception until April 2015, comparing the efficacy of TENS for the treatment of rotator cuff tendinopathy with placebo or any other intervention. | Six studies were included in this review. One placebo-controlled trial reported that a single TENS session provided immediate pain reduction for patients with rotator cuff tendinopathy, but did not follow the participants in the short, medium or long term. | Due to the limited number of studies and the overall high risk of bias of the studies included in this review, no conclusions can be drawn on the efficacy of TENS for the treatment of rotator cuff tendinopathy. | Moderate |
| Ortega-Castillo et al. (Ortega-Castillo & Medina-Porqueres, 2016) | Effectiveness of the eccentric exercise therapy in physically active adults with symptomatic shoulder impingement or lateral epicondylar tendinopathy: A systematic review | Relevant randomized controlled trials (RCTs) were sourced using MEDLINE, SPORT Discus, Physiotherapy Evidence Database (PEDro) and CINAHL databases. | In 11 studies, pain decreased significantly with eccentric exercise, but only in five studies, the reduction was significantly better than in the non-eccentric group (in all or some of the parameters). | Eccentric exercise may reduce pain and improve strength in upper limb tendinopathies, but whether its effectiveness is much better than other forms of treatment remains questionable. | Moderate |
| McKivigan et al. (McKivigan et al., 2017) | A Systematic Review on the Efficacy of Iontophoresis as a Treatment for Lateral Epicondylitis | The following databases were searched for relevant articles: PubMed, Cochrane Library, PEDro, SPORT Discus, Google Scholar, and the APTA library. | Fourteen relevant studies were identified. Twelve were experimental in vivo studies, two were review studies. All studies were published 2002 through 2015, providing a robust overview of treatments over the last 15 years. | Based on this review, it is not conclusive that iontophoresis be recommended as a treatment approach for the management of epicondylitis, however iontophoresis should not be ruled out in treating epicondylitis as it is a dose-response modality. | Low |
| Punnoose et al. (Punnoose et al., 2017) | Extracorporeal Shock Wave Therapy for Achilles and Patellar Tendinopathy: Meta-Analysis and a Systematic Review of the Literature | A systematic review and meta-analysis was conducted using MEDLINE, EMBASE, AMED, CINAHL, PEDro and Cochrane databases and bibliographic searches from inception until April 2013 to identify randomized control trials comparing ESWT with other treatment methods. | Nine studies met the inclusion criteria. Meta-analysis showed no significant differences between the intervention and control group on the pooled VISA-A scores ($p = 0.59$, 95% CI-15.02, 26.51) or load-induced pain ($p = 0.51$, 95% CI-3.15, 1.56) for Achilles tendinopathy and no significant differences between the pooled VISA-P scores for patellar tendinopathy ($p = 0.27$, CI-7.86, 27.87). | The meta-analysis did not demonstrate a statistically significant improvement in symptoms or load induced pain for ESWT compared to other treatments or control. Adequately powered, high quality studies with longer follow-ups are required. | Low |
| Liao et al. (Liao et al., 2018a) | Efficacy of Extracorporeal Shock Wave Therapy for Lower Extremity Tendinopathy: A Meta-analysis of Randomized Controlled Trials | The articles were obtained from the following online databases and search engines: Medline, PubMed, the Excerpta Medica dataBASE, the Cochrane Library Database, the Physiotherapy Evidence Database (PEDro), the China Knowledge Resource Integrated Database, and Google Scholar. | 29 RCTs were included. General ESWT showed significant effects at the immediate follow-up [pain score: standardized mean difference (SMD) = −1.41, 95% confidence interval (CI) = −2.01 to −0.82, $P < 0.00001$; function: SMD − 2.59, 95% CI − 1.54–3.64, $P < 0.00001$] as well as at 3, 6, and ≥12 months. | ESWT exerted a positive overall effect on pain and function for LE tendinopathy. Shock wave types and dosage levels may have different contributions to treatment efficacy. | Moderate |
| Liao et al. (Liao et al., 2018b) | Efficacy of extracorporeal shock wave therapy for knee tendinopathies and other soft tissue disorders: a meta-analysis of randomized controlled trials | A comprehensive search of online databases and search engines was performed up to June 2018. Primary sources were MEDLINE, PubMed, the Excerpta Medica dataBASE, the Cochrane Library, the Physiotherapy Evidence Database (PEDro), the China Knowledge Resource Integrated Database, and Google Scholar. | 19 RCTs were included. ESWT had overall significant effects on the TSR (OR: 3. 36, 95% CI: 1.84–6.12, $P < 0.0001$), pain reduction (SMD: − 1.49, 95% CI: − 2.11 to − 0.87, $P < 0.00001$), and ROM restoration (SMD: 1.76, 95% CI: 1.43–2.09, $P < 0.00001$). | The ESWT exerts an overall effect on the TSR, pain reduction, and ROM restoration in patients with KSTDs. Shock-wave types and application levels have different contributions to treatment efficacy for KSTDs, which must be investigated further for optimizing these treatments in clinical practice. | Moderate |
| Lim et al. (Lim & Wong, 2018) | Effects of isometric, eccentric, or heavy slow resistance exercises on pain and function in individuals with patellar tendinopathy: A systematic review | Academic journals from CINAHL, Embase, MEDLINE, Scopus, SPORTDiscus™, and The Cochrane Library were searched from inception to August 2017. | Fifteen studies (3 isometric, 2 HSR, and 10 eccentric) were included for this review. Nine studies were randomized controlled trials, which provided good Level II evidence; four studies were of satisfactory Level III evidence; and two studies were case series (Level IV evidence). | Findings from isometric exercises can be trusted to guide clinical practice (Grade A), whereas eccentric exercises can be trusted to guide clinical practice in most clinical situations (Grade B). It is recommended that HSR exercises should be applied carefully to individual clinical circumstances (Grade C) and interpreted with care. | Moderate |
| Lucado et al. (Lucado et al., 2018) | Do joint mobilizations assist in the recovery of lateral elbow tendinopathy? A systematic review and meta-analysis | A comprehensive English language literature search was performed using the following computerized databases: CINAHL, | A total of 20 studies met the inclusion criteria; 7 were included in the meta-analysis. Pooled data across all time | There is compelling evidence that joint mobilizations directed at the elbow improve both pain and functional grip scores across all | Low |

**Table 3** (*continued*)

| Studies | Title | Search strategy | Results | Conclusion | OC |
|---|---|---|---|---|---|
| | | PubMed, and PEDro up to June 2017. | periods demonstrated a mean effect size of 0.43 (95% confidence interval [CI]: 0.15–0.71) for MWM on improving pain rating, and 0.31 (95% CI: 0.11–0.51) for MWM on improving grip strength, 0.47 (95% CI: 0.11–0.82) for Mill's manipulation on improving pain rating | time frames compared to control groups in the management of LET. | |
| Wilson et al. (Wilson et al., 2018) | Exercise, orthoses and splinting for treating Achilles tendinopathy: a systematic review with meta-analysis | Medline, CINAHL, Embase, AMED, WHO-ICTRP, Web of Science, PEDro and Cochrane Library from inception to October 2017. | Twenty-two studies were included. Moderate level evidence favoured eccentric exercise over control for improving pain and function in mid-portion tendinopathy. Moderate level evidence favoured eccentric exercise over concentric exercise for reducing pain. There was moderate level evidence of no significant difference in pain or function between eccentric exercise and heavy slow resistance exercise. | We conditionally recommend exercise for improving pain and function in mid-portion Achilles tendinopathy. The balance of evidence did not support recommendation of one type of exercise programme over another. | Moderate |
| Hickey et al. (Hickey et al., 2019) | The Long-Term Effects of Eccentric Exercise Vs. Extracorporeal Shockwave Therapy in Athletes Aged 18-50 with Lower Extremity Tendinopathy: A Meta-Analysis and Systematic Review | Search procedures followed PRISA guidelines using the PubMed, CINAHL, and Cochrane Library databases. | A fixed effects model was used to compare the interventions. Eccentric Exercise was found to have very large effect sizes of 2.363 (1.075, 3.651) and 18.790 (8.604, 28.977) for improving pain and function respectively when compared with extracorporeal shockwave therapy. | Eccentric exercise is the treatment of choice for athletes with patellar tendinopathy, while extracorporeal shockwave therapy is a viable secondary option for patients that fail to respond to eccentrics alone. | Low |
| Murphy et al. (Murphy et al., 2019) | Efficacy of heavy eccentric calf training for treating mid-portion Achilles tendinopathy: a systematic review and meta-analysis | PUBMED, CINAHL (Ovid) and CINAHL (EBSCO) were searched from inception until September 24, 2018. | Seven studies met the inclusion criteria. This review suggests HECT may be superior to both natural history, mean difference (MD) (95% CI) of 20.6 (11.7–29.5, one study) and traditional physiotherapy, MD (95% CI) of 17.70 (3.75–31.66, two studies). | Current evidence suggests that HECT may be superior to natural history and traditional physiotherapy while HECT may be inferior to other exercise interventions. | High |
| Yan et al. (Yan et al., 2019) | A comparative study of the efficacy of ultrasonics and extracorporeal shock wave in the treatment of tennis elbow: a metaanalysis of randomized controlled trials | RCTs published in the PubMed, Embase, Cochrane Library, and SpringerLink databases comparing ESWT and US in treating tennis elbow were identified by a software and manual search. | Five RCTs comprising five patients were included in the present meta-analysis. The results revealed a significantly lower VAS score of pain in the ESWT group (1 month: MD = 4.47, $p = 0.0001$; 3months: MD = 20.32, $p < 0.00001$; and 6 months: MD = 4.32, $p < 0.0001$) compared to US. | Although there was no significant difference in the elbow function evaluation scores between ESWT and US, the superiority of the ESWT group in the VAS of pain (both at 1 month, 3months, and 6 months follow-ups) raised grip strength in ESWT group and the scores for subjective evaluation of efficacy indicated that ESWT offers more effective therapy for lateral epicondylitis than US therapy. | Low |

statistically-significant reduction of pain and improvement of function (Liao et al., 2018a, 2018b) at all follow-up periods (<1 month to >12 months). The clinical significance of these results, nonetheless, was undetermined, since the reported values were not absolute values (standardized mean differences). The beneficial effects of ESWT for calcific tendinitis was supported by two studies (Bannuru et al., 2014; Lee et al., 2011). Shockwave type and application parameters may contribute differently to treatment efficacy (Al-Abbad & Simon, 2013; Liao et al., 2018a, 2018b; Verstraelen, Jansen, & Morrenhof, 2014). The results of the current review are consistent with a systematic review by Burcal et al. (Burcal et al., 2019) regarding the efficacy of ESWT for pain reduction. The efficacy of ESWT in treating tendinopathy has been also reported by other studies (Gerdesmeyer et al., 2015; Ko & Wang, 2018, pp. 27–41), but the optimal parameters of treatment were not identified (Ko & Wang, 2018, pp. 27–41). ESWT was shown to reduce the expression of MMP-1, MMP-13 and IL-6 *in vitro* (Han et al., 2009), but an elevation of IL-6, IL-8, MMP-2, and MMP-9 concentrations were reported immediately after ESWT in vivo (Waugh et al., 2015).

Low-level laser therapy (LLLT) revealed a pooled reduction in pain of 1.53 cm on VAS scale (Tumilty et al., 2010; Haslerud et al., 2015; Boudreault et al., 1017) and an improvement in function; grip strength of 9.59 kg (Tumilty et al., 2010). The reported reduction in pain may be clinically significant, since the MCID has been determined to be a reduction of 1.4 cm in patients with RT (Tashjian et al., 2009). Other studies, however, have stated that the MCID for pain may range between 13 mm and 28 mm, depending on the severity of the condition (Bird & Dickson, 2001; Todd, 2001). The stated improvement in grip strength may also be clinically significant, as the MCID in grip strength was estimated to be 6–6.5 kg (Kim, Park, & Shin, 2014; Nitschke, McMeeken, Burry, & Matyas, 1999). It must be highlighted that these beneficial effects were

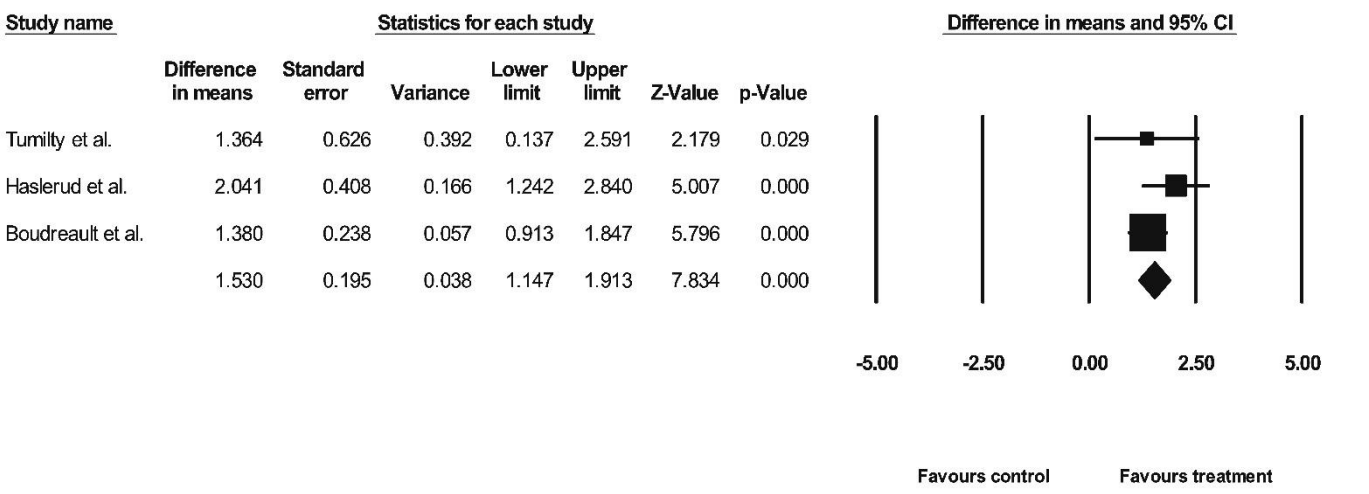



**Fig. 2.** Forest plot of studies assessing the efficacy of LLLT intervention, either alone versus placebo or combined with exercise versus exercise plus placebo, in pain reduction measured on the VAS scale. The follow-up period for LLLT intervention ranged between (2–12 weeks).

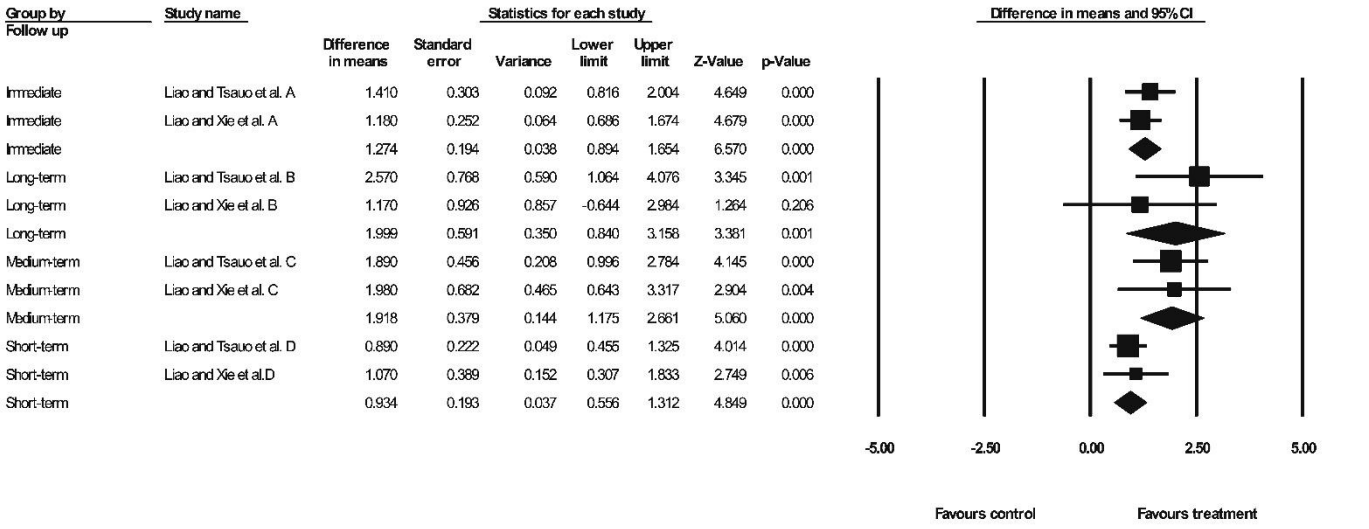



**Fig. 3.** Forest plot of studies evaluating the efficacy of ESWT intervention, either alone or combined with other interventions versus placebo or other interventions, in pain reduction at different follow-up durations (Immediate: ≤1 month; Short-term: > 1 month and ≤3 months; Medium-term > 3 months and ≤6 months; Long-term: ≥ 12 months).

observed on the short-term (2–12 weeks), but the long-term effects are yet to be determined. The recommended doses must be applied to achieve these positive results (Tumilty et al., 2010; Haslerud et al., 2015; Boudreault et al., 1017). Moreover, LLLT was shown to be more effective than US in reducing pain (Desmeules et al., 2015). The results of the current review regarding LLLT are consistent with the guidelines (Carcia et al., 2010). An umbrella review (Mamais, Papadopoulos, Lamnisos, & Stasinopoulos, 2018), nevertheless, found poor results for the efficacy of LLLT in the management of LET. Mechanisms of action of LLLT were proposed in several reviews in literature (Bjordal, Lopes-Martins, & Frigo, 2015; Cotler, Chow, Hamblin, & Carroll, 2015; Posten et al., 2005). LLLT at low-doses was shown to enhance fibroblast proliferation (Lubart, Wollman, Friedmann, Rochkind, & Laulicht, 1992), collagen synthesis, and tensile strength (Woodruff et al., 2004).

Low to moderate level evidence showed that ultrasound therapy did not provide benefit compared to placebo, LLLT, or combined to exercise (Desmeules et al., 2015). Current evidence was insufficient to determine the efficacy of kinesiology taping or non-elastic taping used alone or in conjunction with other interventions (Desjardins-

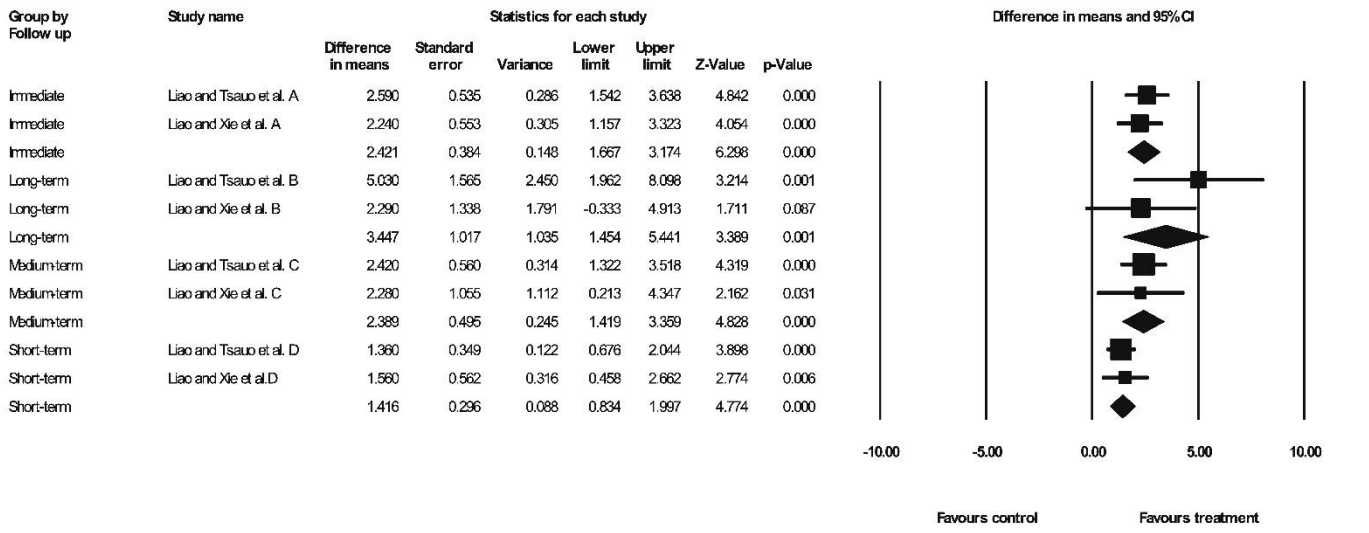



**Fig. 4.** Forest plot of studies evaluating the efficacy of ESWT intervention, either alone or combined with other interventions versus placebo or other interventions, in function improvement at different follow-up durations (Immediate: ≤1 month; Short-term: > 1 month and ≤3 months; Medium-term > 3 months and ≤6 months; Long-term: ≥ 12 months).

Charbonneau et al., 2015) (moderate-quality evidence). The results for manual therapy, orthotic therapy, transcutaneous electric nerve stimulation, and iontophoresis were either inconclusive or contradictory. Clar et al. (Clar, Tsertsvadze, Hundt, Clarke, & Sutcliffe, 2014), similarly, reported limited evidence for the efficacy of manual therapy; particularly mobilization/manipulation with exercise in the treatment of Rotator cuff disorders.

Further research is required as optimal protocols of interventions and the superior intervention remain unknown. Using standardized treatment parameters, outcome measures, and follow-up durations are advised to improve homogeneity and facilitate statistical treatment of results.

### 4.1. Limitations

- The main limitation in the present review and most of the included studies is that the follow-up assessment durations were too short to determine long-term effects of the identified interventions.
- Low methodological quality, within and across studies, may reduce confidence in results. Twenty-three of the identified reviews had a low or critically low rating of overall confidence. Therefore, only studies scoring 9 points or higher on the AMSTAR 2 scale were further analyzed using the GRADE principles in attempt to reach accurate results.
- Statistical pooling of results was not feasible for many of the interventions, due to heterogeneity within and across results regarding outcome measures, treatment protocols and follow-up durations. To reach meaningful results, only meta-analyses with moderate rating of overall confidence or higher and adequate homogeneity were included in the meta-analysis of the present review.
- Despite the reported histopathological similarities (Abate et al., 2009; Järvinen et al., 1997; Maffulli et al., 2004), caution is advised when interpreting results due to possible pathological differences in tendinopathy at each region.
- Some of the identified reviews included the same studies. However, it is unlikely that this has influenced the results since the determining criteria of the overall quality of evidence necessitate at least one systematic review of corresponding quality (Group, 2004).

## Ethical approval

None declared.

## Funding source

This work was supported by an FCT grant - Foundation for Science and Technology (Fundação para Ciência e Tecnologia) - Portugal, with reference number SFRH/BD/144090/2019. The funding source had no involvement in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

## Declaration of competing interest

None declared.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ptsp.2020.08.002.

## References

Abate, M., Silbernagel, K. G., Siljeholm, C., Di Iorio, A., De Amicis, D., Salini, V., et al. (2009). Pathogenesis of tendinopathies: inflammation or degeneration? *Arthritis research & therapy, 11*(3), 1–15.

Ackermann, P. W., & Renström, P. (2012). Tendinopathy in sport. *Sports Health, 4*(3), 193–201. https://doi.org/10.1177/1941738112440957

Al-Abbad, H., & Simon, J. V. (2013). The effectiveness of extracorporeal shock wave therapy on chronic achilles tendinopathy: a systematic review. *Foot & ankle international, 34*(1), 33–41. https://doi.org/10.1177/1071100712464354

Andres, B. M., & Murrell, G. A. (2008). Treatment of tendinopathy: what works,

what does not, and what is on the horizon. *Clinical orthopaedics and related research, 466*(7), 1539–1554. https://doi.org/10.1007/s11999-008-0260-1
Bannuru, R. R., Flavin, N. E., Vaysbrot, E., Harvey, W., & McAlindon, T. (2014). High-energy extracorporeal shock-wave therapy for treating chronic calcific tendinitis of the shoulder: A systematic review. *Annals of Internal Medicine, 160*(8), 542–549. https://doi.org/10.7326/M13-1982
Bird, S. B., & Dickson, E. W. (2001). Clinically significant changes in pain along the visual analog scale. *Annals of Emergency Medicine, 38*(6), 639–643. https://doi.org/10.1067/mem.2001.118012
Bjordal, J. M., Lopes-Martins, R. A. B., & Frigo, L. (2015). Low level laser therapy–mechanism of action: Inflammatory Process. In *Lasers in dentistry: Guide for clinical practice* (pp. 27–33).
Blanchette, M.-A., & Normand, M. C. (2011). Impairment assessment of lateral epicondylitis through electromyography and dynamometry. *Journal of the Canadian Chiropractic Association, 55*(2), 96–106. PMC3095584.
Boudreault J, Desmeules F, Roy J, Dionne C, Frémont P. The efficacy of laser therapy for rotator cuff tendinopathy: A systematic review and meta-analysis. J Nov Physiother Phys Rehabil 3 (1): 001-015 DOI: 1017352/2455. 2016;5487(001). https://doi.org/7352/2455-5487.000029.
Burcal, C. J., Rosen, A. B., Taylor, T., & Nicola, M. (2019). Evidence to practice review: Best practices in patellar tendinopathy management. *Clinical Practice in Athletic Training, 2*(1), 4–10. https://doi.org/10.31622/2019/0001.2
Cacchio, A., De Paulis, F., & Maffulli, N. (2014). Development and validation of a new visa questionnaire (VISA-H) for patients with proximal hamstring tendinopathy. *British Journal of Sports Medicine, 48*(6), 448–452. https://doi.org/10.1136/bjsports-2012-091552
Carcia, C. R., Martin, R. L., Houck, J., Wukich, D. K., Altman, R. D., Curwin, S., et al. (2010). Achilles pain, stiffness, and muscle power deficits: Achilles tendinitis: Clinical practice guidelines linked to the international classification of functioning, disability, and health from the orthopaedic section of the American physical therapy association. *Journal of Orthopaedic & Sports Physical Therapy, 40*(9), A1–A26. https://doi.org/10.2519/jospt.2010.0305
Cardoso, T. B., Pizzari, T., Kinsella, R., Hope, D., & Cook, J. L. (2019). Current trends in tendinopathy management. *Best Practice & Research Clinical Rheumatology*. https://doi.org/10.1016/j.berh.2019.02.001
Carlsson, A. M. (1983). Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. *Pain, 16*(1), 87–101. https://doi.org/10.1016/0304-3959(83)90088-X
Chang, W. D., Wu, J. H., Yang, W. J., & Jiang, J. A. (2010). Therapeutic effects of low-level laser on lateral epicondylitis from differential interventions of Chinese-western medicine: Systematic review. *Photomed Laser Surg, 28*(3), 327–336. https://doi.org/10.1089/pho.2009.2558
Clar, C., Tsertsvadze, A., Hundt, G. L., Clarke, A., & Sutcliffe, P. (2014). Clinical effectiveness of manual therapy for the management of musculoskeletal and non-musculoskeletal conditions: Systematic review and update of UK evidence report. *Chiropractic & Manual Therapies, 22*(1), 12. https://doi.org/10.1186/2045-709X-22-12
Cotler, H. B., Chow, R. T., Hamblin, M. R., & Carroll, J. (2015). The use of low level laser therapy (LLLT) for musculoskeletal pain. *MOJ Orthopedics & Rheumatology, 2*(5). https://doi.org/10.15406/mojor.2015.02.00068
Couppé, C., Svensson, R. B., Silbernagel, K. G., Langberg, H., & Magnusson, S. P. (2015). Eccentric or concentric exercises for the treatment of tendinopathies? *Journal of Orthopaedic & Sports Physical Therapy, 45*(11), 853–863. https://doi.org/10.2519/jospt.2015.5910
Cullinane, F. L., Boocock, M. G., & Trevelyan, F. C. (2014). Is eccentric exercise an effective treatment for lateral epicondylitis? A systematic review. *Clinical Rehabilitation, 28*(1), 3–19. https://doi.org/10.1177/0269215513491974
Dauber, J. A., Ball, M. O., Lewis, A. M., & Neidermeyer, C. R. (2018). Victorian Institute of sport assessment questionnaire, patellar tendon (VISA-P). *Critical Reviews in Physical and Rehabilitation Medicine, 30*(3).
Dean, B. J. F., Gettings, P., Dakin, S. G., & Carr, A. J. (2016). Are inflammatory cells increased in painful human tendinopathy? A systematic review. *British Journal of Sports Medicine, 50*(4), 216–220. https://doi.org/10.1136/bjsports-2015-094754
Desjardins Charbonneau, A., Roy, J.-S., Dionne, C. E., Frémont, P., MacDermid, J. C., & Desmeules, F. (2015). The efficacy of manual therapy for rotator cuff tendinopathy: A systematic review and meta-analysis. *Journal of Orthopaedic & Sports Physical Therapy, 45*(5), 330–350. https://doi.org/10.2519/jospt.2015.5455
Desjardins Charbonneau, A., Roy, J. S., Dionne, C. E., & Desmeules, F. (2015). The efficacy of taping for rotator cuff tendinopathy: A systematic review and meta-analysis. *International Journal of Sports Physical Therapy, 10*(4), 420.
Desmeules, F., Boudreault, J., Dionne, C. E., Frémont, P., Lowry, V., MacDermid, J. C., et al. (2016). Efficacy of exercise therapy in workers with rotator cuff tendinopathy: A systematic review. *Journal of Occupational Health*. https://doi.org/10.1539/joh.15-0103-RA, 15-0103-RA.
Desmeules, F., Boudreault, J., Roy, J.-S., Dionne, C., Frémont, P., & MacDermid, J. C. (2015). The efficacy of therapeutic ultrasound for rotator cuff tendinopathy: A systematic review and meta-analysis. *Physical Therapy in Sport, 16*(3), 276–284. https://doi.org/10.1016/j.ptsp.2014.09.004
Desmeules, F., Boudreault, J., Roy, J. S., Dionne, C. E., Frémont, P., & MacDermid, J. C. (2016). Efficacy of transcutaneous electrical nerve stimulation for rotator cuff tendinopathy: A systematic review. *Physiotherapy, 102*(1), 41–49. https://doi.org/10.1016/j.physio.2015.06.004
Dilger, C. P., & Chimenti, R. L. (2019). Nonsurgical treatment options for insertional achilles tendinopathy. *Foot and Ankle Clinics, 24*(3), 505–513. https://doi.org/10.1016/j.fcl.2019.04.004
Dimitrios, S., Konstantinos, P., & Antonis, K. (2013). Effectiveness of iontophoresis for lateral elbow tendinopathy. *Journal of Novel Physiotherapies S, 2*, 2. https://doi.org/10.4172/2165-7025.S2-005
Dimitrios, S., & Malliaras, P. (2016). It is time to abandon the myth that eccentric training is best practice. *Biology of Exercise, 12*(1), 15–21. https://doi.org/10.4127/jbe.2016.0097
Dingemanse, R., Randsdorp, M., Koes, B. W., & Huisstede, B. M. (2014). Evidence for the effectiveness of electrophysical modalities for treatment of medial and lateral epicondylitis: A systematic review. *British Journal of Sports Medicine, 48*(12), 957–965. https://doi.org/10.1136/bjsports-2012-091513
Dixon, J., & Bird, H. (1981). Reproducibility along a 10 cm vertical visual analogue scale. *Annals of the Rheumatic Diseases, 40*(1), 87–89. https://doi.org/10.1136/ard.40.1.87
Eubank, B. H., Mohtadi, N. G., Lafave, M. R., Wiley, J. P., & Emery, J. H. (2017). Further validation and reliability testing of the rotator cuff quality of life index (RC-QOL) according to the consensus-based standards for the selection of health measurement instruments (COSMIN) guidelines. *Journal of Shoulder and Elbow Surgery, 26*(2), 314–322. https://doi.org/10.1016/j.jse.2016.07.030
Evans, N. A., Adkins, S., Clarke, K., & Cox, W. C. (2018). Functional impairment test-hand and neck/shoulder/arm. *Critical Reviews in Physical and Rehabilitation Medicine, 30*(3). https://doi.org/10.1615/CritRevPhysRehabilMed.2018028936
Evans, J. P., Porter, I., Gangannagaripalli, J. B., Bramwell, C., Davey, A., Smith, C. D., et al. (2019). Assessing patient-centred outcomes in lateral elbow tendinopathy: A systematic review and standardised comparison of English language clinical rating systems. *Sports Medicine-Open, 5*(1), 10. https://doi.org/10.1186/s40798-019-0183-2
Fearon, A., Ganderton, C., Scarvell, J. M., Smith, P. N., Neeman, T., Nash, C., et al. (2015). Development and validation of a VISA tendinopathy questionnaire for greater trochanteric pain syndrome, the VISA-G. *Manual Therapy, 20*(6), 805–813. https://doi.org/10.1016/j.math.2015.03.009
Flodgren, G., Eccles, M. P., Shepperd, S., Scott, A., Parmelli, E., & Beyer, F. R. (2011). An overview of reviews evaluating the effectiveness of financial incentives in changing healthcare professional behaviours and patient outcomes. *Cochrane Database of Systematic Reviews*, (7)https://doi.org/10.1002/14651858.CD009255
Gerdesmeyer, L., Mittermayr, R., Fuerst, M., Al Muderis, M., Thiele, R., Saxena, A., et al. (2015). Current evidence of extracorporeal shock wave therapy in chronic Achilles tendinopathy. *International Journal of Surgery, 24*, 154–159. https://doi.org/10.1016/j.ijsu.2015.07.718
Group, G. W. (2004). Grading quality of evidence and strength of recommendations. *BMJ British Medical Journal, 328*(7454), 1490. https://doi.org/10.1136/bmj.328.7454.1490
Gummesson, C., Ward, M. M., & Atroshi, I. (2006). The shortened disabilities of the arm, shoulder and hand questionnaire (quick DASH): Validity and reliability based on responses within the full-length DASH. *BMC Musculoskeletal Disorders, 7*(1), 44. https://doi.org/10.1186/1471-2474-7-44
Hamilton, A., Balnave, R., & Adams, R. (1994). Grip strength testing reliability. *Journal of Hand Therapy, 7*(3), 163–170. https://doi.org/10.1016/S0894-1130(12)80058-5
Han, S. H., Lee, J. W., Guyton, G. P., Parks, B. G., Courneya, J.-P., & Schon, L. C. (2009). J. Leonard goldner award 2008: Effect of extracorporeal shock wave therapy on cultured tenocytes. *Foot & Ankle International, 30*(2), 93–98. https://doi.org/10.3113/FAI-2009-0093
Haslerud, S., Magnussen, L. H., Joensen, J., Lopes-Martins, R. A., & Bjordal, J. M. (2015). The efficacy of low-level laser therapy for shoulder tendinopathy: A systematic review and meta-analysis of randomized controlled trials. *Physiotherapy Research International, 20*(2), 108–125. https://doi.org/10.1002/pri.1606
Hawker, G. A., Mian, S., Kendzerska, T., & French, M. (2011). Measures of adult pain: Visual analog scale for pain (vas pain), numeric rating scale for pain (nrs pain), mcgill pain questionnaire (mpq), short-form mcgill pain questionnaire (sf-mpq), chronic pain grade scale (cpgs), short form-36 bodily pain scale (sf-36 bps), and measure of intermittent and constant osteoarthritis pain (icoap). *Arthritis Care & Research, 63*(S11), S240–S252. https://doi.org/10.1002/acr.20543
Hickey, C., Walker, D., Lee, S., & Vitato, N. (2019). The long-term effects of eccentric exercise vs. Extracorporeal shockwave therapy in athletes aged 18-50 with patellar tendinopathy: A meta-analysis and systematic review. *Ann Physiother Occup Ther, 2*(3), Article 000130.
Higgins, J. P., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *BMJ British Medical Journal, 327*(7414), 557. https://doi.org/10.1136/bmj.327.7414.557
Hoogvliet, P., Randsdorp, M. S., Dingemanse, R., Koes, B. W., & Huisstede, B. M. (2013). Does effectiveness of exercise therapy and mobilisation techniques offer guidance for the treatment of lateral and medial epicondylitis? A systematic review. *British Journal of Sports Medicine*. https://doi.org/10.1136/bjsports-2012-091990. bjsports-2012-091990.
Huisstede, B. M., Gebremariam, L., van der Sande, R., Hay, E. M., & Koes, B. W. (2011). Evidence for effectiveness of Extracorporal Shock-Wave Therapy (ESWT) to treat calcific and non-calcific rotator cuff tendinosis–a systematic review. *Manual Therapy, 16*(5), 419–433. https://doi.org/10.1016/j.math.2011.02.005
Hutchison, A.-M., Beard, D., Pallister, I., Topliss, C. J., & Williams, P. (2011). Is physiotherapy effective for patients with a chronic mid-body achilles tendinopathy? A systematic review of non-surgical and non-pharmacological interventions. *International Musculoskeletal Medicine, 33*(4), 152–160. https://doi.org/10.1179/1753615411Y.0000000010

Innes, E. (1999). Handgrip strength testing: A review of the literature. *Australian Occupational Therapy Journal, 46*(3), 120–140. https://doi.org/10.1046/j.1440-1630.1999.00182.x

Ioppolo, F., Tattoli, M., Di Sante, L., Venditto, T., Tognolo, L., Delicata, M., et al. (2013). Clinical improvement and resorption of calcifications in calcific tendinitis of the shoulder after shock wave therapy at 6 months' follow-up: A systematic review and meta-analysis. *Archives of Physical Medicine and Rehabilitation, 94*(9), 1699–1706. https://doi.org/10.1016/j.apmr.2013.01.030

Iversen, J. V., Bartels, E. M., & Langberg, H. (2012). The victorian Institute of sports assessment–achilles questionnaire (VISA-A)–a reliable tool for measuring achilles tendinopathy. *International Journal of Sports Physical Therapy, 7*(1), 76.

Järvinen, M., Jozsa, L., Kannus, P., Järvinen, T., Kvist, M., & Leadbetter, W. (1997). Histopathological findings in chronic tendon disorders. *Scandinavian Journal of Medicine & Science in Sports, 7*(2), 86–95.

Jayaseelan, D. J., Mischke, J. J., & Strazzulla, R. L. (2019). Eccentric exercise for achilles tendinopathy: A narrative review and clinical decision-making considerations. *Journal of Functional Morphology and Kinesiology, 4*(2), 34. https://doi.org/10.3390/jfmk4020034

Jester, A., Harth, A., Wind, G., Germann, G., & Sauerbier, M. (2005). Disabilities of the arm, shoulder and hand (DASH) questionnaire: Determining functional activity profiles in patients with upper extremity disorders. *The Journal of Hand Surgery, 30*(1), 23–28. https://doi.org/10.1016/J.JHSB.2004.08.008

Johnson, C. (2005). Measuring pain. Visual analog scale versus numeric pain scale: What is the difference? *Journal of Chiropractic Medicine, 4*(1), 43. https://doi.org/10.1016/S0899-3467(07)60112-8

Joseph, M. F., Taft, K., Moskwa, M., & Denegar, C. R. (2012). Deep friction massage to treat tendinopathy: A systematic review of a classic treatment in the face of a new paradigm of understanding. *Journal of Sport Rehabilitation, 21*(4), 343–353.

Kim, J. K., Park, M. G., & Shin, S. J. (2014). What is the minimum clinically important difference in grip strength? *Clinical Orthopaedics and Related Research, 472*(8), 2536–2541. https://doi.org/10.1007/s11999-014-3727-2

Ko, J.-Y., & Wang, F.-S. (2018). *Extracorporeal shockwave therapy for tendinopathy. Shockwave Medicine*. Karger Publishers.

Krey, D., Borchers, J., & McCamey, K. (2015). Tendon needling for treatment of tendinopathy: A systematic review. *The Physician and Sportsmedicine, 43*(1), 80–86. https://doi.org/10.1080/00913847.2015.1004296

Lee, S. Y., Cheng, B., & Grimmer-Somers, K. (2011). The midterm effectiveness of extracorporeal shockwave therapy in the management of chronic calcific shoulder tendinitis. *Journal of Shoulder and Elbow Surgery, 20*(5), 845–854. https://doi.org/10.1016/j.jse.2010.10.024

Liao, C.-D., Tsauo, J.-Y., Chen, H.-C., & Liou, T.-H. (2018). Efficacy of extracorporeal shock wave therapy for lower-limb tendinopathy: A meta-analysis of randomized controlled trials. *American Journal of Physical Medicine and Rehabilitation, 97*(9), 605–619. https://doi.org/10.1097/PHM.0000000000000925

Liao, C.-D., Xie, G.-M., Tsauo, J.-Y., Chen, H.-C., & Liou, T.-H. (2018). Efficacy of extracorporeal shock wave therapy for knee tendinopathies and other soft tissue disorders: A meta-analysis of randomized controlled trials. *BMC Musculoskeletal Disorders, 19*(1), 278. https://doi.org/10.1186/s12891-018-2204-6

Lim, E. C. W. (2013). Pain free grip strength test. *Journal of Physiotherapy, 59*(1), 59. https://doi.org/10.1016/S1836-9553(13)70152-8

Lim, H. Y., & Wong, S. H. (2018). Effects of isometric, eccentric, or heavy slow resistance exercises on pain and function in individuals with patellar tendinopathy: A systematic review. *Physiotherapy Research International, 23*(4), Article e1721. https://doi.org/10.1002/pri.1721

Littlewood, C., Ashton, J., Chance-Larsen, K., May, S., & Sturrock, B. (2012). Exercise for rotator cuff tendinopathy: A systematic review. *Physiotherapy, 98*(2), 101–109. https://doi.org/10.1016/j.physio.2011.08.002

Littlewood, C., Malliaras, P., & Chance-Larsen, K. (2015). Therapeutic exercise for rotator cuff tendinopathy: A systematic review of contextual factors and prescription parameters. *International Journal of Rehabilitation Research, 38*(2), 95–106. https://doi.org/10.1097/MRR.0000000000000113

Littlewood, C., May, S., & Walters, S. (2013). A review of systematic reviews of the effectiveness of conservative interventions for rotator cuff tendinopathy. *Shoulder & Elbow, 5*(3), 151–167. https://doi.org/10.1111/sae.12009

Loew, L. M., Brosseau, L., Tugwell, P., Wells, G. A., Welch, V., Shea, B., et al. (2014). Deep transverse friction massage for treating lateral elbow or lateral knee tendinitis. *Cochrane Database of Systematic Reviews, 11*. https://doi.org/10.1002/14651858.CD003528.pub2. CD003528.

Lubart, R., Wollman, Y., Friedmann, H., Rochkind, S., & Laulicht, I. (1992). Effects of visible and near-infrared lasers on cell cultures. *Journal of Photochemistry and Photobiology B: Biology, 12*(3), 305–310. https://doi.org/10.1016/1011-1344(92)85032-

Lucado, A. M., Dale, R. B., Vincent, J., & Day, J. M. (2018). Do joint mobilizations assist in the recovery of lateral elbow tendinopathy? A systematic review and meta-analysis. *Journal of Hand Therapy*. https://doi.org/10.1016/j.jht.2018.01.010

Lunsford, B. R., & Perry, J. (1995). The standing heel-rise test for ankle plantar flexion: Criterion for normal. *Physical Therapy, 75*(8), 694–698. https://doi.org/10.1093/ptj/75.8.694

MacDermid, J. (2005). Update: The patient-rated forearm evaluation questionnaire is now the patient-rated tennis elbow evaluation. *Journal of Hand Therapy, 18*(4), 407–410. https://doi.org/10.1197/j.jht.2005.07.002

MacDermid, J. C., & Silbernagel, K. G. (2015). Outcome evaluation in tendinopathy: Foundations of assessment and a summary of selected measures. *Journal of Orthopaedic & Sports Physical Therapy, 45*(11), 950–964. https://doi.org/10.2519/jospt.2015.6054

Maffulli, N. (1998). Overuse tendon conditions: Time to change a confusing terminology. *Arthroscopy, 14*(8), 840–843. https://doi.org/10.1016/S0749-8063(98)70021-0

Maffulli, N., Longo, U. G., Loppini, M., & Denaro, V. (2010). Current treatment options for tendinopathy. *Expert Opinion on Pharmacotherapy, 11*(13), 2177–2186. https://doi.org/10.1517/14656566.2010.495715

Maffulli, N., Testa, V., Capasso, G., Ewen, S. W., Sullo, A., Benazzo, F., et al. (2004). Similar histopathological picture in males with Achilles and patellar tendinopathy. *Medicine & Science in Sports & Exercise, 36*(9), 1470–1475. https://doi.org/10.1249/01.MSS.0000139895.94846.8D

Maffulli, N., Wong, J., & Almekinders, L. C. (2003). Types and epidemiology of tendinopathy. *Clinics in Sports Medicine, 22*(4), 675–692. https://doi.org/10.1016/s0278-5919(03)00004-8

Magnussen, R. A., Dunn, W. R., & Thomson, A. B. (2009). Nonoperative treatment of midportion achilles tendinopathy: A systematic review. *Clinical Journal of Sport Medicine, 19*(1), 54–64. https://doi.org/10.1097/JSM.0b013e31818ef090

Mamais, I., Papadopoulos, K., Lamnisos, D., & Stasinopoulos, D. (2018). Effectiveness of low level laser therapy (LLLT) in the treatment of lateral elbow tendinopathy (LET): An umbrella review. *Laser Therapy, 27*(3), 174–186. https://doi.org/10.5978/islsm.27_18-OR-16

Mani-Babu, S., Morrissey, D., Waugh, C., Screen, H., & Barton, C. (2015). The effectiveness of extracorporeal shock wave therapy in lower limb tendinopathy: A systematic review. *The American Journal of Sports Medicine, 43*(3), 752–761. https://doi.org/10.1177/0363546514531911

Mathiowetz, V., Weber, K., Volland, G., & Kashman, N. (1984). Reliability and validity of grip and pinch strength evaluations. *The Journal of Hand Surgery, 9*(2), 222–226. https://doi.org/10.1016/S0363-5023(84)80146-X

McKivigan, J. M., Yamashita, B., & Smith, D. (2017). A systematic review on the efficacy of iontophoresis as a treatment for lateral epicondylitis. *Research & Investigations in Sports Medicine, 1*(3). https://doi.org/10.31031/RISM.2017.01.000512 [Article RISM. 000512].

Mead, M. P., Gumucio, J. P., Awan, T. M., Mendias, C. L., & Sugg, K. B. (2018). Pathogenesis and management of tendinopathies in sports medicine. *Translational Sports Medicine, 1*(1), 5–13. https://doi.org/10.1002/tsm2.6

Meyer, A., Tumilty, S., & Baxter, G. (2009). Eccentric exercise protocols for chronic non-insertional achilles tendinopathy: How much is enough? *Scandinavian Journal of Medicine & Science in Sports, 19*(5), 609–615. https://doi.org/10.1111/j.1600-0838.2009.00981.x

Mosca, M. J., Rashid, M. S., Snelling, S. J., Kirtley, S., Carr, A. J., & Dakin, S. G. (2018). Trends in the theory that inflammation plays a causal role in tendinopathy: A systematic review and quantitative analysis of published reviews. *BMJ Open Sport & Exercise Medicine, 4*(1), Article e000332. https://doi.org/10.1136/bmjsem-2017-000332

Murphy, M. C., Travers, M. J., Chivers, P., Debenham, J. R., Docking, S. I., Rio, E. K., et al. (2019). Efficacy of heavy eccentric calf training for treating mid-portion achilles tendinopathy: A systematic review and meta-analysis. *British Journal of Sports Medicine, 53*(17), 1070–1077. https://doi.org/10.1136/bjsports-2018-099934

Murtaugh, B., & Ihm, J. M. (2013). Eccentric training for the treatment of tendinopathies. *Current Sports Medicine Reports, 12*(3), 175–182. https://doi.org/10.1249/JSR.0b013e3182933761

Newcomer, K. L., Martinez-Silvestrini, J. A., Schaefer, M. P., Gay, R. E., & Arendt, K. W. (2005). Sensitivity of the patient-rated forearm evaluation questionnaire in lateral epicondylitis. *Journal of Hand Therapy, 18*(4), 400–406. https://doi.org/10.1197/j.jht.2005.07.001

Nitschke, J. E., McMeeken, J. M., Burry, H. C., & Matyas, T. A. (1999). When is a change a genuine change?: A clinically meaningful interpretation of grip strength measurements in healthy and disabled women. *Journal of Hand Therapy, 12*(1), 25–30. https://doi.org/10.1016/S0894-1130(99)80030-1

Nogueira Júnior, A. C., & Júnior, M. J. M. (2015). The effects of laser treatment in tendinopathy: A systematic review. *Acta Ortopedica Brasileira, 23*(1), 47–49. https://doi.org/10.1590/1413-78522015230100513

Ortega-Castillo, M., & Medina-Porqueres, I. (2016). Effectiveness of the eccentric exercise therapy in physically active adults with symptomatic shoulder impingement or lateral epicondylar tendinopathy: A systematic review. *Journal of Science and Medicine in Sport, 19*(6), 438–453. https://doi.org/10.1016/j.jsams.2015.06.007

Overend, T. J., Wuori-Fearn, J. L., Kramer, J. F., & MacDermid, J. C. (1999). Reliability of a patient-rated forearm evaluation questionnaire for patients with lateral epicondylitis. *Journal of Hand Therapy, 12*(1), 31–37. https://doi.org/10.1016/S0894-1130(99)80031-3

ONeill, S., Watson, P. J., & Barry, S. (2015). Why are eccentric exercises effective for achilles tendinopathy? *International Journal of Sports Physical Therapy, 10*(4), 552.

Posten, W., Wrone, D. A., Dover, J. S., Arndt, K. A., Silapunt, S., & Alam, M. (2005). Low-level laser therapy for wound healing: Mechanism and efficacy. *Dermatologic Surgery, 31*(3), 334–340. https://doi.org/10.1111/j.1524-4725.2005.31086

du Prel, J.-B., Hommel, G., Röhrig, B., & Blettner, M. (2009). Confidence interval or p-value?: Part 4 of a series on evaluation of scientific publications. *Deutsches Ärzteblatt International, 106*(19), 335. https://doi.org/10.3238/arztebl.2009.0335

Punnoose, A., Norrish, A., & Pak, K. (2017). Extracorporeal shock wave therapy for achilles and patellar tendinopathy: Meta-analysis and a systematic review of the literature. *Journal of Physiotherapy and Physical Rehabilitation, 2*(1). https://doi.org/10.4172/2573-0312.1000124

Raman, J., MacDermid, J. C., & Grewal, R. (2012). Effectiveness of different methods of resistance exercises in lateral epicondylosis—a systematic review. *Journal of Hand Therapy, 25*(1), 5–26.

Razmjou, H., Bean, A., van Osnabrugge, V., MacDermid, J. C., & Holtby, R. (2006). Cross-sectional and longitudinal construct validity of two rotator cuff disease-specific outcome measures. *BMC Musculoskeletal Disorders, 7*(1), 26. https://doi.org/10.1186/1471-2474-7-26

Renstrom, P., & Woo, S. L. Y. (2008). Tendinopathy: A major medical problem in sport. *Tendinopathy in athletes, 2008*, 1–9.

Robinson, J., Cook, J. L., Purdam, C., Visentini, P., Ross, J., Maffulli, N., et al. (2001). The VISA-A questionnaire: A valid and reliable index of the clinical severity of achilles tendinopathy. *British Journal of Sports Medicine, 35*(5), 335–341. https://doi.org/10.1136/bjsm.35.5.335

Rocourt, M. H., Radlinger, L., Kalberer, F., Sanavi, S., Schmid, N. S., Leunig, M., et al. (2008). Evaluation of intratester and intertester reliability of the Constant-Murley shoulder assessment. *Journal of Shoulder and Elbow Surgery, 17*(2), 364–369. https://doi.org/10.1016/j.jse.2007.06.024

Rompe, J. D., Overend, T. J., & MacDermid, J. C. (2007). Validation of the patient-rated tennis elbow evaluation questionnaire. *Journal of Hand Therapy, 20*(1), 3–11. https://doi.org/10.1197/j.jht.2006.10.003

Ross, M. D., & Fontenot, E. G. (2000). Test–retest reliability of the standing heel-rise test. *Journal of Sport Rehabilitation, 9*(2), 117–123. https://doi.org/10.1123/jsr.9.2.117

Rowe, V., Hemmings, S., Barton, C., Malliaras, P., Maffulli, N., & Morrissey, D. (2012). Conservative management of midportion achilles tendinopathy: A mixed methods study, integrating systematic review and clinical reasoning. *Sports Medicine, 42*(11), 941–967. https://doi.org/10.2165/11635410-000000000-00000

Rutland, M., O'Connell, D., Brismée, J.-M., Sizer, P., Apte, G., & O'Connell, J. (2010). Evidence–supported rehabilitation of patellar tendinopathy. *North American Journal of Sports Physical Therapy: NAJSPT, 5*(3), 166.

Ryan, R., Santesso, N., Lowe, D., Hill, S., Grimshaw, J. M., Prictor, M., et al. (2014). Interventions to improve safe and effective medicines use by consumers: An overview of systematic reviews. *Cochrane Database of Systematic Reviews*, (4) https://doi.org/10.1002/14651858.CD007768.pub3

Scott, L. A., Munteanu, S. E., & Menz, H. B. (2014). Effectiveness of orthotic devices in the treatment of achilles tendinopathy: A systematic review. *Sports Medicine, 45*(1), 95–110. https://doi.org/10.1007/s40279-014-0237-z

Scott, A., Squier, K., Alfredson, H., Bahr, R., Cook, J. L., Coombes, B., et al. (2020). Icon 2019: International scientific tendinopathy symposium consensus: Clinical terminology. *British Journal of Sports Medicine, 54*(5), 260–262. https://doi.org/10.1136/bjsports-2019-100885

Shakespeare, T. P., Gebski, V. J., Veness, M. J., & Simes, J. (2001). Improving interpretation of clinical studies by use of confidence levels, clinical significance curves, and risk-benefit contours. *The Lancet, 357*(9265), 1349–1353. https://doi.org/10.1016/S0140-6736(00)04522-0

Shea, B. J., Bouter, L. M., Peterson, J., Boers, M., Andersson, N., Ortiz, Z., et al. (2007). External validation of a measurement tool to assess systematic reviews (AMSTAR). *PloS One, 2*(12). https://doi.org/10.1371/journal.pone.0001350

Shea, B. J., Grimshaw, J. M., Wells, G. A., Boers, M., Andersson, N., Hamel, C., et al. (2007). Development of AMSTAR: A measurement tool to assess the methodological quality of systematic reviews. *BMC Medical Research Methodology, 7*(1), 10. https://doi.org/10.1186/1471-2288-7-10

Shea, B. J., Hamel, C., Wells, G. A., Bouter, L. M., Kristjansson, E., Grimshaw, J., et al. (2009). AMSTAR is a reliable and valid measurement tool to assess the methodological quality of systematic reviews. *Journal of Clinical Epidemiology, 62*(10), 1013–1020. https://doi.org/10.1016/j.jclinepi.2008.10.009

Shea, B. J., Reeves, B. C., Wells, G., Thuku, M., Hamel, C., Moran, J., et al. (2017). Amstar 2: A critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. *Bmj, 358*, j4008. https://doi.org/10.1136/bmj.j4008

Smidt, N., van der Windt, D. A., Assendelft, W. J., Mourits, A. J., Deville, W. L., de Winter, A. F., et al. (2002). Interobserver reproducibility of the assessment of severity of complaints, grip strength, and pressure pain threshold in patients with lateral epicondylitis. *Archives of Physical Medicine and Rehabilitation, 83*(8), 1145–1150. https://doi.org/10.1053/apmr.2002.33728

Stratford, P. (1987). Extensor carpi radialis tendonitis: A validation of selected outcome measures. *Physiotherapie Canada, 39*, 250–255.

Stratford, P., Lavy, D., & Gowland, C. (1993). Evaluative properties of measures used to assess patients with lateral epicondylitis at the elbow. *Physiotherapie Canada, 45*, 160.

Stratford, P. W., & Levy, D. R. (1994). Assessing valid change over time in patients with lateral epicondylitis at the elbow. *Clinical Journal of Sport Medicine, 4*(2), 88–91. https://doi.org/10.1097/00042752-199404000-00004

Stratford, P. W., Norman, G. R., & McIntosh, J. M. (1989). Generalizability of grip strength measurements in patients with tennis elbow. *Physical Therapy, 69*(4), 276–281. https://doi.org/10.1093/ptj/69.4.276

Sussmilch-Leitch, S. P., Collins, N. J., Bialocerkowski, A. E., Warden, S. J., & Crossley, K. M. (2012). Physical therapies for achilles tendinopathy: Systematic review and meta-analysis. *Journal of Foot and Ankle Research, 5*(1), 15. https://doi.org/10.1186/1757-1146-5-15

Tashjian, R. Z., Deloach, J., Porucznik, C. A., & Powell, A. P. (2009). Minimal clinically important differences (MCID) and patient acceptable symptomatic state (PASS) for visual analog scales (VAS) measuring pain in patients treated for rotator cuff disease. *Journal of Shoulder and Elbow Surgery, 18*(6), 927–932. https://doi.org/10.1016/j.jse.2009.03.021

Thorborg, K., Hölmich, P., Christensen, R., Petersen, J., & Roos, E. M. (2011). The copenhagen hip and Groin outcome score (HAGOS): Development and validation according to the COSMIN checklist. *British Journal of Sports Medicine, 45*(6), 478–491. https://doi.org/10.1136/bjsm.2010.080937

Todd, K. H. (2001). Patient-oriented outcome measures: The promise of definition. *Annals of Emergency Medicine, 38*(6), 672–674. https://doi.org/10.1067/mem.2001.119746

Tumilty, S., Munn, J., McDonough, S., Hurley, D. A., Basford, J. R., & Baxter, G. D. (2010). Low level laser treatment of tendinopathy: A systematic review with meta-analysis. *Photomed Laser Surg, 28*(1), 3–16. https://doi.org/10.1089/pho.2008.2470

Verstraelen, F., Jansen, L., & Morrenhof, J. (2014). High-energy versus low-energy extracorporeal shock wave therapy for calcifying tendinitis of the shoulder: Which is superior? A meta-analysis. *Clinical Orthopaedics and Related Research, 472*(9), 2816–2825.

Vincent, J., & MacDermid, J. C. (2014). Patient-rated tennis elbow evaluation questionnaire. *Journal of Physiotherapy, 60*(4), 240. https://doi.org/10.1016/j.jphys.2014.08.002

Waugh, C., Morrissey, D., Jones, E., Riley, G., Langberg, H., & Screen, H. (2015). In vivo biological response to extracorporeal shockwave therapy in human tendinopathy: Response of tendinopathy to shockwave therapy. *European Cells and Materials, 3*(29), 268–280. https://doi.org/10.22203/ecm.v029a20

Weber, C., Thai, V., Neuheuser, K., Groover, K., & Christ, O. (2015). Efficacy of physical therapy for the treatment of lateral epicondylitis: A meta-analysis. *BMC Musculoskeletal Disorders, 16*, 223. https://doi.org/10.1186/s12891-015-0665-4. PMC4549077

Wilson, F., Walshe, M., O'Dwyer, T., Bennett, K., Mockler, D., & Bleakley, C. (2018). Exercise, orthoses and splinting for treating achilles tendinopathy: A systematic review with meta-analysis. *British Journal of Sports Medicine, 52*(24), 1564–1574. https://doi.org/10.1136/bjsports-2017-098913

Woodruff, L. D., Bounkeo, J. M., Brannon, W. M., Dawes, K. S., Barham, C. D., Waddell, D. L., et al. (2004). The efficacy of laser therapy in wound repair: A meta-analysis of the literature. *Photomedicine and Laser Surgery, 22*(3), 241–247. https://doi.org/10.1089/1549541041438623

Xu, Y., & Murrell, G. A. (2008). The basic science of tendinopathy. *Clinical Orthopaedics and Related Research, 466*(7), 1528–1538.

Yan, C., Xiong, Y., Chen, L., Endo, Y., Hu, L., Liu, M., et al. (2019). A comparative study of the efficacy of ultrasonics and extracorporeal shock wave in the treatment of tennis elbow: A meta-analysis of randomized controlled trials. *Journal of Orthopaedic Surgery and Research, 14*(1). https://doi.org/10.1186/s13018-019-1290-y

Young, J. L., Rhon, D. I., de Zoete, R. M., Cleland, J. A., & Snodgrass, S. J. (2018). The influence of dosing on effect size of exercise therapy for musculoskeletal foot and ankle disorders: A systematic review. *Brazilian Journal of Physical Therapy, 22*(1), 20–32.

# Supplementary data

| Studies | Population | Intervention(s) | Outcome(s) and/or outcome measure(s) | Limitations | OC |
|---|---|---|---|---|---|
| Tumilty et al. [1] | Human participants who had been diagnosed with tendinopathy and exhibited pain and/or functional disability were included. There was no distinction made with regards to age, gender, level of activity or chronicity of the injury. | One group in the controlled trial must have involved participants treated with active LLLT. Comparisons were made with at least one of the following: placebo, no treatment, or other treatments such as medication, exercise therapy or other electrotherapy modalities. Interventions based upon combinations of LLLT and other modalities were not considered for the review. | Trials which assessed pain or function for at least one of the outcome measures were considered for inclusion. Pain: used VAS for both final scores and change in scores; Site of injury: i.e., for lateral epicondylitis, Achilles tendinopathies, rotator cuff injuries; Grip strength. | The clinical application of LLLT was either poorly reported or varied between studies. Poor blinding procedures, lack of randomization, and lack of intention to treat analysis may have increased potential biases. Lack of use of valid and reliable outcome measures, and inadequate detail in the reporting of these measures. We did not perform a hand search of the literature. It might be thought that one would have been beneficial, but the databases targeted and the search strategy used were deemed by the authors to be robust enough. | Moderate |
| Chang et al. [2] | Subjects were diagnosed as having LE of elbow with pain induced by resisted extension of the wrist. | LLLT was used on the inflamed tendons, MTrPs, or acupuncture points as a treatment of LE. The control group must have received a non-laser or placebo laser treatment with zero output. | Pain measurement: VAS. Another method was to rate pain by FPV scale. Grasp or finger grip force: This was measured by a grip or pinch dynamometer or a vigorimeter. Muscle test: resistive tests of forearm movements and the MWP exercise. ROM of the wrist joint. PPT. Questionnaire and treatment effects: Upper extremity function was assessed by the DASH questionnaire. | Some of the assessment tools were considered to be inappropriate after reviewing some studies. Due to the lower number of RCTs in laser acupuncture, we were unable to further explore the effectiveness of LLLT on acupuncture points for LE. Only three papers could be used for the meta-analysis because there were no unified standards for assessment tools. | Low |
| Huisstede et al. [3] | Patients with SIS were included, SIS was not caused by an acute trauma or any systemic disease as described in the definition of CANS. | An intervention for treating SIS was evaluated. ESWT can be subdivided in low-, medium- and high-energy extracorporeal shockwaves. For the present study, we defined shockwaves ≤0.11 mJ/mm2 as low-ESWT, between 0.12 and 0.28 mJ/mm2 as medium-ESWT, and >0.28 mJ/mm2 as high-ESWT. | Results on pain, function or recovery were reported and follow-up period of at least two weeks was reported. | Because of the heterogeneity of the trials, we refrained from statistical pooling of the results of the individual trials. The use of a best evidence synthesis is a next best solution. Secondly, only 56% of the total number of included RCTs was of high quality. | Low |
| Hutchison et al. [4] | Male/female, athletic or sedentary, 18 years and over, chronic midbody Achilles tendinopathy (3 month + duration of symptoms) | Any treatment which is currently part of physiotherapy practice or could be part of physiotherapy (it was not necessary for the intervention to be carried out by a physiotherapist) | N/A | Lack of blinding increases the risk of introducing experimental bias. The variety of choice of control group also raises some concerns. | Low |
| Lee et al. [5] | Adult participants (aged ≥18 years) who had experienced symptoms for at least the past 6 months. | At least one treatment arm involved the use of ESWT for calcific rotator cuff tendinitis. | Any validated outcome measure of pain and shoulder function pertaining to the effect of ESWT on treatment of calcific rotator cuff tendinitis. | We found no reports of an ongoing effect after up to 1 year after the intervention. The methodologic quality of future research could be improved in a number of ways. Assessors should be blinded and there should be larger numbers of participants in the treatment arms. Outcomes should be measured in a standard manner and evaluated at regular follow-up. | Moderate |
| Joseph et al. [6] | N/A | N/A | Pain: VAS. Function: Patient report, grip strength, VAS, and function scales such as the | N/A | Low |

| | | | | | |
|---|---|---|---|---|---|
| | | | Neer Scale and the Tennis Elbow Functional Scale. | | |
| Littlewood et al. [7] | Studies of adult patients presenting with signs and symptoms suggestive of rotator cuff tendinopathy, defined as: 1. Symptom duration greater than three months. 2. Minimal resting pain. 3. Largely preserved range of shoulder motion. 4. Pain exacerbated consistently through resisted testing, usually abduction and/or lateral rotation. 5. No cervical spine involvement. | Any exercise intervention which included loaded (against gravity or resistance) exercise as a component. Initial scoping searches highlighted that it would be unlikely that studies evaluating the effect of loaded exercise alone would be identified. Combined interventions, e.g. exercise and electrotherapy or exercise and manual therapy, which do not enable a judgement about the comparative efficacy of exercise were excluded. | Any clinical outcome including, but not restricted to, measures of pain and disability. | No unpublished studies were identified for inclusion. It has been suggested that identifying unpublished studies for inclusion is important to minimize publication bias. However, others have questioned this suggesting that many unpublished studies eventually become published and truly unpublished studies might have poor or unclear methodology which in turn might serve to introduce bias to the review. | Low |
| Rowe et al. [8] | Subjects were human adults (aged > 18 years) with mid-portion Achilles tendinopathy. Studies with insertional tendinopathies were only included if a sub-group of mid-portion AT participants could be separated from the main group. Studies were required to recruit participants with chronic pain or symptoms (i.e. at least 3 months), to ensure a true tendinopathy was studied. | Conservative modalities were considered as any intervention not involving surgery, injection therapy or orally administered pharmacotherapies. Studies were required to make a comparison between two groups or compare the before and after effects of an intervention. | The primary outcome measure of concern in the literature review was assessment of pain, and pain reduction was the main focus in the majority of studies. Secondary outcomes of function and strength were also assessed. The most valid and reliable outcome measure used was the (VISA-A). | The strength of the evidence presented within the literature review may be questioned by some due to the broad inclusion criteria. The PEDro quality assessment scale was used to compensate for the lack of stringent inclusion criteria. However, it may be possible that some conclusions, notably derived from those studies with a low level of evidence, must be viewed cautiously. | Low |
| Sussmilch-Leitch et al.[9] | Achilles tendinopathy was defined as participants experiencing one or more common signs or symptoms (tenderness on palpation, pain at rest or during activity, stiffness during activity, and impaired function), either in the midportion or insertional region of the Achilles tendon. The diagnosis of AT had to be made by a healthcare or medical practitioner. | Studies eligible for inclusion were RCTs evaluating the effect of at least one non-surgical, non-pharmacological intervention. | Pain and/or altered function associated with AT. | Only English language studies were included, meaning that potentially relevant papers may have been excluded based on publication language. Reviewers who rated studies on the modified PEDro scale were not blinded to author, institution and journal, and only one reviewer extracted study data, which may have introduced biases. Furthermore, the inclusion of studies with mixed locations of tendon pathology prevents this review from making clearer distinctions between the evidence for each entity. | Low |
| Al-Abbad et al. [10] | Male or female adults aged 18 years or older with chronic Achilles tendinopathy (both insertional or noninsertional). | Extracorporeal shock wave therapy<br>In comparison with any conservative treatment or sham. | Pain Foot/ankle or lower extremity function score. | Limitations in our review might be the exclusion of 2 non-English studies that may result in language bias. Other limitations are the relative inconsistencies in adhering to a treatment dosage among all studies, which could have made drawing a strong recommendation easier. | Moderate |
| Cullinane et al. [11] | Patients who had undergone a diagnostic test for lateral | At least one treatment programme involving an eccentric exercise therapy, | At least one functional or disability outcome measure. | Of the 12 studies included in the review, only three were of high quality and only eight involved randomized controlled trials. The most common | Low |

| | | | | | |
|---|---|---|---|---|---|
| | epicondylitis, or had been diagnosed by a General Practitioner. | either exclusively or in conjunction with other treatments. | | methodological weakness was the lack of blinding of participants and treatment providers, with all studies scoring zero for question F and 11 studies scoring 0 for question E on the quality scoring sheet. The blinding of participants and therapists to the exercise therapy intervention is problematic. | |
| Dimitrios et al. [12] | Subjects aged >18 years old treated for LET. Patients suffered from LET for at least one month (4 weeks). | The treatment had to be any type of iontophoresis evaluated against at least one of the following: (i) placebo; (ii) no treatment; (iii) another treatment, conservative (physical therapy intervention or medical) or operative. | Pain (scales or description words), function (scales, tests, or description words), grip strength (pain-free or maximum), and a global measure (overall improvement, proportions of patients recovered, subjective improvement of symptoms. | Methodological shortcomings of this systematic review include searching in English alone, lack of trials selection blinding, and absence of meta-analyses. It may sometimes be difficult for reviewers to decide whether it is clinically relevant to combine the results of a group of studies in a meta-analysis. | C. Low |
| Hoogvliet et al. [13] | Patients with ME or LE were included, epicondylitis was not caused by an acute trauma or any systemic disease as described in the definition of CANS | Interventions for treating epicondylitis were evaluated | Pain, function or recovery were reported | First, we refrained from statistical pooling of the results of the individual trials because of the heterogeneity of the included studies. Secondly, only 7 of the 15 included RCTs were of high quality, which increase the risk of bias. Thirdly, because in several studies the follow-up period was relatively short (sometimes immediately after treatment). Finally, the statistical power of several studies was relatively low owing to the small number of subjects. | Low |
| Ioppolo et al. [14] | Adults >18 years of age, shoulder pain or tenderness from calcific tendonitis in patients with type I or II calcification according to the radiographic classification of Gartner and Simons. | All RCTs that compared ESWT with placebo or no treatment were considered | Outcome measures were clinical improvement, evaluated by shoulder functional scales, and resorption of calcific deposits, defined through radiographic examinations | In general, most studies measure the effects that shock waves have on reabsorption only at a distance of 3 months after the conclusion of treatment, for the simple reason that this phenomenon is not held to be “premature.” Indeed, the studies of this systematic review used a different number of sessions and impulses. | Low |
| Bannuru et al. [15] | Humans that studied treatment of calcific or noncalcific tendinitis of the shoulder | Different energy levels of ESWT compared against each other or placebo or weighed ESWT against other treatments. | The included trials evaluated pain predominantly by using a VAS score. Shoulder function was most frequently evaluated by using the Constant–Murley score, active ROM and shoulder power, the Shoulder Pain and Disability Index, and the function subscale of the UCLA Shoulder Rating Scale. Resolution of calcification was measured radiographically or sonographically. | The treatment application differed greatly among the studies. The trials were small and far too heterogeneous to reasonably estimate the rates of calcification resorption. There are no universally accepted EFD cut-off values, and some of the included studies used different cut-off values. Study grouping by EFD levels did not take into account the frequency of shock waves delivered or total cumulative dose administered. | Moderate |
| Dingemanse et al. [16] | Patients with ME or LE were included, (2) epicondylitis was not caused by an acute trauma or any systemic disease as described in the definition of CANS | Interventions for treating epicondylitis were evaluated. | Results on pain, function or recovery were reported. | In this review we used the Furlan-list to assess the methodological quality of the RCTs. Also, we used the arbitrary limit of 50% to decide whether or not the evaluated study is of high quality; accordingly, 14 of the 20 (70%) RCTs were of high quality. Of | Low |

| | | | | | |
|---|---|---|---|---|---|
| | | | | the RCTs included in our review, only 32% included over 50 patients. | |
| Haslerud et al. [17] | Trials included human participants diagnosed with shoulder tendinopathy or subacromial impingement syndrome, experiencing pain and functional disability. | Treatment with LLLT at wavelengths in the range of 632–1064 nm, targeting the tendon pathology, acupuncture or trigger points, and administered to one group in the controlled trial. | Pain intensity on VAS scale. Improved global health status. Improved shoulder function in Shoulder Pain and Disability Index, Constant Murley Shoulder Score, Shoulder Disability Questionnaire or VAS for function | The result for each comparison and outcome arises from a handful of studies. Outcome measures are based solely on end of treatment data (2–12 weeks) and display the potential short-term effects of LLLT. Only three trials provided post-treatment follow-up data, and no robust conclusions can be drawn regarding the long-term effects. | Moderate |
| Loew et al. [18] | Studies were included if study participants were 18 years of age or older and had been diagnosed with tendinitis (lateral elbow tendinitis and knee tendinitis). Participants had to show no signs of psychiatric conditions and had to demonstrate stable physical and medical status. | Trials comparing deep transverse friction massage versus no therapy or active treatments (such as therapeutic ultrasound, exercise program, or phonophoresis therapy) were accepted, provided they were given equally to all treatment groups. | Mean (or mean change in) pain, Proportion reporting pain relief of 30% or greater, function, quality of life, patient global assessment of success, total number of withdrawals due to adverse effects, number of adverse events, ROM, muscle strength, endurance. | It is important to consider whether all relevant trials were identified (whether or not all pertinent data could be obtained) and if the methods used (search strategy, selection of studies, data collection, analysis) could have introduced potential bias. When selecting studies for inclusion, we did not consider languages other than French and English. | Moderate |
| Mani-Babu et al. [19] | N/A | N/A | N/A | N/A | Low |
| Desjardins-Charbonneau et al. [20] | Participants were aged 18 years or older and presented a diagnosis of RC tendinopathy/ tendinitis/ tendinosis, partial RC tear, impingement syndrome or subacromial bursitis | Efficacy of a taping intervention (NET or KT) was compared to any other type of intervention or to a placebo | the review focus on outcomes related to clinical efficacy such as pain and function | Despite the relatively homogenous taping interventions (two out of four RCT used the same non-elastic taping and three out five RCTs used the same KT), pooling of data was limited because of significant heterogeneity | Moderate |
| Desjardins-charbonneau et al. [21] | Participants were diagnosed with RC tendinopathy/ tendinitis, shoulder impingement syndrome, or subacromial bursitis; participants were adults | MT intervention was compared with another type of treatment, including other MT interventions. Manual therapy interventions that were considered for inclusion had to be specific hands-on interventions | All types of outcome measures were considered for inclusion | Funnel plots were not generated, thus the risk of publication bias was not assessed, which limits the strength of our conclusions | Low |
| Desmeules et al. [22] | Adults participants (>18 years) suffering from RC tendinopathy. Studies including participants suffering from shoulder pain were accepted if it could determine that the majority of the participants were suffering from RC tendinopathy. | US therapy either alone or in conjunction with other interventions was compared to a placebo or to any other therapeutic modality including US | N/A | The primary limitation in this review was the low quality of the RCTs. Further, only two of the eleven included studies could be pooled together for a meta-analysis. The validated Cochrane Methodological Risk of Bias assessment tool was used to determine risk of bias among studies. However, for two of the eight risk of bias items, inter-rater agreement was poor. | Moderate |
| Krey et al. [23] | Tendinosis was defined as chronic tendinopathy or chronic tendonitis lasting ≥ 3 months | Tendon needling was defined as using a needle for intervention into a tendon, either with or without ultrasound use for visualization. | N/A | The search strategy produced only 4 articles meeting the criteria for this review. There are numerous studies of lesser levels of evidence, but our purpose was to examine the highest level evidence to arrive at conclusions regarding tendon needling for tendinopathy. | Low |

| | | | | | |
|---|---|---|---|---|---|
| Nogueira Júnior et al. [24] | N/A | N/A | N/A | N/A | C. Low |
| Scott et al. [25] | Adults (participants over the age of 18 years). Participants could experience symptoms of any duration | Orthotic devices (foot orthoses, heel raises, splinting, footwear, taping or bracing) for the treatment of mid-portion or insertional AT. | At least one outcome measure of pain, function or disability | The conclusions of this review are limited due to the small number of studies, many of which provided low-level evidence and/or were of poor methodological quality. Furthermore, three studies included in this review did not clearly describe if participants had midportion or insertional AT | Moderate |
| Weber et al. [26] | Adults (18 years and older). Patients had to be diagnosed with LE | Studies were eligible if they investigated a physical therapy intervention in comparison to a waiting-list control group, treatment as usual control group or sham-control group. If a study investigated a combination of therapy modalities, the control group would have to match one of the therapy modalities. | All outcomes were considered for inclusion as long as at least three studies used the same outcome measurement | ECSWT results might be relatively stable while non-ECSWT results might change, even with only a few new studies. Patients varied largely in their duration of symptoms, making it impossible to differentiate between studies with only acute or only chronic LE patients. Another distorting factor in this meta-analysis was the rather large difference in treatment durations and sessions per week. | Low |
| Boudreault et al. [27] | Participants suffered from RC tendinopathy or other related diagnostics such as impingement syndrome, long head of the biceps tendinopathy or subacromial bursitis, adult population (≥18 years old). | At least one of the intervention under study was LT either low level or high level. LT could be compared to any other therapeutic modality or to a placebo. | The outcomes of interest included patient reported outcomes such as pain, function, health-related quality of life, as well as performance-based outcomes such as shoulder ROM and muscle strength. | Despite the fact that the reviewers used Cochrane risk of bias assessment tool, there can be some discrepancy in the interpretation and in the scoring. Pooling of results was not always possible, but we were able to summarize evidence concerning the efficacy of LT in individuals suffering from RC tendinopathy despite the fact that there was an heterogeneity across studies. | Moderate |
| Desmeules et al. [28] | Participants were adults (>18 years). Participants suffered from RC tendinopathy or other related diagnostics, such as impingement syndrome, subacromial bursitis, or bicipital tendinitis. | At least one of the interventions under study included exercises for the shoulder and could also include the upper quadrant | The main outcomes of interest were work-related, but other clinical outcomes were also considered, such as pain and disability. | Our study has some limitations that arise from the RCTs included in the review, which had small sample sizes and moderate to high risk of bias. Also, because of the heterogeneity of the results, we could not conduct meta-analyses. The information about return-to-work rate of workers was not available in all the trials, and non-validated and heterogeneous return-to-work outcomes were used. | Low |
| Desmeules et al. [29] | Participants suffered from rotator cuff tendinopathy or other related diagnoses such as subacromial impingement syndrome, bicipital tendinitis or subdeltoid bursitis. Participants were adults (age ≥18 years); | TENS was compared with any other therapeutic modality, placebo or no intervention | N/A | Due to the heterogeneity of the results, it was not possible to pool the results into meta-analyses. The Cochrane methodological risk of bias validated tool was poor for the item on 'other sources of bias' (κ = 0.3). However, the inter-rater agreement was poor for the item on 'other sources of bias' (κ = 0.3). | Moderate |
| Ortega-Castillo et al. [30] | Adult participants with clinical diagnosis of tendinopathy | Eccentric exercise was employed to treat upper extremity tendinopathies | Results regarding pain or strength were assessed | The lack of general agreement with respect to inclusion and exclusion criteria may lessen the validity of the studies. The failure to accurately report exercise protocols and the substantial | Moderate |

| | | | | | |
|---|---|---|---|---|---|
| | | | | variation in exercise parameters made it difficult to assess the effectiveness of each study's ability to isolate an eccentric exercise component and provide a progressive muscle stimulus. | |
| McKivigan et al. [31] | N/A | N/A | N/A | Four studies had low Pedro scores (4 or lower) and moderate to low quality of evidence. Three studies were of healthy adults with no diagnosis of elbow pain. Lastly, although all but one study was randomized or experimental designs, the number of articles reviewed in this study was limited to 12 that met the inclusion criteria. | Low |
| Punnoose et al. [32] | Adult humans with achilles and patellar tendinopathy | ESWT, which can be generated by different methods compared to "standard care" or another intervention | Reduction in pain and increase in function measured at any time after treatment. | Overall, the number of trials in this field is small and studies feature relatively small sample sizes. Excluding studies published in languages other than English could lead to language bias. Additionally, study selection at the title and abstract stage was performed by one researcher (potentially resulting in selection bias). | Low |
| Liao et al. [33] | N/A | control groups received a placebo through a sham shock wave application or underwent active treatment (eg, exercise, injections, or surgery) | primary outcome included pain, which was measured using a quantifiable scale, such as a visual analog scale (VAS), and the ratio of successful treatment was measured using a rank scale, such as the Roles and Maudsley score; the secondary outcomes included physical function or disability | First, only LE tendinopathy was included, and the results may not be generalizable. Heterogeneity among the included studies was noted. Other application parameters which may interfere with the therapeutic response were not considered for comparisons in this current. Other confounding factors which may have contributed to treatment efficacy were not computed for TSR. | Moderate |
| Liao et al. [34] | N/A | RCTs involving KSTDs including tendinopathy and other noncartilage soft tissue disorders; RCTs in which controls received a placebo through sham shock wave application or underwent noninvasive/invasive treatment (e.g., exercise, injections, or surgery); | Primary outcomes included pain that was measured using a quantifiable scale (VAS) and the successful treatment rate that was measured using a ranking scale (e.g., the Roles and Maudsley score or Likert-type scale). Outcomes included physical function and disability that were assessed using questionnaires for patient-reported outcomes (e.g., the VISA-P) or measured using performance-based testing (e.g., the vertical jump test). | First, not all types of KSTDs were included in this meta-analysis. Thus, the results may not be generalizable to other upper or lower limb conditions such as supraspinatus tendinopathy and Achilles' tendinopathy. Second, we observed heterogeneity across the included trials. Third, other application parameters which may interfere with therapeutic response, were not considered in comparisons in this study. Only five RCTs reported performance-based functional outcomes including knee ROM. | Moderate |
| Lim et al. [35] | Participants with tendinopathy | Human interventional studies investigating isometric, eccentric, or HSR training exercises in PT were also included. | Articles reporting at least one of the outcomes of pain and/or function in PT were included | A limitation of this review is the inability to directly compare the effects of isometric, eccentric, and HSR exercises. This is due to the lack of available studies comparing the effects of these interventions. The present review included studies of different study designs and level of evidence based on the NHMRC hierarchy. | Moderate |
| Lucado et al. [36] | Adults aged 18 years and older clinically diagnosed with LET | We defined joint mobilizations as an intervention to LET that includes either | Outcomes related to the purpose of this review, including pain, grip strength, | Some lower/moderate-quality evidence were included and thus have a larger risk of introducing | Low |

| | | | | | |
|---|---|---|---|---|---|
| | | joint mobilizations or joint manipulations to the local region (elbow) or to a distant but related site (cervical spine, thoracic spine, or wrist) | and disability, were of primary interest | study selection bias. There were challenges in the collection and pooling of data. Another barrier observed in homogenizing our findings was in the variability in the clinical presentation of LET. | |
| Wilson et al. [37] | Studies involving participants with a reported diagnosis of Achilles tendinopathy (also described as 'tendinitis' or 'tendinosis') were included. There were no restrictions placed on recruitment setting, gender, age, duration of symptoms or activity levels of participants. Participants with either mid-portion or insertional tendinopathy (or both) were considered. | Trials comparing any of the three categories of interventions (exercise, orthoses or splinting) with no intervention or placebo intervention, and (2) trials comparing different types, applications and modes of delivery of interventions within the three intervention categories | The primary outcomes of interest were functional assessment measures, for example, the VISA-A; measures of pain, for example, the VAS; and quality of life measures, for example, the SF-36. Secondary outcomes included return to previous level of activity, adverse effects, patient satisfaction, recurrence of symptoms and radiographic findings. | Some studies did not state what type of tendinopathy participants had. Some studies had co-interventions that are likely to confound results. There was a paucity of reporting of objective functional measures. Studies examined an active/recreational population, and findings may not apply to other population groups. | Moderate |
| Hickey et al. [38] | Subjects over 18 years of age, a history of exercise-related patellar tendon pain for at least 3 months | The Alfredson protocol, 12-week follow-up data available with both means and standard deviations. | The VISA-P and/or the VAS outcome measures | The lack of research on athletes with either PaT or AT. Some studies also had lower PEDro scores and a there was a shortage of studies that directly compare EE to ESWT. Both of the EE Studies in the meta-analysis had small samples sizes. Lack of parameter consistency and finally, the treatment windows and follow-up times differed for both interventions. | Low |
| Murphy et al. [39] | Both physically active and sedentary participants aged 18 years and over identified as having mid-portion AT for greater than 3 months were included | One study arm used HECT to treat mid-portion AT and the other used a natural history, sham rehabilitation, traditional physiotherapy or other exercise intervention. | The VISA-A | HECT may be superior to natural history, the quality of evidence is very low and we have little confidence in the estimated effect. Furthermore, no studies have investigated the comparison between HECT and sham rehabilitation. HECT may be superior to traditional physiotherapy. However, the quality of evidence is low and our confidence in the estimated effect is limited. | High |
| Yan et al. [40] | N/A | Comparison between ESWT and US on efficacy for treating lateral epicondylitis | Major outcomes measured were the efficacy of pain relief and functional restoration. | Firstly, the number of enrolled trials is small which limits the generalizability and contingency of the results. Secondly, the side effects of ESWT and US were not evaluated during follow-up. The high heterogeneity among the results weakens the reliability of the results. | Low |

LLLT, Low-level laser therapy; LE, Lateral epicondylitis; MTrPs, Myofascial trigger points; VAS, visual analogue scale; FPV, Five Point Verbal scale; MWP, Mercy Wedge Pro exercise; MWM, mobilization with movement; RCTs, randomized controlled trials; SIS, shoulder impingement syndrome; CANS, complaints of the arm, neck and/or shoulder; ESWT, Extracorporeal shockwave therapy; AT, Achilles tendinopathy; PEDro, Physiotherapy evidence database; US; Ultrasound therapy; ME, Medial epicondylitis; EFD, energy flux density; RC, Rotator Cuff; KT, Kinesiology taping; NET, Non-elastic taping; MT, Manual therapy; ROM, Range of motion; PPT, Pain pressure threshold; DASH, Disabilities of the Arm, Shoulder and Hand questionnaire; VISA-P, the Victorian Institute of Sport Assessment-Patella questionnaire; VISA-A, the Victorian Institute of Sport Assessment–Achilles Questionnaire; SF-36, 36-Item Short Form Survey; TSR, treatment success rate; KSTD, Knee soft tissue disorders; HSR, Heavy slow resistance; PT; Physiotherapy; PaT, Patellar tendinopathy, NHMRC, National Health and Medical Research Council; LET, Lateral elbow tendinopathy; EE, Eccentric exercise; HECT, heavy eccentric calf training.

## EXCLUDED FULL-TEXT ARTICLES

### Non-efficacy studies

[1] Murphy M, Travers M, Gibson W, Chivers P, Debenham J, Docking S, et al. Rate of Improvement of Pain and Function in Mid-Portion Achilles Tendinopathy with Loading Protocols: A Systematic Review and Longitudinal Meta-Analysis. Sports Med. 2018;48(8):1875-91. http://dx.doi.org/10.1007/s40279-018-0932-2.
[2] Young JL, Rhon DI, Cleland JA, Snodgrass SJ. The Influence of Exercise Dosing on Outcomes in Patients With Knee Disorders: A Systematic Review. J Orthop Sports Phys Ther. 2018;48(3):146-61. http://dx.doi.org/10.2519/jospt.2018.7637.
[3] Young JL, Rhon DI, de Zoete RMJ, Cleland JA, Snodgrass SJ. The influence of dosing on effect size of exercise therapy for musculoskeletal foot and ankle disorders: a systematic review. Braz J Phys Ther. 2018;22(1):20-32. http://dx.doi.org/10.1016/j.bjpt.2017.10.001. PMC5816081.
[4] Littlewood C, Malliaras P, Chance-Larsen K. Therapeutic exercise for rotator cuff tendinopathy: a systematic review of contextual factors and prescription parameters. Int J Rehabil Res. 2015;38(2):95-106. http://dx.doi.org/10.1097/mrr.0000000000000113.
[5] Verstraelen FU, In den Kleef NJ, Jansen L, Morrenhof JW. High-energy versus low-energy extracorporeal shock wave therapy for calcifying tendinitis of the shoulder: which is superior? A meta-analysis. Clinical orthopaedics and related research. 2014;472(9):2816-25. http://dx.doi.org/10.1007/s11999-014-3680-0. PMC4117900.
[6] Malliaras P, Barton CJ, Reeves ND, Langberg H. Achilles and patellar tendinopathy loading programmes : a systematic review comparing clinical outcomes and identifying potential mechanisms for effectiveness. Sports Med. 2013;43(4):267-86. http://dx.doi.org/10.1007/s40279-013-0019-z.
[7] Raman J, MacDermid JC, Grewal R. Effectiveness of different methods of resistance exercises in lateral epicondylosis--a systematic review. J Hand Ther. 2012;25(1):5-25; quiz 6. http://dx.doi.org/10.1016/j.jht.2011.09.001.

### Protocols of studies

[1] Murphy M, Travers M, Gibson W. Is heavy eccentric calf training superior to wait-and-see, sham rehabilitation, traditional physiotherapy and other exercise interventions for pain and function in mid-portion Achilles tendinopathy? Syst Rev. 2018;7(1):58. http://dx.doi.org/10.1186/s13643-018-0725-6. PMC5899347.
[2] Escriche-Escuder A, Casaña J, Cuesta-Vargas AI. Progression criteria in loading exercise programmes in lower limb tendinopathy: a protocol for a systematic review and meta-analysis. BMJ Open. 2019;9(12):e032940. http://dx.doi.org/10.1136/bmjopen-2019-032940.
[3] dos Santos Franco YR, Miyamoto GC, Franco KFM, de Oliveira RR, Cabral CMN. Exercise therapy in the treatment of tendinopathies of the lower limbs: a protocol of a systematic review. Systematic Reviews. 2019;8(1):142. http://dx.doi.org/10.1186/s13643-019-1058-9.

### Studies including invasive, pharmacologic, surgical interventions

[1] Cavaleri R, Schabrun SM, Te M, Chipchase LS. Hand therapy versus corticosteroid injections in the treatment of de Quervain's disease: A systematic review and meta-analysis. J Hand Ther. 2016;29(1):3-11. http://dx.doi.org/10.1016/j.jht.2015.10.004.
[2] Tsikopoulos K, Tsikopoulos I, Simeonidis E, Papathanasiou E, Haidich AB, Anastasopoulos N, et al. The clinical impact of platelet-rich plasma on tendinopathy compared to placebo or dry needling injections: A meta-analysis. Phys Ther Sport. 2016;17:87-94. http://dx.doi.org/10.1016/j.ptsp.2015.06.003.

[3] Louwerens JK, Veltman ES, van Noort A, van den Bekerom MP. The Effectiveness of High-Energy Extracorporeal Shockwave Therapy Versus Ultrasound-Guided Needling Versus Arthroscopic Surgery in the Management of Chronic Calcific Rotator Cuff Tendinopathy: A Systematic Review. Arthroscopy. 2016;32(1):165-75. http://dx.doi.org/10.1016/j.arthro.2015.06.049.
[4] Mendonça LDM, Leite HR, Zwerver J, Henschke N, Branco G, Oliveira VC. How strong is the evidence that conservative treatment reduces pain and improves function in individuals with patellar tendinopathy? A systematic review of randomised controlled trials including GRADE recommendations. British journal of sports medicine. 2020;54(2):87-93.
[5] Sims SE, Miller K, Elfar JC, Hammert WC. Non-surgical treatment of lateral epicondylitis: a systematic review of randomized controlled trials. Hand. 2014;9(4):419-46.
[6] Sayegh ET, Strauch RJ. Does nonsurgical treatment improve longitudinal outcomes of lateral epicondylitis over no treatment? A meta-analysis. Clinical Orthopaedics and Related Research®. 2015;473(3):1093-107.
[7] Wu Y-C, Tsai W-C, Tu Y-K, Yu T-Y. Comparative effectiveness of nonoperative treatments for chronic calcific tendinitis of the shoulder: a systematic review and network meta-analysis of randomized controlled trials. Archives of physical medicine and rehabilitation. 2017;98(8):1678-92. e6.
[8] Vander Doelen T, Jelley W. Non-surgical treatment of patellar tendinopathy: A systematic review of randomized controlled trials. Journal of science and medicine in sport. 2019.
[9] Wiegerinck J, Kerkhoffs G, Van Sterkenburg M, Sierevelt I, van Dijk C. Treatment for insertional Achilles tendinopathy: a systematic review. Knee Surgery, Sports Traumatology, Arthroscopy. 2013;21(6):1345-55.
[10] Kearney R, Costa ML. Insertional achilles tendinopathy management: a systematic review. Foot & ankle international. 2010;31(8):689-94.
[11] Everhart JS, Cole D, Sojka JH, Higgins JD, Magnussen RA, Schmitt LC, et al. Treatment Options for Patellar Tendinopathy: A Systematic Review. Arthroscopy. 2017;33(4):861-72. http://dx.doi.org/10.1016/j.arthro.2016.11.007.
[12] Larsson ME, Kall I, Nilsson-Helander K. Treatment of patellar tendinopathy--a systematic review of randomized controlled trials. Knee Surg Sports Traumatol Arthrosc. 2012;20(8):1632-46. http://dx.doi.org/10.1007/s00167-011-1825-1.

**Non-English language**

[1] Lorenzen J, Krämer R, Vogt P, Knobloch K. Systematic review about eccentric training in chronic patella tendinopathy. Sportverletzung Sportschaden: Organ der Gesellschaft fur Orthopadisch-Traumatologische Sportmedizin. 2010;24(4):198-203.
[2] Krämer R, Lorenzen J, Vogt P, Knobloch K. Systematic review about eccentric training in chronic achilles tendinopathy. Sportverletzung Sportschaden: Organ der Gesellschaft fur Orthopadisch-Traumatologische Sportmedizin. 2010;24(4):204-11.
[3] Zandt J, Hahn D, Buchmann S, Beitzel K, Schwirtz A, Imhoff A, et al. May eccentric training be effective in the conservative treatment of chronic supraspinatus tendinopathies? A review of the current literature. Sportverletzung Sportschaden: Organ der Gesellschaft fur Orthopadisch-Traumatologische Sportmedizin. 2010;24(4):190-7.
[4] dos Santos FT, Piazza L. Evidências científicas no tratamento fisioterapêutico da tendinopatia patelar: uma revisão sistemática da literatura. ConScientiae Saúde. 2015;14(3):489-96.
[5] de la Fuente A, Valero B, Cuadrado N. Abordaje fisioterápico de la tendinopatía rotuliana: revisión sistemática. Fisioterapia. 2019

**References**

[1] Tumilty S, Munn J, McDonough S, Hurley DA, Basford JR, Baxter GD. Low level laser treatment of tendinopathy: a systematic review with meta-analysis. Photomed Laser Surg. 2010;28(1):3-16. http://dx.doi.org/10.1089/pho.2008.2470.
[2] Chang WD, Wu JH, Yang WJ, Jiang JA. Therapeutic effects of low-level laser on lateral epicondylitis from differential interventions of Chinese-Western medicine: systematic review. Photomed Laser Surg. 2010;28(3):327-36. http://dx.doi.org/10.1089/pho.2009.2558.
[3] Huisstede BM, Gebremariam L, van der Sande R, Hay EM, Koes BW. Evidence for effectiveness of Extracorporal Shock-Wave Therapy (ESWT) to treat calcific and non-calcific rotator cuff tendinosis–a systematic review. Manual therapy. 2011;16(5):419-33. http://dx.doi.org/10.1016/j.math.2011.02.005.
[4] Hutchison A-M, Beard D, Pallister I, Topliss CJ, Williams P. Is physiotherapy effective for patients with a chronic mid-body Achilles tendinopathy? A systematic review of non-surgical and non-pharmacological interventions. International Musculoskeletal Medicine. 2011;33(4):152-60. http://dx.doi.org/10.1179/1753615411Y.0000000010.
[5] Lee SY, Cheng B, Grimmer-Somers K. The midterm effectiveness of extracorporeal shockwave therapy in the management of chronic calcific shoulder tendinitis. J Shoulder Elbow Surg. 2011;20(5):845-54. http://dx.doi.org/10.1016/j.jse.2010.10.024.
[6] Joseph MF, Taft K, Moskwa M, Denegar CR. Deep friction massage to treat tendinopathy: a systematic review of a classic treatment in the face of a new paradigm of understanding. J Sport Rehabil. 2012;21(4):343-53.
[7] Littlewood C, Ashton J, Chance-Larsen K, May S, Sturrock B. Exercise for rotator cuff tendinopathy: a systematic review. Physiotherapy. 2012;98(2):101-9. http://dx.doi.org/10.1016/j.physio.2011.08.002.
[8] Rowe V, Hemmings S, Barton C, Malliaras P, Maffulli N, Morrissey D. Conservative management of midportion achilles tendinopathy: A mixed methods study, integrating systematic review and clinical reasoning. Sports Medicine. 2012;42(11):941-67. http://dx.doi.org/10.2165/11635410-000000000-00000.
[9] Sussmilch-Leitch SP, Collins NJ, Bialocerkowski AE, Warden SJ, Crossley KM. Physical therapies for Achilles tendinopathy: systematic review and meta-analysis. Journal of foot and ankle research. 2012;5(1):15. http://dx.doi.org/10.1186/1757-1146-5-15.
[10] Al-Abbad H, Simon JV. The effectiveness of extracorporeal shock wave therapy on chronic achilles tendinopathy: a systematic review. Foot & ankle international. 2013;34(1):33-41. http://dx.doi.org/10.1177/1071100712464354.
[11] Cullinane FL, Boocock MG, Trevelyan FC. Is eccentric exercise an effective treatment for lateral epicondylitis? A systematic review. Clinical rehabilitation. 2014;28(1):3-19. http://dx.doi.org/10.1177/0269215513491974.
[12] Dimitrios S, Konstantinos P, Antonis K. Effectiveness of Iontophoresis for Lateral Elbow Tendinopathy. J Nov Physiother S. 2013;2:2. http://dx.doi.org/10.4172/2165-7025.S2-005.
[13] Hoogvliet P, Randsdorp MS, Dingemanse R, Koes BW, Huisstede BM. Does effectiveness of exercise therapy and mobilisation techniques offer guidance for the treatment of lateral and medial epicondylitis? A systematic review. British journal of sports medicine. 2013:bjsports-2012-091990. http://dx.doi.org/10.1136/bjsports-2012-091990.
[14] Ioppolo F, Tattoli M, Di Sante L, Venditto T, Tognolo L, Delicata M, et al. Clinical improvement and resorption of calcifications in calcific tendinitis of the shoulder after shock wave therapy at 6 months' follow-up: a systematic review and meta-analysis. Archives of physical medicine and rehabilitation. 2013;94(9):1699-706. http://dx.doi.org/10.1016/j.apmr.2013.01.030.
[15] Bannuru RR, Flavin NE, Vaysbrot E, Harvey W, McAlindon T. High-energy extracorporeal shock-wave therapy for treating chronic calcific tendinitis of the shoulder: a systematic review. Annals of internal medicine. 2014;160(8):542-9. http://dx.doi.org/10.7326/M13-1982.

[16] Dingemanse R, Randsdorp M, Koes BW, Huisstede BM. Evidence for the effectiveness of electrophysical modalities for treatment of medial and lateral epicondylitis: a systematic review. Br J Sports Med. 2014;48(12):957-65. http://dx.doi.org/10.1136/bjsports-2012-091513.
[17] Haslerud S, Magnussen LH, Joensen J, Lopes-Martins RA, Bjordal JM. The efficacy of low-level laser therapy for shoulder tendinopathy: a systematic review and meta-analysis of randomized controlled trials. Physiother Res Int. 2015;20(2):108-25. http://dx.doi.org/10.1002/pri.1606.
[18] Loew LM, Brosseau L, Tugwell P, Wells GA, Welch V, Shea B, et al. Deep transverse friction massage for treating lateral elbow or lateral knee tendinitis. Cochrane Database Syst Rev. 2014(11):CD003528. http://dx.doi.org/10.1002/14651858.CD003528.pub2.
[19] Mani-Babu S, Morrissey D, Waugh C, Screen H, Barton C. The effectiveness of extracorporeal shock wave therapy in lower limb tendinopathy: a systematic review. The American journal of sports medicine. 2015;43(3):752-61. http://dx.doi.org/10.1177/0363546514531911.
[20] Desjardins-Charbonneau A, Roy JS, Dionne CE, Desmeules F. The efficacy of taping for rotator cuff tendinopathy: a systematic review and meta-analysis. International journal of sports physical therapy. 2015;10(4):420.
[21] Desjardins-Charbonneau A, Roy J-S, Dionne CE, Frémont P, MacDermid JC, Desmeules F. The efficacy of manual therapy for rotator cuff tendinopathy: a systematic review and meta-analysis. journal of orthopaedic & sports physical therapy. 2015;45(5):330-50. http://dx.doi.org/10.2519/jospt.2015.5455.
[22] Desmeules F, Boudreault J, Roy J-S, Dionne C, Frémont P, MacDermid JC. The efficacy of therapeutic ultrasound for rotator cuff tendinopathy: A systematic review and meta-analysis. Physical Therapy in Sport. 2015;16(3):276-84. http://dx.doi.org/10.1016/j.ptsp.2014.09.004.
[23] Krey D, Borchers J, McCamey K. Tendon needling for treatment of tendinopathy: a systematic review. The Physician and sportsmedicine. 2015;43(1):80-6. http://dx.doi.org/10.1080/00913847.2015.1004296.
[24] Nogueira Júnior AC, Júnior MdJM. The effects of laser treatment in tendinopathy: a systematic review. Acta ortopedica brasileira. 2015;23(1):47-9. http://dx.doi.org/10.1590/1413-78522015230100513.
[25] Scott LA, Munteanu SE, Menz HB. Effectiveness of Orthotic Devices in the Treatment of Achilles Tendinopathy: A Systematic Review. Sports Medicine. 2014;45(1):95-110. http://dx.doi.org/10.1007/s40279-014-0237-z.
[26] Weber C, Thai V, Neuheuser K, Groover K, Christ O. Efficacy of physical therapy for the treatment of lateral epicondylitis: a meta-analysis. BMC Musculoskelet Disord. 2015;16:223. http://dx.doi.org/10.1186/s12891-015-0665-4. PMC4549077.
[27] Boudreault J, Desmeules F, Roy J, Dionne C, Frémont P. The Efficacy of Laser Therapy for Rotator Cuff Tendinopathy: A Systematic Review and Meta-Analysis. J Nov Physiother Phys Rehabil 3 (1): 001-015 DOI: 1017352/2455. 2016;5487(001). http://dx.doi.org/7352/2455-5487.000029.
[28] Desmeules F, Boudreault J, Dionne CE, Frémont P, Lowry V, MacDermid JC, et al. Efficacy of exercise therapy in workers with rotator cuff tendinopathy: a systematic review. Journal of occupational health. 2016:15-0103-RA. http://dx.doi.org/10.1539/joh.15-0103-RA.
[29] Desmeules F, Boudreault J, Roy JS, Dionne CE, Frémont P, MacDermid JC. Efficacy of transcutaneous electrical nerve stimulation for rotator cuff tendinopathy: A systematic review. Physiotherapy (United Kingdom). 2016;102(1):41-9. http://dx.doi.org/10.1016/j.physio.2015.06.004.
[30] Ortega-Castillo M, Medina-Porqueres I. Effectiveness of the eccentric exercise therapy in physically active adults with symptomatic shoulder impingement or lateral epicondylar tendinopathy: a systematic review. Journal of science and medicine in sport. 2016;19(6):438-53. http://dx.doi.org/10.1016/j.jsams.2015.06.007.
[31] McKivigan JM, Yamashita B, Smith D. A Systematic Review on the Efficacy of Iontophoresis as a Treatment for Lateral Epicondylitis. Research & Investigations in Sports Medicine. 2017;1(3):[Article RISM. 000512]. http://dx.doi.org/10.31031/RISM.2017.01.000512

[32] Punnoose A, Norrish A, Pak K. Extracorporeal Shock Wave Therapy for Achilles and Patellar Tendinopathy: Meta-Analysis and a Systematic Review of the Literature. Journal of Physiotherapy and Physical Rehabilitation. 2017;2(1). http://dx.doi.org/10.4172/2573-0312.1000124.
[33] Liao C-D, Tsauo J-Y, Chen H-C, Liou T-H. Efficacy of Extracorporeal Shock Wave Therapy for Lower-Limb Tendinopathy: A Meta-analysis of Randomized Controlled Trials. American journal of physical medicine & rehabilitation. 2018;97(9):605-19. http://dx.doi.org/10.1097/PHM.0000000000000925.
[34] Liao C-D, Xie G-M, Tsauo J-Y, Chen H-C, Liou T-H. Efficacy of extracorporeal shock wave therapy for knee tendinopathies and other soft tissue disorders: a meta-analysis of randomized controlled trials. BMC musculoskeletal disorders. 2018;19(1):278. http://dx.doi.org/10.1186/s12891-018-2204-6.
[35] Lim HY, Wong SH. Effects of isometric, eccentric, or heavy slow resistance exercises on pain and function in individuals with patellar tendinopathy: A systematic review. Physiotherapy Research International. 2018;23(4):e1721. http://dx.doi.org/10.1002/pri.1721.
[36] Lucado AM, Dale RB, Vincent J, Day JM. Do joint mobilizations assist in the recovery of lateral elbow tendinopathy? A systematic review and meta-analysis. Journal of Hand Therapy. 2018. http://dx.doi.org/10.1016/j.jht.2018.01.010.
[37] Wilson F, Walshe M, O'Dwyer T, Bennett K, Mockler D, Bleakley C. Exercise, orthoses and splinting for treating Achilles tendinopathy: a systematic review with meta-analysis. Br J Sports Med. 2018;52(24):1564-74. http://dx.doi.org/10.1136/bjsports-2017-098913.
[38] Hickey C, Walker D, Lee S, Vitato N. The Long-Term Effects of Eccentric Exercise vs. Extracorporeal Shockwave Therapy in Athletes Aged 18-50 with Patellar Tendinopathy: A Meta-Analysis and Systematic Review. Ann Physiother Occup Ther. 2019;2(3):000130.
[39] Murphy MC, Travers MJ, Chivers P, Debenham JR, Docking SI, Rio EK, et al. Efficacy of heavy eccentric calf training for treating mid-portion Achilles tendinopathy: a systematic review and meta-analysis. British journal of sports medicine. 2019;53(17):1070-7. http://dx.doi.org/10.1136/bjsports-2018-099934.
[40] Yan C, Xiong Y, Chen L, Endo Y, Hu L, Liu M, et al. A comparative study of the efficacy of ultrasonics and extracorporeal shock wave in the treatment of tennis elbow: A meta-analysis of randomized controlled trials. Journal of Orthopaedic Surgery and Research. 2019;14(1). http://dx.doi.org/10.1186/s13018-019-1290-y.

# III

PHYSICAL THERAPY REVIEWS
https://doi.org/10.1080/10833196.2019.1695355

Taylor & Francis
Taylor & Francis Group

Check for updates

# Efficacy of physical therapy interventions for chronic lateral elbow tendinopathy: a systematic review

Beshoy Girgis and José Alberto Duarte

CIAFEL, Faculty of Sport, University of Porto, Porto, Portugal

**ABSTRACT**

**Study Design:** Systematic review.

**Background:** Existing systematic reviews and meta-analyses do not support a specific physical therapy treatment for lateral elbow tendinopathy (LET). The results of these studies, however, may not accurately represent the current state of evidence. This may be due, in part, to methodological limitations in these studies, specifically the lack of utilization of standardized and disease-specific outcome measures.

**Objectives:** To determine the effectiveness of physical therapy interventions in treating chronic lateral elbow tendinopathy as assessed in randomized controlled trials (RCTs) using pain-free grip strength and/or patient-rated tennis elbow evaluation questionnaire as outcome measures.

**Methods:** A search of PubMed, Physiotherapy Evidence Database (PEDro), Scopus database, Google Scholar and bibliography was conducted from inception to November 2018. The methodological quality of the identified RCTs were assessed using the PEDro scale and the modified Sackett scale was used to appraise findings based on the level of evidence.

**Results:** A total of two thousand nine hundred and six citations were identified, however, only twenty-two RCTs matched the eligibility criteria and were included in the review. The identified studies varied in design, investigated diverse physical therapy modalities that were applied for dissimilar periods, assessed outcome measures at unalike periods, and showed inconsistent results.

**Conclusion:** A rigorous review of literature showed insufficient evidence to support or refute the effectiveness of many of the current physical therapy interventions for chronic LET. Nevertheless, the results might suggest immediate efficacy of mobilization with movement of the elbow joint and short-term efficacy of myofascial release technique.

**Level of evidence:** Therapy, level 1a-

**ARTICLE HISTORY**
Received 12 June 2019
Accepted 14 November 2019

**KEYWORDS**
Chronic tendinopathy; chronic tendon disease; conservative management; lateral epicondylitis; tennis elbow

## Introduction

Lateral elbow tendinopathy (LET), commonly known as tennis elbow, was first described in 1873 by Runge and the term was derived from "lawn tennis arm" designated by Morris in 1882 [1,2]. LET was determined to have a relatively constant annual incidence of approximately 15.1 cases per 10,000 patients in the United States [3]. The prevalence of LET in the general population was reported to be 1% to 3% [4,5]. It is commonly diagnosed between ages of 42 to 56 [5,6]. Occupational exposure to non-neutral wrist and elbow postures, forceful and repetitive hand activities were identified as risk factors for the development of LET [7–11]. Other risk factors might include rotator cuff pathology, De Quervain's disease, carpal tunnel syndrome, oral corticosteroid therapy, and previous smoking history [6].

Initially, patients report pain related to activity at the lateral elbow that may be associated with reduced grip strength [12–14]. In the chronic and more severe stages, symptoms may progress so that pain occurs at rest and accompanies any activity which may lead to disability [15–17]. The typical duration of LET episode may range from six months to two years [18] and the majority of patients may spontaneously recover by twelve months [19,20]. Nevertheless, LET symptoms may persist for several years and the condition commonly recurs even after symptom recovery [21–27]. Chronic and severe cases frequently impair activities of daily living and cause disability [28,29]. Severe baseline pain, and long duration of symptoms and disability may be associated with poor prognosis [20,30].

### *Pathophysiology*

Many theories regarding the etiology of LET have been mentioned in the literature [31–35], but it has been historically considered an overuse strain of the

CONTACT Beshoy Girgis begirgis@gmail.com; up201301273@fade.up.pt CIAFEL, Faculty of Sport, University of Porto, Portugal, R. Dr. Plácido Costa, 91, 4200-450 Porto, Portugal

**Table 1.** Inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|---|---|
| • Randomized controlled trials | • Surgical interventions |
| • Chronic lateral elbow tendinopathy (>6 weeks) | • Invasive interventions |
| • Single physical therapy modality | • Pharmacologic interventions |
| • Multiple physical therapy modality(s), accepted if additional intervention was standardized in both groups | • Non-English language studies |
| • Outcomes assessed by PFGS and/or PRTEE questionnaire | • Abstracts only |

origin of wrist extensors [36,37]. It is thought to result from degeneration of the tendons of wrist extensor muscles, primarily the extensor carpi radialis brevis, at their proximal attachment at the lateral epicondyle, which is commonly described as tendinosis [38–40]. However, the pathological cascade of tendinopathy seems to include inflammation and degeneration [33,41]. Indeed, recent systematic reviews [42,43] and narrative reviews [32,44] have supported the role of inflammation in the pathogenesis of tendinopathy. Furthermore, other studies have also indicated that inflammation can promote the development of overuse tendon injury [45–52]. This concept of the involvement of an inflammatory component in tendinopathy is also supported by the animal models' findings, which have demonstrated accumulation of inflammatory cells and cytokines [48,53–56].

### Rationale

Conservative management is considered the first choice of treatment for LET [19,57,58]. Nonetheless, a comprehensive overview of systematic reviews has indicated that many of the available conservative interventions lack supporting evidence [59]; moreover, other systematic reviews and meta-analyses have concluded that existing evidence may not support a specific physical therapy treatment of LET [60–67]. Furthermore, these studies did not limit inclusion criteria to the standardized and disease-specific outcome measures that are recommended by experts in the field. These experts recommend the use of pain-free grip strength (PFGS) and patient-rated tennis elbow evaluation (PRTEE) questionnaire as more valid and responsive measures with respect to impairment and patient-reported outcomes, respectively [68–70]. The results of these reviews, consequently, may not accurately represent the clinical state of patients, thereby reducing the degree of certainty in the results. Indeed, the lack of standardization and disease-specificity of outcome measures hinder interpretation and statistical-treatment of results [59,71].

LET continues to be a condition difficult to properly manage, after more than a century of research and investigation [72,73], and an optimal treatment is yet undefined [74,75]. More than 40 treatment modalities have been described in literature for LET, including ultrasound therapy (US), transcutaneous electrical nerve stimulation (TENS), extracorporeal shockwave therapy (ESWT), low-level laser therapy (LLLT), manipulative therapy and elbow brace. However, none of these treatments has been determined to be better than any of the others [40,76–79]. The lack of head-to-head studies between different modalities may, however, contribute to this dilemma. Therefore, in the light of experts' recommendations to use pain-free grip strength (PFGS) and patient-rated tennis elbow evaluation (PRTEE) questionnaire as better outcome measures, reevaluation of literature for the effectiveness of physical therapy interventions in treating chronic LET is necessary.

### Objectives

This review was conducted to determine the effectiveness of physical therapy interventions in treating chronic lateral elbow tendinopathy as assessed in randomized controlled trials using pain-free grip strength and/or patient-rated tennis elbow evaluation questionnaire as outcome measures.

## Methods

### Eligibility criteria

The review was limited to published randomized controlled trials (RCTs) assessing the efficacy of physical therapy modalities in treating chronic LET (duration of symptoms more than 6 weeks [80–82]). Only studies that used PFGS and/or PRTEE questionnaire (formerly known as the patient-rated forearm evaluation questionnaire; PRFEQ [83]) as outcome measures were included. Studies with multiple physical therapy modality(s) were included only if the additional intervention(s) was standardized in both groups. Abstracts, studies written in non-English language, and studies that investigated surgical, invasive, and pharmacologic interventions were excluded. An overview of the eligibility criteria is shown in Table 1.

### Information sources

Electronic search of the National Institutes of Health (NIH) PubMed, the Physiotherapy Evidence

Database (PEDro), Scopus database, and Google Scholar was performed from inception to November 2018. Inspection of bibliographic references complemented the electronic search. The terms used in the electronic search were: *tennis elbow; lateral elbow tendinopathy; lateral epicondylitis; lateral humeral epicondylitis; lateral epicondylosis; lateral epicondylalgia; chronic lateral elbow tendinopathy; chronic tennis elbow; chronic lateral humeral epicondylitis; chronic lateral epicondylitis; chronic lateral epicondylosis; chronic lateral epicondylalgia.* The full search strategy of PubMed database is provided in the Appendix.

### Study selection process

A standard data sheet was used for data collection and management. Duplicates were identified and removed electronically using EndNote X8.2 software (Bld11343). Full-text articles were reviewed for possible inclusion following removal of duplicates and non-matching studies. Moreover, full-text articles were checked for eligibility in cases of abstracts. None of the authors were contacted for additional data.

### Data items

The data extracted from each study were study design, study inclusion and exclusion criteria, patients' characteristics (sample size, age and gender), duration of symptoms, intervention details, outcome measures, outcome assessment(s) and follow-up, and results. Primary and secondary outcomes were examined for relevant outcome measures.

### Outcome measures

The primary outcome was grip strength, determined through PFGS evaluation, since weakness with hand grip dynamometry is a specific sign of chronic LET [15,84–88]. PFGS measurement is valid, reliable, and more change-sensitive than maximal grip strength [84,89–97]. The position of the patient during strength testing must be standardized as the variability in elbow position was shown to affect grip strength [98–101]. The handle of the dynamometer should be set at the second position [94,102–105], and the mean value of three measurements should be calculated for data analysis [90].

The secondary outcomes were pain intensity and function status, determined using PRTEE questionnaire, since lateral elbow pain and impaired function are frequent in chronic LET [29]. The PRTEE questionnaire is a valid, reliable, and sensitive tool [71,83,106–109]. Moreover, the PRTEE questionnaire was specifically designed to evaluate patients with LET [108]. PRTEE is composed of 15 items, developed to rate elbow pain and arm disability in LET patients. A total score can be calculated on a scale of 100, where the score of 0 represents no pain or disability, while the score of 100 represents worst pain and disability. The results of PFGS and PRTEE questionnaire outcome measures were chronologically classified as immediate, short-term (3 weeks to 3 months after treatment), and long-term (6 months to 1 year after treatment), for practical reasons and simplification.

### Methodological quality assessment

The methodological quality of the identified RCTs was assessed using the Physiotherapy Evidence Database (PEDro) scale. The PEDro scale comprises a total of 11 items, and each satisfied item, with exception of the first item, contributes to 1 point in the total score (range = 0–10 points) [110]. The PEDro scale has showed good inter-rater reliability, and high construct and convergent validity [110–112]. The modified Sackett scale was used to appraise findings based on the level of evidence [113].

## Results

A total of 2906 citations, including title and abstract, were found using the electronic search of databases ($n = 1709$) and other sources ($n = 1197$). 1721 citations were screened for eligibility following the removal of duplicates ($n = 1185$). 71 full-text articles were assessed for eligibility after non-matching studies were excluded ($n = 1650$), and subsequently 49 full-text articles (out of 71) were excluded with reasons; investigated surgical interventions ($n = 12$), invasive interventions ($n = 17$), and pharmacologic interventions ($n = 5$), and written in languages other than English ($n = 15$).

Twenty-two randomized controlled trials (RCTs) matched the eligibility criteria and were included in the review [114–135]; fifteen studies were of parallel-group design [118–125,127,128,130–132,134,135], and seven studies were of cross-over design [114–117,126,129,133]. The identified studies varied in design, investigated diverse physical therapy modalities that were applied for dissimilar periods, assessed outcome measures at unalike periods, and showed inconsistent results. Further details are provided in the supplementary information. The flow of the review is presented according to preferred reporting items for systematic reviews and meta-analyses (PRISMA) in Figure 1.

### Methodological quality assessment results

The risk of bias assessment scores ranged between 3 and 9 (poor to excellent). Studies were then

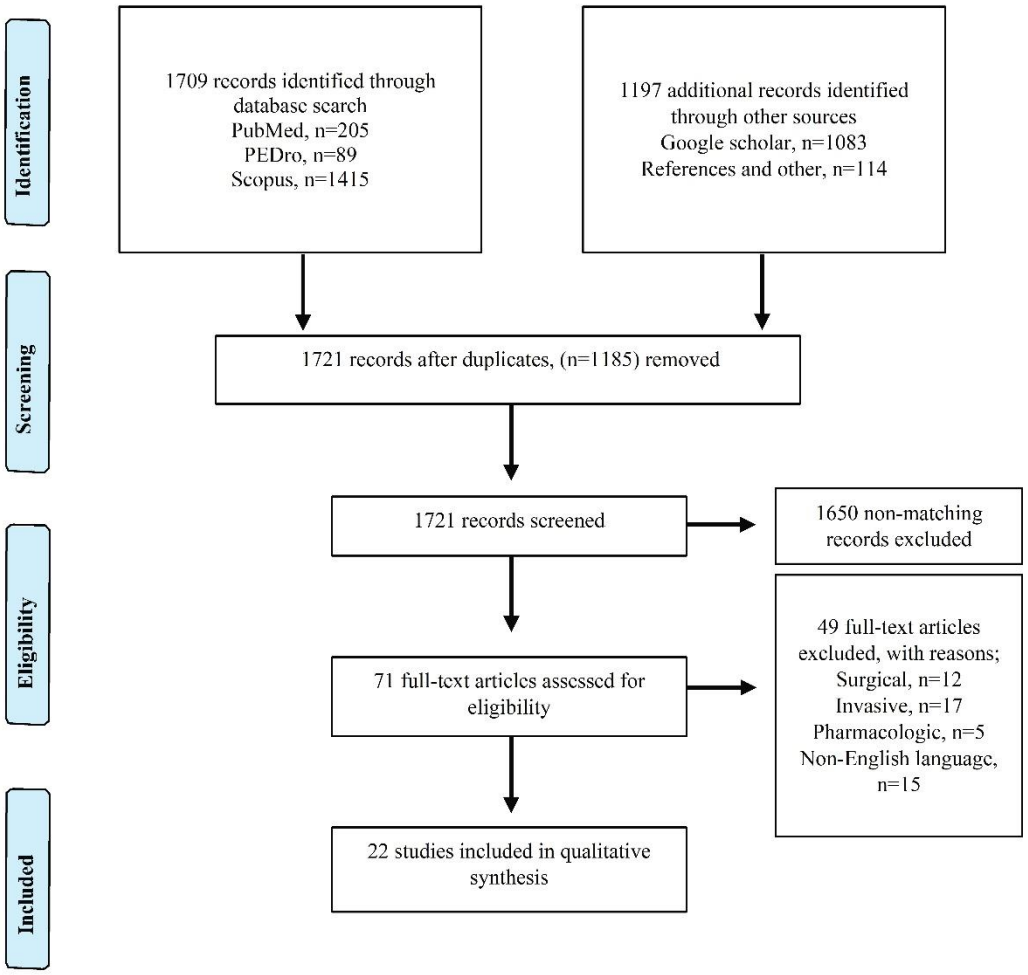



**Figure 1.** Study flow diagram according to preferred reporting items for systematic reviews and meta-analyses (PRISMA).

**Table 2.** Summary of methodological quality assessment of studies.

| PEDro scale | Total | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vicenzino et al. [114] | 4 | No | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| Vicenzino et al. [115] | 5 | Yes | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| Vicenzino et al. [116] | 4 | Yes | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Paungmali et al. [117] | 8 | Yes | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| Spacca et al. [118] | 7 | No | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 |
| Martinez-Silvestrini et al. [119] | 6 | Yes | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| D'Vaz et al. [120] | 7 | No | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| Faes et al. [121] | 6 | Yes | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| Staples et al. [122] | 8 | Yes | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| Coff et al. [123] | 8 | Yes | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| Ajimsha et al. [124] | 6 | Yes | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| Khuman et al. [125] | 6 | Yes | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| Bisset et al. [126] | 8 | Yes | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| Desai et al. [127] | 5 | Yes | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Capan et al. [128] | 6 | No | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 |
| Au et al. [129] | 5 | Yes | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| Ökmen et al. [130] | 7 | Yes | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| López-de-Celis et al. [131] | 9 | Yes | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| Upadhyay et al. [132] | 4 | Yes | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Cho et al. [133] | 5 | Yes | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| Nowotny et al. [134] | 3 | Yes | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Sethi et al. [135] | 5 | Yes | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 |

Total PEDro scale score is calculated out of 10 (first criterion score does not contribute to total score); (0) present, (1) absent. The scale items are 1: Eligibility criteria, 2: Random allocation, 3: Concealed allocation, 4: Baseline comparability, 5: Blind subjects, 6: Blind therapists, 7: Blind assessors, 8: Adequate follow-up, 9: Intention-to-treat analysis, 10: Between-group comparisons, 11: Point estimates and variability.

appraised based on the levels of evidence defined by the modified Sackett scale [113]. Thirteen studies [117–126,128,130,131] achieved a PEDro score of 6 or above and were therefore considered level I evidence, whereas nine studies [114–116,127,129, 132–135] received a PEDro score less than 6 and were consequently regarded as level II evidence. Summary of methodological quality assessment is provided in Table 2.

Concealed allocation was performed in only nine studies [117,121,123,125–128,130,131]. Only four studies included blinding of all subjects [118,120, 122,131] and only one study blinded therapists who administered therapy [120]. Twelve studies blinded assessors who measured outcomes [114,115,117, 120,122–124,126,128,130,131,133], while only eight studies applied "intention to treat" analysis [117–119,122,123,126,131,135]. Eleven studies incorporated placebo intervention [114–118,120,122, 124,128,129,133].

## Results of outcome measures assessment

### Pain-free grip strength

- Immediate

Vicenzino et al. [114] found significant effect of cervical spine manipulative physiotherapy beyond placebo intervention or no-intervention control, and reported a mean improvement in PFGS of 33.2 N ($p < 0.01$). Vicenzino et al. [116] assessed diamond taping technique and found improvement in PFGS by 24% from baseline ($p = 0.028$), and the treatment effect was greater than placebo and control. Vicenzino et al. [115] and Paungmali et al. [117] examined mobilization with movement treatment technique and found significant increase in PFGS of 58% during and 46% immediately after treatment, but not during and after placebo and control. Bisset et al. [126] found no significant difference between forearm-elbow brace, forearm brace, and no-brace ($p = 0.1$). Au et al. [129] assessed facilitatory, inhibitory, sham kinesio taping, and no-taping condition and found no significant difference between interventions ($p = 0.312$). Cho et al. [133] reported no difference between Y-shaped kinesio taping and sham taping ($p = 0.556$).

- Short-term (3 weeks–3 months)

Spacca *et al.* [118] found significant difference in PFGS between radial shockwave therapy intervention and control intervention at 4 weeks ($p < 0.001$), whereas, Staples et al. [122] found no significant difference between extracorporeal shockwave therapy and control intervention at 6 weeks and 3 months, even after adjusting for symptom duration. Martinez-Silvestrini et al. [119] examined eccentric strengthening exercise, concentric strengthening exercise, and stretching and found no significant difference between the three interventions ($p = 0.44$). Faes et al. [121] investigated dynamic extensor brace treatment and found significant improvement in PFGS at 12 weeks ($p < 0.005$), however, Nowotny et al. [134] assessed dynamic extension wrist orthosis and found no difference between groups at 12 weeks. López-de-Celis et al. [131] examined and found a statistically significant difference between diacutaneous fibrolysis and placebo group ($p < 0.01$), and control group ($p < 0.03$) at 3 weeks and 3 months. Sethi et al. [135] found no statistically significant difference between scapular muscles strengthening plus conventional physiotherapy and conventional physiotherapy alone ($p = 0.56$).

**Table 3.** Chronological classification of results as assessed by pain-free grip strength (PFGS).

| Study | PEDro | LOE |
|---|---|---|
| Immediate | | |
| Vicenzino et al. [114] | 4 | II |
| Vicenzino et al. [115] | 5 | II |
| Vicenzino et al. [116] | 4 | II |
| Paungmali et al. [117] | 8 | I |
| Bisset et al. [126] | 8 | I |
| Au at al. [129] | 5 | II |
| Cho et al. [133] | 5 | II |
| Short-term | | |
| Spacca et al. [118] | 7 | I |
| Martinez-Silvestrini et al. [119] | 6 | I |
| Faes et al. [121] | 6 | I |
| Staples et al. [122] | 8 | I |
| López-de-Celis et al. [131] | 9 | I |
| Nowotny et al. [134] | 3 | II |
| Sethi et al. [135] | 5 | II |
| Long-term | | |
| Spacca et al. [118] | 7 | I |
| Faes et al. [121] | 6 | I |
| Staples et al. [122] | 8 | I |
| Nowotny et al. [134] | 3 | II |

Short-term outcome assessment (3 weeks–3 months); long-term outcome assessment (6 months–1 year); PEDro, PEDro scale score; LOE, level of evidence according to the modified Sackett scale [113].

- Long-term (6 months–1 year)

Spacca et al. [118] found significant difference in PFGS between radial shockwave therapy intervention and control intervention at 6 months follow-up ($p < 0.001$), whereas, Staples et al. [122] found no significant difference between extracorporeal shockwave therapy and control intervention at 6 months. Faes et al. [121] investigated dynamic extensor brace treatment and found significant improvement in PFGS at 24 weeks ($p < 0.002$). Nowotny et al. [134] assessed dynamic extension wrist orthosis and found no difference between groups at 12 months. Summary of results as assessed by PFGS is presented in Table 3.

### Patient-rated tennis elbow evaluation questionnaire

- Immediate

None of the studies assessed changes in this outcome measure immediately after intervention.

- Short-term (3 weeks–3 months)

Martinez-Silvestrini et al. [119] found no significant difference between eccentric strengthening exercise, concentric strengthening exercise and stretching ($p = 0.87$). D'Vaz et al. [120] found no significant difference between pulsed low-intensity ultrasound and placebo therapy (7%; 95% CI: −20%–35%)

($p = 0.60$). Faes et al. [121] compared dynamic extensor brace to no brace and found significant difference between groups after 12 weeks. Coff et al. [123] found no significant difference in improvements between InterX® treatment group and conventional physiotherapy group at 3 weeks ($p = 0.61$). Ajimsha et al. [124] found statistically significant increase in PRTEE score in myofascial release technique group compared to sham ultrasound group at 4 weeks and 12 weeks ($p < 0.005$). Moreover, Khuman et al. [125] found statistically significant improvement in the myofascial release technique group compared to the control group (stretching, strengthening, and pulsed ultrasound) at 4 weeks ($p < 0.005$).

Desai et al. [127] assessed medial to lateral taping plus exercise program compared to exercise program alone and found statistically significant difference at 4 weeks ($p = 0.000$). Capan et al. [128] found no significant difference between radial shockwave therapy and sham intervention at 1 months ($p = 0.425$) and 3 months ($p = 0.517$). Ökmen et al. [130] compared peloid therapy plus lateral epicondylitis band to lateral epicondylitis band alone and found no statistically significant difference between groups at 2 weeks but found statistically significant difference at 6 weeks ($p < 0.05$). Upadhyay et al. [132] found statistically significant difference between progressive strengthening exercise plus conventional therapy and conventional therapy alone at 6 weeks ($p = 0.0025$). Nowotny et al. [134] found significant reduction in PRTEE score in dynamic extension wrist orthosis plus physiotherapy group but not in physiotherapy group alone at 12 weeks. Sethi et al. [135] found no statistically significant difference between scapular muscle strengthening plus conventional physiotherapy compared to conventional physiotherapy alone at 6 weeks ($p = 0.95$).

- Long-term (6 months - 1 year)

Faes et al. [121] compared dynamic extensor brace to no brace and found that the significant improvements were sustained at 6 months. Coff et al. [123] found no significant difference between InterX treatment group and conventional physiotherapy group at 9 months. Nowotny et al. [134] found no significant difference between dynamic extension wrist orthosis plus physiotherapy and physiotherapy alone at 12 months. Summary of results as assessed by PRTEE questionnaire is presented in Table 4. Outline of characteristics of studies is provided Table 5.

**Table 4.** Chronological classification of results as assessed by patient-rated tennis elbow evaluation (PRTEE) questionnaire.

| Study | PEDro | LOE |
|---|---|---|
| Immediate | | |
| N/A | N/A | |
| Short-term | | |
| Martinez-Silvestrini et al. [119] | 6 | I |
| D'Vaz et al. [120] | 7 | I |
| Faes et al. [121] | 6 | I |
| Coff et al. [123] | 8 | I |
| Ajimsha et al. [124] | 6 | I |
| Khuman et al. [125] | 6 | I |
| Desai et al. [127] | 5 | II |
| Capan et al. [128] | 6 | I |
| Ökmen et al. [130] | 7 | I |
| Upadhyay et al. [132] | 4 | II |
| Nowotny et al. [134] | 3 | II |
| Sethi et al. [135] | 5 | II |
| Long-term | | |
| Faes et al. [121] | 6 | I |
| Coff et al. [123] | 8 | I |
| Nowotny et al. [134] | 3 | II |

Short-term outcome assessment (3 weeks–3 months); long-term outcome assessment (6 months–1 year); PEDro, PEDro scale score; LOE, level of evidence according to the modified Sackett scale [113]; N/A, not applicable.

## Discussion

The aim of this study was to review the literature to determine the efficacy of physical therapy interventions in treating chronic lateral elbow tendinopathy. Twenty-two studies matched the eligibility criteria and were included in the present review. However, most of these studies did not assess the effects of physical therapy modalities beyond the short-term, and each intervention was evaluated in a small number of studies. Moreover, these studies demonstrated significant clinical, methodological, and statistical heterogeneity. Clinical heterogeneity was owed to differences in study population, treatment protocols, and timings of outcome assessment. Methodological heterogeneity was due to differences in study design, concealed allocation, blinding, inclusion of placebo intervention, and risk of bias. Statistical heterogeneity was due to inconsistency between study results. Additionally, potential prognostic factors, such as severe baseline pain and long duration of symptoms, were rarely considered. It is well known that the main advantage of meta-analysis is to provide objective, replicable, and systematic methods of summarizing outcomes of studies. However, due to the significant heterogeneity observed in the results, as previously discussed, statistical synthesis of data was not performed as per recommendations [136–141].

Nonetheless, results of the current review might suggest immediate efficacy of mobilization with movement of the elbow joint and short-term efficacy of myofascial release technique. Vicenzino et al. [115] and Paungmali et al. [117] assessed the immediate effect of mobilization with movement, compared pre to post intervention changes in PFGS, and reported an increase of 44.24 N (4.51 kg), and

**Table 5.** Summary of characteristics of studies.

| Study | Study design | Sample size | Age of subjects | Duration of symptoms | Intervention(s) | Outcome measure(s) | Outcome assessment(s) | Result(s) |
|---|---|---|---|---|---|---|---|---|
| Vicenzino et al. [114] | Randomized, double blind, placebo-controlled, repeated measures | Total:15<br>8F, 7M | 44 ± 2 years<br>(range 22.5–62 years) | 8 ± 2 months (range 2–36 months) | Cervical spine manipulative physiotherapy, placebo intervention and control (no intervention). All interventions applied in lots of 30s with 60s rest. One intervention per day in random order over three days. | • PPT<br>• PFGS<br>• ULTT2b<br>• Pain and function | Immediately before and after each intervention | Significant intervention effect beyond placebo or control for ULTT2b, PFGS, PPT and 24h pain scores ($p < 0.01$). Mean improvement in PFGS of 33.2 N. |
| Vicenzino et al. [115] | Randomized, repeated measures, double blind, placebo-controlled trial | Total:24<br>10F, 14M | 46.43 ± 1.68<br>(range 34–66 years) | 8.33 ± 1.71 months (range, 2–36 months) | Mobilization with movement treatment technique of the elbow, placebo technique, and control (no intervention). All interventions applied in random order, one per day, over three days at least 48h apart. | • PFGS<br>• PPT | • PFGS before, during and after each intervention.<br>• PPT before and after each intervention. | Significant increase in PFGS from pre-application values during (58%) and after (46%) treatment but not during and after placebo or control. PPT increased by approx. 10 % after treatment. |
| Vicenzino et al. [116] | Single blind, placebo-controlled, randomized, crossover study | Total:16<br>4F, 12M | 45.8 ± 10.2 years (range 24–61 years) | 13.1 ± 9.9 months | Diamond taping technique, placebo taping technique, and a no-tape control.<br>Patients were randomly assigned to a sequence of the three intervention conditions. | • PFGS<br>• PPT | Pretreatment, immediately post-intervention, and 30 min after intervention | The taping technique improved PFGS significantly by 24% from baseline ($p = 0.028$). Treatment effect was greater than placebo and control. PPT changes were not statistically significant. |
| Paungmali et al. [117] | Placebo-controlled repeated measures study | Total:24<br>7F, 17M | 48.5 ± 7.2 years | 8.9 ± 8.4 months | Mobilization with movement of the elbow, a placebo condition, and a control condition. Ten repetitions of the treatment technique were applied, with approximately 15s rest intervals between repetitions. All interventions applied in random order, one per day, over three days at least 48h apart. | • PFGS<br>• PPT<br>• TPT | Pretreatment, during application of treatment (PFGS) and immediately after intervention | PFGS increased during (37%) and after (47.5%) application of MWM treatment technique ($p = 0.001$), but not during or following the placebo or control. No change in PPT. Reduction in TPT in control. |
| Spacca et al. [118] | Prospective randomized controlled single-blind study | Total:62<br>30F, 32M | TG:46.82 ± 9.52 years<br>CG:47.03 ± 9.15 years | TG:12 ± 4.95 months<br>CG:13 ± 5.03 months | TG: 4 RSWT sessions at weekly intervals for 4 weeks, 2000 impulses each; 15 mm applicator, 1.2 bar and 4 Hz for 500 impulses and 1 bar and 10 Hz for 1500 impulses. | • VAS<br>• DASH<br>• PFGS | Pretreatment, end of treatment (4 weeks), and follow-up (6 months) | Significant difference in VAS, PFGS, and DASH in TG vs CG at end of treatment |

(*continued*)

**Table 5.** Continued.

| Study | Study design | Sample size | Age of subjects | Duration of symptoms | Intervention(s) | Outcome measure(s) | Outcome assessment(s) | Result(s) |
|---|---|---|---|---|---|---|---|---|
| | | | | | CG: 4 RSWT sessions at weekly intervals for 4 weeks, 20 impulses each; 15 mm applicator, 1.2 bar and 4 Hz for 5 impulses and 1 bar and 10 Hz for 15 impulses. | • Subjective satisfaction and NNT | | ($p < 0.001$) and at 6 months follow-up. Higher satisfaction in TG compared to CG. |
| Martinez-Silvestrini et al. [119] | Randomized controlled trial | Total:94<br>44F, 50M | 45.5 ± 7.7 years<br>TG1:46.6 years<br>TG2:47.0 years<br>CG:43.1 years | N/A | TG1: Eccentric strengthening home program for 6 weeks + Stretching and other conservative therapy home program<br>TG2: Concentric strengthening home program for 6 weeks + Stretching and other conservative therapy home program<br>CG: Stretching and other conservative therapy home program for 6 weeks | • PFGS<br>• VAS<br>• PRFEQ<br>• DASH<br>• SF-36 | Pretreatment and 6 weeks after treatment | No significant differences in outcome measures between the three groups (PFGS; $p = 0.44$) (PRFEQ; $p = 0.87$). |
| D'Vaz et al. [120] | Randomized, double-blind, placebo-controlled trial | Total:59<br>25F, 24M | TG:51 years (range 45–57)<br>CG:50 years (range 46–57) | TG:8 months (range 4–36)<br>CG:9 months (range 3–48) | TG: Pulsed LIUS, self-administered; 1.5MHz ultrasound signal modulated by on/Off square function, 20 min, daily for 12 weeks<br>CG: Placebo therapy, self-administered; devices did not emit ultrasound signal, 20 min, daily for 12 weeks | • VAS<br>• PRFEQ<br>• Grip strength<br>• Summary status | Baseline, 6 weeks and 12 weeks | No statistically significant difference between groups (7%; 95% CI: −20%–35%) ($p = 0.60$) |
| Faes et al. [121] | Randomized controlled trial, followed by crossover | Total:63<br>35F, 28M | TG:46 ± 8 years<br>CG:48 ± 9 years | TG:5.5 months (range 2–89)<br>CG:4 months (range 2–221) | TG: Dynamic extensor brace treatment, used throughout the day; for 12 weeks, followed by no brace period of 12 weeks<br>CG: No brace treatment, for 12 weeks, followed by brace treatment for 12 weeks | • VAS<br>• PRFEQ<br>• PFGS<br>• MGS | Baseline, and 6,121,824 weeks | Brace treatment resulted in significant pain reduction and improvement in PFGS ($p < 0.005$) and arm functionality after 12 weeks, and were sustained for another 12 weeks ($p < 0.002$) |
| Staples et al. [122] | Randomized, double-blind, placebo-controlled trial | Total:68<br>28F, 40M | TG:49.8 ± 7.4 years<br>CG:49.1 ± 8.8 years | TG:52.6 ± 64.3 weeks<br>CG:68 ± 98.8 weeks | TG: 3 ESWT sessions at weekly intervals; 2000 impulses per week, 240 per min, the energy level set as maximally tolerated<br>CG: 3 sub-therapeutic ESWT sessions at weekly intervals, 100 impulses per week, 90 per min, the energy did not exceed 0.03 mJ/mm$^2$ | • VAS<br>• Function<br>• DASH<br>• SF-36<br>• PFGS<br>• MGS<br>• PET | Baseline, 6 weeks,<br>3 months, and 6 months | Both groups improved significantly in almost all outcome measures. However, there were no differences between the groups even after adjusting for duration of symptoms |

(*continued*)

**Table 5.** Continued.

| Study | Study design | Sample size | Age of subjects | Duration of symptoms | Intervention(s) | Outcome measure(s) | Outcome assessment(s) | Result(s) |
|---|---|---|---|---|---|---|---|---|
| Coff et al. [123] | Randomized controlled trial | Total:26<br>13F, 13M | TG:44.8 ± 12 years<br>CG:43.6 ± 12 years | TG: range 2–120 months<br>CG: range 3–60 months | TG: InterX® treatment for 15–20 min, twice weekly for three weeks, plus intervention as in CG<br>CG: Cross friction and soft tissue massage, 3–5 min ultrasound, education regarding stretching and strengthening, twice weekly for three weeks | • VAS<br>• PRTEE<br>• Grip strength | Baseline, after 3 weeks (all outcomes), and 9 months (VAS and PRTEE only) | No significant difference in improvements between groups (PRTEE at 3 weeks; $p = 0.61$) |
| Ajimsha et al. [124] | Randomized, controlled, single-blind trial | Total:65<br>38F, 27M | TG:30.5 ± 4.9 years<br>CG:29.3 ± 4.9 years | TG:8.1 ± 2.8 months<br>CG:7.9 ± 3.2 months | TG: Myofascial release technique performed for 30 min, 3 times per week for 4 weeks<br>CG: Sham ultrasound therapy for 30 min, 3 times per week for 4 weeks | • PRTEE | Baseline, 4 weeks (after treatment), and 12 weeks (follow-up) | The myofascial release technique group scored more than control group at weeks 4 (78.7%) and 12 (63.1%) ($p < 0.005$) |
| Khuman et al. [125] | Randomized controlled study | Total:30<br>13F, 17M | TG:37.2 ± 3.35 years<br>CG:37.7 ± 2.79 years | TG:8.3 ± 2.05 months<br>CG:8.8 ± 2.44 months | TG: Myofascial release technique performed for 30 min/ session, 3 times a week for 4 weeks, plus intervention as in CG<br>CG: stretching, strengthening exercise, and pulsed ultrasound; 1MHz, 1.5 W/ $cm^2$, 1:4 ratio, for 5 min, 3 sessions per week for 4 weeks | • NPRS<br>• PRTEE<br>• Grip strength | Baseline and 4 weeks | Significant decrease in pain and improvement in function and grip strength in both groups. Myofascial release technique group improved significantly compared to control group ($p < 0.05$). |
| Bisset et al. [126] | Cross-over, double-blind randomized controlled trial | Total:34<br>16F, 18M | 47.8 ± 8.5 years (range 28–67) | 64.6 ± 137.4 weeks (range 6–570) | Forearm-elbow brace, forearm brace, and no-brace control condition were applied in randomized order, in 3 testing sessions, with at least 48 hours between each session. | • PFGS<br>• PPT<br>• Wrist angle during PFG | Immediately before and after each intervention | No significant difference between braces or the control condition for any outcome (PFGS; $p = 0.1$) |
| Desai et al. [127] | Randomized controlled trial | Total:40<br>21F, 19M | TG:41.15 ± 6.51 years<br>CG:39.45 ± 5.12 years | N/A | TG: Medial to lateral taping plus intervention as in CG<br>CG: Exercise program including stretching and eccentric exercise; 3 sessions per week for 4 weeks. | • VAS<br>• PRTEE | Baseline and 4 weeks | Statistically significant difference between groups in VAS and PRTEE scores ($p = 0.000$) after 4 weeks |
| Capan et al. [128] | Double-blind, randomized, placebo-controlled trial | Total:56<br>44F, 12M | TG:48.4 ± 9 years<br>CG:46.2 ± 7.4 years | TG:7.9 ± 5.2 months<br>CG:7.7 ± 5.2 months | TG: RSWT was applied once weekly for three consecutive weeks (three sessions total); 2000 impulses, 10Hz, 1.8 bar in each session, 15mm applicator.<br>CG: Sham RSWT; the same contact gel was applied to the same area without contact of applicator with the skin | • VAS<br>• RM<br>• PRTEE<br>• MGS | Baseline, 1 month, and 3 months | No significant difference between groups at 1 month ($p = 0.425$) and 3 months ($p = 0.517$) post-treatment |

(*continued*)

**Table 5.** Continued.

| Study | Study design | Sample size | Age of subjects | Duration of symptoms | Intervention(s) | Outcome measure(s) | Outcome assessment(s) | Result(s) |
|---|---|---|---|---|---|---|---|---|
| Au at al. [129] | Deceptive, crossover trial | Total:30<br>25F, 5M | 41.4 ± 11.6 years | N/A | Patients were randomly allocated into different sequences of four taping conditions: facilitatory KT, inhibitory KT, sham KT, and no tape condition | • NPRS<br>• PFGS<br>• MGS<br>• EMG | Immediately after each intervention | No significant differences in pain intensity, PFGS ($p = 0.312$), MGS, and EMG were recorded between interventions |
| Ökmen et al. [130] | Randomized, controlled, single-blind study | Total:65<br>37F, 24M | TG:43.9 ± 11.62 years<br>CG:45.63 ± 11.38 years | TG:5 months (range 3–24)<br>CG:5 months (range 3–48) | TG: Peloid therapy; five consecutive days a week for the first 2 weeks, for a total of 10 sessions, 47 °C peloid applied to the elbow for 20 min, plus intervention as in CG<br>CG: LEB; neoprene LEB during the day for 6 weeks, 2–2.5 cm distally to lateral epicondyle. No additional treatment | • PRTEE<br>• NHP | Baseline, 2 weeks and 6 weeks | No statistically significant difference between groups at 2 weeks ($p > 0.05$), but a statistically significant difference was found in favor of TG for all outcomes at 6 weeks ($p < 0.05$) |
| López-de-Celis et al. [131] | Double-blind randomized controlled trial | Total:54<br>33F, 21M | TG:46.72 ± 7.97 years<br>PG:47.89 ± 8.16 years<br>CG:50.67 ± 9.84 years | TG:8.1 ± 5.53 months<br>PG:14.8 ± 11.79 months<br>CG:8.4 ± 8.55 months | TG: Diacutaneous fibrolysis; 6 sessions (2 per week on non-consecutive days), 10 min each, plus intervention as in CG<br>PG: Sham diacutaneous fibrolysis; 6 sessions (2 per week on non-consecutive days), plus intervention as in CG<br>CG: Physical therapy protocol including therapeutic ultrasound, TENS, and stretching, daily sessions from Monday to Friday during three weeks | • VAS<br>• PFG<br>• PPT<br>• DASH | Baseline, 3 weeks (end of treatment), and 3 months | A statistically significant difference between TG and PG ($p < 0.01$) and TG and CG ($p < 0.03$) in pain-free grip strength at three months, and in function compared to CG ($p < 0.01$) |
| Upadhyay et al. [132] | Randomized controlled trial | Total:30<br>15F, 15M | TG:43.06 ± 6.57 years<br>CG:41 ± 7.67 years | TG:(range 6–9) months<br>CG:(range 7–8) months | TG: Progressive strengthening exercise; 4 set program, 10 repetitions in 2 or 3 series each, for 6 weeks plus intervention as in CG<br>CG: Conventional therapy including ultrasound, deep transverse friction massage, and stretching exercise; for 6 weeks | • VAS<br>• MGS<br>• PRTEE | Baseline and 6 weeks (end of treatment) | Statistically significant improvement in TG compared to CG in VAS ($p = 0.0003$), MGS ($p = 0.0131$), and PRTEE ($p = 0.0025$) |
| Cho et al. [133] | Randomized, double-blind, cross-over study | Total:15<br>10F, 5M | 52.3 ± 8.8 years | 4.9 ± 2.7 months | Patients received two taping sessions in a random order with a 3-day interval in between, one with two Y-shaped Kinesio taping strips and the other with sham taping | • NRS<br>• PFGS<br>• PPT | Before and 15 min after intervention | Both taping sessions significantly improved PFGS and significantly reduced NRS. Kinesio taping exhibited superiority in NRS compared to sham taping ($p = 0.03$) |

(*continued*)

**Table 5.** Continued.

| Study | Study design | Sample size | Age of subjects | Duration of symptoms | Intervention(s) | Outcome measure(s) | Outcome assessment(s) | Result(s) |
|---|---|---|---|---|---|---|---|---|
| Nowotny et al. [134] | Randomized controlled trial | Total:61<br><br>35F, 26M | TG:47 years<br><br>CG:46 years | N/A | TG: Dynamic extension wrist orthosis plus intervention as in CG<br><br>CG: Physiotherapy; daily eccentric strengthening exercise, 3 times a day for 10 min, 10 repetitions | • PRTEE<br>• VAS<br>• ROM<br>• PFGS<br>• MGS<br>• Placzek score | Baseline, 12 weeks and 12 months | PFGS improved significantly in both groups at 3 and 12 months. There was a statistically significant difference in PRTEE score in TG only at 12 weeks and improvement in both groups at 12 months |
| Sethi et al. [135] | Randomized controlled trial | Total:26<br><br>13F, 13M | TG:44.92 ± 10.84 years<br><br>CG:47.77 ± 9.44 years | TG:5.3 ± 2.49 months<br><br>CG:4.46 ± 1.66 months | TG: Scapular muscles strengthening; 3 sets of 10 repetitions, 3 times a week for 6 weeks, plus intervention as in CG<br><br>CG: Conventional physiotherapy; pulsed ultrasound, stretching, and eccentric strengthening exercise, 3 sessions per week for 6 weeks | • PRTEE<br>• VAS<br>• PFGS<br>• LSST<br>• EMG | Baseline and 6 weeks | Statistically significant group effect on EMG, but no statistically significant difference on VAS ($p = 0.09$), PFGS ($p = 0.56$), PRTEE ($p = 0.95$), scapular muscle strength, and LSST. |

F, female; M, Male; TG, treatment group; CG, control group; PG: placebo group; PPT, pressure-pain threshold; PFGS, pain-free grip strength; ULTT2b, upper limb tension test 2 b; TPT, thermal pain threshold; VAS, visual analogue scale; DASH, disabilities of the arm shoulder and hand questionnaire; NNT, number needed to treat; PRFEQ, patient-rated forearm evaluation questionnaire; RSWT, radial shockwave therapy; LIUS, low-intensity ultrasound therapy; MGS, maximum grip strength; SF-36, medical outcomes study short form-36 health survey; PET, problem elicitation technique; PRTEE, patient rated tennis elbow evaluation questionnaire; KT, Kinesio tape; NPRS, numerical pain rated scale; RM, roles and maudsley score; LEB, lateral epicondylitis band; NHP, Nottingham health profile; NRS, numerical rating scale; ROM, range of motion; N/A, not applicable; LSST, lateral scapular slide test; EMG, electromyography.

47 N (4.79 kg), respectively. These values, however, may not be clinically significant as the minimum clinically important difference (MCID) in grip strength was estimated at 58.83–63.74 N (6–6.5 kg) [142,143]. Ajimsha et al. [124] and Khuman et al. [125] examined the short-term effect of myofascial release technique and compared pre to post intervention changes in PRTEE questionnaire score. Ajimsha et al. [124] reported a reduction of 78.8% at 4 weeks and 63.3% at 12 weeks in the experimental group, and 6.8% at 4 weeks and increase in score by 2.17% at 12 weeks in the control group. Khuman et al. [125] reported a reduction of 82% at 4 weeks in the experimental group and 47.6% in the control group. These values may be clinically significant as the MCID in total PRTEE questionnaire score was estimated at 22% reduction of baseline score for clinical significance described as "a little better", and 37% reduction of baseline score for clinical significance described as "much better" or "completely recovered" [144]. Studies assessing the efficacy of extracorporeal shockwave therapy [118, 122, 128] and dynamic extensor orthosis [121,134] showed contradictory results.

The results of the present review are consistent with other systematic reviews and meta-analyses that examined the efficacy of physical therapy interventions for lateral elbow tendinopathy [60–67]. Bisset et al. [61] conducted a systematic review and meta-analysis, and concluded that evidence for long-term benefit of physical interventions is lacking and that extracorporeal shockwave therapy is ineffective in treating tennis elbow. This meta-analysis compared effect sizes of studies with different designs, nevertheless, different experimental designs may not be directly comparable unless adjustments are made [138,145–147]. A meta-analysis by Weber et al. [60] was conducted to evaluate the efficacy of physical therapy for the treatment of lateral epicondylitis, and concluded that differences between treatments were smaller than differences between treatment and control groups. However, pre-post effect sizes were used in this meta-analysis, a design that may lead to biased and inaccurate results [145,148,149]. Sayegh et al. [64] assessed, in a meta-analysis, the efficacy of nonsurgical treatments of lateral epicondylitis compared with observation or placebo treatments, and concluded that evidence for intermediate to long term clinical benefit of nonsurgical treatments is lacking. These meta-analyses, nonetheless, have pooled effect sizes of various conservative treatments with different protocols and outcome assessment timings, which may not be methodologically sound [137,150,151]. In the present review, a meta-analysis was not performed due to significant heterogeneity of the results.

Further systematic reviews have comparable conclusions to the results of the current review regarding specific interventions. These reviews, however, did not limit inclusion criteria to the recommended outcome measures [68–70]. Findings of Herd and Meserve [152] indicated the effectiveness of mobilization with movement, but they stated that their results should be generalized with care. Conversely, Hoogvliet et al. [153] concluded that evidence to support mobilization with movement is limited. Laimi et al. [154] mentioned that existing evidence on myofascial release therapy is insufficient to confirm its effectiveness for the treatment of chronic musculoskeletal pain. Reviews on the effectiveness of extracorporeal shockwave therapy had contradictory results. Stasinopoulos and Johnson [155] and Buchbinder et al. [156] showed that relevant studies had conflicting results. In addition, Rompe and Maffulli [157] concluded that qualitative evidence for the effectiveness of extracorporeal shockwave therapy exists under restrictive conditions only. Strujis et al. [158] demonstrated that definitive conclusions concerning the effectiveness of orthotic devices for LET could not be drawn. Other systematic reviews [159–165] have assessed the effectiveness of non-physical therapy interventions, however, these interventions are beyond the scope of the present review.

The multitude and diversity of the studies that investigated potential remedies for lateral elbow tendinopathy reflect the seriousness of this condition, and indicate that it may not be simply regarded as a self-limiting disease. Recent advances concerning the role of inflammation in the pathophysiology of this disease challenge traditional theories, and may yield breakthroughs in the management of this condition in the future.

### Limitations

- The restrictive criteria, of including published studies that were only written in English language and that used PFGS and/or PRTEE questionnaire as outcome measures, may have narrowed the scope of this review. These eligibility criteria were, however, chosen in order to enhance the methodological rigor and therefore facilitate reaching clinically relevant conclusions.
- Selective outcome reporting may have influenced the results, since most of the included studies used outcome measures other than PFGS and/or PRTEE questionnaire, nevertheless, the data of these measures were excluded from the review. However, the chosen outcomes represent more valid, reliable, and sensitive outcomes, and are also recommended by experts in the field.

- Studies in the present review were not blindly assessed which might have led to evaluation inaccuracy. Blinded assessment of methodological quality of studies was shown to result in more accurate and consistent evaluation scores [166], however another study challenged this conclusion [167].
- Other limitations inherent in the identified studies may include the following: Few studies assessed long term efficacy of physical therapy interventions. Procedures of PFGS measurement were inadequately reported, and patient positioning varied across studies. The effect sizes of mobilization with movement [115,117] and myofascial release technique [124,125] may be overestimated [168,169] as both subjects and therapists in these studies were not blinded. Intention to treat analysis was not performed in three of the studies [115,124,125], and allocation of patients was not concealed in two studies [115,124]. One of the included studies used only one part, the function part, of the PRTEE questionnaire [123].

### *Conclusions*

The literature assessment revealed insufficient evidence to support or refute the effectiveness of many of the current physical therapy interventions for chronic lateral elbow tendinopathy. Significant heterogeneity, inconsistency, and paucity of studies assessing each intervention hindered reaching conclusive results. Nonetheless, the results might support immediate efficacy of mobilization with movement of the elbow joint and short-term efficacy of myofascial release technique. Further research should assess long-term effects of interventions, implement standardized and recommended outcome measures, and have more rigorous procedures.

## Disclosure statement

No potential conflict of interest was reported by the authors

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Notes on contributors

*Beshoy Girgis* is a physical therapist. Currently he is a third-year PhD student in the Doctoral Program in Physiotherapy at the Faculty of Sport, University of Porto.

*José Alberto Duarte* is a full professor of physiology and the Head of the Laboratory of Biochemistry and Experimental Morphology at the Faculty of Sport, University of Porto; in this school, he also directs the PhD Course in Physiotherapy. He has published about two hundred scientific manuscripts in peer-reviewed journals and registered three international patents.

## ORCID

Beshoy Girgis http://orcid.org/0000-0003-3202-8609
José Alberto Duarte http://orcid.org/0000-0003-4756-5917

## References

1. Morris H. The rider's sprain. The Lancet. 1882; 120:133–134.
2. Runge F. Zur genese und behandlung des schreibekrampfes. Berl Klin Wochenschr. 1873;10: 245–248.
3. Degen RM, Conti MS, Camp CL, et al. Epidemiology and disease burden of lateral epicondylitis in the USA: analysis of 85,318 patients. HSS J. 2018;14:9–14.
4. Shiri R, Viikari-Juntura E, Varonen H, et al. Prevalence and determinants of lateral and medial epicondylitis: a population study. Am J Epidemiol. 2006;164:1065–1074.
5. Walker-Bone K, Palmer KT, Reading I, et al. Occupation and epicondylitis: a population-based study. Rheumatology 2012;51:305–310.
6. Titchener A, Fakis A, Tambe A, et al. Risk factors in lateral epicondylitis (tennis elbow): a case-control study. J Hand Surg Eur. 2013;38:159–164.
7. Haahr J, Andersen J. Physical and psychosocial risk factors for lateral epicondylitis: a population based case-referent study. Occup Environ Med. 2003;60:322–329.
8. Herquelot E, Guéguen A, Roquelaure Y, et al. Work-related risk factors for incidence of lateral epicondylitis in a large working population. Scand J Work Environ Health. 2013;39:578–588.
9. Fan ZJ, Silverstein BA, Bao S, et al. The association between combination of hand force and forearm posture and incidence of lateral epicondylitis in a working population. Hum Factors. 2014;56:151–165.
10. Seidel D, Ditchen D, Hoehne-Hückstädt U, et al. Quantitative measures of physical risk factors associated with work-related musculoskeletal disorders of the elbow: a systematic review. IJERPH. 2019; 16:130.
11. van Rijn RM, Huisstede BM, Koes BW, et al. Associations between work-related factors and specific disorders at the elbow: a systematic literature review. Rheumatology 2008;48:528–536.
12. Whaley AL, Baker CL. Lateral epicondylitis. Clin Sports Med. 2004;23:677–691.
13. Geoffroy P, Yaffe MJ, Rohan I. Diagnosing and treating lateral epicondylitis. Can Fam Physician. 1994;40:73.
14. Brummel J, Baker CL, III, Hopkins R, et al. Epicondylitis: lateral. Sports Med Arthrosc Rev. 2014;22:e1–e6.

15. Pienimäki TT, Siira PT, Vanharanta H. Chronic medial and lateral epicondylitis: a comparison of pain, disability, and function. Arch Phys Med Rehabil. 2002;83:317–321.
16. Pienimäkil T, Siiral P, Vanharantal H. Widespread pain in chronic epicondylitis. Eur J Pain. 2011;15: 921–927.
17. Noteboom T, Cruver R, Keller J, et al. Tennis elbow: a review. J Orthop Sports Phys Ther. 1994; 19:357–366.
18. Cyriax JH. The pathology and treatment of tennis elbow. J Bone Joint Surg Am. 1936;18:921–940.
19. Smidt N, Van Der Windt DA, Assendelft WJ, et al. Corticosteroid injections, physiotherapy, or a wait-and-see policy for lateral epicondylitis: a randomised controlled trial. The Lancet. 2002;359: 657–662.
20. Smidt N, Lewis M, Van Der Windt D, et al. Lateral epicondylitis in general practice: course and prognostic indicators of outcome. J Rheumatol. 2006;33:2053–2059.
21. Binder A, Hazleman B. Lateral humeral epicondylitis—a study of natural history and the effect of conservative therapy. Rheumatology 1983;22: 73–76.
22. Bisset L, Beller E, Jull G, et al. Mobilisation with movement and exercise, corticosteroid injection, or wait and see for tennis elbow: randomised trial. BMJ 2006;333:939.
23. Coombes BK, Bisset L, Brooks P, et al. Effect of corticosteroid injection, physiotherapy, or both on clinical outcomes in patients with unilateral lateral epicondylalgia: a randomized controlled trial. JAMA 2013;309:461–469.
24. Hudak PL, Cole DC, Haines AT. Understanding prognosis to improve rehabilitation: the example of lateral elbow pain. Arch Phys Med Rehabil. 1996;77:586–593.
25. Murtagh J. Tennis elbow. Aust Fam Physician. 1988;17:90–91.
26. Bot SDM, van der Waal JM, Terwee CB, et al. Course and prognosis of elbow complaints: a cohort study in general practice. Ann Rheum Dis. 2005;64:1331–1336.
27. Hamilton PG. The prevalence of humeral epicondylitis: a survey in general practice. J R Coll Gen Pract. 1986;36:464–465.
28. Solheim E, Hegna J, Øyen J. Extensor tendon release in tennis elbow: results and prognostic factors in 80 elbows. Knee Surg Sports Traumatol Arthrosc. 2011;19:1023–1027.
29. Lai WC, Erickson BJ, Mlynarek RA, et al. Chronic lateral epicondylitis: challenges and solutions. OAJSM. 2018;9:243.
30. Haahr J, Andersen J. Prognostic factors in lateral epicondylitis: a randomized trial with one-year follow-up in 266 new cases treated with minimal occupational intervention or the usual approach in general practice. Rheumatology 2003;42:1216–1225.
31. Cook J, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. Br J Sports Med. 2009;43:409–416.
32. Fu S-C, Rolf C, Cheuk Y-C, et al. Deciphering the pathogenesis of tendinopathy: a three-stages process. BMC Sports Sci Med Rehabil. 2010;2:30.
33. Abate M, Silbernagel KG, Siljeholm C, et al. Pathogenesis of tendinopathies: inflammation or degeneration? Arthritis Res Ther. 2009;11:235.
34. Arnoczky SP, Lavagnino M, Egerbacher M. The mechanobiological aetiopathogenesis of tendinopathy: is it the over-stimulation or the under-stimulation of tendon cells? Int J Exp Pathol. 2007;88: 217–226.
35. Leadbetter W. Cell-matrix response in tendon injury. Clin Sports Med. 1992;11:533–578.
36. Nirschl RP, Pettrone FA. Tennis elbow. The surgical treatment of lateral epicondylitis. J Bone Joint Surg Am. 1979;61:832–839.
37. Coonrad RW, Hooper WR. Tennis elbow: its course, natural history, conservative and surgical management. JBJS 1973;55:1177–1182.
38. Ernst E. Conservative therapy for tennis elbow. Br J Clin Pract. 1992;46:55.
39. Putnam MD, Cohen M. Painful conditions around the elbow. Orthop Clin North Am. 1999;30: 109–118.
40. Thurston A. Conservative and surgical treatment of tennis elbow: a study of outcome. ANZ J Surg. 1998;68:568–572.
41. Alfredson H, Cook J. A treatment algorithm for managing Achilles tendinopathy: new treatment options. Br J Sports Med. 2007;41:211–216.
42. Dean BJF, Gettings P, Dakin SG, et al. Are inflammatory cells increased in painful human tendinopathy? A systematic review. Br J Sports Med. 2016; 50:216–220.
43. Mosca MJ, Rashid MS, Snelling SJ, et al. Trends in the theory that inflammation plays a causal role in tendinopathy: a systematic review and quantitative analysis of published reviews. BMJ Open Sport Exerc Med. 2018;4:e000332.
44. Rees JD, Stride M, Scott A. Tendons–time to revisit inflammation. Br J Sports Med. 2013;47: e2.60.
45. Backman L, Andersson G, Wennstig G, et al. Endogenous substance P production in the Achilles tendon increases with loading in an in vivo model of tendinopathy: peptidergic elevation preceding tendinosis-like tissue changes. J Musculoskelet Neuronal Interact. 2011;11:133–140.
46. Fedorczyk JM, Barr AE, Rani S, et al. Exposure-dependent increases in IL-1$\beta$, substance P, CTGF, and tendinosis in flexor digitorum tendons with upper extremity repetitive strain injury. J Orthop Res. 2010;28:298–307.
47. Gao HG, Fisher PW, Lambi AG, et al. Increased serum and musculotendinous fibrogenic proteins following persistent low-grade inflammation in a rat model of long-term upper extremity overuse. PLoS One. 2013;8:e71875.
48. Kietrys DM, Barr-Gillespie AE, Amin M, et al. Aging contributes to inflammation in upper extremity tendons and declines in forelimb agility in a rat model of upper extremity overuse. PloS One. 2012;7:e46954.
49. Kragsnaes MS, Fredberg U, Stribolt K, et al. Stereological quantification of immune-competent cells in baseline biopsy specimens from Achilles tendons: results from patients with chronic tendinopathy followed for more than 4 years. Am J Sports Med. 2014;42:2435–2445.

50. Millar NL, Hueber AJ, Reilly JH, et al. Inflammation is present in early human tendinopathy. Am J Sports Med. 2010;38:2085–2091.
51. Spiesz EM, Thorpe CT, Chaudhry S, et al. 98 Early inflammatory response of tenocytes to overload. Br J Sports Med. 2014;48:A63.2–A64.
52. Sullo A, Maffulli N, Capasso G, et al. The effects of prolonged peritendinous administration of PGE1 to the rat Achilles tendon: a possible animal model of chronic Achilles tendinopathy. J Orthop Sci. 2001;6:349–357.
53. Pingel J, Wienecke J, Kongsgaard M, et al. Increased mast cell numbers in a calcaneal tendon overuse model. Scand J Med Sci Sports. 2013;23: e353–e360.
54. Millar N, Wei A, Molloy T, et al. Cytokines and apoptosis in supraspinatus tendinopathy. Bone Joint J. 2009;91:417–424.
55. Dohnert MB, Venâncio M, Possato JC, et al. Gold nanoparticles and diclofenac diethylammonium administered by iontophoresis reduce inflammatory cytokines expression in Achilles tendinitis. Int J Nanomed. 2012;7:1651.
56. Legerlotz K, Jones G, Screen H, et al. Cyclic loading of tendon fascicles using a novel fatigue loading system increases interleukin-6 expression by tenocytes. Scand J Med Sci Sports. 2013;23:31–37.
57. Newcomer KL, Laskowski ER, Idank DM, et al. Corticosteroid injection in early treatment of lateral epicondylitis. Clin J Sport Med. 2001;11: 214–222.
58. Abat F, Alfredson H, Cucchiarini M, et al. Current trends in tendinopathy: consensus of the ESSKA basic science committee. Part II: treatment options. J Exp Orthop. 2018;5:38.
59. Long L, Briscoe S, Cooper C, et al. What is the clinical effectiveness and cost-effectiveness of conservative interventions for tendinopathy? An overview of systematic reviews of clinical effectiveness and systematic review of economic evaluations. Health Technol Assess. 2015;19:1–134.
60. Weber C, Thai V, Neuheuser K, et al. Efficacy of physical therapy for the treatment of lateral epicondylitis: a meta-analysis. BMC Musculoskelet Disord. 2015;16:223.
61. Bisset L, Paungmali A, Vicenzino B, et al. A systematic review and meta-analysis of clinical trials on physical interventions for lateral epicondylalgia. Br J Sports Med. 2005;39:411–422.
62. Dingemanse R, Randsdorp M, Koes BW, et al. Evidence for the effectiveness of electrophysical modalities for treatment of medial and lateral epicondylitis: a systematic review. Br J Sports Med. 2014;48:957–965.
63. Kohia M, Brackle J, Byrd K, et al. Effectiveness of physical therapy treatments on lateral epicondylitis. J Sport Rehabil. 2008;17:119–136.
64. Sayegh ET, Strauch RJ. Does nonsurgical treatment improve longitudinal outcomes of lateral epicondylitis over no treatment? A meta-analysis. Clin Orthop Relat Res. 2015;473:1093–1107.
65. Sims SE, Miller K, Elfar JC, et al. Non-surgical treatment of lateral epicondylitis: a systematic review of randomized controlled trials. Hand (New York, NY). 2014;9:419–446.
66. Smidt N, Assendelft W, Arola H, et al. Effectiveness of physiotherapy for lateral epicondylitis: a systematic review. Ann Med. 2003; 35:51–62.
67. Trudel D, Duley J, Zastrow I, et al. Rehabilitation for patients with lateral epicondylitis: a systematic review. J Hand Ther. 2004;17:243–266.
68. Bisset LM, Vicenzino B. Physiotherapy management of lateral epicondylalgia. J Physiother. 2015; 61:174–181.
69. MacDermid JC, Silbernagel KG. Outcome evaluation in tendinopathy: foundations of assessment and a summary of selected measures. J Orthop Sports Phys Ther. 2015;45:950–964.
70. Coombes BK, Bisset L, Vicenzino B. Management of lateral elbow tendinopathy: one size does not fit all. J Orthop Sports Phys Ther. 2015;45:938–949.
71. Evans JP, Porter I, Gangannagaripalli JB, et al. Assessing patient-centred outcomes in lateral elbow tendinopathy: a systematic review and standardised comparison of English language clinical rating systems. Sports Med Open. 2019;5:10.
72. Hong QN, Durand MJ, Loisel P. Treatment of lateral epicondylitis: where is the evidence? Joint Bone Spine. 2004;71:369–373.
73. Lipman K, Wang C, Ting K, et al. Tendinopathy: injury, repair, and current exploration. DDDT. 2018;12:591.
74. Alizadehkhaiyat O, Fisher AC, Kemp GJ, et al. Assessment of functional recovery in tennis elbow. J Electromyogr Kinesiol. 2009;19:631–638.
75. Bhabra G, Wang A, Ebert JR, et al. Lateral elbow tendinopathy: development of a pathophysiology-based treatment algorithm. Orthop J Sports Med. 2016;4(11):2325967116670635.
76. Chard M, Hazleman B. Tennis elbow—a reappraisal. New York (NY): Oxford University Press; 1989.
77. Khan KM, Cook JL, Bonar F, et al. Histopathology of common tendinopathies. Sports Med. 1999;27: 393–408.
78. Labelle H, Guibert R, Joncas J, et al. Lack of scientific evidence for the treatment of lateral epicondylitis of the elbow. An attempted meta-analysis. J Bone Joint Surg Br. 1992;74:646–651.
79. Dion S, Wong JJ, Côté P, et al. Are passive physical modalities effective for the management of common soft tissue injuries of the elbow: a systematic review by the Ontario protocol for traffic injury management (OPTIMa) collaboration. Clin J Pain. 2017;33:71–86.
80. Fredberg U, Stengaard-Pedersen K. Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation. Scand J Med Sci Sports. 2008;18:3–15.
81. Järvinen M, Jozsa L, Kannus P, et al. Histopathological findings in chronic tendon disorders. Scand J Med Sci Sports. 1997;7:86–95.
82. Childress MA, Beutler A. Management of chronic tendon injuries. Am Fam Phys. 2013;87:486–490.
83. MacDermid J. Update: the patient-rated forearm evaluation questionnaire is now the patient-rated tennis elbow evaluation. J Hand Ther. 2005;18: 407–410.
84. Stratford P, Lavy D, Gowland C. Evaluative properties of measures used to assess patients with lateral epicondylitis at the elbow. Physiother Can. 1993;45:160–164.

85. Pienimäki T, Siira P, Vanharanta H. Muscle function of the hand, wrist and forearm in chronic lateral epicondylitis. Eur J Phys Med Rehabil. 1997;7: 171–178.
86. Coombes BK, Bisset L, Vicenzino B. Elbow flexor and extensor muscle weakness in lateral epicondylalgia. Br J Sports Med. 2012;46:449–453.
87. Alizadehkhaiyat O, Fisher AC, Kemp GJ, et al. Upper limb muscle imbalance in tennis elbow: a functional and electromyographic assessment. J Orthop Res. 2007;25:1651–1657.
88. Chourasia AO, Buhr KA, Rabago DP, et al. Effect of lateral epicondylosis on grip force development. J Hand Ther. 2012;25:27–37.
89. Stratford PW, Levy DR. Assessing valid change over time in patients with lateral epicondylitis at the elbow. Clin J Sport Med. 1994;4:88–91.
90. Lim E. Pain free grip strength test. J Physiother. 2013;59:59.
91. Blanchette M-A, Normand MC. Impairment assessment of lateral epicondylitis through electromyography and dynamometry. J Can Chiropr Assoc. 2011;55:96–106.
92. Hamilton A, Balnave R, Adams R. Grip strength testing reliability. J Hand Ther. 1994;7:163–170.
93. Innes E. Handgrip strength testing: a review of the literature. Aust Occ Ther J. 1999;46:120–140.
94. Mathiowetz V, Weber K, Volland G, et al. Reliability and validity of grip and pinch strength evaluations. J Hand Surg. 1984;9:222–226.
95. Smidt N, van der Windt DA, Assendelft WJ, et al. Interobserver reproducibility of the assessment of severity of complaints, grip strength, and pressure pain threshold in patients with lateral epicondylitis. Arch Phys Med Rehabil. 2002;83:1145–1150.
96. Stratford PW, Norman GR, McIntosh JM. Generalizability of grip strength measurements in patients with tennis elbow. Phys Ther. 1989;69: 276–281.
97. Stratford P. Extensor carpi radialis tendonitis: a validation of selected outcome measures. Physiother Can. 1987;39:250–255.
98. Ee F. Grip strength. Clinical assessment recommendations. 2nd ed. Chicago (IL): American Society of Hand Therapists; 1992.
99. Balogun JA, Akomolafe CT, Amusa LO. Grip strength: effects of testing posture and elbow position. Arch Phys Med Rehabil. 1991;72:280–283.
100. Dorf ER, Chhabra AB, Golish SR, et al. Effect of elbow position on grip strength in the evaluation of lateral epicondylitis. J Hand Surg. 2007;32: 882–886.
101. Mathiowetz V, Rennells C, Donahoe L. Effect of elbow position on grip and key pinch strength. J Hand Surg. 1985;10:694–697.
102. Ashton L, Myers S. Serial grip strength testing-its role in assessment of wrist and hand disability. Internet J Surg. 2004;5:1–12.
103. Oxford KL. Elbow positioning for maximum grip performance. J Hand Ther. 2000;13:33–36.
104. Su C-Y, Lin J-H, Chien T-H, et al. Grip strength in different positions of elbow and shoulder. Arch Phys Med Rehabil. 1994;75:812–815.
105. Firrell JC, Crain GM. Which setting of the dynamometer provides maximal grip strength? J Hand Surg. 1996;21:397–401.
106. Overend TJ, Wuori-Fearn JL, Kramer JF, et al. Reliability of a patient-rated forearm evaluation questionnaire for patients with lateral epicondylitis. J Hand Ther. 1999;12:31–37.
107. Rompe JD, Overend TJ, MacDermid JC. Validation of the patient-rated tennis elbow evaluation questionnaire. J Hand Ther. 2007;20:3–11.
108. Newcomer KL, Martinez-Silvestrini JA, Schaefer MP, et al. Sensitivity of the patient-rated forearm evaluation questionnaire in lateral epicondylitis. J Hand Ther. 2005;18:400–406.
109. Vincent J, MacDermid JC. Patient-rated tennis elbow evaluation questionnaire. J Physiother. 2014; 60:240.
110. Maher CG, Sherrington C, Herbert RD, et al. Reliability of the PEDro scale for rating quality of randomized controlled trials. Phys Ther. 2003;83: 713–721.
111. de Morton NA. The PEDro scale is a valid measure of the methodological quality of clinical trials: a demographic study. Aust J Physiother. 2009;55: 129–133.
112. Yamato TP, Maher C, Koes B, et al. The PEDro scale had acceptably high convergent validity, construct validity, and interrater reliability in evaluating methodological quality of pharmaceutical trials. J Clin Epidemiol. 2017;86:176.
113. Straus SR, Glasziou P, Haynes RB. Evidence-based medicine: how to practice and teach EBM. 3rd ed. Toronto: Elsevier Churchill Livingstone; 2005.
114. Vicenzino B, Collins D, Wright A. The initial effects of a cervical spine manipulative physiotherapy treatment on the pain and dysfunction of lateral epicondylalgia. Pain 1996;68:69–74.
115. Vicenzino B, Paungmali A, Buratowski S, et al. Specific manipulative therapy treatment for chronic lateral epicondylalgia produces uniquely characteristic hypoalgesia. Man Ther. 2001;6: 205–212.
116. Vicenzino B, Brooksbank J, Minto J, et al. Initial effects of elbow taping on pain-free grip strength and pressure pain threshold. J Orthop Sports Phys Ther. 2003;33:400–407.
117. Paungmali A, O'Leary S, Souvlis T, et al. Hypoalgesic and sympathoexcitatory effects of mobilization with movement for lateral epicondylalgia. Phys Ther. 2003;83:374–383.
118. Spacca G, Necozione S, Cacchio A. Radial shock wave therapy for lateral epicondylitis: a prospective randomised controlled single-blind study. Eura Medicophys. 2005;41:17–25.
119. Martinez-Silvestrini JA, Newcomer KL, Gay RE, et al. Chronic lateral epicondylitis: comparative effectiveness of a home exercise program including stretching alone versus stretching supplemented with eccentric or concentric strengthening. J Hand Ther. 2005;18:411–419; quiz 420.
120. D'vaz A, Ostor A, Speed C, et al. Pulsed low-intensity ultrasound therapy for chronic lateral epicondylitis: a randomized controlled trial. Rheumatology. 2005;45:566–570.
121. Faes M, van den Akker B, de Lint JA, et al. Dynamic extensor brace for lateral epicondylitis. Clin Orthop Relat Res. 2006;442:149–157.
122. Staples MP, Forbes A, Ptasznik R, et al. A randomized controlled trial of extracorporeal

shock wave therapy for lateral epicondylitis (tennis elbow). J Rheumatol. 2008;35:2038–2046.
123. Coff L, Massy-Westropp N, Caragianis S. Randomized controlled trial of a new electrical modality (InterX) and soft tissue massage, stretching, ultrasound and exercise for treating lateral epicondylitis. Hand Ther. 2009;14:46–52.
124. Ajimsha M, Chithra S, Thulasyammal RP. Effectiveness of myofascial release in the management of lateral epicondylitis in computer professionals. Arch Phys Med Rehabil. 2012;93:604–609.
125. Khuman PR, Trivedi P, Devi S, et al. Myofascial release technique in chronic lateral epicondylitis: a randomized controlled study. Int J Health Sci Res. 2013;3:45–52.
126. Bisset LM, Collins NJ, Offord SS. Immediate effects of 2 types of braces on pain and grip strength in people with lateral epicondylalgia: a randomized controlled trial. J Orthop Sports Phys Ther. 2014;44:120–128.
127. Desai BD. Effectiveness of medial to lateral taping with exercise programme in subjects with lateral epicondylitis. Int J Physiother. 2014;1:83–90.
128. Capan N, Esmaeilzadeh S, Oral A, et al. Radial extracorporeal shock wave therapy is not more effective than placebo in the management of lateral epicondylitis: a double-blind, randomized, placebo-controlled trial. Am J Phys Med Rehabil. 2016;95:495–506.
129. Au IPH, Fan PCP, Lee WY, et al. Effects of Kinesio tape in individuals with lateral epicondylitis: a deceptive crossover trial. Physiother Theory Pract. 2017;33:914–919.
130. Ökmen BM, Eröksüz R, Altan L, et al. Efficacy of peloid therapy in patients with chronic lateral epicondylitis: a randomized, controlled, single blind study. Int J Biometeorol. 2017;61:1965–1972.
131. López-de-Celis C, Barra-López M-E, González-Rueda V, et al. Effectiveness of diacutaneous fibrolysis for the treatment of chronic lateral epicondylalgia: a randomized clinical trial. Clin Rehabil. 2018;32:644–653.
132. Upadhyay S, Shukla Y, Patel KK. Effects of progressive strengthening exercises in chronic lateral epicondylitis. Int J Health Sci Res. 2017;7:244–257.
133. Cho Y-T, Hsu W-Y, Lin L-F, et al. Kinesio taping reduces elbow pain during resisted wrist extension in patients with chronic lateral epicondylitis: a randomized, double-blinded, cross-over study. BMC Musculoskelet Disord. 2018;19:193.
134. Nowotny J, El-Zayat B, Goronzy J, et al. Prospective randomized controlled trial in the treatment of lateral epicondylitis with a new dynamic wrist orthosis. Eur J Med Res. 2018;23:43.
135. Sethi K, Noohu MM. Scapular muscles strengthening on pain, functional outcome and muscle activity in chronic lateral epicondylalgia. J Orthop Sci. 2018;23:777–782.
136. Melsen W, Bootsma M, Rovers M, et al. The effects of clinical and statistical heterogeneity on the predictive values of results from meta-analyses. Clin Microbiol Infect. 2014;20:123–129.
137. Slavin RE. Best evidence synthesis: an intelligent alternative to meta-analysis. J Clin Epidemiol. 1995;48:9–18.
138. Morris SB, DeShon RP. Combining effect size estimates in meta-analysis with repeated measures and independent-groups designs. Psychol Methods. 2002;7:105.
139. Victor N. The challenge of meta-analysis": discussion, indications and contra-indications for meta-analysis. J Clin Epidemiol. 1995;48:5–8.
140. Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. Ann Intern Med. 1997;127:820–826.
141. Bender R, Friede T, Koch A, et al. Methods for evidence synthesis in the case of very few studies. Res Synth Methods 2018;9:382–392.
142. Nitschke JE, McMeeken JM, Burry HC, et al. When is a change a genuine change: a clinically meaningful interpretation of grip strength measurements in healthy and disabled women. J Hand Ther. 1999;12:25–30.
143. Kim JK, Park MG, Shin SJ. What is the minimum clinically important difference in grip strength? Clin Orthop Relat Res. 2014;472:2536.
144. Poltawski L, Watson T. Measuring clinically important change with the patient-rated tennis elbow evaluation. Hand Ther. 2011;16:52–57.
145. Ray JW, Shadish WR. How interchangeable are different estimators of effect size? J Consult Clin Psychol. 1996;64:1316.
146. Glass GV, Smith ML, McGaw B. Meta-analysis in social research. Beverly Hills: Sage Publications, Incorporated; 1981.
147. Morris SB, DeShon RP. Correcting effect sizes computed from factor analysis of variance for use in meta-analysis. Psychol Methods. 1997;2:192.
148. Cuijpers P, Weitz E, Cristea I, et al. Pre-post effect sizes should be avoided in meta-analyses. Epidemiol Psychiatr Sci. 2017;26:364–368.
149. Morris SB. Estimating effect sizes from pretest-posttest-control group designs. Org Res Methods. 2008;11:364–386.
150. Durlak JA. How to select, calculate, and interpret effect sizes. J Pediatr Psychol. 2009;34:917–928.
151. Vacha-Haase T, Thompson B. How to estimate and interpret various effect sizes. J Counsel Psychol. 2004;51:473.
152. Herd CR, Meserve BB. A systematic review of the effectiveness of manipulative therapy in treating lateral epicondylalgia. J Man Manip Ther. 2008;16:225–237.
153. Hoogvliet P, Randsdorp MS, Dingemanse R, et al. Does effectiveness of exercise therapy and mobilisation techniques offer guidance for the treatment of lateral and medial epicondylitis? A systematic review. Br J Sports Med. 2013;47:1112.
154. Laimi K, Makila A, Barlund E, et al. Effectiveness of myofascial release in treatment of chronic musculoskeletal pain: a systematic review. Clin Rehabil. 2018;32:440–450.
155. Stasinopoulos D, Johnson M. Effectiveness of extracorporeal shock wave therapy for tennis elbow (lateral epicondylitis). Br J Sports Med. 2005;39:132–136.
156. Buchbinder R, Assendelft WJ, Barnsley L, et al. Shock wave therapy for lateral elbow pain. Cochrane Database Syst Rev. 2002;4:CD003524.
157. Rompe JD, Maffulli N. Repetitive shock wave therapy for lateral elbow tendinopathy (tennis elbow): a systematic and qualitative analysis. Br Med Bull. 2007;83:355–378.

158. Struijs P, Smidt N, Arola H, et al. Orthotic devices for tennis elbow: a systematic review. Br J Gen Pract. 2001;51:924–929.
159. Assendelft W, Hay EM, Adshead R, et al. Corticosteroid injections for lateral epicondylitis: a systematic overview. Br J Gen Pract. 1996;46: 209–216.
160. de Vos R-J, Windt J, Weir A. Strong evidence against platelet-rich plasma injections for chronic lateral epicondylar tendinopathy: a systematic review. Br J sports Med. 2014;48:952–956.
161. Mattie R, Wong J, McCormick Z, et al. Percutaneous needle tenotomy for the treatment of lateral epicondylitis: a systematic review of the literature. PM R 2017;9:603–611.
162. Olaussen M, Holmedal O, Lindbaek M, et al. Treating lateral epicondylitis with corticosteroid injections or non-electrotherapeutical physiotherapy: a systematic review. BMJ Open. 2013;3: e003564.
163. Saltychev M, Laimi K, Virolainen P, et al. Effectiveness of platelet-rich plasma in treatment of lateral epicondylitis–a systematic review and meta-analysis. PRM 2018;1:7–16.
164. Smidt N, Assendelft WJ, van der Windt DA, et al. Corticosteroid injections for lateral epicondylitis: a systematic review. Pain 2002;96:23–40.
165. Kalichman L, Bannuru RR, Severin M, et al. Injection of botulinum toxin for treatment of chronic lateral epicondylitis: systematic review and meta-analysis. Semin Arthritis Rheum. 2011;40: 532–538.
166. Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials. 1996;17: 1–12.
167. Morissette K, Tricco AC, Horsley T, et al. Blinded versus unblinded assessments of risk of bias in studies included in a systematic review. Cochrane Database Syst Rev. 2011;9:MR000025.
168. Jüni P, Altman DG, Egger M. Systematic reviews in health care: assessing the quality of controlled clinical trials. BMJ 2001;323:42–46.
169. Schulz KF, Chalmers I, Hayes RJ, et al. Empirical evidence of bias: dimensions of methodological quality associated with estimates of treatment effects in controlled trials. JAMA 1995;273: 408–412.

## Appendix: Search strategy

((((((((((((((tennis elbow[MeSH Major Topic]) OR tennis elbow[Title/Abstract]) OR lateral epicondylitis[Title/Abstract]) OR lateral elbow tendinopathy[Title/Abstract]) OR lateral humeral epicondylitis[Title/Abstract]) OR lateral epicondylosis[Title/Abstract]) OR lateral epicondylalgia [Title/Abstract]) OR chronic lateral epicondylitis[Title/Abstract]) OR chronic lateral elbow tendinopathy[Title/Abstract]) OR chronic lateral humeral epicondylitis[Title/Abstract]) OR chronic lateral epicondylosis[Title/Abstract]) OR chronic tennis elbow[Title/Abstract]) OR chronic lateral epicondylalgia [Title/Abstract]) AND randomized controlled trial[Publication Type]) AND English[Language]))

# Supplemental material

| Study | Study design | Inclusion criteria | Exclusion criteria | Limitations |
|---|---|---|---|---|
| **Vicenzino et al. (1996) [1]** | Randomized, double blind, placebo controlled, repeated measures | Lateral elbow pain reproduced by at least three of the following tests: palpation over the lateral epicondyle region, stretch of the forearm extensor muscles, resisted static contraction of the wrist extensors or extensor carpi radialis brevis, or static contraction in gripping | Subjects with sequelae of previous pain, dysfunction or impaired sensation in the forequarter (i.e. cervical spine to hand) necessitating treatment were excluded. Recent steroid injection(s) into the elbow also excluded subjects | N/A |
| **Vicenzino et al. (2001) [2]** | Randomized, repeated measures, double blind, placebo-controlled trial | Pain provoked on digital palpation over the lateral epicondyle and on gripping a hand dynamometer. In addition, pain had to be experienced over the lateral epicondyle in at least one of the following tests: resisted static contraction of the wrist extensors or extensor carpi radialis brevis; or stretching of the extensor muscles of the forearm | Subjects were excluded if they had concomitant problems in the neck or upper limbs rather than lateral epicondylalgia. Neurological impairment, previous experience of manipulative therapy to the elbow joint, aversion to manual contact as assessed on a pre-experimental physical examination, health conditions which preclude manipulative therapy (e.g. osteoporosis, diabetes, hemophilia) and concurrent use of certain medications such as analgesic or anti-inflammatory drugs | The time course of therapeutic effects following mobilization-with-movement treatment remains to be evaluated |
| **Vicenzino et al. (2003) [3]** | Single blind, placebo controlled, randomized, crossover study | Pain over the lateral epicondyle, pain on grip strength testing, and pain with 1 of the following tests: extensor carpi radialis test (i.e., resisted middle-finger extension), resisted wrist extension, or passive stretch of the wrist extensors. In addition, to be included, participants had to abstain from any other form of treatment during their involvement in this study | Allergies to adhesive tape, a recent steroid injection (6 weeks), any upper-limb neurological abnormalities on physical examination (e.g., cervical neuropathy), a concomitant upper limb orthopedic condition, or any concurrent treatment | N/A |
| **Paungmali et al. (2003) [4]** | Placebo controlled repeated measures study | Pain over the lateral side of the elbow that was provoked by palpation of the lateral epicondyle region and gripping tasks. In addition, pain had to be experienced over the lateral epicondyle during at least one of the following: resisted static contraction of the wrist extensors or extensor carpi radialis brevis muscle or stretching of the forearm extensor muscles | Cervical spine or upper-limb problems (e.g., referred pain, conditions other than lateral epicondylalgia). Other exclusion criteria included neurological impairments, neuromuscular diseases, cardiovascular diseases, health conditions that would have precluded treatment (e.g., osteoporosis, malignancies, hemophilia, diabetes), recent steroid injection, using prescription medications such as beta-adrenoceptor blocking agents or anti-inflammatory or analgesic drugs, aversion to manual contact, and previous therapy for the elbow joint | Local effects, such as possible changes in the relationships of bones and soft tissues about the joint and possible changes in local neural receptors in soft tissues at the time of treatment, may account for the measured changes |
| **Spacca et al. (2005) [5]** | Prospective randomized controlled single-blind study | Clinical diagnosis of lateral epicondylitis of the elbow confirmed by diagnostic imaging (ultrasound or magnetic resonance imaging), duration of symptoms for at least 10 months; pain with a visual analog scale | Pregnancy, implanted pacemaker, blood coagulation disorders, assumption of anticoagulant drugs, skeletally immature patients, inflammatory or neoplastic disorders, pathologies in the shoulder and/or neck, and | Although the lack of a true placebo-group can represent a limit of the study, the results showed that the RSWT are comparable, both in the effects that in the effectiveness to low-energy ESWT, in the treatment of tennis |

| | | | | |
|---|---|---|---|---|
| | | (VAS) score 3 cm, failed previous conservative treatments included nonsteroidal anti-inflammatory drugs, cortisone injection, physical therapy, exercise programs, and the use of functional elbow brace | treatment with corticosteroid injections in the last 4 weeks | elbow. Also, as shown for low-energy ESWT, RSWT have the advantage that can be done without local anesthesia |
| **Martinez-Silvestrini et al. (2005) [6]** | Randomized controlled trial | Pain localized to the lateral elbow (one or both arms), continuous symptoms present for more than three months, and maximal tenderness localized to the lateral epicondyle and pain with two of the following three maneuvers: resisted wrist extension, resisted middle finger extension, or chair lift test | Substantial osteoarthritis, rheumatoid arthritis, or inflammatory arthropathy affecting the elbow or wrist; connective tissue disease; diffuse pain syndrome (e.g., generalized tension myalgia or fibromyalgia); carpal tunnel syndrome; cervical radiculopathy; history of fracture of the radius, ulna, or humerus with resultant deformity of the affected extremity; numbness or tingling of the dorsum of the forearm or hand of the involved extremity; pending litigation (other than workers' compensation) related to elbow pain; inability to perform the strengthening exercises because of excessive pain; and 10) unable to return for follow-up evaluation | Because subjects with lateral epicondylitis do improve over time, the authors cannot be sure that the observed improvement was not due to the natural course of the condition regardless of treatment intervention. Similarly, because all subjects received stretching, icing, and education, the authors cannot be sure that these treatments did not affect outcome |
| **D'Vaz et al. (2005) [7]** | Randomized, double-blind, placebo-controlled trial | Patients with LET of at least 6 weeks' duration were recruited from general practice, physiotherapy and rheumatology clinics, and had to have failed at least one first-line treatment including nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroid injection | | The selection criteria required patients to have failed at least one first-line therapy. This may have biased the study to include a more recalcitrant group. Spontaneous resolution is said to occur most commonly by 12 months; thus, the patients may have improved regardless of intervention. Therefore, this trial may not have provided a true reflection of the usefulness of LIUS |
| **Faes et al. (2006) [8]** | Randomized controlled trial, followed by crossover | Physician's diagnosis of lateral epicondylitis; recurrence of symptoms after initial treatment; persistent symptoms despite alternative treatments for lateral epicondylitis; age between 18 and 70 years; and ability to complete questionnaires in Dutch | Bilateral lateral epicondylitis; duration of symptoms less than 6 weeks (to avoid spontaneous recovery); neurologic or rheumatic disorders to the lower arm or wrist; and a history of muscular, neural, or bone injuries to the lower arm or wrist apart from lateral epicondylitis | N/A |
| **Staples et al. (2008) [9]** | Randomized, double-blind, placebo-controlled trial | Eligible patients were at least 18 years old, with lateral elbow pain of at least 6 weeks' duration, normal anteroposterior and lateral radiographs of the elbow, and reproducibility of pain by 2 or more of the following tests: palpation of the lateral epicondyle and/or the common extensor origin of the elbow; resisted wrist extension (dorsiflexion) and pronation with the elbow | Bilateral epicondylitis, generalized inflammatory arthritis such as rheumatoid arthritis, concurrent shoulder and/or neck pain and/or pain proximal to the elbow on the affected side, any wound or skin lesion on the lateral side of the affected elbow, neurological symptoms or abnormal neurological findings in the affected arm, pregnancy, | The study failed to meet its recruitment target of 65 per group, leaving it with insufficient power to detect the specified difference of 10 mm in pain or function measured on a 100-mm VAS. The authors chose to deliver a small shock wave dose to the placebo group rather than sham therapy to limit the likelihood that patients would determine their treatment allocation. It is possible, but |

| | | | | |
|---|---|---|---|---|
| | | in extension; and pain reproduced by static stretching of the pronated wrist in palmar flexion with the elbow in extension. | severe infection, known malignancy, bleeding disorder, pacemaker, previous surgery to the elbow, oral and/or topical NSAID in the previous 2 weeks, local corticosteroid injection in the previous month, oral glucocorticosteroids within the previous 6 weeks, lack of informed consent, or any other reason thought likely to result in inability to complete the trial | unlikely, that this small dose had a therapeutic effect |
| **Coff et al. (2009) [10]** | Randomized controlled trial | Clinical diagnosis of lateral epicondylitis, diagnosed by referring physician, newly diagnosed lateral epicondylitis, or an exacerbation of long-term lateral epicondylitis. Patients needed to speak and understand English and to be able to communicate their perceived pain in terms of a visual analogue scale (VAS) | Infection, pacemaker, pregnant, cervical radiculopathy, existing pathology in the affected elbow, which would have affected the results, or were receiving other treatment to their elbow | Participants with histories ranging from acute to chronic exacerbations were included and provided with the same treatment protocol because they reflect clinical referral patterns. Another limitation of this study is that it was not possible to blind participants to treatment Implemented. |
| **Ajimsha et al. (2012) [11]** | Randomized, controlled, single-blind trial | Pain lasting _1 day in the last 7 days in the lateral elbow region, tenderness over the lateral elbow region, and pain occurring over the lateral elbow region during resisted active extension of the wrist; pain lasting at least 3 months; those working with a personal computer, computer terminal, or equivalent device with a computer mouse; those using a computer for 50% or more of the work day; and those who had completed a baseline Patient-Rated Tennis Elbow Evaluation (PRTEE) scale. | Those with a history of trauma to the affected elbow in the preceding 6 weeks, history of elbow instability, previous elbow surgery, any other pathology involving the affected upper limb or cervical spine, use of oral/ systemic steroids, use of analgesics on more than 10 days a month, and any other treatment for LE during the previous 6 months were excluded from the study | One limitation of this trial was that practitioners could not be blinded. A slight improvement over time occurred in the control group at week 4; this could be due to a "meaning response". |
| **Khuman et al. (2013) [12]** | Randomized controlled study | Age 30-45 years, both gender, CLE> 3 months, unilateral involvement, NPRS score 4 to 8 | History of trauma, surgery, acute infections, any systemic disorders, cervical spine and upper limb dysfunction, neurological impairments, cardiovascular diseases, osteoporosis, recent steroid infiltration, ossification and calcification of soft tissue, malignancies, athletes, recently underwent physiotherapy interventions in least 3 months, unwillingness to attend all treatment sessions & assessments | The study has certain limitations like no blinding procedure performed, the sample size were small and long-term improvements in the pain, functional performance and grip strength with MFR technique were not recorded. The intervention was of only 4 weeks' duration in a small sample size |
| **Bisset et al. (2014) [13]** | Cross-over, double-blind randomized controlled trial | Pain over the lateral elbow for a minimum of 6 weeks that increased with palpation of the lateral epicondyle, gripping, or resisted extension of the wrist or the second or third finger | Bilateral elbow symptoms; cervical radiculopathy; any other elbow joint pathology or peripheral nerve involvement; past history of elbow surgery, dislocation, fracture, or tendon rupture; shoulder, wrist, or hand pathology; systemic or neurological disorders; treatment for elbow pain by a health care practitioner within the preceding 3 | It appears that the assessor blinding was not completely successful, as the assessor correctly guessed the intervention on more occasions than would be expected by chance alone. Furthermore, while patients typically wear an elbow brace to improve pain-free function, the impact of the test braces on general upper-limb function was not assessed |

| | | | | |
|---|---|---|---|---|
| | | | months; and corticosteroid injection for elbow pain within the previous 6 months | in this study. Finally, this study only evaluated the immediate effects of the forearm braces, with no longer-term follow-up |
| **Desai et al. (2014) [14]** | Randomized controlled trial | Subjects with pain at the lateral epicondyle of elbow from past 6 weeks, pain increasing during palpation, gripping, and resisted wrist and finger extension, positive Mill's and Cozen's test | Concomitant bony, neurological impairments, neurological diseases, previous trauma to the elbow region, previous surgery to the elbow region, cervical radiculopathy, systemic diseases, allergic to tape | Subjects with wide range group between 30 to 50 years of age were considered for the study, thus results cannot be generalized to individual age. Follow-up was not done therefore long-term effects were not found. Only pain and functional ability were studied. Measurements such as sensitivity to pain, range of motion and quality of life were not studied |
| **Capan et al. (2016) [15]** | Double-blind, randomized, placebo-controlled trial | Epicondyle pain and epicondyle tenderness and pain on resisted extension of the wrist with symptom duration of more than 3 months who were unresponsive to previous treatments were enrolled in this study | History of trauma at the affected elbow and presence of posterior interosseous nerve entrapment, systemic rheumatic disease, systemic infection, pregnancy, malignancies, coagulopathies, anticoagulant use, cardiac pacemaker, and unwillingness to participate in the study | The number of dropouts, duration of follow-up, and clinical diagnosis of lateral epicondylitis might be considered as limitations of this study |
| **Au at al. (2017) [16]** | Deceptive, crossover trial | All enlisted patients were free of musculoskeletal or neurological conditions, which may have caused pain over the elbow area. Patients were also free of any other joint pain and previous surgery in the tested upper extremity | Patients were asked to list all the prophylactic and rehabilitation equipment they could think of. Those who were able to name "tape", "adhesive plaster", "adhesive ribbon", or anything equivalent were excluded. Patients who had previously received physiotherapy treatment for LE were also excluded | In order to collect the muscle activity from the taped muscles, two circular holes were cut through the tape to allow for EMG electrode placement. This might have caused a substantive change in the structure of the tape and therefore is one potential limitation. Additionally, only the immediate effects of KT placement were measured |
| **Ökmen et al. (2017) [17]** | Randomized, controlled, single-blind study | More than 3 months elbow pain and had a diagnosis of LET. The diagnosis of LET was made if patients complained about the pain on the elbow that was aggravated by pressure on the lateral epicondyle of the humerus and by resisted dorsiflexion of the wrist | Patients who received physical therapy, were injected or with a history of surgical intervention within a year of elbow area, with malignancy, uncontrolled diabetes and hypertension, cervical radiculopathy, polyneuropathy, and inflammatory rheumatic disease were excluded. | The authors decided that it would be unethical to include a no-treatment control group in our methodology in the wake of the clinical condition of the patients. The authors also could not form a placebo peloid group because a mud preparation completely devoid of minerals was not available. These two factors together with the lack of a much longer follow-up period comprise the limitations of the study |
| **López-de-Celis et al. (2017) [18]** | Double-blind randomized controlled trial | Individuals over 18 years old, with a clinical diagnosis of chronic lateral epicondylalgia who had signed the informed consent form. They were considered to have chronic lateral epicondylalgia if they presented epicondyle pain with a history of more than three months, increasing with gripping or pressing activities, and a positive result in the Cozen test or the Mill test | Other concomitant pathologies in the same extremity (e.g. cervicobrachialgia), previous surgery in the affected elbow, recent injection (3 previous months) in the elbow region, local acute inflammatory processes, diacutaneous fibrolysis contraindications (wounds or skin disorders in the elbow region, vascular alterations, receiving anticoagulant treatment), involvement in pending litigation or difficulties (due to | One limitation of this study is that sample size was small and, as some patients had bilateral involvement, data are likely to be correlated between the two elbows of the same patient weakening the statistical power of this study. Another limitation is that we have evaluated short-term results in a condition in which flare-ups are common and studies with a long-term follow-up period are required. |

| | | | | |
|---|---|---|---|---|
| | | | language or other reasons) with understanding the informed consent | |
| **Upadhyay et al. (2017)[19]** | Randomized controlled trial | Patients between Age: 30 – 60 years, Sex: both sex, local tenderness on palpation over the lateral epicondyle, clinically diagnosed patients of lateral epicondylitis with minimum duration of 3 months and Positive Mill's test | Patients having cubital osteoarthritis, carpal tunnel syndrome, rheumatic arthritis, cervical radicular syndrome, severe cervical spondylitis, painful shoulder or rotator cuff tendonitis, previous trauma, fracture or surgical procedure around the elbow, history of immobilization of elbow, any neurological disorder like stroke, head injury, restriction of the wrist motions, osteoporosis or pathological disorder like malignancy or referred pain | The sample size was small so the results cannot be generalized to population, long term follow up was not carried out to provide results about endurance, and home program taught to the patients was not supervised |
| **Cho et al. (2018) [20]** | Randomized, double-blind, cross-over study | The criteria for the diagnosis of chronic lateral epicondylitis was based on the clinical presentation and included: (1) typical pain over the lateral epicondyle elicited by resisted wrist extension; (2) tenderness at the lateral epicondyle; and (3) symptoms lasting for at least 2 months | Patients were excluded if they previously had had experience with KT treatment, had had a steroid injection for lateral epicondylitis within the past 3 months, were suspected of having elbow arthritis, or had a wound where the taping was to be applied. If pain was reported in both elbows, the one with more-severe pain was used in experiment | First, the sample size was small. Second, only the immediate effects of KT were evaluated. Whether these effects lasted beyond 15 min is unknown. Third, only the effects on pain elicited by isometric wrist extension with 1-kg resistance were explored, so whether KT is effective at higher resistance is also unknown. These issues are important to consider before applying taping in the sport field. Fourth, placebo effect of any kind of taping that the subjects might expect benefit on that cannot be ruled out. And finally, patient selection was not limited to athletes, therefore, projecting results onto people who sustain lateral epicondylitis from sport injuries was not possible |
| **Nowotny et al. (2018) [21]** | Randomized controlled trial | Symptoms for more than 3 months, a Placzek score greater than 4 and written consent of the patient. | Previous surgery, rheumatic arthritis, elbow instability, former fracture of the elbow, higher-grade extensor tendon ruptures (more than 50%) or a cervical radiculopathy | The present study has some limitations: one is the high drop-out rate of about 50% for the 12 weeks and approximately two-thirds during the 12 month follow-up. Due to the natural course of LE and the overall better and good results after 12 weeks and 12 months, the motivation for further long-time follow-up in the clinic and clinical examination maybe reduced. Overall, the drop out was similar in both study groups. One bias is the heterogeneity of the investigators and therapists, which is due to the multi-center approach. Although patients were interviewed about their compliance during the follow-up, the potential irregular implementation of the physiotherapeutic self-exercises can influence the results |

| | | | | |
|---|---|---|---|---|
| **Sethi et al. (2018) [22]** | Randomized controlled trial | Symptoms of LE from at least past 3 months, pain greater than 3 on VAS scale on the lateral epicondyle when palpated; pain in at least two of the following four tests, Tomsen test, Maudsley test, Mill's test, handgrip dynamometer test. None of subjects participated in the study were consuming<br>any oral medications such as NSAID's or using any other method for pain relief during the study period | Dysfunction in the shoulder, neck or thoracic region, local or generalized arthritis, local or generalized neurological deficit, radial nerve entrapment, bilateral elbow pain, history of surgery in the affected elbow, had undergone any treatment for LE in the past 4 weeks | There was no follow up for the study and future studies with follow up for symptoms is recommended with scapular muscle strengthening in LE |

RSWT, radial shockwave therapy; N/A, not applicable

## EXCLUDED FULL-TEXT ARTICLES

### Invasive interventions

Fink, M., Wolkenstein, E., Luennemann, M., Gutenbrunner, C., Gehrke, A., & Karst, M. (2002). Chronic epicondylitis: effects of real and sham acupuncture treatment: a randomised controlled patient-and examiner-blinded long-term trial. *Complementary Medicine Research*, *9*(4), 210-215.

Massy-Westropp, N., Simmonds, S., Caragianis, S., & Potter, A. (2012). Autologous blood injection and wrist immobilisation for chronic lateral epicondylitis. *Advances in orthopedics*, *2012*.

Arik, H. O., Kose, O., Guler, F., Deniz, G., Egerci, O. F., & Ucar, M. (2014). Injection of autologous blood versus corticosteroid for lateral epicondylitis: a randomised controlled study. *Journal of Orthopaedic Surgery*, *22*(3), 333-337.

Petrella, R. J., Cogliano, A., Decaria, J., Mohamed, N., & Lee, R. (2010). Management of tennis elbow with sodium hyaluronate periarticular injections. *BMC Sports Science, Medicine and Rehabilitation*, *2*(1), 4.

Fink, M., Wolkenstein, E., Karst, M., & Gehrke, A. (2002). Acupuncture in chronic epicondylitis: a randomized controlled trial. *Rheumatology*, *41*(2), 205-209.

Glanzmann, M. C., & Audigé, L. (2015). Platelet-rich plasma for chronic lateral epicondylitis: Is one injection sufficient?. *Archives of orthopaedic and trauma surgery*, *135*(12), 1637-1645.

Åkermark, C., Crone, H., Elsasser, U., & Forsskåhl, B. (1995). Glycosaminoglycan polysulfate injections in lateral humeral epicondylalgia: a placebo-controlled double-blind trial. *International journal of sports medicine*, *16*(03), 196-200.

Küçük en, S., Yilmaz, H., Sall , A., & U urlu, H. (2013). Muscle energy technique versus corticosteroid injection for management of chronic lateral epicondylitis: randomized controlled trial with 1-year follow-up. *Archives of physical medicine and rehabilitation*, *94*(11), 2068-2074.

Koh, C., & Seffinger, M. A. (2016). Muscle Energy Technique Improves Chronic Lateral Epicondylitis. *The Journal of the American Osteopathic Association*, *116*(1), 58-58.

Krogh, T. P., Fredberg, U., Stengaard-Pedersen, K., Christensen, R., Jensen, P., & Ellingsen, T. (2013). Treatment of lateral epicondylitis with platelet-rich plasma, glucocorticoid, or saline: a randomized, double-blind, placebo-controlled trial. *The American journal of sports medicine*, *41*(3), 625-635.

Murtezani, A., Ibraimi, Z., Vllasolli, T. O., Sllamniku, S., Krasniqi, S., & Vokrri, L. (2015). Exercise and Therapeutic Ultrasound Compared with Corticosteroid Injection for Chronic Lateral Epicondylitis: A Randomized Controlled Trial. *Ortopedia, traumatologia, rehabilitacja*, *17*(4), 351-357.

Oh, D. S., Kang, T. H., & Kim, H. J. (2016). Pulsed radiofrequency on radial nerve under ultrasound guidance for treatment of intractable lateral epicondylitis. *Journal of anesthesia*, *30*(3), 498-502.

Placzek, R., Drescher, W., Deuretzbacher, G., Hempfing, A., & Meiss, A. L. (2007). Treatment of chronic radial epicondylitis with botulinum toxin A: a double-blind, placebo-controlled, randomized multicenter study. *JBJS*, *89*(2), 255-260.

Rabago, D., Lee, K. S., Ryan, M., Chourasia, A. O., Sesto, M. E., Zgierska, A., ... & Miller, D. (2013). Hypertonic dextrose and morrhuate sodium injections (prolotherapy) for lateral epicondylosis (tennis elbow): Results of a single-blind, pilot-level randomized controlled trial. *American journal of physical medicine & rehabilitation/Association of Academic Physiatrists*, *92*(7), 587.

Raeissadat, S. A., Rayegani, S. M., Hassanabadi, H., Rahimi, R., Sedighipour, L., & Rostami, K. (2014). Is platelet-rich plasma superior to whole blood in the management of chronic tennis elbow: one year randomized clinical trial. *BMC Sports Science, Medicine and Rehabilitation*, *6*(1), 12.

Shiple, B. J. (2013). How effective are injection treatments for lateral epicondylitis?. *Clinical Journal of Sport Medicine*, *23*(6), 502-503.

Valera-Garrido, F., Minaya-Muñoz, F., & Medina-Mirapeix, F. (2014). Ultrasound-guided percutaneous needle electrolysis in chronic lateral epicondylitis: short-term and long-term results. *Acupuncture in Medicine*, acupmed-2014.

**Pharmacologic interventions**

Frizziero, A., Causero, A., Bernasconi, S., Papalia, R., Longo, M., Sessa, V., ... & Rovati, S. (2016). Efficacy of betamethasone valerate medicated plaster on painful chronic elbow tendinopathy: a double-blind, randomized, placebo-controlled trial. *Muscles, ligaments and tendons journal*, *6*(1), 131.

Paoloni, J. A., Murrell, G. A., Burch, R. M., & Ang, R. Y. (2009). Randomised, double-blind, placebo-controlled clinical trial of a new topical glyceryl trinitrate patch for chronic lateral epicondylosis. *British journal of sports medicine*, *43*(4), 299-302.

Burnham, R., Gregg, R., Healy, P., & Steadward, R. (1998). The effectiveness of topical diclofenac for lateral epicondylitis. *Clinical journal of sport medicine*, *8*, 78-81.

Paoloni, J. A., Appleyard, R. C., Nelson, J., & Murrell, G. A. (2003). Topical nitric oxide application in the treatment of chronic extensor tendinosis at the elbow. *The American Journal of Sports Medicine*, *31*(6), 915-920.

Ozden, R., Uruc, V., Do ramaci, Y., Kalaci, A., & Yengil, E. (2014). Management of tennis elbow with topical glyceryl trinitrate. *Acta Orthop Traumatol Turc*, *48*(2), 175-80.

**Surgical interventions**

Almquist, E. E., Necking, L., & Bach, A. W. (1998). Epicondylar resection with anconeus muscle transfer for chronic lateral epicondylitis. *Journal of Hand Surgery*, *23*(4), 723-731.

Bigorre, N., Raimbeau, G., Fouque, P. A., Saint Cast, Y., Rabarin, F., & Cesari, B. (2011). Lateral epicondylitis treatment by extensor carpi radialis fasciotomy and radial nerve decompression: Is outcome influenced by the occupational disease compensation aspect?. *Orthopaedics & Traumatology: Surgery & Research*, *97*(2), 159-163.

Grewal, R., MacDermid, J. C., Shah, P., & King, G. J. (2009). Functional outcome of arthroscopic extensor carpi radialis brevis tendon release in chronic lateral epicondylitis. *The Journal of hand surgery*, *34*(5), 849-857.

Kroslak, M., & Murrell, G. A. (2018). Surgical Treatment of Lateral Epicondylitis: A Prospective, Randomized, Double-Blinded, Placebo-Controlled Clinical Trial. *The American journal of sports medicine*, *46*(5), 1106-1113.

Kwon, B. C., Kim, J. Y., & Park, K. T. (2017). The Nirschl procedure versus arthroscopic extensor carpi radialis brevis debridement for lateral epicondylitis. *Journal of shoulder and elbow surgery*, *26*(1), 118-124.

Luchetti, R., Atzei, A., Brunelli, F., & Fairplay, T. (2005). Anconeus muscle transposition for chronic lateral epicondylitis, recurrences, and complications. *Techniques in hand & upper extremity surgery*, *9*(2), 105-112.

Lungu, E., Grondin, P., Tétreault, P., Desmeules, F., Cloutier, G., Choinière, M., & Bureau, N. J. (2018). Ultrasound-guided tendon fenestration versus open-release surgery for the treatment of chronic lateral epicondylosis of the elbow: protocol for a prospective, randomised, single blinded study. *BMJ open*, *8*(6), e021373.

Matache, B. A., Berdusco, R., Momoli, F., Lapner, P. L., & Pollock, J. W. (2016). A randomized, double-blind sham-controlled trial on the efficacy of arthroscopic tennis elbow release for the management of chronic lateral epicondylitis. *BMC musculoskeletal disorders*, *17*(1), 239.

Posch, J. N., Goldberg, V. M., & Larrey, R. (1978). Extensor fasciotomy for tennis elbow: a long-term follow-up study. *Clinical orthopaedics and related research*, (135), 179-182.

Rose, N. E., Forman, S. K., & Dellon, A. L. (2013). Denervation of the lateral humeral epicondyle for treatment of chronic lateral epicondylitis. *The Journal of hand surgery*, *38*(2), 344-349.

Terra, B. B., Rodrigues, L. M., Nahssen Filho, A., de Almeida, G. D. B., Cavatte, J. M., & De Nadai, A. (2015). Arthroscopic treatment for chronic lateral epicondylitis. *Revista Brasileira de Ortopedia (English Edition)*, *50*(4), 395-402.

Thornton, S. J., Rogers, J. R., Prickett, W. D., Dunn, W. R., Allen, A. A., & Hannafin, J. A. (2005). Treatment of recalcitrant lateral epicondylitis with suture anchor repair. *The American journal of sports medicine*, *33*(10), 1558-1564.

**Non-English Language**

Karst, M., Lünnemann, M., Wolkenstein, E., & Fink, M. (2004). Akupunktur bei chronischer Epicondylopathia lateralis humeri–eine prospektive, randomisierte und kontrollierte, klinische Studie. *Deutsche Zeitschrift für Akupunktur*, *47*(4), 20-27.

Bieler, G & Hoffmann, W & Skripitz, W. (1983). Ultrasound therapy as the sole treatment in epicondylitis - an alternative. 19. 609-610.

Forthomme, B., Croisier, J. L., Foidart, M., & Crielaard, J. M. (2004). Exploration isocinétique de l'avant-bras et du poignet Méthodologie et application à une pathologie tendineuse. *Journal de traumatologie du sport*, *21*(2), 80-87.

Geldschläger, S. (2004). Osteopathische versus orthopädische Behandlung der chronischen Epicondylopathia humeri radialis: Eine randomisiert kontrollierte Untersuchung. *Complementary Medicine Research*, *11*(2), 93-97.

Grewe, H. E. (1955). Conservative treatment of epicondylitis and other chronic inflammations. *Zentralblatt fur Chirurgie*, *80*(30), 1210-1214.

Haake, M., Jensen, K., Prinz, H., & Willenberg, T. (2000). Design einer Multizenterstudie zum Wirksamkeitsnachweis der Extrakorporalen Stoßwellentherapie (ESTW) bei Epicondylitis humeri radialis. *Zeitschrift für Orthopädie und ihre Grenzgebiete*, *138*(02), 99-103.

Heyd, R & Schopohl, B & Kirchner, J & Strassmann, Gerd & D. Böttcher, H. (1997). Strahlentherapie der Epicondylopathia humeri. Deutsche Medizinische Wochenschrift - DEUT MED WOCHENSCHR. 122. 247-252. 10.1055/s-2008-1047604.

Knobloch, K., Sophia, S., & Vogt, P. M. (2012). Innovative multimodale Therapie der chronischen lateralen Epikondylitis. *Obere Extremität*, *7*(3), 158-165.

Krischek, O., Rompe, J. D., Hopf, C., Vogel, J., Herbsthofer, B., Nafe, B., & Bürger, R. (1998). Die extrakorporale Stoßwellentherapie bei Epicondylitis humeri ulnaris oder radialis-Eine prospektive, kontrollierte, vergleichende Studie. *Zeitschrift für Orthopädie und ihre Grenzgebiete*, *136*(01), 3-7.

Müller, U., & Moll, G. (1983). Treatment of epicondylitis with locally injected orgotein (double blind study). *Zeitschrift fur Rheumatologie*, *42*(1), 21-24.

Placzek, R., Lindner, M. S., Deuretzbacher, G., & Meiss, A. L. (2004). Therapie der chronischen Epicondylopathia humeri radialis mit Botulinumtoxin A. *Zeitschrift für Orthopädie und ihre Grenzgebiete*, *142*(06), 701-705.

Richter, D., Ekkernkamp, A., & Muhr, G. (1995). Extracorporeal shock wave therapy--an alternative concept for the treatment of epicondylitis of the humerus and radius?. *Der Orthopade*, *24*(3), 303-306.

Ries, C., Franke, S., Dietrich, F., Jakubowitz, E., Dehlinger, F., & Hollinger, B. (2013). Die transossäre Refixation der Extensoren bei chronischer radialer Epikondylopathie mit und ohne Rekonstruktion des LUCL-Komplexes–eine retrospektive Analyse von 101 Patienten. *Zeitschrift für Orthopädie und Unfallchirurgie*, *151*(03), 296-301.

Saudan, Y. (1977). Non-surgical treatment of the acute and chronic epicondylitis (tennis elbow)(author's transl). *Therapeutische Umschau. Revue therapeutique*, *34*(2), 81.

Terra, B. B., Rodrigues, L. M., Nahssen Filho, A., de Almeida, G. D. B., Cavatte, J. M., & De Nadai, A. (2015). Tratamento artroscópico da epicondilite lateral crônica. *Revista Brasileira de Ortopedia*, *50*(4), 395-402.

## References


1. Vicenzino B, Collins D, Wright A. The initial effects of a cervical spine manipulative physiotherapy treatment on the pain and dysfunction of lateral epicondylalgia. Pain. 1996;68(1):69-74.
2. Vicenzino B, Paungmali A, Buratowski S, et al. Specific manipulative therapy treatment for chronic lateral epicondylalgia produces uniquely characteristic hypoalgesia. Manual therapy. 2001;6(4):205-212.
3. Vicenzino B, Brooksbank J, Minto J, et al. Initial effects of elbow taping on pain-free grip strength and pressure pain threshold. Journal of Orthopaedic & Sports Physical Therapy. 2003;33(7):400-407.
4. Paungmali A, O'Leary S, Souvlis T, et al. Hypoalgesic and sympathoexcitatory effects of mobilization with movement for lateral epicondylalgia. Physical therapy. 2003;83(4):374-383.
5. Spacca G, Necozione S, Cacchio A. Radial shock wave therapy for lateral epicondylitis: a prospective randomised controlled single-blind study. Europa Medicophysica. 2005;41(1):17-25.
6. Martinez-Silvestrini JA, Newcomer KL, Gay RE, et al. Chronic lateral epicondylitis: comparative effectiveness of a home exercise program including stretching alone versus stretching supplemented with eccentric or concentric strengthening. Journal of Hand Therapy. 2005;18(4):411-420.
7. D'vaz A, Ostor A, Speed C, et al. Pulsed low-intensity ultrasound therapy for chronic lateral epicondylitis: a randomized controlled trial. Rheumatology. 2005;45(5):566-570.
8. Faes M, van den Akker B, de Lint JA, et al. Dynamic extensor brace for lateral epicondylitis. Clinical orthopaedics and related research. 2006;442:149-157.
9. Staples MP, Forbes A, Ptasznik R, et al. A randomized controlled trial of extracorporeal shock wave therapy for lateral epicondylitis (tennis elbow). The Journal of rheumatology. 2008;35(10):2038-2046.
10. Coff L, Massy-Westropp N, Caragianis S. Randomized controlled trial of a new electrical modality (InterX) and soft tissue massage, stretching, ultrasound and exercise for treating lateral epicondylitis. Hand Therapy. 2009;14(2):46-52.
11. Ajimsha M, Chithra S, Thulasyammal RP. Effectiveness of myofascial release in the management of lateral epicondylitis in computer professionals. Archives of physical medicine and rehabilitation. 2012;93(4):604-609.
12. Khuman PR, Trivedi P, Devi S, et al. Myofascial release technique in chronic lateral epicondylitis: a randomized controlled study. Int J Health Sci Res. 2013;3(7):45-52.
13. Bisset LM, Collins NJ, Offord SS. Immediate effects of 2 types of braces on pain and grip strength in people with lateral epicondylalgia: a randomized controlled trial. journal of orthopaedic & sports physical therapy. 2014;44(2):120-128.
14. Desai BD. Effectiveness of medial to lateral taping with exercise programme in subjects with lateral epicondylitis. International Journal of Physiotherapy. 2014;1(2):83-90.
15. Capan N, Esmaeilzadeh S, Oral A, et al. Radial extracorporeal shock wave therapy is not more effective than placebo in the management of lateral epicondylitis: a double-blind, randomized, placebo-controlled trial. American journal of physical medicine & rehabilitation. 2016;95(7):495-506.
16. Au IPH, Fan PCP, Lee WY, et al. Effects of Kinesio tape in individuals with lateral epicondylitis: A deceptive crossover trial. Physiother Theory Pract. 2017 Dec;33(12):914-919.
17. Ökmen BM, Eröksüz R, Altan L, et al. Efficacy of peloid therapy in patients with chronic lateral epicondylitis: a randomized, controlled, single blind study. International journal of biometeorology. 2017;61(11):1965-1972.

18. López-de-Celis C, Barra-López M-E, González-Rueda V, et al. Effectiveness of diacutaneous fibrolysis for the treatment of chronic lateral epicondylalgia: a randomized clinical trial. Clinical rehabilitation. 2018;32(5):644-653.
19. Upadhyay S, Shukla Y, Patel KK. Effects of Progressive Strengthening Exercises in Chronic Lateral Epicondylitis. International Journal of Health Sciences and Research April. 2017;7(4):244-257.
20. Cho Y-T, Hsu W-Y, Lin L-F, et al. Kinesio taping reduces elbow pain during resisted wrist extension in patients with chronic lateral epicondylitis: a randomized, double-blinded, cross-over study. BMC Musculoskeletal Disorders. 2018;19(1):193.
21. Nowotny J, El-Zayat B, Goronzy J, et al. Prospective randomized controlled trial in the treatment of lateral epicondylitis with a new dynamic wrist orthosis. European journal of medical research. 2018;23(1):43.
22. Sethi K, Noohu MM. Scapular muscles strengthening on pain, functional outcome and muscle activity in chronic lateral epicondylalgia. Journal of Orthopaedic Science. 2018;23(5):777-782.

# Chapter III

## *Original manuscripts*

# IV

Journal of Tissue Viability 27 (2018) 274–284

Contents lists available at ScienceDirect

Journal of Tissue Viability

journal homepage: www.elsevier.com/locate/jtv

# High Voltage Monophasic Pulsed Current (HVMPC) for stage II-IV pressure ulcer healing. A systematic review and meta-analysis

Beshoy Girgis*, José Alberto Duarte

*CIAFEL, Faculty of Sports, University of Porto, Portugal*

ARTICLE INFO

*Keywords:*
Pressure ulcer
Decubitus ulcer
Electrical stimulation
Chronic wound healing
Wound care

ABSTRACT

This review was conducted to determine and quantify the efficacy of high-voltage monophasic pulsed current (HVMPC) in the treatment of stage II-IV pressure ulcers (PrUs), identify the details of HVMPC intervention parameters and the superior protocol, and ascertain other potential benefits and the safety of HVMPC intervention. Eleven studies, nine randomized controlled trials (RCTs) and two case series studies, matched the criteria and were included in the systematic review, whereas, only level 1 evidence RCTs were included in the meta-analysis. The percentage of wound surface area reduction per week was 12.39%; 95% CI, [10.43–14.37] for HVMPC plus standard wound care (SWC) and 6.96%; 95% CI, [5.56–8.38] for SWC alone or SWC plus sham HVMPC. The net effect of HVMPC was 5.4% per week (an increase of 78% greater than SWC alone or SWC plus sham HVMPC). Level 1, 2 and 4 evidence studies have consistently indicated that HVMPC plus SWC were more effective than SWC alone or SWC plus sham HVMPC in treating stage II-IV PrUs. Level 1 evidence studies showed that HVMPC intervention improved the healing of PrUs (reduced wound surface area), and combined with SWC, increased the probability of complete healing and almost eliminated the probability of worsening of healing. HVMPC intervention was shown to be relatively safe, with rare adverse reactions.

## 1. Introduction

Pressure ulcers (PrUs) are a common cause of severe patient discomfort and frequently result in life threatening infections [1]. Due to pain, long institutional stay and treatment, PrUs may reduce quality of life and may also, in some patients, contribute to premature mortality [1–3]. A recent overview of systematic reviews has indicated that evidence for usage of any non-pharmacologic therapy to increase wound healing rate was inconclusive, and only electrotherapy was supported by low quality evidence [4]. Nonetheless, other systematic reviews and meta-analyses corroborated the use of electrotherapy for treating chronic wounds [5–9].

### 1.1. Rationale

A recent review of reviews by Houghton [10] has reported that well-conducted systematic reviews provided results that strongly support electrical stimulation therapy for various chronic wound types and PrUs in particular. The guidelines recommend electrical stimulation (ES) for the management of stage II to IV PrUs [11–14]. These guidelines, however, do not specify neither the current type nor the parameters (duration, frequency, amplitude) of ES to be applied. In addition, lack of conclusive evidence may be a contributing factor to this dilemma. Indeed, this may be confusing for therapists as the choice of the most effective ES type and parameters becomes their responsibility, which may explain the limited clinical implementation of ES therapy for PrUs [8,15]. This is especially true because of the considerable diversity in ES forms and parameters; moreover, not all forms and parameters demonstrate positive results and a specific dosage range may be required to enhance wound healing [7,16–20].

Koel and Houghton [8] in a systematic review and meta-analysis have concluded that there is a clear reason to apply monophasic ES specifically for PrUs treatment. Moreover, Kawasaki et al. [9] has indicated that moderate evidence levels support ES efficacy in treatment of PrUs and that high-voltage monophasic pulsed current (HVMPC) seems to be a better choice than direct current (DC) stimulation for pressure ulcer healing. HVMPC is a type of current with a twin-peaked monophasic pulsed waveform [17,21,22]. Based on these studies and the corresponding characteristics of the current, we chose to conduct this review on HVMPC specifically to reach a conclusion regarding its effectiveness in the healing of PrUs.

* Corresponding author. CIAFEL, Faculty of Sports, University of Porto, Portugal, R. Dr. Plácido, Costa, 91, 4200-450, Porto, Portugal.
*E-mail addresses:* begirgis@gmail.com, up201301273@fade.up.pt (B. Girgis), jarduarte@fade.up.pt (J.A. Duarte).

https://doi.org/10.1016/j.jtv.2018.08.003
Received 15 March 2018; Received in revised form 7 July 2018; Accepted 10 August 2018
0965-206X/ © 2018 Tissue Viability Society. Published by Elsevier Ltd. All rights reserved.

**Table 1**
Inclusion and Exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|---|---|
| • Published trials on human subjects | • Ulcer types other than pressure ulcer |
| • No study design restriction | • Forms of electrical stimulation therapy other than HVMPC |
| • Treatment of pressure ulcers | • Studies written in languages other than English |
| • HVMPC application | • Abstracts |

### 1.2. Objectives

- To determine and quantify the efficacy of high-voltage monophasic pulsed current in the treatment of stage II-IV pressure ulcers.
- To identify the details of HVMPC intervention parameters and the superior protocol.
- To ascertain other potential benefits and the safety of HVMPC intervention.

## 2. Methods

### 2.1. Eligibility criteria

The systematic review was restricted to published trials done on human subjects, focusing only on HVMPC application for the treatment of PrUs, and written in English language. Abstracts and studies written in languages other than English were excluded. Inclusion and exclusion criteria are shown in Table 1.

### 2.2. Information sources

Electronic search of the United States National Library of Medicine (NLM) and National Institutes of Health (NIH) PubMed, the Physiotherapy Evidence Database PEDro, and Scopus database from year 1960 up to 24 December 2017 was performed. The electronic search was complemented by inspection of bibliographic references. The search terms used were: High Voltage Pulsed Current (HVPC), High Voltage Electrical Stimulation (HVES), High Voltage Pulsed Galvanic Stimulation (HVPGS), High Voltage Monophasic Pulsed Current (HVMPC), Pressure Ulcer, Pressure Sore, Decubitus Ulcer, and Bedsore.

### 2.3. Data items

The extracted data from each study were study design, study inclusion and exclusion criteria, subjects' characteristics (number, age, and gender), pressure ulcer characteristics (number, stage, baseline surface area, duration, and location), details of HVMPC protocol parameters (voltage, frequency, pulse interval (pulse spacing), intervention duration, and frequency of sessions per week), details of electrode arrangement (electrode polarity, placement, material and size, and distance between electrodes), details of standard wound care (SWC) intervention(s), and intervention(s) period. Primary and secondary endpoint outcomes were also analyzed for relevant outcome measures.

### 2.4. Outcome measures

The primary outcome was pressure ulcer healing, determined through the reduction in wound surface area (WSA). The secondary outcomes were the incidences of complete healing and worsening of healing, determined through the number of PrUs that completely healed and that increased in surface area relative to baseline, respectively. Other secondary outcomes were adverse reactions to HVMPC intervention, morbidity and pain status.

### 2.5. Methodological quality assessment

The PEDro (Physiotherapy Evidence Database) scale was used to assess the methodological quality of the identified randomized controlled trials (RCTs). The PEDro scale has showed good inter-rater reliability, and high construct and convergent validity [23–25]. Alternatively, the Downs and Black scale was used to assess the methodological quality of non-randomized and/or non-controlled studies [26]. Sackett et al. developed a scale to appraise findings based on the level of evidence [27]. This scale has been modified by Straus et al. into five levels of evidence, integrating PEDro scores into the levels of evidence [28]. In order to reach the most accurate and reliable evidence, only RCTs with PEDro score of 6 or above (level 1 evidence - according to Straus et al. [28]) were included in the meta-analysis.

### 2.6. Summary measures

The reduction in WSA was determined through the endpoint mean percentage of reduction in WSA from baseline (mean ↓WSA %) reported in each sample. Nevertheless, due to the variation in intervention duration across studies, another variable, the mean percentage area reduction per week (PARW), had to be introduced for the meta-analytic procedures to be plausible. The PARW was calculated for each treatment and control sample, and then weighted by corresponding sample size to get the average of percentage area reduction per week (APARW). The total of averages of percentage area reduction per week (TAPARW) across treatment and across control samples were consequently calculated and compared. The number of completely healed ulcers was subject to descriptive and risk ratio analysis, while the number of ulcers that worsened was subject to descriptive analysis only. Synthesis of results is provided in the supplementary information (SI).

### 2.7. Subgroup analysis

A subgroup analysis of two HVMPC protocols, cathodal and cathodal-anodal, was performed to determine the superior protocol. The net effect of the cathodal protocol, in which the cathode was used as the active electrode throughout the study period, was calculated. Moreover, the net effect of the cathodal-anodal protocol, in which the cathode was used as the active electrode for the first 5 days followed by the anode for the remainder of the study period, was also computed. The net effects of both protocols were then compared.

## 3. Results

### 3.1. Results of the systematic review

Eleven studies matched the eligibility criteria and were included in the review; nine randomized controlled trials (RCTs) [29–37] and two case series studies [38,39]. Regardless of design, all of these studies consistently reported positive effects of HVMPC application expressed by the increase in mean percentage area reduction per week and/or the incidence of complete ulcer healing. Characteristics of studies included in the systematic review are provided in the SI. The flow of the review is presented in a diagram according to PRISMA (Fig. 1).

### 3.2. Methodological quality assessment results

The assessment scores of the RCTs using the PEDro scale ranged from 4 to 9 (fair to excellent), with the overall quality improving in recently conducted studies. Moreover, the evaluation of the two case series studies using the Downs and Black scale revealed poor to fair scores (12 and 17) (Tables 2 and 3). According to the previously mentioned levels of evidence by Straus et al. [28], five RCTs [32,33,35–37] achieved a PEDro score of 6 or above and were regarded as level 1 evidence, four RCTs [29–31,34] received a PEDro score of less

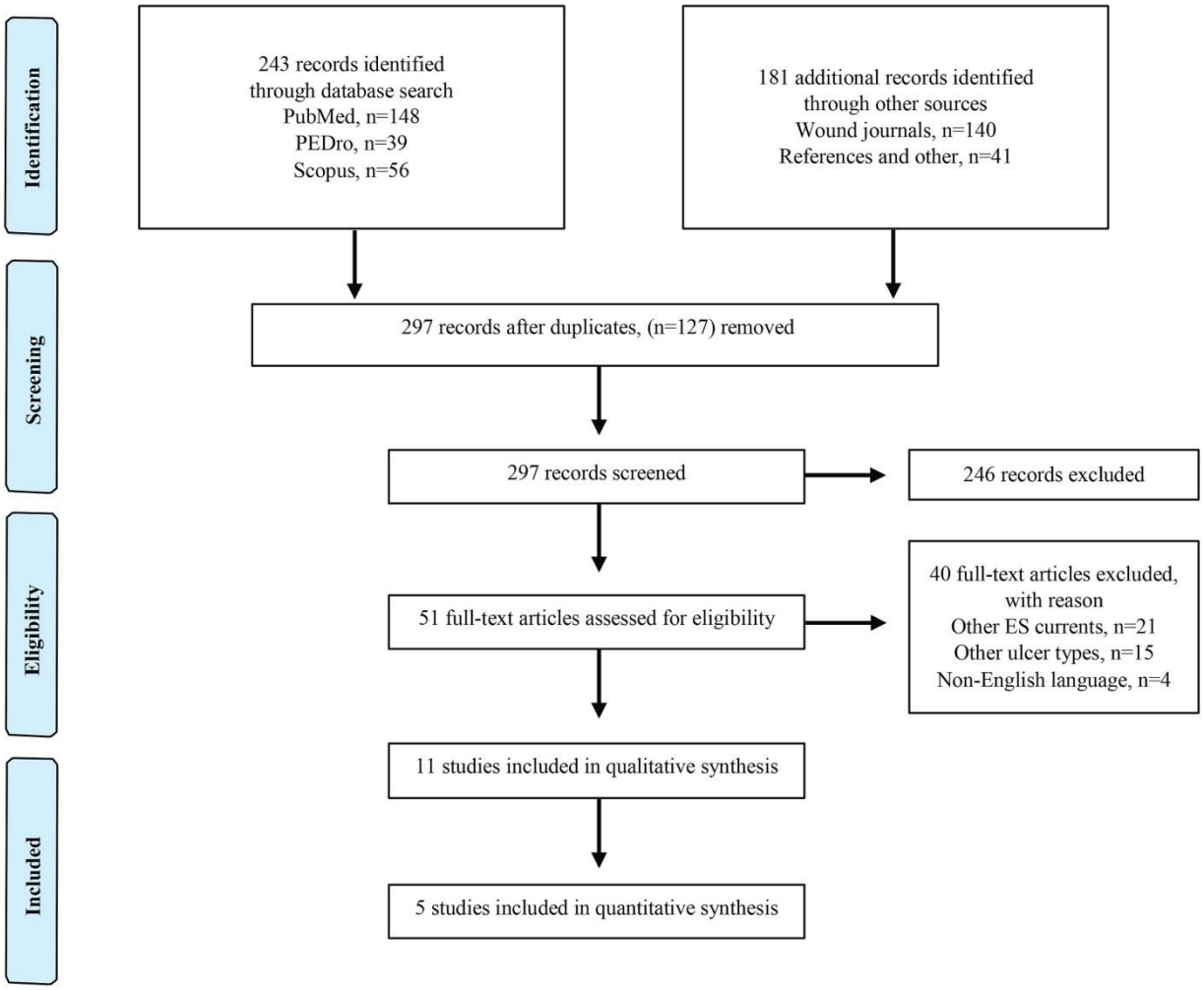



**Fig. 1.** Study flow diagram (PRISMA).

**Table 2**
**Methodological quality assessment scores of RCTs.** Total PEDro scale score is calculated out of 10 (first criterion score does not contribute to total score); (0) present, (1) absent. The scale items are 1: Eligibility criteria, 2: Random allocation, 3: Concealed allocation, 4: Baseline comparability, 5: Blind subjects, 6: Blind therapists, 7: Blind assessors, 8: Adequate follow-up, 9: Intention-to-treat analysis, 10: Between-group comparisons, 11: Point estimates and variability.

| PEDro scale: | Total | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kloth and Feeder (1988) [30] | **4** | No | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Griffin et al. (1991) [29] | **4** | Yes | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| Ahmad (2008) [31] | **4** | No | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Houghton et al. (2010) [32] | **8** | Yes | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| Franek et al. (2011) [33] | **6** | No | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| Franek et al. (2012) [34] | **4** | No | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Polak et al. (2016) [35] | **7** | Yes | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 |
| Polak and Kloth et al. (2016) [36] | **7** | No | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 |
| Polak and Kloth et al. (2017) [37] | **9** | No | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |

than 6 and were considered as level 2 evidence, and two case series studies [38,39] were deemed as level 4 evidence.

### 3.3. Results of the meta-analysis

The calculated TAPARW of the treatment arm was 12.39%; 95% CI, [10.43–14.37], while the control arm TAPARW was 6.96%; 95% CI, [5.56–8.38] (Fig. 2). The difference between the treatment and the control arms showed that HVMPC intervention net effect was 5.4% per week (an increase of 78% greater than the control arm) (Table 4). Meta-analytic calculations are provided in the SI.

### 3.4. Characteristics of studies included in the meta-analysis

The studies included in the meta-analysis involved 256 patients (264 PrUs) in total. The treatment arm received HVMPC plus SWC and included 137 patients (142 PrUs), while the control arm received SWC alone or SWC plus sham HVMPC and included 119 patients (122 PrUs). The mean age of subjects in the treatment arm was 71.4 ± 12.9 years, while in the control arm was 68.6 ± 12.5 years. The treatment arm incorporated 46 males and 91 females, whereas the control arm involved 36 males and 83 females.

The number of PrUs, according to stage, included in the treatment arm was I:7, II:67, III:51, IV:15, X:2, while in the control arm was I:8, II:64, III:38, IV:12, X:0. The mean ulcer duration (pre-intervention) in the treatment arm was 20.1 ± 20.83 weeks and in the control arm was 40.99 ± 64.52 weeks. The mean baseline WSA in the treatment arm was 7.14 ± 2.8 $cm^2$ and in the control arm was 7.11 ± 3.11 $cm^2$. The ulcers in both arms were commonly located in the pelvic region (sacrum/coccyx, ischial tuberosity/trochanter major), and also in the lower extremity (leg, ankle, foot), trunk, hand, and elbow (only in one control sample) regions. Further details are provided in the SI.

HVMPC protocol parameters were as follows: The voltage ranged between 50-150 V and most commonly was 100 V [33,35–37]. The

**Table 3**
**Methodological quality assessment scores of case series studies.** Total Downs and Black scale score is calculated out of 27; (0) present, (1) absent.

| Downs and Black scale: | Total | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Recio, A. C. et al. (2012) [38] | 12 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| | | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | |
| | | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | |
| Bora Karsli, P. et al. (2017) [39] | **17** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| | | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 |
| | | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | |
| | | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 3 | |

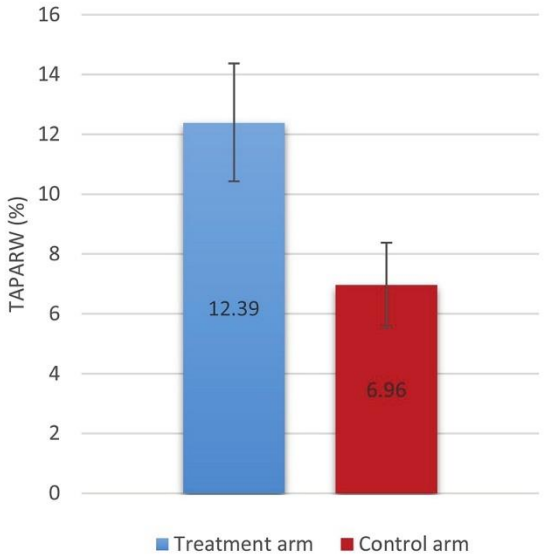



**Fig. 2. Results of the meta-analysis; all studies (PEDro score ≥6).** TAPARW, total of averages of percentage area reduction per week; Error bars represent the 95% confidence intervals.

**Table 4**
**Results of the meta-analysis.** TAPARW, total of averages of percentage area reduction per week.

| All Studies (PEDro score ≥6) | Treatment arm | Control arm | Net effect |
|---|---|---|---|
| Number of subjects | 137 | 139 | **5.4** |
| Number of ulcers | 142 | 142 | |
| TAPARW (%) | 12.39 | 6.96 | |
| Standard deviation | 2.46 | 1.76 | |
| Standard error of mean | 1 | 0.72 | |
| 95% Confidence interval | 10.43–14.37 | 5.56–8.38 | |

frequency used in all studies was 100HZ [32,33,35–37]. The pulse interval ranged between 50-100 μs [32,33,35–37]. The intervention duration and frequency of sessions were between 50 min for 5 days a week to 40 min, 8 times a day for 7 days a week. The most frequently applied intervention was 50 min, once a day for 5 days a week [33,35–37]. The mean intervention period in the treatment arm was 7 ± 2.44 weeks and in the control arm was 7.2 ± 2.68 weeks.

The monopolar technique was commonly used, in which the active electrode was placed over the ulcer in all of the studies (except for five patients in one study [32]), but the polarity differed across studies. One study [35] and one group in another study [37] used negative polarity (cathode) for the first 5 days replaced by positive polarity (anode) for the remainder of intervention. Another study used the cathode for the first two weeks replaced by the anode for the remainder of intervention [33]. Moreover, one study [36] and one group in another study [37] used the cathode throughout the treatment period. Polarity was also reversed weekly starting by the cathode initially in one study [32].

The dispersive electrode was often placed 20 cm from the active electrode [32,35–37]. One study did not mention the distance between electrodes [33]. The electrodes were usually made of conductive carbon-rubber [35–37], but also silver or conductive carbon-rubber electrodes were used [33]. One study did not report the material of the used electrodes [32]. The size of electrodes were identical in three studies [35–37], the active electrode was 5 × 10 cm and the dispersive electrode was 10 × 10 cm. In another study 11 of 16 patients used a 4.8 × 10.2 cm active electrode and a 12.7 × 20.3 cm dispersive electrode in the treatment group, while two patients used two equally sized 4.8 × 10.2 cm active and dispersive electrodes (bipolar technique), and three patients used electroconductive socks [32]. The size of electrodes was not mentioned in one study [33]. Standard wound care (SWC) was identical in three studies [35–37]. Pressure redistribution surfaces were used in four studies [32,35–37]. Enzymatic debridement was used in three studies [35–37]. Local bath and pharmacological agents were included in one study only [33]. Silver-containing dressings were used in one study (except for 4 patients in the control group) [32]. Summary of the characteristics of identified studies is reported in Table 5.

### 3.5. Subgroup analysis

The cathodal HVMPC protocol demonstrated a net effect of 5.5% per week (an increase of 68% greater than the control arm). The TAPARW of the treatment arm was 13.54%; 95% CI, [13.32–13.77] and the control arm TAPARW was 8.04%; 95% CI, [6.42–9.66]. Alternatively, the cathodal-anodal HVMPC protocol revealed a net effect of 4.77% per week (an increase of 62% more than the control arm). The TAPARW of the treatment arm was 12.33%; 95% CI, [11.72–12.94], while the control arm TAPARW was 7.61%; 95% CI, [6.66–8.56] (Fig. 3). The calculated difference between the net effects of both protocols proved the cathodal HVMPC protocol superior by 0.7% per week (an increase of 15% more than the cathodal-anodal protocol) (Tables 6 and 7).

### 3.6. Secondary outcome measures

#### 3.6.1. Number of completely healed PrUs

In all identified studies, 74 ulcers in the treatment arm healed completely, compared to 25 ulcers in the control arm. In the studies included in the meta-analysis, 61 ulcers in the treatment arm healed completely, in contrast with 23 ulcers in the control arm. The risk ratio (RR) analysis of the number of completely healed PrUs in treatment and control arms demonstrated that the probability of complete healing was 1.93 ($p = 0.002$) folds higher in the treatment arm; 95% CI, [1.26–2.93] (the results were homogenous $I^2 = 0\%$, $p = 0.461$) (Fig. 4).

#### 3.6.2. Number of PrUs that increased in surface area (worsened)

In all identified studies, 1 ulcer in the treatment arm increased in surface area, in comparison with 14 ulcers in the control arm. In the studies included in the meta-analysis, 1 ulcer in the treatment arm increased in surface area, versus 10 ulcers in the control arm. Descriptive

**Table 5**
Summary of the characteristics of identified studies.

| Study | Study design | Number of subjects | Mean age of subjects | Ulcer stage(s) | Intervention (s) | Stimulation parameters | Electrode arrangement | Intervention period | Results | PARW (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Kloth and Feeder (1988) [30] | RCT, no blinding | Total:16<br>TG:9<br>CG:7 | TG:70.13 ± 20.9<br>CG:65.61 ± 21.1 | IV | TG:<br>HVMPC +SWC<br>CG:<br>Sham HVMPC +SWC | 100–175 V, 105HZ, 50 μs for 45 min, once a day, 5 days a week. At 100 V, 100 μs, the total delivered electric charge was 342 μC/s | Positive electrode over wound (polarity maintained unless a plateau is reached), 15 cm cephalad to negative electrode | TG:7.3 weeks (Mean)<br>CG:7.4 weeks (Mean) | **Mean ↓ WSA (%)**<br>TG:100 ± 0<br>CG: 6.65 ± 23.2 (area increased) | **TG:13.69**<br>**CG: 0.89** |
| Griffin et al. (1991)* [29] | RCT, modified double-blind | Total:17<br>TG:8 M<br>CG:9 M | TG:34<br>CG:34 | II-IV<br>TG: II = 2 III = 5 IV = 1<br>CG:II = 2 III = 6 IV = 1 | TG: HVMPC+SWC<br>CG:<br>Sham HVMPC +SWC | 200 V, 100HZ, 75 μs for 60 min, once a day, 7 days a week. The total delivered electric charge was 500 μC/s | Negative electrode over wound and positive electrode distally. Active electrode was a piece of heavy-duty aluminum foil slightly larger than ulcer perimeter. Dispersive electrode was 20 × 25 cm, placed over medial thigh | 20 consecutive days | **Mean ↓ WSA (%)**<br>TG:78<br>CG:54.5 | **TG:27.3**<br>**CG:19.1** |
| Ahmad (2008)* [31] | RCT, no blinding | Total:60<br>TGI:15 (6 M/9 F)<br>TGII:15 (7 M/8 F)<br>TGIII:15 (8 M/7 F)<br>CG:15 (9 M/6 F) | TGI:38.4 ± 6.82<br>TGII:38.5 ± 1.68<br>TGIII:39.4 ± 1.74<br>CG:39.4 ± 1.69 | II | TGI:<br>HVMPC 45min<br>TGII:<br>HVMPC 60min<br>TGIII:<br>HVMPC 120min<br>CG:<br>Sham HVMPC 45min + SWC | 100–175 V, 120HZ, 50 μs for 45/60/ 120 min once a day, 7 days a week | Negative electrode over wound for first 3 days then positive electrode maintained until healing (repeated in plateaus). Active electrode was a piece of heavy-duty aluminum foil slightly larger than ulcer perimeter. Dispersive electrode was strapped over the medial thigh | 5 weeks | **Measured WSA Baseline ($cm^2$):**<br>TGI:7.12 ± 1.63<br>TGII:7.12 ± 1.62<br>TGIII:7.14 ± 1.57<br>CG:7.21 ± 1.54<br>**After 3 weeks:**<br>TGI:6.40 ± 1.53<br>TGII:3.46 ± 0.82<br>TGIII:3.68 ± 0.79<br>CG:6.65 ± 1.47<br>**After 5 weeks:**<br>TGI:5.10 ± 1.73<br>TGII:0.60 ± 0.35<br>TGIII:0.64 ± 0.61<br>CG:5.39 ± 1.79 | **TGI:5.67**<br>**TGII:18.31**<br>**TGIII:18.2**<br>**CG:5.05** |
| Houghton et al. (2010) [32] | RCT, parallel group, single blind | Total:34<br>TG:16 (8 M/8 F)<br>CG:18 (12 M/ 6 F) | TG:50.3 ± 17.3<br>CG:50.8 ± 11.6 | II-IV<br>TG:<br>II = 1,III = 6, IV = 7,X = 2<br>CG:<br>II = 4,III = 4, IV = 10,X = 0 | TG:<br>HVMPC +SWC<br>CG:<br>SWC | 50–150 V, 100HZ, 100 μs, for 20 min then 10HZ for 20 min and 20 min off cycle, repeated 8 times a day, 7 days a week | Negative electrode initially and reversed weekly. In 11of 16 cases, the active electrode was 4.8 × 10.2 cm over wound and dispersive electrode was 12.7 × 20.3 cm (monopolar) 20 cm between electrodes | 12 weeks or until healing | **Mean ↓ WSA (%)**<br>TG:70 ± 25<br>CG:36 ± 61 | **TG:5.83**<br>**CG:3** |
| Franek et al. (2011) [33] | RCT, no blinding | Total:58<br>TG:29 (19 M/ 10 F)<br>CG:29 (11 M/ 18 F) | TG:59.9 ± 8.88<br>CG:60 ± 9.97 | I-III<br>TG: I = 7, II = 13 III = 9<br>CG: I = 8, II = 13 III = 8 | TG:<br>HVMPC + PSWC<br>CG:<br>PSWC | 100 V, 100HZ, 50 μs for 50 min once daily, 5 days a week | Negative electrode over wound for first 2 weeks, altered to positive for remainder of intervention. Active electrode size was matched to ulcer size, dispersive electrode was on intact peri-wound skin. Conductive carbon rubber or silver electrodes | 6 weeks | **Mean ↓ WSA (%)**<br>TG: 85.38<br>CG:40.08<br>**Mean ↓ cavity volume (%)**<br>TG:20.69<br>CG:9.39 | **TG:14.23**<br>**CG:6.68** |
| Franek et al. (2012) [34] | RCT, no blinding<br><br>RCT, single blind | Total:50<br>TG:26 (18 M/8 F)<br>CG:24 (10 M/ 14 F) | TG:59 ± 18.16<br>CG:56.2 ± 19.70 | II-III<br>TG: IIA = 5, IIB = 12, III = 9<br>CG: IIA = 5, IIB = 11, III = 8 | TG: HVMPC + SWC<br>CG:<br>SWC | 100–150 V, 100HZ, 50 μs for 50 min once daily, 5 days a week | Negative electrode over wound for first 1–2 weeks, altered to positive for remainder of intervention, 20 cm between electrodes. Conductive carbon rubber electrodes | Until healing for maximum of 6 weeks | **Mean ↓ WSA (%)**<br>TG: 88.90 ± 14<br>CG:44.40 ± 63<br>**Mean ↓ cavity volume (%)**<br>TG:100 ± 0<br>CG:54 ± 39.4 | **TG:14.82**<br>**CG:7.4** |

(*continued on next page*)

**Table 5** (*continued*)

| Study | Study design | Number of subjects | Mean age of subjects | Ulcer stage(s) | Intervention (s) | Stimulation parameters | Electrode arrangement | Intervention period | Results | PARW (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Polak et al. (2016) [35] | | Total:77 (88PrUs)<br>US:25 (8 M/17 F) (28 PrUs)<br>ES:24 (5 M/19 F) (29 PrUs)<br>CG:28 (4 M/24 F) (31 PrUs) | US:77.11 ± 10.36<br>ES:79.33 ± 10.21<br>CG:79.13 ± 9.57 | II-IV<br>US: II = 18, III = 8 IV = 2<br>ES: II = 19, III = 7 IV = 3<br>CG: II = 23, III = 7 IV = 1 | US:<br>US + SWC<br>ES:<br>HVMPC + SWC<br>CG:<br>SWC | 100 V, 100HZ, 77 μs for 50 min once daily, 5 days a week. The total delivered electric charge was 250 μC/s | Negative electrode over wound for first 5 days then positive electrode for the remainder of intervention. The active electrode was 5 × 10 cm and dispersive electrode is 10 × 10 cm. 20 cm between electrodes. Conductive carbon rubber electrodes | 6 weeks or until wound closure | **Mean ↓ WSA (%)**<br>US:77.48 ± 11.59<br>ES:76.19 ± 32.83<br>CG:48.97 ± 53.42<br>ES group had significantly more PrUs that decreased in area by at least 50% | **US:12.91**<br>**ES:12.70**<br>**CG:8.16** |
| Polak and Kloth et al. (2016) [36] | RCT, double blind | Total:49<br>TG:25 (6 M/19 F)<br>CG:24 (6 M/18 F) | TG:79.92 ± 8.50<br>CG:76.33 ± 12.74 | II-III<br>TG: II = 11. III = 14<br>CG: II = 11, III = 13 | TG:<br>HVMPC + SWC<br>CG:<br>Sham HVMPC + SWC | 100 V, 100HZ, 77 μs for 50 min once daily, 5 days a week. The total delivered electric charge was 250 μC/s | Negative electrode over wound, The active electrode is 5 × 10 cm and dispersive electrode was 10 × 10 cm. 20 cm between electrodes. Conductive carbon rubber electrodes | 6 weeks or until wound closure | **Mean ↓ WSA (%)**<br>After 1 week<br>TG:35 ± 30.5<br>CG:17.07 ± 34.13<br>After 6 weeks<br>TG:80.31 ± 29.02<br>CG:54.65 ± 42.65 | **TG:13.39**<br>**CG:9.11** |
| Polak and Kloth et al. (2017) [37] | RCT single blind | Total:63<br>CAT:23 (6 M/17 F)<br>CAN:20 (2 M/18 F)<br>CG:20 (3 M/17 F) | CAT:79.35 ± 8.48<br>CAN:79.65 ± 11.4<br>CG:76.75 ± 12.24 | II-IV<br>CAT: II = 12, III = 9, IV = 2<br>CAN: II = 11, III = 6, IV = 3<br>CG: II = 13, III = 6 IV = 1 | CAT:<br>Cathode HVMPC + SWC<br>CAN:<br>Cathode + Anode HVMPC + SWC<br>CG:<br>Sham HVMPC + SWC | 100 V, 100HZ, 77 μs for 50 min once daily, 5 days a week. The total delivered electric charge was 250 μC/s | CAT: Negative electrode over wound<br>CAN: Negative electrode over wound for first 5 days then positive electrode, The active electrode was 5 × 10 cm and dispersive electrode was 10 × 10 cm. 20 cm between electrodes. Conductive carbon rubber electrodes | 6 weeks | **Mean ↓ WSA (%)**<br>After 6 weeks<br>CAT:82.34 ± 28.41<br>CAN:70.77 ± 36.89<br>CG:40.53 ± 36.1 | **CAT:13.72**<br>**CAN:11.80**<br>**CG:6.76** |
| Recio, A. C. et al. (2012) [38] | Retrospective case series | 3 (3 M) | 43 | III-IV<br>PtA: IV<br>PtB: IV<br>PtC: III | HVMPC + SWC | 100HZ for 60 min, 3–5 times a week | Negative electrode over wound initially, polarity alternated once weekly, 2 inch rounded electrode into wound bed and dispersive electrode placed proximal to wound | PtA:16 weeks<br>PtB: 22 weeks<br>PtC: 6.5 weeks | **Mean ↓ WSA (%)**<br>PtA:95.58<br>PtB:96.25<br>PtC:100 | **PtA:5.97**<br>**PtB:4.37**<br>**PtC:15.38** |
| Bora Karsli, P. et al. (2017) [39] | Prospective, randomized case series | 27 (22 M/ 5 F)<br>TG1:15 (25 PrUs)<br>TG2:12 (22 PrUs) | 32.63 ± 15.96 | II-IV<br>HVMPC:<br>II = 5, III = 13, IV = 7<br>US:II = 9, III = 13, IV = 0 | TG1:<br>HVMPC + SWC<br>TG2:<br>US + SWC | 50–150 V, 100HZ, 10-/ 50-/100-μs, 2 s ramp-up time, for 60 min, 3 times a week | Negative electrode initially, polarity altered at weekly intervals (electrode placement was not provided) | 4–12 weeks | **Measured WSA Baseline ($cm^2$):**<br>TG1:25.25 ± 17.77<br>TG2:15.17 ± 16.85<br>**WSA After intervention**<br>TG1:16.71 ± 15.53<br>TG2:7.88 ± 16.05<br>**Mean ↓ WSA (%)**<br>TG1: 43%<br>TG2:63% | **TG1:3.58**<br>**TG2:5.25** |

HVMPC, high-voltage monophasic pulsed current; SWC, standard wound care; US, ultrasound; ES, electrical stimulation; CAT, cathode group; CAN, cathode and anode group; PrUs, pressure ulcers; TG, treatment group; CG, control group; M, male; F, female; V, volts; HZ, Hertz; μs, microseconds; PSWC, pharmacologic agents + standard wound care, PARW, mean percentage area reduction per week; *, mean ↓WSA was calculated for this sample; PtA, patient A; PtB, patient B; PtC, patient C.

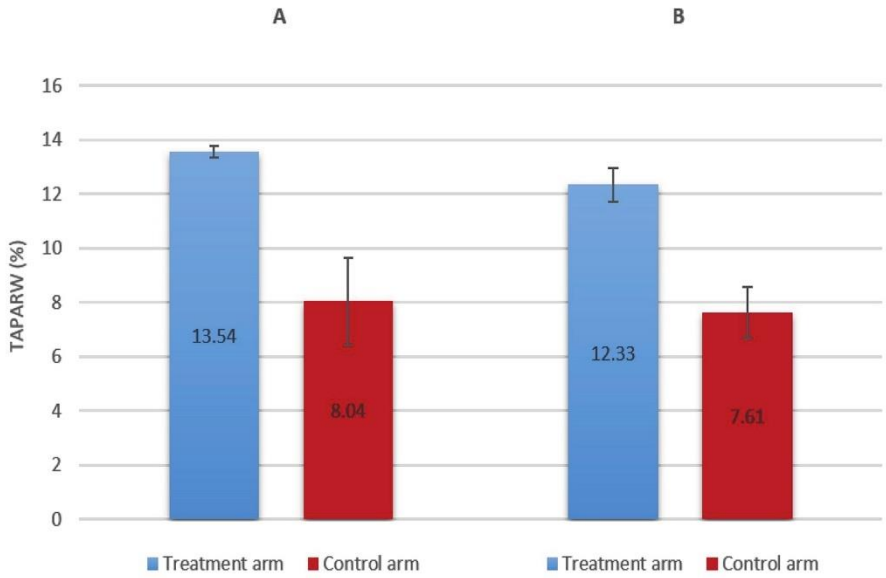



**Fig. 3. Subgroup analysis of the cathodal HVMPC protocol (A) and the cathodal-anodal HVMPC protocol (B).** TAPARW, total of averages of percentage area reduction per week; Error bars represent the 95% confidence intervals.

**Table 6**
**Subgroup analysis of the cathodal HVMPC protocol.** TAPARW, total of averages of percentage area reduction per week; *, APARW (average of percentage area reduction per week).

| Cathodal HVMPC Protocol | Treatment arm | Control arm | Net effect |
|---|---|---|---|
| Number of ulcers | 48 | 44 | **5.5** |
| Polak and Kloth et al. (2016)* [36] | 6.97 | 4.96 | |
| Polak and Kloth et al. (2017)* [37] | 6.57 | 3.07 | |
| TAPARW (%) | 13.54 | 8.04 | |
| Standard deviation | 0.164 | 1.17 | |
| Standard error of mean | 0.116 | 0.827 | |
| 95% Confidence interval | 13.32–13.77 | 6.42–9.66 | |

**Table 7**
**Subgroup analysis of the cathodal-anodal HVMPC protocol.** TAPARW, total of averages of percentage area reduction per week; *, APARW (average of percentage area reduction per week).

| Cathodal-Anodal HVMPC Protocol | Treatment arm | Control arm | Net effect |
|---|---|---|---|
| Number of ulcers | 49 | 51 | **4.77** |
| Polak et al. (2016)* [35] | 7.51 | 4.96 | |
| Polak and Kloth et al. (2017)* [37] | 4.81 | 2.65 | |
| TAPARW (%) | 12.33 | 7.61 | |
| Standard deviation | 0.44 | 0.68 | |
| Standard error of mean | 0.31 | 0.48 | |
| 95% Confidence interval | 11.72–12.94 | 6.66–8.56 | |

analysis of these results is presented in the SI.

### 3.6.3. Adverse reactions to intervention

Five studies stated that HVMPC intervention had no adverse reactions and patients did not complain of any discomfort [29,34–37]. One study reported minor and rare adverse reactions to treatment caused by contact dermatitis to the dispersive electrode and was resolved by using non-adhesive carbon electrode, also one patient had a persistent (>24 h) red area or burn under the active electrode and was remedied by reducing the current intensity [32]. Three studies indicated that HVMPC is a safe intervention [31,32,34].

### 3.6.4. Morbidity and pain status

None of the studies reported pain status or pain relief associated to treatment, however, two studies stated that HVMPC did not cause pain or discomfort [29,34]. HVMPC intervention promoted complete healing of stage III-IV recalcitrant PrUs that were unresponsive to previous treatments including SWC [30,38]. One study mentioned that some patients in the control group received HVMPC as part of SWC during the follow-up period and did not achieve complete healing [32]. This was, however, attributed to premature termination of HVMPC intervention [32]. Significant reduction in WSA occurred in each and all PrUs stages (II-IV) in response to HVMPC intervention [39]. Wound healing progressed consistently and steadily in patients who received HVMPC intervention [29,33,34]. Four out of nine patients in the

| Study | Risk Ratio (RR) [95% CI] | p-value | Relative weight (%) | Treatment arm | Control arm | RR and 95% CI |
|---|---|---|---|---|---|---|
| Houghton et al. (2010)[32] | 1.35[0.5-3.58] | 0.547 | 18.52 | 6/16 | 5/18 | |
| Franek et al. (2011)[33] | 2[0.67-5.91] | 0.210 | 15.05 | 8/29 | 4/29 | |
| Polak et al. (2016)[35] | 2.29[1.09-4.8] | 0.028 | 32.25 | 15/29 | 7/31 | |
| Polak and Kloth et al. (2016)[36] | 1.65[0.78-3.46] | 0.19 | 31.86 | 12/25 | 7/24 | |
| Polak and Kloth et al. (2017)[37] | 19.57[1.24-308] | 0.034 | 2.33 | 20/43 | 0/20 | |
| **Overall effect** | **1.93[1.26-2.93]** | **0.002** | | | | |
| **Heterogeneity: $I^2$=0%, $p$=0.461** | | | | | | |
| **Model: Random** | | | | | | |



**Fig. 4. Forest plot of RR analysis.** RR < 1, the probability of complete healing is lower in treatment arm; RR > 1, the probability of complete healing is higher in treatment arm.

treatment group of one study reached a healing plateau with anodal stimulation, the polarity was changed to cathode initially and alternated daily if a second healing plateau occurred [30]. Significant reduction in wound exudate was reported in two studies [33,38].

## 4. Discussion

Wound surface area reduction is a valid endpoint regarding wound healing, however, the minimal clinically relevant benefit of additional reduction in wound area has not been established [40–42]. The results of the current review show that HVMPC plus SWC have increased the percentage of WSA reduction more than SWC alone or SWC plus sham HVMPC. Additionally, the results suggest that healing progressed more consistently as only one ulcer in the treatment arm increased in WSA in contrast with 10 ulcers in the control arm. The U.S. Food and Drug Administration (FDA) has indicated that complete wound closure is one of the most objective and clinically meaningful efficacy endpoints [40,42]. In the present review, level 1 evidence studies revealed that 61 ulcers (out of 142, 43%) healed completely in the treatment arm, whereas in the control arm, 23 ulcers (out of 122, 19%) healed completely. Pain status as well as pain relief associated to treatment were not discussed in any of the studies. This was, however, unexpected considering that pressure ulcer-related pain is common and that even the SWC is painful [43–46]. Moreover, HVMPC intervention was shown to be effective in reducing pain in other conditions [47–50].

The mechanisms behind the observed effects of HVMPC intervention may be explained by various studies that examined the effects of the current and the electrical properties of wounds. HVMPC was determined to enhance fibroblast proliferation (near cathode) [51–53] and keratinocyte proliferation [54], have antibacterial effects [55–60], increase collagen synthesis [51,61,62], and augment blood flow [63,64]. Another theory that may explain the results is the galvanotaxis theory (cell migration towards positive or negative electric charge). Fibroblasts [65–70] and keratinocytes [71–77] were shown to move towards the cathode. Conversely, the anode was demonstrated to attract macrophages, neutrophils, epithelial cells, and fibroblasts (slow) [76,78–80].

Since each electric charge attract different cells, it is not unexpected to observe differences between the two polarities in regard to wound healing properties. Cathodal ES was reported to enhance tensile strength of wounds [81–84], while anodal ES was shown to improve epithelialization but not tensile strength [85–87]. Two studies used cathodal-anodal HVMPC (cathode for first 3 days followed by anode for the remainder of intervention, similar to the cathodal-anodal HVMPC protocol discussed in the current review) in rabbits and guinea-pigs and concluded that HVMPC in the described manner enhanced wound epithelialization but did not augment the tensile strength [88,89]. A study found that using the anode for the first three days followed by the cathode increased the tensile strength [90], however, another study did not detect differences in tensile strength related to either polarities [91]. Other poorly designed studies reached conclusions inconsistent with these results [92–95].

Burr et al. [96] measured and described electrical correlates during wound healing in humans. The wound potential was reported to be positive in the first duration of some four days and then it became and remained negative until complete healing [96]. The same phenomena were observed in guinea-pigs and mice, in which the positive charge duration coincided with the inflammatory phase of healing and lack of tensile strength, whereas the negative charge duration corresponded with the proliferative phase of healing and improvement in wound tensile strength [96–99]. Based on these studies, the results suggest that the electrical correlates identified during wound healing may not be simple epiphenomena and may have a role in wound healing, since augmenting them through external electrical stimulation may lead to corresponding changes in tensile strength. Consequently, cathodal ES seems to be a more convenient option for chronic wound healing, considering that its application may attract fibroblasts and keratinocytes to the wound site, may increase wound tensile strength which may result in a better healing quality, and resembles the typically occurring negative charge duration coinciding with the proliferative healing phase.

The results of the current review principally agree with other reviews in literature. Gardner et al. [6] reported that ES combined with SWC increased the percentage of healing per week (PHW) in pressure ulcer patients by 16.63% per week, although this effect may be overestimated and inaccurate, since the control samples were underrepresented. The results of the present review show that HVMPC combined with SWC increased the percentage of WSA reduction by 12.39% per week; 95% CI, [10.43–14.37], and 13.54% per week; 95% CI, [13.32–13.77] using the cathodal HVMPC protocol.

Lala et al. [5] has recently reported that ES increased wound closure by 1.55 ($p = 0.01$); 95% CI, [1.12–2.15] times more than SWC alone or SWC plus sham ES. However, these results were due to the effects of ES and SWC combined and not ES treatment alone. The results of the current review show that the probability of complete wound healing was 1.96 ($p = 0.002$); 95% CI, [1.26–2.93] times higher in patients treated with HVMPC plus SWC when compared to SWC alone or SWC plus sham HVMPC.

### 4.1. Limitations

- The main limitation in this review and most of the included studies is that the follow-up duration was not long enough for all ulcers to completely heal. Two studies, however, continued until complete healing of all ulcers and stated that HVMPC intervention promoted complete healing of stage III-IV recalcitrant PrUs that did not respond to previous treatments including SWC [30,38].
- The design may have resulted in reporting bias, as unpublished studies and studies written in languages other than English were not included. Nevertheless, the inclusion was not limited to a specific study design in attempt to compensate for this bias.
- Inadequate randomization and concealed allocation may lead to overestimation of treatment effects [100–102]. All level 1 evidence studies applied appropriate randomization and allocation concealment. Placebo effect may not be significant in objective outcomes [103] and blinding was shown to help reach more accurate results [101,104]. Blinding and placebo or sham HVMPC were rarely applied in most studies, except for two level 1 evidence studies [36,37] that used sham HVMPC, and blinded patients and assessors but did not blind therapists. Nonetheless, the objective nature of WSA measurement may compensate for the lack of both placebo and blinding effects. Moreover, the features of ES may not permit blinded application.
- Blinded assessment of the methodological quality of studies was shown to produce more accurate and consistent evaluation scores than open assessment [105], however this was challenged by another study [106]. The studies in this review were not blindly assessed, which might have led to evaluation inaccuracy.
- Two level 1 evidence studies were of long duration (3–4 years), which may have introduced confounding factors to these studies [33,35].
- The FDA defined complete wound closure and recommended that adequate follow-up should succeed complete wound healing to ascertain that actual healing had occurred [42]. However, all level 1 evidence studies did not precisely define it [32,33,35–37] and only one study followed patients for at least three months after treatment had ended [32].
- SWC was customized for each patient to match their individual needs [32,35–37], which might have influenced the differences in wound healing between arms.
- Two studies in the meta-analysis included a small number of stage I and X PrUs [32,33], these were, however, out of the scope of this

review (stage II-IV).
- The method used to measure WSA (acetate tracing) has a margin of error, however, it was used for ulcer measurement in both arms, which may have equalized this error.

### 4.2. Conclusions

Level 1, 2 and 4 evidence studies have consistently indicated that HVMPC plus SWC were more effective than SWC alone or SWC plus sham HVMPC in treating stage II-IV PrUs. Level 1 evidence studies showed that HVMPC intervention increased the percentage of WSA reduction and therefore improved the healing of PrUs. Moreover, HVMPC plus SWC not only increased the probability of complete ulcer healing, but also almost eliminated the probability of worsening or increase in WSA. Cathodal HVMPC protocol was determined to be superior to the cathodal-anodal HVMPC protocol. HVMPC intervention was shown to be relatively safe, with rare adverse reactions.

### 4.3. Future research

- Further investigation should focus on identifying the exact underlying mechanisms behind the beneficial effects of HVMPC intervention.
- Further research should investigate the efficacy of HVMPC in the treatment of ulcers of other etiologies, especially diabetic and venous leg ulcers.

## Funding and conflict of interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare no conflict of interest.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jtv.2018.08.003.

## References

[1] Russo CA, Steiner C, Spector W. Hospitalizations related to pressure ulcers among adults 18 years and older. 2006. statistical brief# 64. 2006. PMC21595131.
[2] Berlowitz DR, Brandeis GH, Anderson J, Du W, Brand H. Effect of pressure ulcers on the survival of long-term care residents. J Gerontol A Biol Sci Med Sci 1997;52(2):M106–10https://doi.org/10.1093/gerona/52A.2.M106.
[3] Thomas DR, Goode PS, Tarquine PH, Allman RM. Hospital-acquired pressure ulcers and risk of death. J Am Geriatr Soc 1996;44(12):1435–40https://doi.org/10.1111/j.1532-5415.1996.tb04067.x.
[4] Velez-Diaz-Pallares M, Lozano-Montoya I, Abraha I, Cherubini A, Soiza RL, O'Mahony D, et al. Nonpharmacologic interventions to heal pressure ulcers in older patients: an overview of systematic reviews (the SENATOR-ONTOP series). J Am Med Dir Assoc 2015;16(6):448–69https://doi.org/10.1016/j.jamda.2015.01.083.
[5] Lala D, Spaulding SJ, Burke SM, Houghton PE. Electrical stimulation therapy for the treatment of pressure ulcers in individuals with spinal cord injury: a systematic review and meta-analysis. Int Wound J 2016;13(6):1214–26https://doi.org/10.1111/iwj.12446.
[6] Gardner SE, Frantz RA, Schmidt FL. Effect of electrical stimulation on chronic wound healing: a meta-analysis. Wound Repair Regen 1999;7(6):495–503https://doi.org/10.1046/j.1524-475X.1999.00495.x.
[7] Liu LQ, Moody J, Traynor M, Dyson S, Gall A. A systematic review of electrical stimulation for pressure ulcer prevention and treatment in people with spinal cord injuries. J Spinal Cord Med 2014;37(6):703–18https://doi.org/10.1179/2045772314Y.0000000226.PMC4231958.
[8] Koel G, Houghton PE. Electrostimulation: current status, strength of evidence guidelines, and meta-analysis. Adv Wound Care 2014;3(2):118–26https://doi.org/10.1089/wound.2013.0448.PMC3928827.
[9] Kawasaki L, Mushahwar VK, Ho C, Dukelow SP, Chan LL, Chan KM. The mechanisms and evidence of efficacy of electrical stimulation for healing of pressure ulcer: a systematic review. Wound Repair Regen 2014;22(2):161–73https://doi.org/10.1111/wrr.12134.
[10] Houghton PE. Electrical stimulation therapy to promote healing of chronic wounds: a review of reviews. Chron Wound Care Manag Res 2017;55(4):25–44https://doi.org/10.2147/CWCMR.S101323.
[11] National Pressure Ulcer Advisory Panel EPUAPaPPPIA. haesler Emily, editor. Prevention and treatment of pressure ulcers: quick reference guide. Perth, Australia: Cambridge Media; 2014. 2014.
[12] Keast DH, Parslow N, Houghton PE, Norton L, Fraser C. Best practice recommendations for the prevention and treatment of pressure ulcers: update 2006. Adv Skin Wound Care 2007;20(8):447–60. quiz 61-2 https://doi.org/10.1097/01.ASW.0000284922.69932.c5.
[13] Gould L, Stuntz M, Giovannelli M, Ahmad A, Aslam R, Mullen-Fortino M, et al. Wound Healing Society 2015 update on guidelines for pressure ulcers. Wound Repair Regen 2016;24(1):145–62https://doi.org/10.1111/wrr.12396.
[14] Whitney J, Phillips L, Aslam R, Barbul A, Gottrup F, Gould L, et al. Guidelines for the treatment of pressure ulcers. Wound Repair Regen 2006;14(6):663–79https://doi.org/10.1111/j.1524-475X.2006.00175.x.
[15] Thomason SS, Evitt CP, Harrow JJ, Love L, Moore DH, Mullins MA, et al. Providers' perceptions of spinal cord injury pressure ulcer guidelines. J Spinal Cord Med 2007;30(2):117–26https://doi.org/10.1080/10790268.2007.11753922.PMC2031945.
[16] Adunsky A, Ohry A. Decubitus direct current treatment (DDCT) of pressure ulcers: results of a randomized double-blinded placebo controlled study. Arch Gerontol Geriatr 2005;41(3):261–9https://doi.org/10.1016/j.archger.2005.04.004.
[17] Kloth LC. Electrical stimulation technologies for wound healing. Adv Wound Care 2014;3(2):81–90https://doi.org/10.1089/wound.2013.0459.PMC3929255.
[18] Ontario HQ. Management of chronic pressure ulcers: an evidence-based analysis. Ontario health technology assessment series 2009;9(3):1. PMC3377577.
[19] Houghton PE. Clinical trials involving biphasic pulsed current, microcurrent, and/or low-intensity direct current. Adv Wound Care 2014;3(2):166–83https://doi.org/10.1089/wound.2013.0446.
[20] Polak A, Franek A, Taradaj J. High-voltage pulsed current electrical stimulation in wound treatment. Adv Wound Care 2014;3(2):104–17https://doi.org/10.1089/wound.2013.0445.
[21] Newton R. High-voltage pulsed galvanic stimulation: theoretical bases and clinical application. In: Nelson R, Currier D, editors. Clinical electrotherapy. Norwalk, CT: Appleton & Lange; 1987. p. 165–82.
[22] Johnson MI. Transcutaneous Electrical Nerve Stimulation (TENS) and TENS-like devices: do they provide pain relief? Pain Rev 2001;8(3):121https://doi.org/10.1191/0968130201pr182ra.
[23] de Morton NA. The PEDro scale is a valid measure of the methodological quality of clinical trials: a demographic study. Aust J Physiother 2009;55(2):129–33https://doi.org/10.1016/S0004-9514(09)70043-1.
[24] Maher CG, Sherrington C, Herbert RD, Moseley AM, Elkins M. Reliability of the PEDro scale for rating quality of randomized controlled trials. Phys Ther 2003;83(8):713–21https://doi.org/10.1093/ptj/83.8.713.
[25] Yamato TP, Maher C, Koes B, Moseley A. The PEDro scale had acceptably high convergent validity, construct validity, and interrater reliability in evaluating methodological quality of pharmaceutical trials. J Clin Epidemiol 2017https://doi.org/10.1016/j.jclinepi.2017.03.002.
[26] Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community 1998;52(6):377–84https://doi.org/10.1136/jech.52.6.377.
[27] Sackett DS S, Richardson W, et al. Evidence-based medicine: how to practice and teach EBM. second ed. Edinburgh: Churchill Livingstone; 2000.
[28] Straus SERW, Glasziou P, Haynes RB. Evidence-based medicine: how to practice and teach EBM. third ed. Toronto: Elsevier Churchill Livingstone; 2005.
[29] Griffin JW, Tooms RE, Mendius RA, Clifft JK, Vander Zwaag R, el-Zeky F. Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. Phys Ther 1991;71(6):433–42. discussion 42-4 https://doi.org/10.1093/ptj/71.6.433.
[30] Kloth LC, Feedar JA. Acceleration of wound healing with high voltage, monophasic, pulsed current. Phys Ther 1988;68(4):503–8https://doi.org/10.1093/ptj/68.4.503.
[31] Ahmad ET. High-voltage pulsed galvanic stimulation: effect of treatment duration on healing of chronic pressure ulcers. Ann Burns Fire Disasters 2008;21(3):124–8.PMC3188162.
[32] Houghton PE, Campbell KE, Fraser CH, Harris C, Keast DH, Potter PJ, et al. Electrical stimulation therapy increases rate of healing of pressure ulcers in community-dwelling people with spinal cord injury. Arch Phys Med Rehabil 2010;91(5):669–78https://doi.org/10.1016/j.apmr.2009.12.026.
[33] Franek A, Kostur R, Taradaj J, Błaszczak E, Szlachta Z, Dolibog P, et al. Effect of high voltage monophasic stimulation on pressure ulcer healing: results from a randomized controlled Trial. 2011.
[34] Franek A, Kostur R, Polak A, Taradaj J, Szlachta Z, Blaszczak E, et al. Using high-voltage electrical stimulation in the treatment of recalcitrant pressure ulcers: results of a randomized, controlled clinical study. Ostomy/Wound Manag 2012;58(3):30.
[35] Polak A, Taradaj J, Nawrat-Szoltysik A, Stania M, Dolibog P, Blaszczak E, et al. Reduction of pressure ulcer size with high-voltage pulsed current and high-frequency ultrasound: a randomised trial. J Wound Care 2016;25(12):742–54https://doi.org/10.12968/jowc.2016.25.12.742.
[36] Polak A, Kloth LC, Blaszczak E, Taradaj J, Nawrat-Szoltysik A, Walczak A, et al. Evaluation of the healing progress of pressure ulcers treated with cathodal high-voltage monophasic pulsed current: results of a prospective, double-blind, randomized clinical trial. Adv Skin Wound Care 2016;29(10):447–59https://doi.org/10.1097/01.ASW.0000493164.75337.de.
[37] Polak A, Kloth LC, Blaszczak E, Taradaj J, Nawrat-Szoltysik A, Ickowicz T, et al. The efficacy of pressure ulcer treatment with cathodal and cathodal-anodal high-

voltage monophasic pulsed current: a prospective, randomized, controlled clinical trial. Phys Ther 2017;97(8):777–89https://doi.org/10.1093/ptj/pzx052.
[38] Reeio AC, Felter CE, Schneider AC, McDonald JW. High-voltage electrical stimulation for the management of Stage III and IV pressure ulcers among adults with spinal cord injury: demonstration of its utility for recalcitrant wounds below the level of injury. The journal of spinal cord medicine 2012;35(1):58–63https://doi.org/10.1179/2045772311Y.0000000044.
[39] Bora Karsli P, Gurcay E, Karaahmet OZ, Cakci A. High-voltage electrical stimulation versus ultrasound in the treatment of pressure ulcers. Adv Skin Wound Care 2017;30(12):565–70https://doi.org/10.1097/01.ASW.0000526606.72489.99.
[40] Gottrup F, Apelqvist J, Price P. European Wound Management Association Patient Outcome G. Outcomes in controlled and comparative studies on non-healing wounds: recommendations to improve the quality of evidence in wound management. J Wound Care 2010;19(6):237–68https://doi.org/10.12968/jowc.2010.19.6.48471.
[41] Flanagan M. Wound measurement: can it help us to monitor progression to healing? J Wound Care 2003;12(5):189–94https://doi.org/10.12968/jowc.2003.12.5.26493.
[42] Food, Administration D. Guidance for industry. Chronic cutaneous ulcer and burn wounds—developing products for treatment. Food and Drug Administration 2006:1–18.
[43] Szor JK, Bourguignon C. Description of pressure ulcer pain at rest and at dressing change. Journal of WOCN 1999;26(3):115–20https://doi.org/10.1016/S1071-5754(99)90028-1.
[44] Stotts N, Puntillo K, Morris A, Stanik-hutt J, Thompson C, White C, et al. How painful is wound care? Wound Repair Regen 2005;13(2):A4https://doi.org/10.1111/j.1067-1927.2005.130215b.x.
[45] Pieper B, Langemo D, Cuddigan J. Pressure ulcer pain: a systematic literature review and national pressure ulcer advisory panel white paper. Ostomy/Wound Manag 2009;55(2):16–31.
[46] Dallam L, Smyth C, Jackson BS, Krinsky R, O'Dell C, Rooney J, et al. Pressure ulcer pain: assessment and quantification. J Wound, Ostomy Cont Nurs 1995;22(5):211–5. discussion 7-8 https://doi.org/10.1097/00152192-199509000-00007.
[47] Morris L, Newton RA. Use of high voltage pulsed galvanic stimulation for patients with levator ani syndrome. Phys Ther 1987;67(10):1522–5https://doi.org/10.1093/ptj/67.10.1522.
[48] Ross C, Segal D. High voltage galvanic stimulation: an aid to post-operative healing. Curr Podiatry 1981;30(5):19–25.
[49] Nathalie M, Paris BD, Herand A, Cintron JR, Zavala A, Singer M. Efficacy of electrogalvanic stimulation in treatment of levator ani syndrome revisited. J Chir 2014;10(2)https://doi.org/10.7438/1584-9341-10-2-12.
[50] Sohn N, Weinstein MA, Robbins RD. The levator syndrome and its treatment with high-voltage electrogalvanic stimulation. Am J Surg 1982;144(5):580–2https://doi.org/10.1016/0002-9610(82)90586-4.
[51] Bourguignon GJ, Bourguignon LY. Electric stimulation of protein and DNA synthesis in human fibroblasts. Faseb J 1987;1(5):398–402https://doi.org/10.1096/fasebj.1.5.3678699.
[52] Bourguignon GJ, Jy W, Bourguignon LY. Electric stimulation of human fibroblasts causes an increase in Ca2+ influx and the exposure of additional insulin receptors. J Cell Physiol 1989;140(2):379–85https://doi.org/10.1002/jcp.1041400224.
[53] Cruz NI, Bayrón FE, Suárez AJ. Accelerated healing of full-thickness burns by the use of high-voltage pulsed galvanic stimulation in the pig. Ann Plast Surg 1989;23(1):49–55https://doi.org/10.1097/00000637-198907000-00009.
[54] Lee J-H, Lee J-S, Kil E-Y. Keratinocyte proliferation in aged rat skin by high voltage pulsed current stimulation. 대한의생명과학회지. 2004;10(4):361–6.
[55] Kuykendall CA, Hutchinson TL, Bloedaum AP. 112 effects of high voltage pulsed current on bacterial viability: an in vitro study. Wound Repair Regen 2004;12(2) https://doi.org/10.1111/j.1067-1927.2004.0abstractdf.x.
[56] Szuminsky NJ, Albers AC, Unger P, Eddy JG. Effect of narrow, pulsed high voltages on bacterial viability. Phys Ther 1994;74(7):660–7https://doi.org/10.1093/ptj/74.7.660.
[57] Merriman HL, Hegyi CA, Albright-Overton CR, Carlos Jr. J, Putnam RW, Mulcare JA. A comparison of four electrical stimulation types on Staphylococcus aureus growth in vitro. J Rehabil Res Dev 2004;41(2):139–46https://doi.org/10.1682/JRRD.2004.02.0139.
[58] Herminawaty D, Defi I, Probowo T, Parwati T. Promising treatment for pressure ulcers using high voltage pulsed current stimulation. Gerontechnology 2014;13(2):202https://doi.org/10.4017/gt.2014.13.02.266.00.
[59] Gomes RC, Brandino HE, de Sousa NT, Santos MF, Martinez R, Guirro RR. Polarized currents inhibit in vitro growth of bacteria colonizing cutaneous ulcers. Wound Repair Regen 2015;23(3):403–11https://doi.org/10.1111/wrr.12296.
[60] Kincaid CB, Lavoie KH. Inhibition of bacterial growth in vitro following stimulation with high voltage, monophasic, pulsed current. Phys Ther 1989;69(8):651–5https://doi.org/10.1093/ptj/69.8.651.
[61] Kim TH, Cho H-y, Lee SM. High-voltage pulsed current stimulation enhances wound healing in diabetic rats by restoring the expression of collagen, α-smooth muscle actin, and TGF- 1. Tohoku J Exp Med 2014;234(1):1–6https://doi.org/10.1620/tjem.234.1.
[62] Falanga V. Electrical stimulation increases the expression of fibroblasts receptors for transforming growth factor-beta. J Invest Dermatol 1987;88. [A].
[63] Mohr T, Akers TK, Wessman HC. Effect of high voltage stimulation on blood flow in the rat hind limb. Phys Ther 1987;67(4):526–33https://doi.org/10.1093/ptj/67.4.526.
[64] Hecker B, Carron H, Schwartz DP. Pulsed galvanic stimulation: effects of current frequency and polarity on blood flow in healthy subjects. Arch Phys Med Rehabil 1985;66(6):369–71.
[65] Erickson CA, Nuccitelli R. Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. J Cell Biol 1984;98(1):296–307https://doi.org/10.1083/jcb.98.1.296.
[66] Dunn MG, Doillon CJ, Berg RA, Olson RM, Silver FH. Wound healing using a collagen matrix: effect of DC electrical stimulation. J Biomed Mater Res 1988;22(S13):191–206https://doi.org/10.1002/jbm.820221310.
[67] Uemura M, Maeshige N, Koga Y, Ishikawa-Aoyama M, Miyoshi M, Sugimoto M, et al. Monophasic pulsed 200- A current promotes galvanotaxis with polarization of actin filament and integrin 2 1 in human dermal fibroblasts. Eplasty 2016;16. PMC4724796.
[68] Sugimoto M, Maeshige N, Honda H, Yoshikawa Y, Uemura M, Yamamoto M, et al. Optimum microcurrent stimulation intensity for galvanotaxis in human fibroblasts. J Wound Care 2012;21(1):5–6. 8,10; discussion -1 https://doi.org/10.12968/jowc.2012.21.1.5.
[69] Nuccitelli R, Erickson CA. Embryonic cell motility can be guided by physiological electric fields. Exp Cell Res 1983;147(1):195–201https://doi.org/10.1016/0014-4827(83)90284-7.
[70] Finkelstein E, Chang W, Chao PH, Gruber D, Minden A, Hung CT, et al. Roles of microtubules, cell polarity and adhesion in electric-field-mediated motility of 3T3 fibroblasts. J Cell Sci 2004;117(Pt 8):1533–45https://doi.org/10.1242/jcs.00986.
[71] Nishimura KY, Isseroff RR, Nuccitelli R. Human keratinocytes migrate to the negative pole in direct current electric fields comparable to those measured in mammalian wounds. J Cell Sci 1996;109(1):199–207.
[72] Farboud B, Nuccitelli R, Schwab IR, Isseroff RR. DC electric fields induce rapid directional migration in cultured human corneal epithelial cells. Exp Eye Res 2000;70(5):667–73https://doi.org/10.1006/exer.2000.0830.
[73] Sheridan DM, Isseroff RR, Nuccitelli R. Imposition of a physiologic DC electric field alters the migratory response of human keratinocytes on extracellular matrix molecules. J Invest Dermatol 1996;106(4):642–6https://doi.org/10.1111/1523-1747.ep12345456.
[74] Pullar CE, Baier BS, Kariya Y, Russell AJ, Horst BA, Marinkovich MP, et al. 4 integrin and epidermal growth factor coordinately regulate electric field-mediated directional migration via Rac 1. Mol Biol Cell 2006;17(11):4925–35https://doi.org/10.1091/mbc.E06-05-0433.
[75] Zhao M, Song B, Pu J, Wada T, Reid B, Tai G, et al. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-γ and PTEN. Nature 2006;442(7101):457–60https://doi.org/10.1038/nature04925.
[76] Guo A, Song B, Reid B, Gu Y, Forrester JV, Jahoda CA, et al. Effects of physiological electric fields on migration of human dermal fibroblasts. J Invest Dermatol 2010;130(9):2320–7https://doi.org/10.1038/jid.2010.96.PMC2952177.
[77] Fang KS, Farboud B, Nuccitelli R, Isseroff RR. Migration of human keratinocytes in electric fields requires growth factors and extracellular calcium. J Invest Dermatol 1998;111(5):751–6https://doi.org/10.1046/j.1523-1747.1998.00366.x.
[78] Orida N, Feldman JD. Directional protrusive pseudopodial activity and motility in macrophages induced by extracellular electric fields. Cell Motil 1982;2(3):243–55https://doi.org/10.1002/cm.970020305.
[79] Eberhardt A, Szczypiorski P, Korytowski G. Effect of transcutaneous electrostimulation on the cell composition of skin exudate. Acta Physiol Pol 1986;37(1):41–6.
[80] Mertz P. Electrical stimulation: acceleration of soft tissue repair by varying the polarity. Wounds 1993;5:153–9.
[81] Assimacopoulos D. Wound healing promotion by the use of negative electric current. Am Surg 1968;34(6):423–31https://doi.org/10.1097%2F00006534-197001000-00053.
[82] Assimacopoulos D. Low intensity negative electric current in the treatment of ulcers of the leg due to chronic venous insufficiency: preliminary report of three cases. Am J Surg 1968;115(5):683–7https://doi.org/10.1016/0002-9610(68)90101-3.
[83] Bigelow J, Al-Husseini S, Von Recum A, Park J. Effect of electrical stimulation on canine skin and percutaneous device: skin interface healing. Skin interface healing and electrical properties of bone and cartilage. New York: Grune & Stratton; 1979. p. 289.
[84] Konikoff JJ. Electrical promotion of soft tissue repairs. Ann Biomed Eng 1976;4(1):1–5https://doi.org/10.1007/BF02363553.
[85] Alvarez OM, Mertz PM, Smerbeck RV, Eaglstein WH. The healing of superficial skin wounds is stimulated by external electrical current. J Invest Dermatol 1983;81(2):144–8https://doi.org/10.1111/1523-1747.ep12543498.
[86] Bach S, Bilgrav K, Gottrup F, Jorgensen TE. The effect of electrical current on healing skin incision. An experimental study. Eur J Surg 1991;157(3):171–4.
[87] Brown M, McDonnell MK, Menton DN. Electrical stimulation effects on cutaneous wound healing in rabbits. A follow-up study. Phys Ther 1988;68(6):955–60https://doi.org/10.1093/ptj/68.6.955.
[88] Brown M, Gogia PP, Sinacore DR, Menton DN. High-voltage galvanic stimulation on wound healing in Guinea pigs: longer-term effects. Arch Phys Med Rehabil 1995;76(12):1134–7https://doi.org/10.1016/S0003-9993(95)80122-7.
[89] Brown M, McDonnell MK, Menton DN. Polarity effects on wound healing using electric stimulation in rabbits. Arch PM&R (Phys Med Rehabil) 1989;70(8):624–7.
[90] Mehmandoust FG, Torkaman G, Firoozabadi M, Talebi G. Anodal and cathodal pulsed electrical stimulation on skin wound healing in Guinea pigs. J Rehabil Res Dev 2007;44(4):611–8.
[91] Wu KT, Go N, Dennis C, Enquist I, Sawyer PN. Effects of electric currents and interfacial potentials on wound healing. J Surg Res 1967;7(3):122–8https://doi.org/10.1016/0022-4804(67)90096-0.
[92] Bayat M, Asgari-Moghadam Z, Maroufi M, Rezaie F-S. Experimental wound healing using microamperage electrical stimulation in rabbits. JRRD (J Rehabil

Res Dev) 2006;43(2):219.
[93] Demir H, Balay H, Kirnap M. A comparative study of the effects of electrical stimulation and laser treatment on experimental wound healing in rats. J Rehabil Res Dev 2004;41(2):147–54.
[94] Stromberg BV. Effects of electrical currents on wound contraction. Ann Plast Surg 1988;21(2):121–3.
[95] Taskan I, Ozyazgan I, Tercan M, Kardas HY, Balkanli S, Saraymen R, et al. A comparative study of the effect of ultrasound and electrostimulation on wound healing in rats. Plast Reconstr Surg 1997;100(4):966–72.
[96] Burr HS, Taffel M, Harvey SC. An electrometric study of the healing wound in man. Yale J Biol Med 1940;12(5):483–5. PMC2602323.
[97] Howes EL, Sooy JW, Harvey SC. The healing of wounds as determined by their tensile strength. JAMA, J Am Med Assoc 1929;92(1):42–5https://doi.org/10.1001/jama.1929.02700270046011.
[98] Burr HS, Harvey SC, Taffel M. Bio-electric correlates of wound healing. Yale J Biol Med 1938;11(2):103–7. PMC2601960.
[99] Taffel M, Harvey SC. Effect of absolute and partial vitamin C deficiency on healing of Wounds. PSEBM (Proc Soc Exp Biol Med) 1938;38(4):518–25https://doi.org/10.3181/00379727-38-9918.
[100] Wood L, Egger M, Gluud LL, Schulz KF, Juni P, Altman DG, et al. Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. BMJ 2008;336(7644):601–5https://doi.org/10.1136/bmj.39465.451748.AD. PMC2267990.
[101] Schulz KF, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. J Am Med Assoc 1995;273(5):408–12https://doi.org/10.1001/jama.1995.03520290060030.
[102] Moher D, Jones A, Cook DJ, Jadad AR, Moher M, Tugwell P, et al. Does quality of reports of randomised trials affect estimates of intervention efficacy reported in meta-analyses? Lancet 1998;352(9128):609–13https://doi.org/10.1016/S0140-6736(98)01085-X.
[103] Hróbjartsson A, Gøtzsche PC. Is the placebo powerless? N Engl J Med 2001;2001(344):1594–602https://doi.org/10.1056/NEJM200105243442106.
[104] Day SJ, Altman DG. Statistics notes: blinding in clinical trials and other studies. BMJ 2000;321(7259):504https://doi.org/10.1136/bmj.321.7259.504. PMC1118396.
[105] Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Contr Clin Trials 1996;17(1):1–12https://doi.org/10.1016/0197-2456(95)00134-4.
[106] Morissette K, Tricco AC, Horsley T, Chen MH, Moher D. Blinded versus unblinded assessments of risk of bias in studies included in a systematic review. Cochrane Database Syst Rev 2011;9. MR000025 https://doi.org/10.1002/14651858.MR000025.pub2.

# Supplementary data

**Methods**

**Synthesis of results**

-The mean percentage of reduction in wound surface area from baseline (mean WSA %) was used as reported in each study. If not reported, while the mean surface area at baseline and at the end of study were reported, the mean WSA % was calculated using the following equation: the difference between baseline and the last post-treatment percentage of mean WSA was calculated and then divided by the baseline percentage of mean WSA.

[Mean ↓WSA % $=\frac{(initial\ WSA - final\ WSA)\times 100}{initial\ WSA}$].

If the median, instead of the mean, percentage of reduction in wound surface area was reported, the mean WSA % was estimated using this equation:

[$\bar{x} \approx \frac{a+2m+b}{4}$] [1].

-The PARW was calculated for each treatment and control sample using the following equation: Mean WSA % of each sample was divided by the intervention period in weeks.

[PARW$=\frac{Mean\downarrow WSA\%}{N.of\ weeks}$].

-The APARW was calculated for each treatment and control sample using the following equation: The PARW was weighted by the corresponding sample size and divided by the total number of sample sizes in the equivalent arm to get the APARW of this sample. [$APARW_x = \sum n_x PARW_x / \sum n_z$] (*x* represents one sample, *z* represents corresponding arm).

-The APARW of each sample in one arm were then summed to get the TAPARW of that arm.

-The variance of the PARW was weighted by the corresponding sample size as well and calculated using the following equation:

[$S^2_{PARWx} = \sum n_{x\,(PARWx - TAPARWz)^2} / \sum n_z$].

-The $S^2_{PARWx}$ of all samples in one arm were then summed to get the $TS^2_{PARWz}$.

-Standard deviation was then calculated as follows: [$SD_z = TS^2_{PARWz}$].

-Standard error of mean was calculated using this equation: the standard deviation of each arm was divided by the square root of total number of samples in that arm.

[SE of $mean_z = SD_z / n_z$] (*n* represents the number of samples).

-Since it is statistically valid, in multiple group studies, to compare more than one treatment group to a common control group; if a study contained two different HVMPC groups, these groups were thus added to the treatment arm as two separate samples, and consequently the common control group of that study was added twice to the control arm as two samples so that the analysis is statistically valid.

-To determine the net effect of HVMPC treatment, the control arm TAPARW was subtracted from the treatment arm TAPARW. The 95% confidence interval was also calculated for both arms as follows: [95% $CI_z$ = $TAPARW_z \pm (SE_z \times 1.96)$]. Confidence intervals, in contrast to p-value, reflect the direction and the strength of the studied effect [2, 3]. Notable medical science international journals, such as the British Medical Journal and the Lancet, recommend the use of confidence intervals [2, 4].

-Risk ratio (RR) analysis of the number of completely healed PrUs and 95% confidence interval (CI) were performed using Comprehensive Meta Analysis V.2.2.064 (CMA; Biostat, NJ). The interpretation of RR was as follows: RR>1, probability of complete healing is higher in treatment arm; RR=1, no difference in probability of complete healing between treatment and control arms; RR<1, probability of complete healing is lower in treatment arm. The heterogeneity was calculated using $I^2$ and p-value; $I^2$ values of 25%, 50%, 75% correspond to low, moderate, high heterogeneity respectively [5]. A random-effects meta-analysis model was used as it represents a more plausible option when assessing published studies [6].

## Results

### Characteristics of studies included in the systematic review

The identified studies included a total of 454 patients (485 PrUs). Two groups in two studies (37 patients with 50 PrUs) [7, 8] received another intervention modality (ultrasound treatment), and therefore these groups were excluded. The number of patients included in the systematic review was 417 patients (435 PrUs), 243 patients in the treatment arm (258 PrUs) received either HVMPC alone or HVMPC plus SWC, while 174 patients in the control arm (177 PrUs) received either SWC alone or SWC plus sham HVMPC. The mean age of patients in the treatment arm was 55.96±18.74 years and in the control arm was 59.8±16.36 years. The treatment arm included 118 males and 128 females, while the control arm had 64 males and 103 females. The number of PrUs, according to stage, included in the treatment arm was I:7, II:136, III:79, IV:34, X:2, whereas in the control arm was I:8, II:97, III:52, IV:20, X:0. The mean ulcer duration (pre-intervention) in the treatment arm was 21.46±15.64 weeks and in the control arm was 30.55±50.91 weeks. The mean baseline WSA in the treatment arm was 8.11±5.53 $cm^2$ and in the control arm was 7.06±3.01 $cm^2$. The ulcers in both arms were commonly located in the pelvic region (sacrum/coccyx, ischial tuberosity/ trochanter major), and also in the lower extremity (leg, ankle, foot), trunk, hand, and elbow (only in one control sample) regions.

HVMPC stimulation protocol parameters were as follows: The voltage ranged between 50-200V and most commonly ranged between 100-175V (a voltage just below that elicits visible muscle contraction) [7, 9-15]. Only one study used a higher voltage of 200V, however, it did not cause visible muscle contraction in any of the patients [16]. Two studies used lower voltage range between 50-150V [8, 17]. The frequency ranged between 100-120HZ, and most commonly was 100HZ [7, 8, 11-17]. One study used 105HZ [9] and another used 120HZ [10]. The pulse interval ranged between 50-100μs, and most commonly between 50-77μs [7, 9-14, 16]. Two studies used 100μs pulse interval [8, 17]. The intervention duration and frequency of sessions were between 45-60 minutes for 3-7 days a week to 40 minutes, 8 times a day for 7 days a week. The frequently applied intervention was 45-50 minutes, once a day for 5 days a week [7, 9, 11-14], however a longer and more frequent intervention of 60 minutes, once a day for 7 days a week was used in two studies [10, 16]. The mean intervention period in the treatment arm was 6.83±3 weeks and in the control arm was 6.36±2.44 weeks.

The monopolar technique was frequently used, as the active electrode was placed over the ulcer in all of the studies (except for five patients in one study [17]), but the polarity differed across studies. Four studies [7, 10-12] and one group in another study [14] used negative polarity (cathode) initially (first 3 days to two weeks) replaced by positive polarity (anode) for the remainder of intervention. Nonetheless, the cathode was used throughout the intervention duration of two studies [13, 16] and one group in another study [14]. Polarity was also reversed weekly starting by the cathode initially in three studies [8, 15, 17]. Only one study used and maintained the anode unless a healing plateau was reached, the polarity was then switched to negative [9].

The dispersive electrode was often placed 15 to 20 cm from the active electrode [7, 9, 12-14, 17]. Five studies did not mention the distance between electrodes [8, 10, 11, 15, 16]. The electrodes were usually made of conductive carbon-rubber [7, 12-14], but also silver or conductive carbon-rubber [11], and heavy-duty aluminum foil (active electrode) were used [10, 16]. Four studies did not report the material of the electrodes used [8, 9, 15, 17].

The sizes of electrodes were identical in three studies [7, 13, 14], in which the active electrode was 5×10 cm and dispersive electrode was 10×10 cm. Four studies matched the size of the active electrode to ulcer surface area [10, 16]. One study used a 2 inch rounded active electrode [15] and in another study 11 of 16 patients used a 4.8×10.2 cm active electrode and a 12.7×20.3 cm dispersive electrode in the treatment group, while two patients used two equally sized 4.8×10.2 cm active and dispersive electrodes (bipolar technique), and three patients used electroconductive socks [17]. The sizes of utilized electrodes were not mentioned in four studies [8, 9, 11, 12].

Standard wound care (SWC) varied across studies, however, it was identical in three studies [7, 13, 14]. Pressure redistribution surfaces were used in five studies [7, 9, 13, 14, 17]. Enzymatic debridement was used in four studies [7, 9, 13, 14], while mechanical debridement was done in three studies [9, 12, 16]. Whirlpool therapy or local bath were performed in two studies [10, 11]. Wound care consisted of cleansing with saline in two studies [8, 15]. Pharmacological agents were included in one study only [11]. Silver-containing dressings were used in one study (except for 4 patients in the control group) [17].

| Studies | Treatment arm | | | | Control arm | | | | Net effect |
|---|---|---|---|---|---|---|---|---|---|
| | Ulcers | PARW | APARW | S² PARW | Ulcers | PARW | APARW | S² PARW | |
| Houghton et al. (2010) [17] | 16 | 5.83 | 0.6569 | 4.8606 | 18 | 3 | 0.3802 | 1.9975 | |
| Franek et al. (2011) [11] | 29 | 14.23 | 2.9061 | 0.6854 | 29 | 6.68 | 1.3642 | 0.0171 | |
| Polak et al. (2016) [7] | 29 | 12.70 | 2.5936 | 0.0186 | 31 | 8.16 | 1.7814 | 0.3093 | |
| Polak and Kloth et al. (2016) [13] | 25 | 13.39 | 2.3573 | 0.1732 | 24 | 9.11 | 1.5397 | 0.7742 | |
| Polak and Kloth et al. (2017) CAT [14] | 23 | 13.72 | 2.2222 | 0.283 | 20 | 6.76 | 0.9521 | 0.0061 | |
| Polak and Kloth et al. (2017) CAN [14] | 20 | 11.8 | 1.6619 | 0.0503 | 20 | 6.76 | 0.9521 | 0.0061 | |
| **Total** | 142 | 71.67 | 12.398 | 6.0711 | 142 | 40.47 | 6.9697 | 3.1103 | **5.4283 77.88%** |
| **Standard deviation** | | | | 2.4639 | | | | 1.7636 | |
| **Standard error of mean** | | | | 1.0059 | | | | 0.72 | |
| **95% Confidence interval** | | | 10.4265-14.3695 | | | | 5.5585-8.3809 | | |

**Table 1:** Results of the meta-analysis. CAT, cathode group; CAN, cathode and anode group.

| Studies | Treatment arm | | | | Control arm | | | | Net effect |
|---|---|---|---|---|---|---|---|---|---|
| | Ulcers | PARW | APARW | S² PARW | Ulcers | PARW | APARW | S² PARW | |
| Polak and Kloth et al. (2016) [13] | 25 | 13.39 | 6.9739 | 0.0130 | 24 | 9.11 | 4.969 | 0.6233 | |
| Polak and Kloth et al. (2017) [14] | 23 | 13.72 | 6.5741 | 0.0141 | 20 | 6.76 | 3.072 | 0.7458 | |
| **Total** | 48 | 27.11 | 13.548 | 0.0271 | 44 | 15.87 | 8.041 | 1.3691 | **5.507 68.48%** |
| **Standard deviation** | | | | 0.1646 | | | | 1.1700 | |
| **Standard error of mean** | | | | 0.1163 | | | | 0.8273 | |
| **95% Confidence interval** | | | 13.3200-13.7759 | | | | 6.4194-9.6625 | | |

**Table 2:** Subgroup analysis of the cathodal HVMPC protocol.

| Studies | Treatment arm | | | | Control arm | | | | Net effect |
|---|---|---|---|---|---|---|---|---|---|
| | Ulcers | PARW | APARW | S² PARW | Ulcers | PARW | APARW | S² PARW | |
| Polak et al. (2016) [7] | 29 | 12.70 | 7.5163 | 0.0801 | 31 | 8.16 | 4.96 | 0.1838 | |
| Polak and Kloth et al. (2017) [14] | 20 | 11.80 | 4.8163 | 0.1155 | 20 | 6.76 | 2.65 | 0.2833 | |
| **Total** | 49 | 24.5 | 12.332 | 0.1956 | 51 | 14.92 | 7.61 | 0.4671 | **4.772 62.04%** |
| **Standard deviation** | | | | 0.4422 | | | | 0.6834 | |
| **Standard error of mean** | | | | 0.3126 | | | | 0.4832 | |
| **95% Confidence interval** | | | 11.7193-12.9446 | | | | 6.6629-8.5570 | | |

**Table 3:** Subgroup analysis of the cathodal-anodal HVMPC protocol.

| Study | Ulcers according to stage | | Ulcers that healed completely | | Ulcers that increased in surface area | |
|---|---|---|---|---|---|---|
| | Treatment arm | Control arm | Treatment arm | Control arm | Treatment arm | Control arm |
| Kloth and Feeder (1988)[9] | IV=9 | IV=7 | IV=9 | 0 | 0 | IV=4 |
| Griffin et al. (1991)[16] | II=2, III=5, IV=1 | II=2, III=6, IV=1 | II=2, III=1 | II=2 | 0 | 0 |
| Ahmad (2008)[10] | II=45 | II=15 | N/A | N/A | N/A | N/A |
| Houghton et al. (2010)[17] | II=1, III=6, IV=7, X=2 | II=4, III=4,IV=10,X=0 | II=1, III/IV/X=5 | II=4, III/IV/X=1 | 0 | 4 |
| Franek et al. (2011)[11] | I=7, II=13, III=9 | I=8, II=13, III=8 | 8 | 4 | N/A | N/A |
| Franek et al. (2012)[12] | II=17, III=9 | II=16, III=8 | N/A | N/A | N/A | N/A |
| Polak et al. (2016)[7] | II=19, III=7, IV=3 | II=23, III=7, IV=1 | 15 | 7 | 0 | 2 |
| Polak and Kloth et al. (2016)[13] | II=11, III=14 | II=11, III=13 | II=9, III=3 | II:6, III=1 | 0 | III=2 |
| Polak and Kloth et al. (2017)[14] | CAT:II=12, III=9,IV=2<br>CAN:II=11,III=6,IV=3 | II=13, III=6, IV=1 | CAT:11<br>CAN:9 | 0 | CAT:0<br>CAN:1 | 2 |
| Recio, A.C., et al. (2012)[15] | III=1, IV=2 | N/A | III=1 | N/A | 0 | N/A |
| Bora Karsli, P. et al. (2017)[8] | II=5, III=13, IV=7 | N/A | N/A | N/A | N/A | N/A |
| **Total** | 258<br>I:7<br>II:136<br>III:79<br>IV:34<br>X:2 | 177<br>I:8<br>II:97<br>III:52<br>IV:20<br>X:0 | 74 | 25 | 1 | 14 |

**Table 4:** Descriptive analysis of the number of PrUs that completely healed and that increased in surface area of all studies included in the systematic review. WSA, wound surface area; N/A, not available; ulcer stages (I, II, III, IV, X); CAT, cathode group; CAN, cathode and anode group.

201

| Study | Ulcers according to stage | | Ulcers that healed completely | | Ulcers that increased in surface area | |
|---|---|---|---|---|---|---|
| | Treatment arm | Control arm | Treatment arm | Control arm | Treatment arm | Control arm |
| Houghton et al. (2010)[17] | II=1, III=6, IV=7, X=2 | II=4, III=4,IV=10,X=0 | II=1, III/IV/X=5 | II=4, III/IV/X=1 | 0 | 4 |
| Franek et al. (2011)[11] | I=7, II=13, III=9 | I=8, II=13, III=8 | 8 | 4 | N/A | N/A |
| Polak et al. (2016)[7] | II=19, III=7, IV=3 | II=23, III=7, IV=1 | 15 | 7 | 0 | 2 |
| Polak and Kloth et al. (2016)[13] | II=11, III=14 | II=11, III=13 | II=9, III=3 | II:6, III=1 | 0 | III=2 |
| Polak and Kloth et al. (2017)[14] | CAT:II=12, III=9,IV=2<br>CAN:II=11,III=6,IV=3 | II=13, III=6, IV=1 | CAT:11<br>CAN:9 | 0 | CAT:0<br>CAN:1 | 2 |
| **Total** | 142<br>I:7<br>II:67<br>III:51<br>IV:15<br>X:2 | 122<br>I:8<br>II:64<br>III:38<br>IV:12<br>X:0 | 61 | 23 | 1 | 10 |

**Table 5:** Descriptive analysis of the number of PrUs that completely healed and that increased in surface area of the studies included in the meta-analysis. WSA, wound surface area; N/A, not available; ulcer stages (I, II, III, IV, X); CAT, cathode group; CAN, cathode and anode group.

| Studies | Subjects | № Ulcers | Age | Gender | Ulcer duration (week) | Baseline WSA (cm²) | Intervention(s) period (week) |
|---|---|---|---|---|---|---|---|
| Kloth and Feeder (1988) [9] | 9 | 9 | 70.13 | N/A | N/A | 4.08 | 7.3 |
| Griffin et al. (1991)* [16] | 8 | 8 | 34 | 8M | 31.75 | 4.053 | 2.85 |
| Ahmad (2008) I [10] | 15 | 15 | 38.4 | 6M, 9F | 19.16 | 7.12 | 5 |
| Ahmad (2008) II [10] | 15 | 15 | 38.47 | 7M, 8F | 19.11 | 7.12 | 5 |
| Ahmad (2008) III [10] | 15 | 15 | 39.4 | 8M, 7F | 19.16 | 7.14 | 5 |
| Houghton et al. (2010) [17] | 16 | 16 | 50.3 | 8M, 8F | 62.57 | 3.38 | 12 |
| Franek et al. (2011) [11] | 29 | 29 | 59.9 | 19M, 10F | 13.77442 | 4.45 | 6 |
| Franek et al. (2012) [12] | 26 | 26 | 59 | 18M, 8F | 13.77442 | 4.54 | 6 |
| Polak et al. (2016) [7] | 24 | 29 | 79.33 | 5M, 19F | 11.25418 | 7.48 | 6 |
| Polak and Kloth et al. (2016) [13] | 25 | 25 | 79.92 | 6M, 19F | 11.03692 | 10.58 | 6 |
| Polak and Kloth et al. (2017) CAT [14] | 23 | 23 | 79.35 | 6M, 17F | 10.47204 | 9.59 | 6 |
| Polak and Kloth et al. (2017) CAN [14] | 20 | 20 | 79.65 | 2M, 18F | 11.51489 | 7.37 | 6 |
| Recio, A. C., et al. (2012) [15] | 3 | 3 | 43 | 3M | 43.4524 | 11.5 | 14.5 |
| Bora Karsli, P. et al. (2017) [8] | 15 | 25 | 32.63 | 22M, 5F | 11.99287 | 25.25 | 8 |
| **Total** | 243 | 258 | | 118M, 128F | | | |
| **Mean** | | | 55.96286 | | 21.46324154 | 8.118071429 | 6.832142857 |
| **Standard deviation** | | | ± 18.74754 | | ± 15.64152296 | ± 5.537433708 | ± 3.002153531 |

**Table 6:** Characteristics of patients and ulcers in the treatment arm of all studies included in the systematic review. N/A, not available; *, mean values were calculated for this sample; CAT, cathode group; CAN, cathode and anode group.



| Studies | Subjects | № Ulcers | Age | Gender | Ulcer duration (week) | Baseline WSA (cm²) | Intervention(s) period (week) |
|---|---|---|---|---|---|---|---|
| Kloth and Feeder (1988) [9] | 7 | 7 | 65.61 | N/A | N/A | 5.2 | 7.4 |
| Griffin et al. (1991)* [16] | 9 | 9 | 34 | 9M | 9.25 | 11.629 | 2.85 |
| Ahmad (2008) [10] | 15 | 15 | 39.4 | 9M, 6F | 17.92 | 7.21 | 5 |
| Houghton et al. (2010) [17] | 18 | 18 | 50.8 | 12M, 6F | 156.42 | 2.73 | 12 |
| Franek et al. (2011) [11] | 29 | 29 | 60 | 11M, 18F | 12.1667 | 4.93 | 6 |
| Franek et al. (2012) [12] | 24 | 24 | 56.2 | 10M, 14F | 12.29704 | 3.97 | 6 |
| Polak et al. (2016) [7] | 28 | 31 | 79.13 | 4M, 24F | 11.03692 | 9.31 | 6 |
| Polak and Kloth et al. (2016) [13] | 24 | 24 | 76.33 | 6M, 18F | 12.21013 | 9.71 | 6 |
| Polak and Kloth et al. (2017) [14] | 20 | 20 | 76.75 | 3M, 17F | 13.16609 | 8.9 | 6 |
| Recio, A. C., et al. (2012) [15] | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Bora Karsli, P. et al. (2017) [8] | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| **Total** | 174 | 177 | | 64M, 103F | | | |
| **Mean** | | | 59.8022 | | 30.55836 | 7.065444444 | 6.361111111 |
| **Standard deviation** | | | ± 16.3697 | | ± 50.91574407 | ± 3.013762064 | ± 2.442050596 |

**Table 7:** Characteristics of patients and ulcers in the control arm of all studies included in the systematic review. N/A, not available; *, mean values were calculated for this sample.

| Studies | Subjects | № Ulcers | Age | Gender | Ulcer duration (week) | Baseline WSA (cm²) | Intervention(s) period (week) |
|---|---|---|---|---|---|---|---|
| Houghton et al. (2010) [17] | 16 | 16 | 50.3 | 8M, 8F | 62.57 | 3.38 | 12 |
| Franek et al. (2011) [11] | 29 | 29 | 59.9 | 19M, 10F | 13.77442 | 4.45 | 6 |
| Polak et al. (2016) [7] | 24 | 29 | 79.33 | 5M, 19F | 11.25418 | 7.48 | 6 |
| Polak and Kloth et al. (2016) [13] | 25 | 25 | 79.92 | 6M, 19F | 11.03692 | 10.58 | 6 |
| Polak and Kloth et al. (2017) CAT [14] | 23 | 23 | 79.35 | 6M, 17F | 10.47204 | 9.59 | 6 |
| Polak and Kloth et al. (2017) CAN [14] | 20 | 20 | 79.65 | 2M, 18F | 11.51489 | 7.37 | 6 |
| **Total** | 137 | 142 | | 46M, 91F | | | |
| **Mean** | | | 71.4083 | | 20.10374167 | 7.141666667 | 7 |
| **Standard deviation** | | | ± 12.9938 | | ± 20.83508318 | ± 2.806360039 | ± 2.449489743 |

**Table 8:** Characteristics of subjects and ulcers in the treatment arm of studies included in the meta-analysis. CAT, cathode group; CAN, cathode and anode group.



| Studies | Subjects | № Ulcers | Age | Gender | Ulcer duration (week) | Baseline WSA (cm²) | Intervention(s) period (week) |
|---|---|---|---|---|---|---|---|
| Houghton et al. (2010) [17] | 18 | 18 | 50.8 | 12M, 6F | 156.42 | 2.73 | 12 |
| Franek et al. (2011) [11] | 29 | 29 | 60 | 11M, 18F | 12.1667 | 4.93 | 6 |
| Polak et al. (2016) [7] | 28 | 31 | 79.13 | 4M, 24F | 11.03692 | 9.31 | 6 |
| Polak and Kloth et al. (2016) [13] | 24 | 24 | 76.33 | 6M, 18F | 12.21013 | 9.71 | 6 |
| Polak and Kloth et al. (2017) [14] | 20 | 20 | 76.75 | 3M, 17F | 13.16609 | 8.9 | 6 |
| **Total** | 119 | 122 | | 36M, 83F | | | |
| **Mean** | | | 68.602 | | 40.999968 | 7.116 | 7.2 |
| **Standard deviation** | | | ± 12.52853 | | ± 64.526167 | ± 3.112102183 | ± 2.683281573 |

**Table 9:** Characteristics of subjects and ulcers in the control arm of studies included in the meta-analysis.

| Study | Ulcer location | Inclusion criteria | Exclusion criteria | HVMPC intervention | SWC intervention(s) |
|---|---|---|---|---|---|
| Kloth and Feeder (1988) [9] | -Ankle and other unspecified | -Intact peripheral nervous system<br>-Stage IV ulcers | N/A | 100-175V, 105HZ, 50μs for 45 min, once a day, 5 days a week. Positive electrode over wound (polarity maintained unless a plateau is reached), 15 cm cephalad to negative electrode. At 100V with a pulse interval of 100 μs, the total delivered electric charge was calculated at 342 μC/s | -Pressure-relieving devices<br>-High-protein dietary supplement.<br>-Manual debridement<br>-Proteolytic enzyme (Elase®) twice daily for first three days<br>-Collagenase enzyme (Biozyme-C®) on fourth day<br>-Transparent dressing (Tegaderm®) applied over wound |
| Griffin et al. (1991) [16] | -Pelvic pressure ulcer (over either the sacral/ coccygeal or gluteal/ ischial regions) | -Male<br>-Complete or incomplete SCI<br>-Pelvic pressure ulcer | -Severe cardiac disease<br>-Cardiac arrhythmia<br>-Uncontrolled autonomic dysreflexia<br>-Pacemaker use | 200V, 100HZ, 75μs for 60 min, once a day, 7 days a week. Negative electrode over wound and positive electrode distally. Active electrode was a piece of heavy-duty aluminum foil slightly larger than ulcer perimeter. Dispersive electrode was 20×25 cm, placed over medial thigh. The total delivered electric charge was 500 μC/s | -Ulcer cleansing twice a day (Cara-klenz®) followed by Carrington gel application and dry dressing<br>-Mechanical debridement<br>-Enzymatic debridement was not used<br>-Pressure relief schedule |
| Ahmad (2008) [10] | | | -Cardiac pacemaker<br>-Peripheral vascular disease<br>-Pregnant<br>-Receiving long-term radiation therapy, steroid therapy, or chemotherapy | 100-175V, 120HZ, 50μs for 45/60/120 min once a day, 7 days a week. Negative electrode over wound for first 3 days then positive electrode maintained until healing (repeated in plateaus). Active electrode was a piece of heavy-duty aluminum foil slightly larger than ulcer perimeter. Dispersive electrode was strapped over the medial thigh | -Debridement<br>-Wet dressing<br>-Whirlpool therapy for 4-5 times a week |
| Houghton et al. (2010) [17] | -Buttock region (Ischial tuberosity, sacrum, coccyx, hip)<br>-Leg (Foot, ankle, knee) | -Paraplegia or quadriplegia caused by SCI<br>-Over 18 years old and living in the community<br>- Stage II-Iv pressure ulcer 1-20 $cm^2$ present for at least 3 months<br>-Able to participate for at least 3 months in a SWC program<br>-No serious or multiple medical conditions that limit healing<br>-No contraindications for EST (cardiac pacemaker, osteomyelitis, pregnancy, cancer) | N/A | 50-150V, 100HZ, 100μs for 20min then 10HZ for 20min and 20min off cycle, repeated 8 times a day, 7 days a week. Negative electrode initially and reversed weekly. In 11of 16 cases, the active electrode was 4.8×10.2 cm over wound and dispersive electrode was 12.7×20.3 cm (monopolar) 20cm between electrodes | -Tailored SWC program customized to each subject included nutritional intervention, optimization of wound dressing protocol, and continence management<br>-Comprehensive pressure management program<br>-Activity schedule modification<br>-In most cases, high-profile air filled cushion for use in wheelchairs<br>-Silver dressing in all but 4 patients in the control group |
| Franek et al. (2011) [11] | -Lower leg, foot, ankle<br>-Gluteal, Ischial<br>-Hand | -Stage I, II, III pressure ulcers | -Spinal cord injuries or loss of sensation<br>-Chronic venous insufficiency<br>-Arteriosclerosis (ABPI < 0.9)<br>-Diabetes<br>-Ventricular arrhythmia<br>-Cardiac pacemakers<br>-Metal implants<br>-Pregnancy<br>-Post-steroid therapy | 100V, 100HZ, 50μs for 50min once daily, 5 days a week. Negative electrode over wound for first 2 weeks, altered to positive for rest of treatment. Active electrode size was matched to ulcer size, dispersive electrode was on intact peri-wound skin. Conductive carbon rubber or silver electrodes | -Pharmacological agents included local wound cleansing with potassium permanganate, ulcer base covered with compresses of fibrolan, colistin, and iruxol and wet dressings of 10% sodium chloride<br>-Dressings were changed daily<br>-Local bath<br>-Compresses<br>-Wet dressings |

**Table 10:** Details of ulcer location, inclusion and exclusion criteria, HVMPC intervention, and standard wound care (SWC) intervention(s). N/A, not available.

| Study | Ulcer location | Inclusion criteria | Exclusion criteria | HVMPC intervention | SWC intervention(s) |
|---|---|---|---|---|---|
| Franek et al. (2012) [12] | -Lower extremity pressure ulcers<br>-Legs, lateral and medial ankles, and greater femoral trochanter | N/A | -Ankle-brachial pressure index (ABPI < 0.9)<br>-Diabetes mellitus<br>-Systemic sclerosis<br>-Cancer<br>-Paresis and paralysis caused by central or peripheral nervous system injury<br>-Pressure ulcers requiring surgical intervention | 100-150V, 100HZ, 50μs for 50min once daily, 5 days a week. Negative electrode over wound for first 1-2 weeks, altered to positive for rest of treatment, 20cm between electrodes. Conductive carbon rubber electrodes | -Standard topical care included cleansing with potassium permanganate followed by covering ulcer base with dressing<br>-Dressings were tailored to each subject<br>-Non-adherent gauze pads, dressings moistened with 0.9% sodium chloride, hydrogel, propolis extractum, and solcoseryl<br>-If infection suspected, desoxyribonucleasum plus fibrinolysinum, ethacridine lactate, and colistinum were applied<br>-Sharp debridement was performed for relatively few patients |
| Polak et al. (2016) [7] | -Buttock region/ trunk<br>-Elbow (control group only)<br>-Lower extremity | -Patients with smaller PrUs of shorter duration | -Contraindications to ES: cancer, electronic implants, necrotic and tunneling wounds, osteomyelitis, PrUs in need of surgical intervention, metal implants in ulcer area<br>-Diabetes patients (HbA1C>7.0%)<br>-Venous and lymphatic insufficiency<br>-Critical infection<br>-Alcoholism<br>-Allergy to standard treatment | 100V, 100HZ, 77μs for 50min once daily, 5 days a week Negative electrode over wound for first 5 days then positive electrode for the rest of treatment. The active electrode was 5×10 cm and dispersive electrode is 10×10 cm. 20cm between electrodes. Conductive carbon rubber electrodes. The total delivered electric charge was 250 μC/s | -Pressure redistribution surfaces, devices and pillows as needed<br>-Tissue debridement, infection and inflammation control, maintaining moisture balance, and monitoring wound edges and epithelialization<br>-Wounds were cleaned with antiseptics or covered with hydrogel and alginates or hydrocolloid or polyurethane foam dressing and 0.9% sodium chloride<br>-Necrotic tissue was cleaned enzymatically and infected wounds rinsed with antiseptics |
| Polak and Kloth et al. (2016) [13] | -Sacrum, coccyx/ Ischial tuberosity / trochanter major | -Stage II and III PrUs only, located on the pelvic girdle (sacrum, coccyx, ischial tuberosity, trochanter major) | -ES contraindications including cancer, electronic implants, malignant, tunneling and necrotic wounds<br>-Osteomyelitis, PrUs requiring surgical intervention, and metal implants in the PrUs area<br>-Diagnoses that might interference with wound healing, such as diabetes (HbA1C > 7%), venous insufficiency, critical infection, alcoholism, and allergy to standard wound treatment | 100V, 100HZ, 77μs for 50 min once daily, 5 days a week. Negative electrode over wound, The active electrode is 5×10 cm and dispersive electrode was 10×10 cm. 20cm between electrodes. Conductive carbon rubber electrodes. The total delivered electric charge was 250 μC/s | -Pressure redistribution surfaces, devices, and pillows as needed<br>- Tissue debridement, infection and inflammation control, maintaining moisture balance, and monitoring wound edges and epithelialization<br>-Wounds were cleaned with antiseptics or covered with hydrogel and alginates or hydrocolloid or polyurethane foam dressing and 0.9% sodium chloride<br>-Necrotic tissue was cleaned enzymatically and infected wounds rinsed with antiseptics |

**Table 10: (continued).**

| Study | Ulcer location | Inclusion criteria | Exclusion criteria | HVMPC intervention | SWC intervention(s) |
|---|---|---|---|---|---|
| Polak and Kloth et al. (2017) [14] | -Sacrum, ischial tuberosity, trochanter major | Chronic PrUs in elderly persons who frequently have co-morbidities impeding normal wound healing | ES contraindications including cancer, electronic implants, malignant, tunneling and necrotic wounds<br>-Osteomyelitis, PrUs requiring surgical intervention, and metal implants in the PrUs area<br>-Diagnoses that might interference with wound healing, such as diabetes (HbA1C > 7%), venous insufficiency, critical infection, alcoholism, and allergy to standard wound treatment<br>-Factors that could disturb wound healing or inhibit the use of ES or pharmacological therapy | 100V, 100HZ, 77μs for 50 min once daily, 5 days a week.<br>CAT: Negative electrode over wound<br>CAN: Negative electrode over wound for first 5 days then positive electrode, The active electrode was 5×10 cm and dispersive electrode was 10×10 cm. 20cm between electrodes. Conductive carbon rubber electrodes. The total delivered electric charge was 250 μC/s | -Pressure redistribution surfaces, devices, and pillows as needed<br>- Tissue debridement, infection and inflammation control, maintaining moisture balance, and monitoring wound edges and epithelialization<br>-Wounds were cleaned with antiseptics or covered with hydrogel and alginates or hydrocolloid or polyurethane foam dressing and 0.9% sodium chloride<br>-Necrotic tissue was cleaned enzymatically and infected wounds rinsed with antiseptics |
| Recio, A. C., et al. (2012) [15] | -Left heel<br>-Left Ischial wound<br>-Right ischial wound | Paraplegia<br>SCI | N/A | -100HZ, for 60 min, 3-5 times a week. Negative electrode over wound initially, polarity alternated once weekly, 2 inch rounded electrode into wound bed and dispersive electrode placed proximal to wound | -Wound care consisted of cleansing with saline and dressing with Telfa padding secured with Hypafix |
| Bora Karsli, P. et al. (2017) [8] | -Sacral, ischial, trochanter, heel, lateral malleolus, head of fibula | Stage II-Iv PrUs | -Patients with a cardiac dysrhythmia or pacemaker, epilepsy, osteomyelitis, pregnancy, malignancy, and/ or uncontrolled autonomic dysreflexia | 50-150V, 100HZ, 10/50/100μs, 2 second ramp-up time, for 60 min, 3 times per week. Negative electrode initially, polarity altered at weekly intervals (electrode placement was not provided) | -Wound care consisted of cleansing with saline |

**Table 10: (continued).**

| Study | Pain status | Adverse reactions to treatment | Morbidity | Limitations |
|---|---|---|---|---|
| Kloth and Feeder (1988) [9] | N/A | N/A | -Ulcers in both groups had been unresponsive to previous treatments and healed completely after HVMPC treatment<br>-Three ulcers in the control group did not heal and were reassigned to the treatment group and healed completely in 8.33 weeks (mean) | N/A |
| Griffin et al. (1991) [16] | N/A | -No patient complained of any discomfort during treatment | -All grade III ulcers in the HVMPC group showed consistent patterns of decreased WSA over the treatment period, the grade IV ulcer showed 67% decrease in WSA, while two grade III ulcers in the placebo group showed periods of increase in WSA, grade IV ulcer showed little change over treatment period (15% decrease) | -Possible limitation was that placebo group had the greatest number of sacral/coccygeal ulcer and HVMPC group had the most gluteal/ ischial ulcers<br>-Another possible limitation was that the chance of inclusion of two patients older than 70 years old, however, they had normal healing rates<br>-Length of study (2.5) years |
| Ahmad (2008) [10] | N/A | -The results indicate that HVMPC in the study manner and dosage is safe and effective treatment for stage II PrUs | N/A | N/A |
| Houghton et al. (2010) [17] | N/A | -Minor and rare adverse reactions<br>-Most common reaction to HVMPC treatment was red, raised, itchy skin beneath large dispersive electrode, this was attributed to contact dermatitis. A non-adhesive carbon electrode was substituted in these cases<br>-One patient had a persistent (>24h) red area or burn under the active electrode, it resolved within 48 hours and was remedied by turning down the intensity of treatment<br>-Relatively safe approach | -People were less likely to have wounds that increased in size (worsened) when their wound treatment included EST<br>-Recurrent or new pressure ulcers developed in 8 subjects in each group within 4 months of closure, however, these were similar to other pressure ulcer recurrence rates<br>-HVMPC administered at low levels in or around the wound bed can accelerate wound healing but do not prevent recurrence or development of new PrUs elsewhere on the body<br>-Only 7 (39%) of 18 of subjects who received SWC achieved complete wound closure by 6 months. This was despite the fact that some of these individuals received EST treatment as part of SWC available in this region<br>-A key reason EST did not produce complete wound closure in study subjects was that they elected to stop using the EST machine prematurely, before the wound was healed | -The study involved a relatively small number of subjects<br>-The single blind study was not set up in a manner that blinded subjects receiving EST. However, the objective nature of wound healing outcomes may not be affected by lack of blinding<br>-Combination of silver dressing and EST may have led to the positive wound healing outcomes observed in the EST+SWC group. However, all but 4 subjects in the SWC group used silver dressing |
| Franek et al. (2011) [11] | N/A | N/A | -The wound healing process progressed more steadily and significantly in patients treated with electrical stimulation | -4 year period study, leading to wide variety of patients and ulcers |
| Franek et al. (2012) [12] | N/A | -No adverse events were observed in this study, indicating that HVMPC treatment is safe<br>-The treatment does not cause pain | -Weekly changes in wound surface areas indicated that in the HVMPC treatment group, wounds healed evenly, whereas average wound areas in the control group were greater after 2 weeks of treatment compared to baseline | -Length of study (4 years) could have introduced variability in methods and procedures<br>-Follow-up was not long enough for all PrUs to completely heal<br>-Absence of blinding and placebo ES in the control group may affect generalizability of findings |

**Table 11:** Details of pain status, adverse reactions to treatment, morbidity, and the limitations of each study. N/A, not available.

| Study | Pain status | Adverse reactions to treatment | Morbidity | Limitations |
|---|---|---|---|---|
| Polak et al. (2016) [7] | N/A | -No adverse effects were observed with HVMPC therapy | -In all patients, wounds healed the fastest in the first 4 weeks, with significantly higher percentage of area reduction in HVMPC group<br>-In the next 2 weeks, PrUs continued to heal, but at a slower rate, the percentage of area reduction of HVMPC group was significantly higher as well<br>-Patients had comorbid diseases that may have contributed to the development of PrUs or may have impeded the healing of existing ulcer, therefore, it was not expected for heal completely during the 6 week intervention period | -Study lasted 3 years<br>-No blinding of patients<br>-No sham stimulation<br>-Customization of treatment may have contributed to differences in PU healing between groups<br>-An aggregate result for all PrUs is reported as the efficacy of HVMPC could not be evaluated by wound severity (stages)<br>-Not long enough study for all ulcers to completely heal |
| Polak and Kloth et al. (2016) [13] | N/A | -No adverse effects were observed with HVMPC therapy | -In all patients, WSA decreased the fastest in the first 4 weeks of treatment, while in weeks 5 and 6, the PrUs surface area decreased at slower rate<br>- Patients had comorbid diseases that may have contributed to the development of PrUs or may have impeded the healing of existing ulcer, therefore, it was not expected for heal completely during the 6 week intervention period | -Focused on stage II and III PrUs only, the results do not predict stage IV PrUs treatment<br>- Not long enough study for all ulcers to completely heal<br>- Customization of treatment may have contributed to differences in PU healing between groups |
| Polak and Kloth et al. (2017) [14] | N/A | -No adverse effects were observed with HVMPC therapy | -In both HVMPC groups, PrUs decreased by half faster and more closed by the end of treatment | -The major limitation is the study period was too short for all PrUs to close<br>-Monitoring patients following the trial was not possible<br>- Specific solutions addressed the needs of individual patients |
| Recio, A. C., et al. (2012) [15] | N/A | N/A | -Significant reduction in drainage and WSA of PtA<br>-Significant reduction in drainage and WSA of PtB<br>-PtC ulcer completely healed<br>-HVMPC treatment enhanced stage III-IV ulcer healing in recalcitrant wounds that persisted for more than 11 months and previously failed to respond to standard wound care | N/A |
| Bora Karsli, P. et al. (2017) [8] | N/A | N/A | -Significant improvement in WSA occurred in all PrUs stages (II/III/IV) | -Inability to provide intergroup homogeneity in terms of PrUs stage and WSA because of randomization<br>-Lack of control group receiving SWC only |

**Table 11: (continued).**

## References


[1] Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. BMC medical research methodology. 2005;5(1):13. http://dx.doi.org/10.1186/1471-2288-5-13.

[2] du Prel J-B, Hommel G, Röhrig B, Blettner M. Confidence interval or p-value?: part 4 of a series on evaluation of scientific publications. Deutsches Ärzteblatt International. 2009;106(19):335. http://dx.doi.org/10.3238/arztebl.2009.0335.

[3] Shakespeare TP, Gebski VJ, Veness MJ, Simes J. Improving interpretation of clinical studies by use of confidence levels, clinical significance curves, and risk-benefit contours. The Lancet. 2001;357(9265):1349-53. http://dx.doi.org/10.1016/S0140-6736(00)04522-0.

[4] Gardner MJ, Altman DG. Confidence intervals rather than P values: estimation rather than hypothesis testing. Br Med J (Clin Res Ed). 1986;292(6522):746-50. http://dx.doi.org/10.1136/bmj.292.6522.746.

[5] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ: British Medical Journal. 2003;327(7414):557. http://dx.doi.org/10.1136/bmj.327.7414.557.

[6] Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. Research Synthesis Methods. 2010;1(2):97-111. http://dx.doi.org/10.1002/jrsm.12.

[7] Polak A, Taradaj J, Nawrat-Szoltysik A, Stania M, Dolibog P, Blaszczak E, et al. Reduction of pressure ulcer size with high-voltage pulsed current and high-frequency ultrasound: a randomised trial. J Wound Care. 2016;25(12):742-54. http://dx.doi.org/10.12968/jowc.2016.25.12.742.

[8] Bora Karsli P, Gurcay E, Karaahmet OZ, Cakci A. High-Voltage Electrical Stimulation Versus Ultrasound in the Treatment of Pressure Ulcers. Adv Skin Wound Care. 2017;30(12):565-70. http://dx.doi.org/10.1097/01.ASW.0000526606.72489.99.

[9] Kloth LC, Feedar JA. Acceleration of wound healing with high voltage, monophasic, pulsed current. Phys Ther. 1988;68(4):503-8. http://dx.doi.org/10.1093/ptj/68.4.503.

[10] Ahmad ET. High-voltage pulsed galvanic stimulation: effect of treatment duration on healing of chronic pressure ulcers. Ann Burns Fire Disasters. 2008;21(3):124-8. PMC3188162.

[11] Franek A, Kostur R, Taradaj J, B aszczak E, Szlachta Z, Dolibog P, et al. Effect of High Voltage Monophasic Stimulation on Pressure Ulcer Healing: Results From a Randomized Controlled Trial2011.

[12] Franek A, Kostur R, Polak A, Taradaj J, Szlachta Z, Blaszczak E, et al. Using high-voltage electrical stimulation in the treatment of recalcitrant pressure ulcers: results of a randomized, controlled clinical study. Ostomy-Wound Management. 2012;58(3):30.

[13] Polak A, Kloth LC, Blaszczak E, Taradaj J, Nawrat-Szoltysik A, Walczak A, et al. Evaluation of the Healing Progress of Pressure Ulcers Treated with Cathodal High-Voltage Monophasic Pulsed Current: Results of a Prospective, Double-blind, Randomized Clinical Trial. Adv Skin Wound Care. 2016;29(10):447-59. http://dx.doi.org/10.1097/01.ASW.0000493164.75337.de.

[14] Polak A, Kloth LC, Blaszczak E, Taradaj J, Nawrat-Szoltysik A, Ickowicz T, et al. The Efficacy of Pressure Ulcer Treatment With Cathodal and Cathodal-Anodal High-Voltage Monophasic Pulsed Current: A Prospective, Randomized, Controlled Clinical Trial. Phys Ther. 2017;97(8):777-89. http://dx.doi.org/10.1093/ptj/pzx052.

[15] Recio AC, Felter CE, Schneider AC, McDonald JW. High-voltage electrical stimulation for the management of Stage III and IV pressure ulcers among adults with spinal cord injury: Demonstration of its utility for recalcitrant wounds below the level of injury. The journal of spinal cord medicine. 2012;35(1):58-63. http://dx.doi.org/10.1179/2045772311Y.0000000044.

[16] Griffin JW, Tooms RE, Mendius RA, Clifft JK, Vander Zwaag R, el-Zeky F. Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. Phys Ther. 1991;71(6):433-42; discussion 42-4. http://dx.doi.org/10.1093/ptj/71.6.433.

[17] Houghton PE, Campbell KE, Fraser CH, Harris C, Keast DH, Potter PJ, et al. Electrical stimulation therapy increases rate of healing of pressure ulcers in community-dwelling people with spinal cord injury. Arch Phys Med Rehabil. 2010;91(5):669-78. http://dx.doi.org/10.1016/j.apmr.2009.12.026.

Excluded studies

Other ES currents


1. Akers TK, Gabrielson AL: The effect of high voltage galvanic stimulation on the rate of healing of decubitus ulcers. Biomed Sci Instrum 1984; 20:99-100.
2. Adegoke BO, Badmos KA. Acceleration of pressure ulcer healing in spinal cord injured patients using interrupted direct current. African journal of medicine and medical sciences. 2001 Sep;30(3):195-7.
3. Adunsky A, Ohry A. Decubitus direct current treatment (DDCT) of pressure ulcers: results of a randomized double-blinded placebo controlled study. Archives of gerontology and geriatrics. 2005 Nov 1;41(3):261-9.
4. Allen J, Houghton PE. A case study for electrical stimulation on a stage III pressure ulcer. Wound Care Canada. 2003;2(1):34-6.
5. Asbjornsen G, Hernaes B, Molvaer G. THE EFFECT OF TRANSCUTANEOUS ELECTRICAL NERVE-STIMULATION ON PRESSURE SORES IN GERIATRIC-PATIENTS. Journal of Clinical and Experimental Gerontology. 1990 Jan 1;12(4):209-14.
6. Baker LL, Rubayi S, Villar F, Demuth SK. Effect of electrical stimulation waveform on healing of ulcers in human beings with spinal cord injury. Wound Repair and Regeneration. 1996 Jan;4(1):21-8.
7. Carley PJ, Wainapel SF. Electrotherapy for acceleration of wound healing: Low intensity direct current. Arch Phys Med Rehabil 1985; 66:443-446.
8. Feedar JA, Kloth LC, Gentzkow GD. Chronic dermal ulcer healing enhanced with monophasic pulsed electrical stimulation. Physical Therapy. 1991 Sep 1;71(9):639-49.
9. Gault WR, Gatens Jr PF. Use of low intensity direct current in management of ischemic skin ulcers. Physical Therapy. 1976 Mar 1;56(3):265-9.
10. Gentzkow GD, Pollack SV, Kloth LC, Stubbs HA. Improved healing of pressure ulcers using Dermapulse, a new electrical stimulation device. Wounds. 1991.
11. Hampton S, Collins F. Treating a pressure ulcer with bio-electric stimulation therapy. British journal of nursing. 2006 Mar 23;15(Sup1):S14-8.
12. Sosa IJ, Reyes O, Kuffler DP. Elimination of a pressure ulcer with electrical stimulation-a case study. Puerto Rico health sciences journal. 2008;27(2).
13. Jercinovic A, Karba R, Vodovnik L, Stefanovska A, Kroselj P, Turk R, Dzidic I, Benko H, Savrin R. Low frequency pulsed current and pressure ulcer healing. IEEE transactions on rehabilitation engineering. 1994 Dec;2(4):225-33.
14. Karba R, Benko H, Savrin R, Vodovnik L. Combination of occlusive dressings and electrical stimulation in pressure ulcer treatment. Medical science research. 1995 Oct 1;23(10):671-4.
15. Karba R, Šemrov D, Vodovnik L, Benko H, Savrin R. DC electrical stimulation for chronic wound healing enhancement Part 1. Clinical study and determination of electrical field distribution in the numerical wound model. Bioelectrochemistry and bioenergetics. 1997 Aug 1;43(2):265-70.
16. Lippert-Grüner M. Gluteal neuromuscular stimulation in therapy and prophylaxis of recurrent sacral pressure ulcers. Spinal Cord. 2003 Jun;41(6):365.
17. Pollack SF, Ragnarsson KT, Djikers M. The Effect of Electrically Induced Lower Extremity Ergometry an an Ischial Pressure Ulcer: A Case Study. The journal of spinal cord medicine. 2004 Jan 1;27(2):143-7.

18. Stefanovska A, Vodovnik L, Benko H, Turk R. Treatment of chronic wounds by means of electric and electromagnetic fields. Medical and Biological Engineering and Computing. 1993 May 1;31(3):213-20.
19. Trontelj K, Karaba R, Vodovnik L, Savrin R, Strukelj MP. Treatment of chronic wounds by low frequency pulsed electrical current. Journal of Tissue Viability. 1994 Oct 1;4(4):105-9.
20. Ullah MO. A study to detect the efficacy of microcurrent electrical therapy on decubitus wound. J Med Sci. 2007;7(8):1320-4.
21. Wood JM, Evans PE, Schallreuter KU, Jacobson WE, Sufit R, Newman J, White C, Jacobson M. A multicenter study on the use of pulsed low-intensity direct current for healing chronic stage II and stage III decubitus ulcers. Archives of Dermatology. 1993 Aug 1;129(8):999-1009.

Other ulcer types

1. B aszczak E, Franek A, Taradaj J, Dolibog P. Evaluation of healing process dynamics of the leg venous ulcers treated by means of selected physical methods. Physiotherapy/Fizjoterapia. 2007 Mar 1;15(1).
2. Silva ED, Martins CC, Guirro EC, Guirro RR. High voltage electrical stimulation as an alternative treatment for chronic ulcers of the lower limbs. Anais brasileiros de dermatologia. 2010 Aug;85(4):567-9.
3. Franek A, Polak A, Kucharzewski M. Modern application of high voltage stimulation for enhanced healing of venous crural ulceration. Medical engineering & physics. 2000 Nov 1;22(9):647-55.
4. Franek A, Taradaj J, Cierpka L, Blaszczak E. High voltage stimulation for healing acceleration of venous leg ulcers. Phlebologie. 2005;34(05):255-60.
5. Franek A, Taradaj J, Polak A, Cierpka L, Blaszczak E. Efficacy of high voltage stimulation for healing of venous leg ulcers in surgically and conservatively treated patients. Phlebologie. 2006;35(03):127-33.
6. Goldman RJ, Brewley BI, Golden MA. Electrotherapy reoxygenates inframalleolar ischemic wounds on diabetic patients: a case series. Advances in skin & wound care. 2002 May 1;15(3):112-20.
7. Goldman RJ, Brewley BI, Cohen R, Rudnick M. Use of electrotherapy to reverse expanding cutaneous gangrene in end-stage renal disease. Advances in skin & wound care. 2003 Dec 1;16(7):363-6.
8. Goldman R, Brewley B, Zhou L, Golden M. Electrotherapy reverses inframalleolar ischemia: a retrospective, observational study. Advances in skin & wound care. 2003 Mar 1;16(2):79-89.
9. Goldman R, Rosen M, Brewley B, Golden M. Electrotherapy promotes healing and microcirculation of infrapopliteal ischemic wounds: a prospective pilot study. Advances in skin & wound care. 2004 Jul 1;17(6):284-94.
10. Houghton PE, Kincaid CB, Lovell M, Campbell KE, Keast DH, Woodbury MG, Harris KA. Effect of electrical stimulation on chronic leg ulcer size and appearance. Physical therapy. 2003 Jan 1;83(1):17-28.
11. Lasko J, Kochik J, Serena T. Combining acoustic pressure wound therapy with electrical stimulation for treatment of chronic lower-extremity ulcers: A case series. Advances in skin & wound care. 2010 Oct 1;23(10):446-9.

12. Sandoval Ortíz MC, Herrera Villabona E, Camargo Lemos DM, Castellanos R. Effects of low level laser therapy and high voltage stimulation on diabetic wound healing. Revista de la Universidad Industrial de Santander. Salud. 2014 Aug;46(2):107-17.
13. Peters EJ, Lavery LA, Armstrong DG, Fleischli JG. Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial. Archives of physical medicine and rehabilitation. 2001 Jun 1;82(6):721-5.
14. Zhou K, Krug K, Stachura J, Niewczyk P, Ross M, Tutuska J, Ford G. Silver-Collagen Dressing and High-voltage, Pulsed-current Therapy for the Treatment of Chronic Full-thickness Wounds: A Case Series. Ostomy/wound management. 2016 Mar;62(3):36-44.
15. Zhou K, Schenk R, Brogan MS. The wound healing trajectory and predictors with combined electric stimulation and conventional care: one outpatient wound care clinic's experience. European journal of clinical investigation. 2016 Dec;46(12):1017-23.

Non-English language

1. Materniak K, Nowak-Wró yna A, Kawecki M, Nowak M. Elektrostymulacja wysokonapi ciowa w leczeniu trudno goj cych si ran i obrz ków. Leczenie Ran. 2012 Jan 1;9(1).
2. Polak A, Franek A, Hu ka-Zurawi ska W, Bendkowski W, Kucharzewski M, Swist D. High voltage electrical stimulation in leg ulcer's treatment. Wiadomosci lekarskie (Warsaw, Poland: 1960). 2000;53(7-8):417-26.
3. Polak A, Taradaj J, Walczak DM, Augustak A. Elektrostymulacja wysokonapi ciowa we wspomaganiu leczenia odle yn: wyniki randomizowanego, kontrolowanego eksperymentu klinicznego–doniesienie wst pne. Leczenie Ran. 2013 Jan 1;10(1):13-22.
4. Santos RP, Nascimento CA, De Andrade EN. Uso da eletroestimulação de alta voltagem na cicatrização de úlceras venosas. Fisioterapia em Movimento. 2017 Sep 4;22(4).

# V

Check for updates

Received: 13 June 2022 | Revised: 4 October 2022 | Accepted: 8 November 2022

DOI: 10.1111/wrr.13063

Wound Repair and Regeneration — WILEY

SYSTEMATIC REVIEW

# The effect of high-voltage monophasic pulsed current on diabetic ulcers and their potential pathophysiologic factors: A systematic review and meta-analysis

Beshoy Girgis PT, MSc[1] | Davide Carvalho MD, PhD[2,3] | José Alberto Duarte MD, PhD[1]

[1]CIAFEL, Faculty of Sport, University of Porto, Porto, Portugal

[2]Department of Endocrinology, Diabetes and Metabolism, Centro Hospitalar S. João, Faculty of Medicine, University of Porto, Porto, Portugal

[3]Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal

**Correspondence**
Beshoy Girgis, CIAFEL, Faculty of Sport, University of Porto, R. Dr. Plácido Costa, 91, 4200-450 Porto, Portugal.
Email: begirgis@gmail.com; up201301273@fade.up.pt

**Funding information**
Fundação para a Ciência e a Tecnologia, Grant/Award Number: SFRH/BD/144090/2019

## Abstract

The present review was conducted to determine the efficacy of high-voltage monophasic pulsed current (HVMPC) in treating diabetic ulcers, assess its effect on skin lesions with each of the pathophysiologic factors potentially contributing to diabetic ulcers, evaluate its safety, and identify treatment parameters. Electronic search of PubMed, Scopus, PEDro and Google Scholar databases was conducted. The revised tool for assessing risk of bias in randomised trials (RoB 2), the risk of bias in non-randomised studies-of interventions (ROBINS-I) and the Joanna Briggs Institute (JBI) critical appraisal tool were used to assess risk of bias and methodological quality. Overall quality of evidence was determined using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) principles. Thirty-two studies matched the eligibility criteria, and included 1061 patients with 1103 skin lesions of selected aetiologies; 12 randomised controlled trials were included in quantitative synthesis. HVMPC plus standard wound care (SWC) likely increased the probability of complete wound healing of pressure ulcers (PrUs) compared with sham/no stimulation plus SWC; relative risk (RR) 2.08; 95% CI: [1.42, 3.04], $p = 0.0002$; $I^2 = 0\%$, $p = 0.61$; eight studies, 358 ulcers. Although conclusive evidence regarding the effect of HVMPC on diabetic ulcers was not found, collateral evidence might suggest a potential benefit. Direct evidence, with moderate certainty, may support its efficacy in treating PrUs, albeit few adverse reactions were reported. Other observations, moreover, might indicate that this efficacy may not be limited to PrUs. Nonetheless, several aspects remain to be clarified for safe and effective application of electrical stimulation for wound healing.

**KEYWORDS**
diabetic ulcers, electrical stimulation, pressure ulcers, neuropathic ulcers, ischemic ulcers, wound healing, high-voltage pulsed current

## 1 | INTRODUCTION

It is beyond doubt that different, albeit not fully understood, pathophysiology may contribute to skin lesions.[1] The structural and functional integrity of these lesions, nonetheless, may be restored through the same *cell types* and following the same *phases of healing*.[2] Cellular damage, proinflammatory cytokine response, and tissue loss are *common features in all wounds* regardless of aetiology.[2,3] As a matter of fact, immediately after injury, extravasated blood cells invade damaged skin, marking the initiation of the

 © 2022 The Wound Healing Society. 

inflammatory phase and the healing process. Cytokine release from leukocytes and platelets may orchestrate the activity of resident skin cells.[4] Fibroblasts and keratinocytes, in particular, may have a crucial role in the formation of granulation tissue and reepithelialization, respectively. An intricate continuum of events is typically incorporated into the classic wound healing phases.[4] The entire healing process may, consequently, be disrupted due to dysregulation of any of these phases.

Failure to proceed through an orderly and timely reparative process that results in sustained restoration of anatomic and functional integrity may characterise chronic wounds, and may differentiate them from acute wounds.[5] Although a subject of debate, an agreement has been reached to consider a wound as chronic in the case of failure to progress toward healing within 4 weeks, when standard of care has been applied.[6] Chronic wounds may have many causes and types, and may include the entire category of skin ulcers; however, the common chronic wounds are pressure ulcers, diabetic ulcers, and venous ulcers.[7] While these ulcers might have similar initiating factors (e.g., trauma), the underlying disease processes may vary; ulcers most commonly occur due to ischemia, venous insufficiency, pressure injury, and neuropathy.[7,8] An ulcer is defined as 'a break in skin or mucous membrane with loss of surface tissue, disintegration, and necrosis of epithelial tissue, and often, purulent exudate'.[9]

The pathogenesis of skin ulcers in patients with diabetes mellitus is complex, and usually includes co-existing factors. The main intrinsic factors are peripheral neuropathy and ischemia, which may lead to loss of protective sensation and impairment of wound healing, respectively.[10] The main extrinsic factors, however, are skin injuries which may result from pressure (abnormal loading due to deformities, ill-fitting shoes, callus, etc.), acute wounds (caused by acute mechanical injury, trauma, etc.), and thermal injuries (burns).[10] These factors combined may lead to the formation of wounds that are difficult to heal or ulcers.[11] Infection may be simultaneously present, which may further complicate the disease process.[12]

Although all chronic wounds commence as acute, time may not be the only difference between acute and chronic wounds. Cellular and molecular abnormalities in chronic diabetic wounds may include compromised or reduced fibroblast and keratinocyte migration and proliferation, collagen deposition, angiogenesis, growth factor production and extracellular matrix remodelling.[13] The pathophysiology of diabetic ulcers may moreover include hyperglycaemia which in turn may lead to oxidative stress, neuropathy, ischaemia and chronic inflammation.[14,15] Infection-induced inflammation may further contribute to the altered microenvironment, possibly resulting in a vicious cycle of inflammation and tissue damage.[16]

### 1.1 | Rationale

Recent evidence has demonstrated the efficacy of electrical stimulation (ES) in improving wound healing,[17,18] and highlighted it as a potential adjunctive therapy in the management of diabetic ulcers.[19–21] Nevertheless, the current state of evidence may not be strong enough to recommend ES for the treatment of hard-to-heal diabetic foot ulcers (DFUs).[22] Existing studies in literature suggest the efficacy of high-voltage monophasic pulsed current (HVMPC) in treating ulcers of various aetiologies.[17,18,23,24] Nonetheless, the efficacy of HVMPC in treating skin ulcers in patients with diabetes mellitus has not been systematically studied to date, to the best of our knowledge.

The definitions of pressure ulcers (PrUs) and DFUs might overlap,[25,26] since any pressure ulcer on the foot of a patient with diabetes is defined as a diabetic foot ulcer.[27] Moreover, a common factor in the development of both PrUs and DFUs is increased pressure,[26] and both are managed by reducing pressure (offloading).[8,25,28] Increased plantar pressure is a significant risk factor for the formation of DFUs,[29,30] and diabetes mellitus may constitute a risk factor for the development of foot PrUs.[31,32]

Failure of chronic wounds to heal might have a common molecular pathophysiologic origin, despite the heterogeneity in their aetiology.[2,33] Persistent inflammatory state due to repeated trauma, low-level bacterial contamination or ischemia might constitute a common underlying mechanism in chronic wounds.[2,3] Elevated matrix metalloproteinases activity, proinflammatory cytokines and reduced growth factor activity might be present in both pressure and diabetic ulcers.[2,3,34]

### 1.2 | Objectives

#### 1.2.1 | Primary objective

Determine the efficacy of HVMPC in treating diabetic ulcers.

#### 1.2.2 | Secondary objective

Assess the effect of HVMPC on skin lesions with each of the pathophysiologic factors potentially contributing to the development of diabetic ulcers, in particular; neuropathic ulcers, ischemic ulcers, pressure ulcers, and acute wounds.

#### 1.2.3 | Tertiary objectives

Evaluate the safety of HVMPC, and identify treatment parameters.

## 2 | METHODS

### 2.1 | Eligibility criteria

The systematic review included studies published in peer-reviewed journals, regardless of design, focusing on HVMPC application for the treatment of diabetic ulcers, neuropathic ulcers, ischemic ulcers, pressure ulcers and acute wounds. A *diabetic ulcer* was defined as 'an open sore or wound that occurs in patients with diabetes mellitus, and is



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

commonly located on the bottom of the foot'; 'Patients with diabetes develop wounds secondary to neuropathy with or without biomechanical abnormalities, peripheral vascular disease with ischemia, or both'.[35] A *neuropathic ulcer* was defined as 'a form of chronic ulceration which develops in anaesthetic skin, appearing on areas subject to trauma or pressure'.[36] An *ischemic ulcer* was defined as 'ulceration that occurs as the result of arterial occlusive disease when non-pressure related disruption or blockage of the arterial blood flow to an area causes tissue necrosis'.[37] A *pressure ulcer* was defined as 'localized damage to the skin and/or underlying tissue, as result of pressure or pressure in combination with shear'.[38] An *acute wound* was defined as 'breakdown of the integrity of the soft tissue envelope surrounding any portion of the body',[39] and including, but not limited to: trauma, closed puncture wounds, lacerations, contusion/blunt trauma, surgical incisions, grafts, and burns.[40,41] Abstracts, editorials, reviews, animal studies, and studies written in languages other than English or Portuguese were excluded.

## 2.2 | Information sources

An electronic search of the following databases was conducted; United States National Library of Medicine (NLM) and National Institutes of Health (NIH) PubMed, Scopus, Physiotherapy Evidence Database PEDro and Google Scholar, to retrieve relevant studies, from inception until the 8th of July (PubMed, PEDro and Scopus) and the 9 July (Google Scholar) 2021. The electronic search of PubMed was repeated on the 22 April 2022. Inspecting bibliographic references complemented the electronic search. The search terms were: High-Voltage Monophasic Pulsed Current (HVMPC), High-Voltage Pulsed Current (HVPC), High-Voltage Pulsed Galvanic Stimulation (HVPGS), High-Voltage Electrical Stimulation (HVES), Diabetic Ulcer, Diabetic Wound, Ischemic Ulcer, Ischemic Wound, Arterial Ulcer, Pressure Ulcer, Pressure Sore, Pressure Wound, Decubitus Ulcer, Bedsore, Skin Wounds, Skin Ulcers, Skin Injuries, Skin Lesions, and Burns. The full search strategies for all databases are available in the Supporting Information.

## 2.3 | Selection process

Search results were imported to EndNote X9.3.3 (Clarivate, London, UK; Build 13966), which was used for the entire review process. Titles and abstracts were screened for matching articles, following identification of duplicates, and full-texts were subsequently retrieved for all eligible articles.

## 2.4 | Data collection process

The following data were extracted from each study: study design, study inclusion and exclusion criteria, participants' characteristics (sample size, age, and gender), wound/ulcer properties (number, type, stage, baseline surface area, duration, and location), HVMPC protocol parameters (voltage, frequency, pulse interval (pulse spacing), intervention duration, and frequency of sessions per week), electrode arrangement (electrode polarity, placement, material and size, and distance between electrodes), standard wound care (SWC) intervention(s), intervention(s) period and funding source(s). Primary and secondary endpoint outcomes were also investigated for relevant outcome measures. Attempts to obtain missing outcome data, through contacting corresponding authors by email, were unsuccessful.

## 2.5 | Outcomes measures

### 2.5.1 | Primary outcomes

a. Incidence of complete healing (ICH), defined as "100% reepithelialization of the wound surface with no discernable exudate and without drainage or dressing, confirmed at two consecutive study visits 2 weeks apart"[42,43]: time to complete healing, and proportion of completely healed ulcers.[44,45]
b. Wound-healing status (WHS), defined as "reduction in wound surface area (WSA) relative to baseline": percent WSA reduction at 4–8 weeks.[44,45]
c. Incidence of worsening of healing (IWH), defined as "increase in WSA relative to baseline": proportion of worsened ulcers.

### 2.5.2 | Secondary outcomes

a. Physical function and ambulation,[44,45] wound-related pain status,[44–46] infection,[44,45] recurrence,[46] amputation[46] and morbidity: respective outcome measures as reported in each study.
b. *Ischemic ulcers*: *cutaneous microcirculation*; skin perfusion pressure, transcutaneous oxygen tension ($TcPO_2$), magnetic resonance angiography (MRA), computed tomographic angiography, segmental volume plethysmography (pulse volume recording), laser doppler flow, pulse oximetry, ankle–brachial index (ABI), and other relevant measures as reported in each study.
c. *Neuropathic ulcers*: *nerve function* Nerve conduction testing, vibration perception threshold, Semmes-Weinstein monofilament testing, symptoms (pain: visual analogue scale (VAS), neuropathic pain scale (NPS), Brief Pain Inventory for Diabetic Peripheral Neuropathy (BPI-DPN), Short-form Mc-Gill Pain Questionnaire (SF-MPQ), Neuropathic Pain Questionnaire (NPQ), and numbness), the Berg Balance Scale (BBS), the Functional Gait Assessment (FGA), and the Activities-specific Balance Confidence Scale (ABC Scale), and other relevant measures as reported in each study.
d. *Acute wounds*: dehiscence, hematoma, seroma formation and other relevant measures as reported in each study.

### 2.5.3 | Tertiary outcomes

a. Reporting of adverse reactions to HVMPC intervention.



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

## 2.6 | Risk of bias assessment

Studies were assessed for their risk of bias and methodological quality using the appropriate scale depending on study design. Randomised controlled trials were assessed using the revised tool for assessing risk of bias in randomised trials (RoB 2),[47] while non-randomised studies were assessed using the risk of bias in non-randomised studies-of interventions (ROBINS-I) tool.[48] Uncontrolled trials were evaluated using the JBI critical appraisal tool.[49] Overall appraisal of studies evaluated using the JBI tool was based on question responses, as follows; high quality (1 'No' or 2 'Unclear'), moderate quality (2 'No' or 2–4 'Unclear'), low quality (>2 'No' or >4 'Unclear').

## 2.7 | Summary measures

Only randomised controlled trials were included in the meta-analysis. Incidences of complete healing and worsening of healing were subject to descriptive and relative risk (RR) analyses along with 95% confidence intervals (CI). The interpretation of RR of complete healing was as follows: RR > 1, probability of complete healing is higher in treatment arm; RR = 1, no difference in probability of complete healing between treatment and control arms; RR < 1, probability of complete healing is lower in treatment arm. Conversely, the RR of worsening of healing was interpreted as follows: RR < 1 denoted lower probability of worsening of healing in the treatment arm, and vice versa. The weighted mean difference (WMD) and 95% CI of the percent reduction in WSA between the treatment and control arms were also calculated.

## 2.8 | Synthesis of results

Studies were categorised, based on wound/ulcer aetiology, into five groups; diabetic ulcers, ischemic/neuropathic ulcers in diabetic patients, pressure ulcers, acute wounds (postoperative and burns), and multiple aetiology wounds and ulcers. All of the studies that included wounds/ulcers of the same aetiology, assessed wound healing using the same outcome measure, and either presented the actual results or sufficient data to calculate them were eligible for synthesis. Forest plots, within which studies were ordered from low to high risk of bias, were used to display results, as recommended.[47] Random-effects model was used due to between-study variability in design. Heterogeneity was assessed using the $I^2$ statistic and the chi-squared test; $I^2$ values of 25%, 50%, and 75% correspond to low, moderate, and high heterogeneity, respectively.[50]

The mean percent reduction in WSA from baseline (mean WSAR %) was included as reported in each study. If not reported, while the mean surface area at baseline and at the end of study were reported, the mean WSAR % was calculated using the following equation: the difference between baseline and the last post-treatment percent of mean WSA was calculated and then divided by the baseline percent of mean WSA.

$$\left[\text{Mean WSAR}\% = \frac{(\text{initial WSA} - \text{final WSA}) \times 100}{\text{initial WSA}}\right]$$

Missing standard deviations were imputed using the following formula:

$$\left[\text{SD} = \sqrt{\left(\frac{(\text{initial WSA } SD - \text{final WSA } SD) \times 100}{\text{initial WSA } SD}\right)^2}\right]$$

If the median, instead of the mean, percent of reduction in WSA was reported, the mean WSAR % was estimated using this equation[51]:

$$\left[\bar{x} \approx \frac{a + 2m + b}{4}\right]$$

In this case, missing standard deviations were estimated using the following equation[51]:

$$\left[\text{SD} = \sqrt{S^2} = \frac{1}{12}\left(\frac{(a - 2m + b)^2}{4} + (b - a)^2\right)\right]$$

Control groups of studies with multiple intervention groups (i.e., multi-arm studies) were counted once, as per the recommendation of the Cochrane handbook,[52] in the analyses of dichotomous variables; proportion of complete and worsening of healing. Nevertheless, combining intervention groups in the analysis of continuous variables; percent WSA reduction, was inconvenient since it may mask true heterogeneity between intervention groups, thereby introducing measurement error. Optimal information sizes were, however, calculated based on the actual number of participants (biological sample size), with subjects in the control group counted only once.

Statistical synthesis was conducted using Review Manager (RevMan) [Computer program]. Version 5.4.1, The Cochrane Collaboration, 2020. RevMan was also used to create funnel plots, while further tests for publication bias were done using STATA® software (Stata/MP 16.0; StataCorp LLC, College Station, TX). Moreover, R software (R studio 2022.02.0, Build 443; 250 Northern Ave, Boston, MA) was utilised to produce risk-of-bias summary tables.

## 2.9 | Subgroup analysis

Subgroup analysis based on risk of bias was performed. Moreover, studies implementing similar HVMPC protocols and intervention durations were assessed. The analysis of intervention durations was performed to explore possible causes of statistical heterogeneity.



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

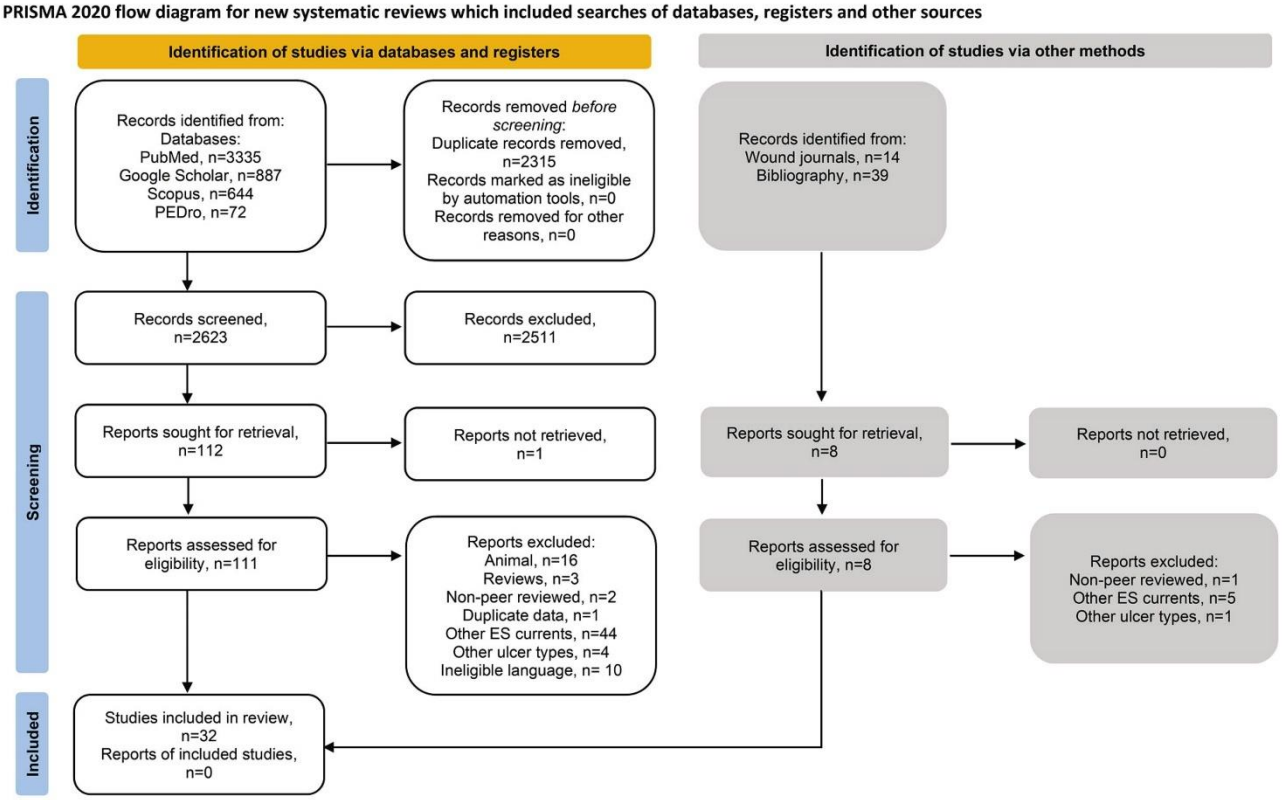



**FIGURE 1** Flow diagram of review process

## 2.10 | Sensitivity analysis

The impacts of excluding studies with missing data and studies with high risk-of-bias rating were assessed. In addition, fixed-effects model meta-analysis was performed.

## 2.11 | Reporting bias assessment

Funnel plots were utilised to assess for asymmetry, moreover, other methods to check for publication bias were used, these included; contour-enhanced funnel plot, Egger's test, Begg's test, and nonparametric trim-and-fill analysis of publication bias.[52]

## 2.12 | Certainty assessment

Results were further analysed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) principles to determine the overall quality of evidence.[53] The GRADE handbook for grading quality of evidence and strength of recommendations (updated October 2013) was consulted.[54] The five GRADE considerations (risk of bias, inconsistency, indirectness, imprecision, and risk of publication bias) were used as described in the handbook. The certainty of evidence was graded as high, moderate, low, very low. Summary of findings table was prepared using the GRADEpro GDT software.[55] Justifications to all decisions to downgrade the certainty of evidence were provided in the explanations section of the table.

# 3 | RESULTS

## 3.1 | Results of the systematic review

The database search identified 4938 records; following removal of 2315 duplicate records, 2623 records were screened, from which 111 were assessed for eligibility, and ultimately 31 full-text articles were included.[56–86] Complementary search of bibliography and wound journals yielded 53 records, and resulted in the additional inclusion of 1 full-text article.[87] The repeated search of PubMed produced 88 records; however, none of these studies were eligible for inclusion. An overview of the flow of the review is provided in Figure 1. A list of all excluded studies is available in the Supporting Information.

## 3.2 | Study characteristics

Thirty-two studies, enrolled 1061 patients who had a total of 1103 skin lesions of selected aetiologies; diabetic ulcers (115 ulcers), ischemic/neuropathic ulcers in diabetic patients (37 ulcers), PrUs (567 ulcers), acute wounds (75 wounds), and multiple



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

aetiology wounds and ulcers (309 wounds and ulcers). The identified studies varied in design; 17 studies were randomised controlled trials,[56,59,60,63,66,67,69,70,72,74-80,87] two studies were non-randomised controlled trials,[57,62] 10 studies were case series studies,[61,65,68,71,73,81,82,84-86] and three studies were case reports.[58,64,83] The sources of funding were as follows: research funded by universities and other academic institutions[59,62,65,76,77,79,80]; research funded by other organisations[61,66,69,70,75,82,85,86]; funding and/or ES units provided, loaned, or donated by manufacturers[61,62,65,67,70,72,73,76]; and no financial disclosure.[68,71,73]

## 3.3 | Risk of bias assessment results

Similarities, in domain ratings, were noted across outcomes in the assessment results of randomised controlled trials using the RoB 2 tool. Bias due to deviations from intended intervention; loss to follow-up and failure to adhere to intention-to-treat analysis were concerns in some of the studies,[56,59,67,72,78,80] while bias due to selective reporting of results was suspected in mostly the same studies[56,59,60,72] (Figures S1–S3). All outcomes examined using ROBINS-I were at a critical risk of bias; mainly due to confounding, classification of interventions, measurement of outcomes and selective reporting[57,62] (Figure S4).

None of the studies evaluated with the JBI tool were excluded from the review, regardless of their quality. Three case series studies,[61,65,85] and one case report[58] were of high quality; two case series studies,[82,86] and one case report[64] were of moderate quality; and five case series studies,[68,71,73,81,84] and one case report[83] were of low quality (Tables S1 and S2). Full details of risk-of-bias judgements and methodological quality appraisals are available in the Supporting Information.

## 3.4 | Results of individual studies

Most of the identified studies, reported that HVMPC, either alone,[56,57,83,84] or in addition to SWC,[58,59,61-67,69-72,74-82,87] and/or other interventions,[57,60,68,73,85,86] had a positive effect on wound healing. Nonetheless, two studies[57,75] did not find statistically significant differences between groups that received HVMPC and groups that did not. Further details are presented in the Supporting Information.

## 3.5 | Meta-analysis results

Twelve randomised controlled trials were included in the quantitative synthesis.[56,59,60,67,69,72,75-80] Data of each study were directly included as reported, however, results had to be calculated/imputed for three studies.[56,60,67] The number of participants is slightly inflated by ($n$ = 8); since one study included patients with more than one ulcer.[80]

### 3.5.1 | Diabetic ulcers

*Incidence of complete healing*

The characteristics of studies included in the meta-analysis were as follows; the treatment arm included 30 participants (with 35 ulcers), while the control arm included 29 participants (with 33 ulcers). Mean age in the treatment arm was 56.85 ± 3.46 years old, and in the control was 59.6 ± 0.42 years old. Ulcer duration was 19.55 ± 0.63 weeks in the treatment arm, while it was 36.45 ± 20.43 weeks in the control arm. Mean baseline WSA was 3.09 ± 2.07 $cm^2$ in the treatment arm, while it was 8.7 ± 7.29 $cm^2$ in the control arm. Intervention period was 14 ± 2.82 weeks. The pooled result was RR 1.43; 95% CI: [0.92, 2.24], $p$ = 0.11; $I^2$ = 7%, $p$ = 0.30 (Figure 2).

### 3.5.2 | Pressure ulcers

*Incidence of complete healing*

The characteristics of studies included in the meta-analysis were as follows; the treatment arm included 195 participants (76 males, and 110 females; with 200 ulcers), while the control arm included 155 participants (57 males, and 91 females; with 158 ulcers). Mean age in the treatment arm was 64.14 ± 15.97 years old, and in the control was 61.89 ± 15.73 years old. Ulcer duration was 19.76 ± 17.37 weeks in the treatment arm, while it was 32.15 ± 54.80 weeks in the control arm. Mean baseline WSA was 8.81 ± 5.69 $cm^2$ in the treatment arm, while it was 9.69 ± 6.90 $cm^2$ in the control arm. Intervention period was 6.81 ± 2.33 weeks. The pooled result was RR 2.08; 95% CI: [1.42, 3.04], $p$ = 0.0002; $I^2$ = 0%, $p$ = 0.61 (Figure 3).

*Wound-healing status*

The characteristics of studies included in the meta-analysis were as follows; the treatment arm included 233 participants (89 males, and 144 females; with 238 ulcers), while the control arm included 160 participants (59 males, and 101 females; with 163 ulcers). Mean age in the treatment arm was 60.20 ± 17.17 years old, and in the control was 62.90 ± 14.99 years old. Ulcer duration was 14.07 ± 3.46 weeks in the treatment arm, while it was 12.80 ± 2.38 weeks in the control arm. Mean baseline WSA was 9.32 ± 4.91 $cm^2$ in the treatment arm, while it was 9.87 ± 7.07 $cm^2$ in the control arm. Intervention period was 6.09 ± 1.04 weeks. Statistical synthesis of all studies (percent WSA reduction at 20 days to 12 weeks) was undertaken (Figure 4), and the pooled result was MD 32.97; 95% CI: [21.86, 44.07], $p$ < 0.00001; $I^2$ = 72%, $p$ < 0.00001. However, the pooled result of percent WSA reduction at 4–8 weeks (Figure 5) was MD 33.76; 95% CI: [21.38, 46.15], $p$ < 0.00001; $I^2$ = 76%, $p$ < 0.00001.



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

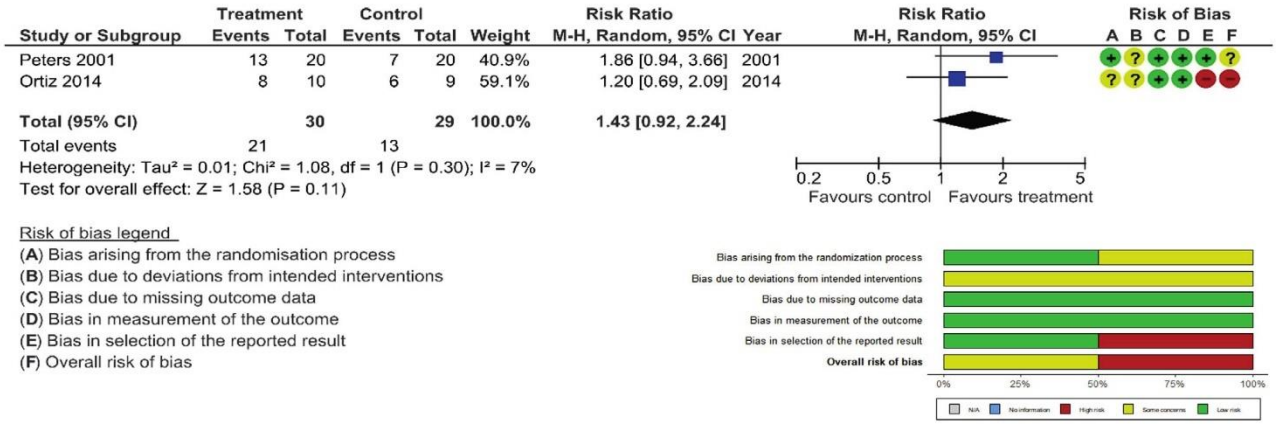



**FIGURE 2** Forest plot of relative risk (RR) analysis of proportion of completely healed diabetic ulcers; RR > 1, probability of complete healing is higher in treatment arm; RR = 1, no difference in probability of complete healing between treatment and control arms; RR < 1, probability of complete healing is lower in treatment arm

*Incidence of worsening of healing*

The characteristics of studies included in the meta-analysis were as follows; the treatment arm included 117 participants (27 males, and 81 females; with 122 ulcers), while the control arm included 97 participants (25 males, and 65 females; with 100 ulcers). Mean age in the treatment arm was 73.11 ± 11.79 years old, and in the control was 69.72 ± 11.79 years old. Ulcer duration was 21.36 ± 23.03 weeks in the treatment arm, while it was 48.20 ± 72.14 weeks in the control arm. Mean baseline WSA was 7.08 ± 2.88 $cm^2$ in the treatment arm, while it was 7.17 ± 3.06 $cm^2$ in the control arm. Intervention period was 7.21 ± 2.40 weeks. The pooled result was RR 0.18; 95% CI: [0.06, 0.60], $p = 0.005$; $I^2 = 0\%$, $p = 0.97$ (Figure 6).

## 3.6 | Secondary outcomes

### 3.6.1 | Morbidity, infection, recurrence and amputation

Ulcers that were unresponsive to previous treatment completely healed following HVMPC intervention.[62,64,68,72,73,81,85] Other studies reported consistent and steady patterns of wound healing.[58–60,63,65–67,69–71,77,78,80] Reduced oedema and improved function were noted following HVMPC intervention in one study.[74] No significant differences in quality of life were observed in one study.[75]

Patients with infected wounds were frequently excluded from studies.[66,68,74–80,82,85] Alternatively, HVMPC intervention was either suspended or discontinued if infection occurred during studies.[74,76,85] Antibiotic therapy was administered in few studies.[58,70] Antiseptics were used to disinfect wounds in some studies.[59,77–80] One study reported that no signs of infection were detected throughout the study.[83]

One study documented recurrence or development of new ulcers, within 4 months of complete healing, in eight subjects in each group.[69] Two patients, who had pre-existing contralateral major amputation and received HVMPC, underwent amputation in one study.[65] Two major amputations in the treatment group, compared with three in the control group, were reported in another study.[62]

### 3.6.2 | Wound-related pain status

Similar pain improvement in both the treatment and control groups was found in two studies.[63,75] Early reduction in postoperative wound pain was observed following HVMPC intervention.[66] Another study also concluded that HVMPC intervention reduced pain in patients with chronic ulcers.[84] Treatment was discontinued following a complaint of increased pain by a patient who had severe Raynaud's syndrome.[85]

## 3.7 | Tertiary outcomes

### 3.7.1 | HVMPC intervention parameters

The parameters ranged between 50–360 V, 8–120 HZ, 15–179 μs, 10–320 min a day, 2–7 days a week, for up to 12 months. Negative electrode over wound (cathodal), positive electrode over wound (anodal) and negative electrode over wound replaced by positive electrode (cathodal–anodal) were all used. Polarity was reversed if healing plateau was reached. The size of active electrode ranged from 5 × 5 cm to 5 × 10 cm, while dispersive electrode was between 9 × 10 cm and 20 × 25 cm. Bipolar, instead of monopolar, technique was applied to two patients who used two equally sized 4.8 × 10.2 cm active and dispersive electrodes. More than one active electrode was sometimes used depending on WSA. The dispersive electrode was placed 5–25 cm from the active electrode. The active electrode was made of heavy-duty aluminium foil, carbon-rubber, Dacron-mesh silver nylon stocking, sterile conductive hydrogel sheet, silver, Metalline® gauze and stainless-steel.



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

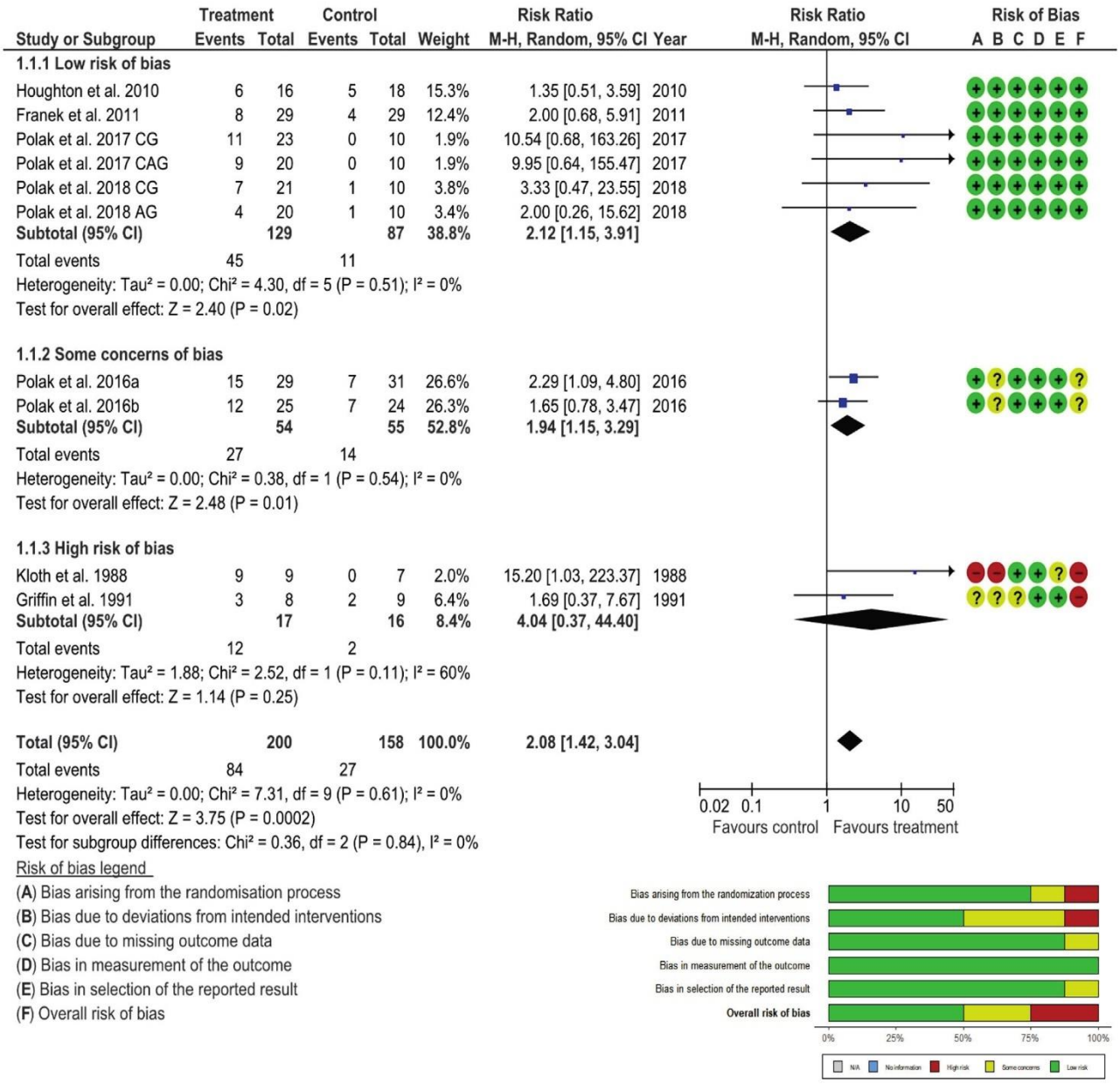



**FIGURE 3** Forest plot of relative risk (RR) analysis of proportion of completely healed pressure ulcers; RR > 1, probability of complete healing is higher in treatment arm; RR = 1, no difference in probability of complete healing between treatment and control arms; RR < 1, probability of complete healing is lower in treatment arm. The number of participants is slightly inflated by ($n = 8$) as one study included participants with more than one ulcer. AG, anodal group; CAG, cathodal–anodal group; CG, cathodal group

The commonly used parameters were 100–150 V (did not induce muscle contraction),[58–61,64,66,68,70,73,75,77–80,82–84,87] 100 HZ,[59–61,63,64,66–71,75,77–83,87] 77–100 μs,[58,69,70,75–80,82,83] 50–60 min a day,[56,58–60,62–67,71,73,77–81,87] 5–7 days a week.[56,58–60,62–65,67,69,72,76–80,87] Negative electrode (active) was usually placed over wound, and positive electrode (dispersive) placed at least 20 cm away from the active electrode[59,70,77–80,82]; polarity was commonly reversed as part of the protocol or if healing plateau was reached.[56,58–60,65,69,71,73,75,77,80,81,83,87] The active electrode was usually 5 × 10 cm, while the dispersive electrode was 10 × 10 cm.[61,62,65,69,77–80] Conductive carbon-rubber electrodes were frequently used.[59–61,63,77–80]

### 3.7.2 | Adverse reactions

Many of the identified studies did not report on adverse reactions. No adverse reactions were observed in a number of studies.[56,58,59,67,70,77–80] Other studies, however, documented minor and rare reactions such as red, raised, itchy skin beneath the dispersive electrode that was attributed to contact dermatitis, and was resolved by using a non-adhesive carbon electrode, or applying hydrocortisone 2.5% ointment to the affected area for 1–2 weeks.[63,69] One patient had a persistent red area or burn (>24 h) that resolved within 48 h, and was remedied by turning down the intensity of stimulation.[69]



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

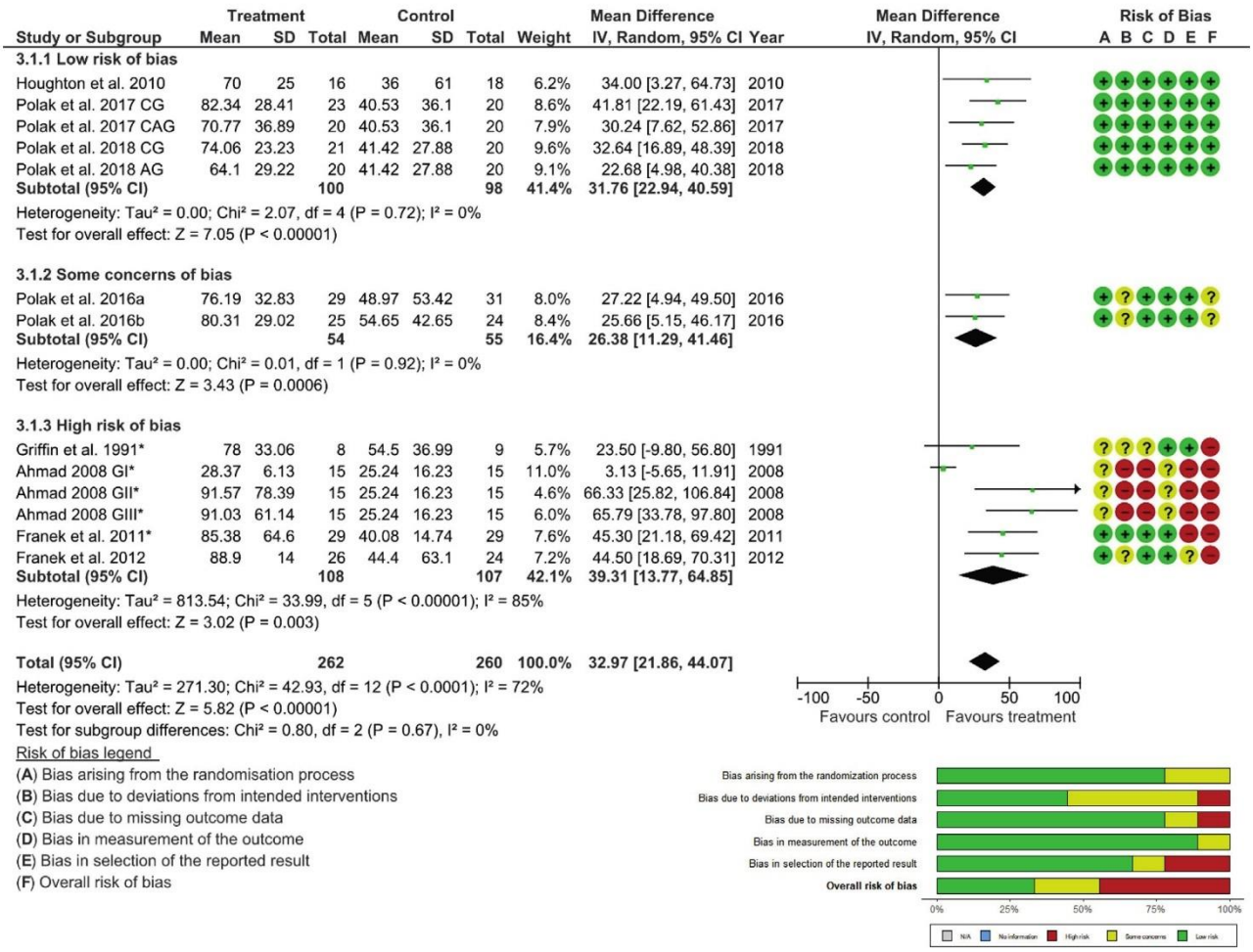



**FIGURE 4** Forest plot of mean difference (MD) analysis of percent wound surface area reduction of pressure ulcers at 20 days–12 weeks; values smaller than zero favours control arm, whereas values greater than zero favours treatment arm. The biological sample size (*n*) is 452 participants; experimental arm ($n = 262$), and control arm ($n = 190$); the number of participants is slightly inflated by ($n = 8$) as one study included participants with more than one ulcer. AG, anodal group; CAG, cathodal–anodal group; CG, cathodal group; *mean and/or standard deviation values were calculated and/or imputed for this sample

Complaints of stimulation intensity by at least one patient were recorded in another study, and were resolved by reducing it.[57] A patient with severe Raynaud's syndrome complained of increased pain following two treatment sessions.[85] Serious adverse events, though unlikely that any were related to the HVMPC intervention, occurred in one study; two participants experienced loss of haemodialysis access requiring brief hospitalisation, and one of these participants developed systemic calciphylaxis and died 1 week later.[63] One patient was diagnosed with chronic osteomyelitis, following the development of excessive exudate, a strong foul odour, and significant redness upon dressing removal.[85] Summary tables are presented in Supporting Information.

## 3.8 | Subgroup analyses results

Possible causes of heterogeneity, mainly in the results concerning the WHS outcome (percent WSA reduction at 4–8 weeks), were investigated. The analyses, however, may not appropriately explain the observed statistical heterogeneity. Significant heterogeneity was found in the results of cathodal–anodal HVMPC protocol, without subgroup differences, suggesting true heterogeneity (Table S3). Moreover, even-though excluding the only study (particularly the only group) with intervention duration of <50 min in the WHS outcome[56] significantly reduced heterogeneity, it may not fully explain the heterogeneity, since other studies with similar intervention duration showed variable results.[70,72,75] Some heterogeneity, although insignificant as confidence intervals overlap, was also observed, within and between protocols, in the results of the outcomes of complete and worsening of healing incidences.

## 3.9 | Sensitivity analyses results

The sensitivity analyses suggest the robustness of the results, with exception of the outcome of WHS; due to a significant difference in



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

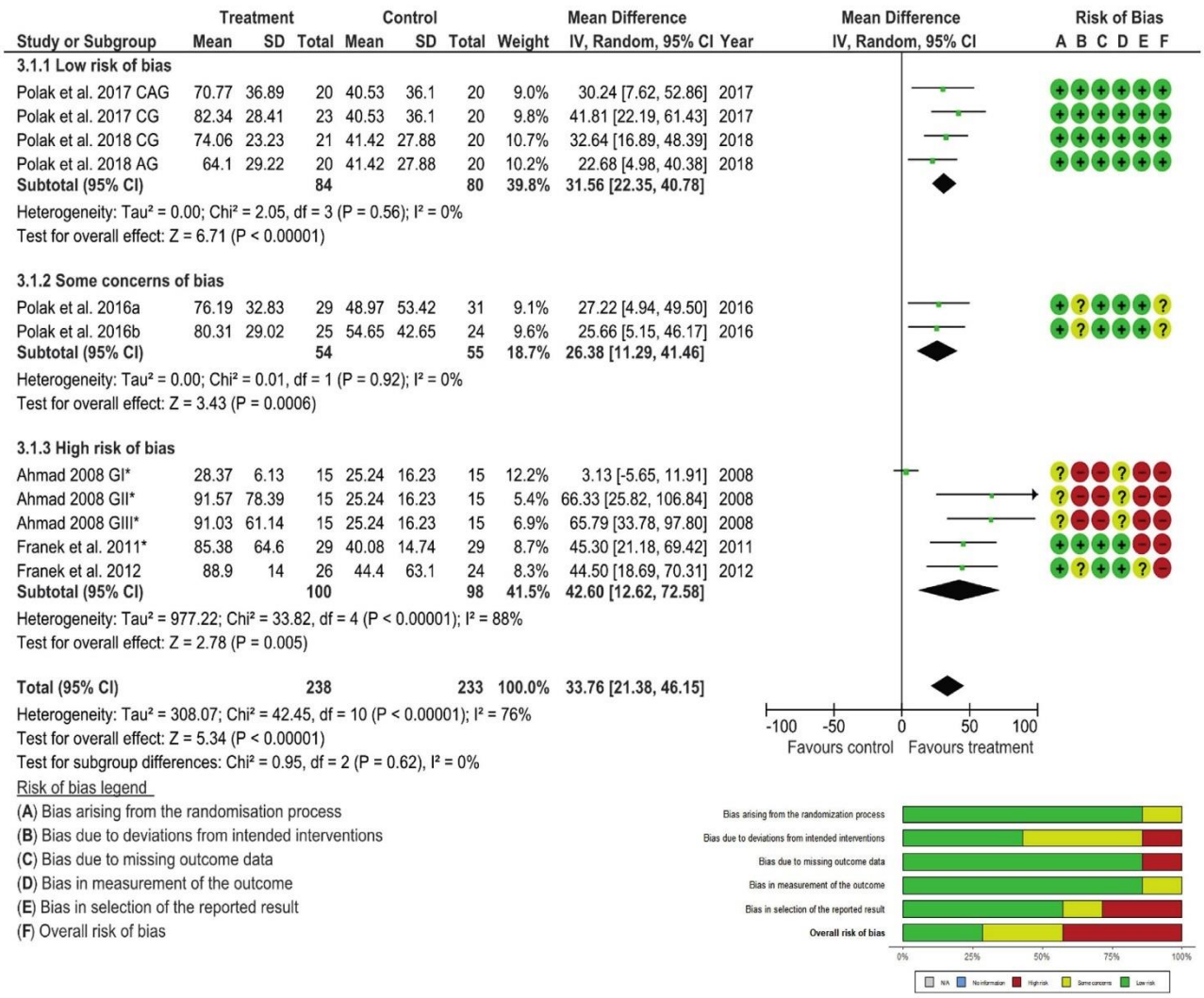



**FIGURE 5** Forest plot of mean difference (MD) analysis of percent wound surface area reduction of pressure ulcers at 4–8 weeks; values smaller than zero favours control arm, whereas values greater than zero favours treatment arm. The biological sample size ($n$) is 401 participants; experimental arm ($n = 238$), and control arm ($n = 163$); the number of participants is slightly inflated by ($n = 8$) as one study included participants with more than one ulcer. AG, anodal group; CAG, cathodal–anodal group; CG, cathodal group; *mean and/or standard deviation values were calculated and/or imputed for this sample

the estimates as assessed using fixed- and random-effects models (Table S4). The only two studies with missing data were rated as at high risk of bias in the WHS outcome;[56,60] excluding them along with other studies with high risk of bias reduced the overall estimate and improved precision.

## 3.10 | Risk of reporting biases

Publication bias was strongly suspected due to small studies, and positive tests for funnel plot asymmetry; Egger's test, Begg's test and non-parametric trim-and-fill analysis of publication bias. Non-reporting bias may, in part, explain the asymmetry, since studies seemed to be lacking in areas where results would be statistically non-significant and unfavourable to treatment (Figure S5). Contour-enhanced funnel plots further suggested non-reporting bias as a plausible explanation for the asymmetry. Further details are provided in the Supporting Information.

## 3.11 | Certainty of evidence

Moderate-certainty evidence indicated that HVMPC plus SWC likely increased the probability of complete healing, and likely reduced the probability of worsening of healing, compared with sham/no stimulation plus SWC. Evidence was downgraded once for strong suspicion of publication bias. Details are provided in the summary of findings table.



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

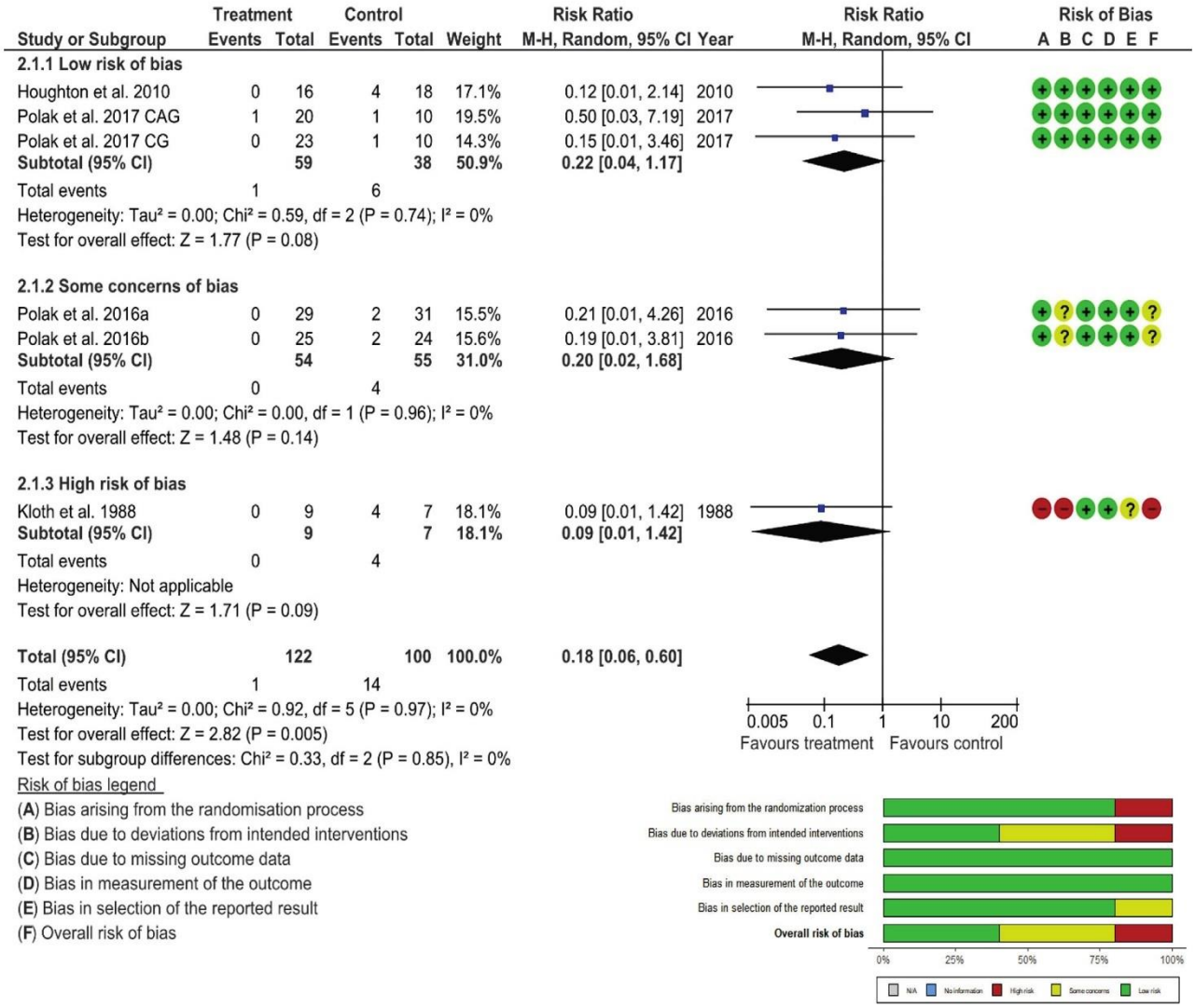



**FIGURE 6** Forest plot of relative risk (RR) analysis of proportion of worsened pressure ulcers; RR > 1, probability of worsening of healing is higher in control arm; RR = 1, no difference in probability of worsening of healing between treatment and control arms; RR < 1, probability of worsening of healing is lower in treatment arm. The number of participants is slightly inflated by ($n = 8$) as one study included participants with more than one ulcer. CAG, cathodal–anodal group; CG, cathodal group

## 4 | DISCUSSION

Although conclusive, direct evidence regarding the efficacy of HVMPC in the treatment of diabetic ulcers was not found in the present review, collateral evidence might suggest a potential benefit. The majority of identified studies reported positive effects of HVMPC in treating wounds and ulcers, regardless of aetiology. Nonetheless, the highest certainty evidence is concerning complete healing of pressure ulcers. Collectively, the evidence on the efficacy of HVMPC in treating skin lesions, with many of the pathophysiologic factors potentially contributing to diabetic ulcers, might justify further research.

HVMPC plus SWC likely increased the probability of complete wound healing of PrUs compared with sham/no stimulation plus SWC (RR 2.08; 95% CI: [1.42, 3.04], $p = 0.0002$; $I^2 = 0\%$, $p = 0.61$; 8 studies, 358 ulcers; moderate-certainty evidence). A main limitation relevant to this finding is that the treatment period of many of the included studies was insufficient for all ulcers to completely heal. However, study durations were prespecified in the protocols of some of the included studies.[77-80] The definition of complete healing, as stated by the U.S. Food and Drug Administration (FDA),[43] was met by only one study which followed patients up to 6 months after discontinuation of treatment.[69] The objective nature of complete wound healing may compensate for this limitation, nevertheless, the potential for recurrence remains not adequately investigated. This is especially true, since the same study reported recurrence in eight patients in each group within 4 months of complete healing, however, without further details.[69] Other systematic reviews on ES[88,89] and HVMPC,[24,90] found similar results suggesting the efficacy of ES, and HVMPC in particular, in treating pressure ulcers.

Percent WSA reduction at 4–8 weeks, as an outcome measure of wound healing, might fulfil FDA requirements for suitability as a primary clinical endpoint.[44,45] HVMPC plus SWC may have increased



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

percent WSA reduction at 4–8 weeks compared with sham/no stimulation plus SWC, but the evidence is very uncertain (MD 33.76%; 95% CI: [21.38, 46.15], $p < 0.00001$; $I^2 = 76\%$, $p < 0.00001$; 7 studies, 401 ulcers; very low-certainty evidence). HVMPC plus SWC, moreover, likely reduced the probability of worsening of healing compared with sham/no stimulation plus SWC (RR 0.18; 95% CI: [0.06, 0.60], $p = 0.005$; $I^2 = 0\%$, $p = 0.97$; 5 studies, 222 ulcers; moderate-certainty evidence). Nonetheless, percent area reduction was criticised for its dependence on initial ulcer size, which may lead to bias.[91–93] Measuring percent area reduction may also present challenges in predicting time to complete healing, since chronic wound healing may not follow a linear trajectory.[93,94]

Most of the reported adverse reactions to HVMPC intervention were not serious, though publication bias remains a concern. Goldman et al.[63] applied the highest intensity (up to 360 V), and reported a rash in one patient. Houghton et al.[69] administered the largest dose (≈448 h of active stimulation in total), and documented a red area or burn under the active electrode in one patient. Lack of intact sensation may significantly increase the risk of adverse reactions to ES.[95] Careful assessment of patient discomfort, and frequent checks for potential damage are, therefore, advised if sensation is compromised.[95] Moreover, caution is advised when treating patients with Raynaud's syndrome, due to a record of increased pain in one patient.[85]

Positive effects of HVMPC were observed in a number of in vivo animal studies, which largely mirrored the findings in human studies. These studies included wounds of variable aetiologies; diabetic wounds, burn wounds and surgical wounds. Potential explanations for these effects may be provided by studies which concluded that HVMPC may increase fibroblast and keratinocyte proliferation, enhance collagen synthesis, augment blood flow, and have antibacterial effects (References are provided in the Supporting Information).

The mechanisms behind the reported effects of HVMPC may be further explained by several in vitro studies. An increase in the influx of calcium ions was observed within the first min, which reached a maximum level by 3 min of HVMPC ES of human dermal fibroblasts.[96] Moreover, a sharp increase in insulin binding to fibroblasts began during the second min, and reached close to a maximum level after 3 min of stimulation.[96] The same phenomena were reported following HVMPC ES of mouse splenic T-lymphocytes.[97,98] Additionally, exposure to electric fields was shown to induce calcium influx in fibroblasts, keratinocytes and other cell types. Calcium signalling pathways are well-known regulators of cell differentiation and proliferation. The messenger role of calcium may be carried through a number of calcium-binding proteins which function as buffers to modulate cellular responses.

Indeed, a calcium and calmodulin-mediated contractile system was suggested to be involved in the exposure of interleukin-2 (IL-2) receptors, and the subsequent increase in protein and DNA synthesis in response to HVMPC. The exposure of IL-2 receptors might be directly upregulated by IL-2, which might be produced through the calcium–calcineurin–NFAT signalling pathway. IL-2 receptors were identified on the membrane of human fibroblasts and keratinocytes. It was reported that IL-2 may promote growth and stimulate DNA synthesis of fibroblasts. Moreover, T-lymphocytes, in particular, may have a modulatory role in wound healing, and their growth and proliferation may be regulated by IL-2. Decreased synthesis of IL-2 in T-lymphocytes, possibly due to an intrinsic defect, was reported in insulin-dependent diabetes mellitus (IDDM). Nevertheless, the exact function of IL-2 in T-lymphocytes remains unknown since a paradoxical immunosuppressive effect was suggested.

The rise in insulin binding following HVMPC was attributed to an increase in the number of insulin receptors rather than an increase in receptor binding affinity. The exposure of insulin receptors might occur through the calcium-calmodulin-dependent protein kinases types II/IV-CREB, and the calcium-calcineurin-NFAT signalling pathways. Human dermal fibroblasts and keratinocytes were found to have insulin receptors. Insulin may impact glucose entry, growth, migration and proliferation of these cells. High-glucose levels in the microenvironment of fibroblasts and keratinocytes, nonetheless, may negatively influence various cellular functions. Fibroblasts derived from diabetic ulcers were shown to have reduced growth, migration and proliferation. Furthermore, hyperproliferation with inhibited migration of keratinocytes, obtained from the edges of chronic diabetic wounds, were reported (References are provided in Supporting Information).

Some aspects need to be carefully considered for safe and effective application of ES for wound healing. Multiple mechanisms may be involved with regulating the influx of calcium, which in turn may regulate many cellular activities and cell death.[99,100] Identifying the processes responsible for modulating intracellular calcium concentration in response to ES may facilitate determining the exact parameters to control cellular functions, thus avoiding inadvertent injury.[101-103] In addition, the main cells involved in dermal repair, particularly fibroblasts, keratinocytes, T-lymphocytes, and macrophages, are not a homogenous population, and are known to have numerous phenotypes.[104-110] It is, however, yet to be known whether all or some of these phenotypes may respond to ES, how and to what extent.

Therefore, additional exploration of the precise mechanisms involved in HVMPC ES is of significant value. Further research is needed to clarify the aforementioned factors in order to either corroborate or refute the use of HVMPC as a therapeutic intervention.

### 4.1 | Limitations

- The primary limitation in the present review and many of the included studies is that the treatment period was insufficient for all ulcers to completely heal. Complete healing, however, may require long treatment periods, thus augmenting the overall length and cost of studies, which may not always be feasible.[93]
- The minimum clinically important difference (MCID) has not been established for wound healing outcome measures. It remains, consequently, unknown whether the results of the current review are clinically significant. Nonetheless, the large effects, in increasing



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

the probability of complete healing and reducing the probability of worsening of healing, may suggest clinical significance.

- Many of the identified studies did not explicitly provide definitions of ulcers that were studied, although the eligibility criteria of these studies were clear enough to meet the definitions set forth in the present review.
- Variability, between-studies and within-studies, with respect to SWC protocols and ulcer locations might have led to systematic differences, but it is unlikely that these differences contributed significantly to the pooled results.
- Patients with significant clinical comorbidities are usually excluded from randomised controlled trials, which may limit the generalizability of results; however, case series and case reports studies might provide complementary data relative to these conditions, which was the rationale behind including them.
- One study defined complete healing as closure of at least 75% or more of the sum of areas of all ulcers per patient,[75] which is different from the FDA's definition.
- A significant difference in ulcer duration and/or baseline WSA between groups was found in two studies,[69,75] which has influenced the overall characteristics of studies.
- A number of patients without ulcers, but had a history of ulceration and were at risk for ulceration, were included in one study.[61] One study included 4 patients who were previously included in an earlier study.[62] Another study included subjects less than 18 years of age, who may react differently to treatment.[87]
- Studies with multiple aetiology wounds and ulcers included patients with venous leg ulcers, which are out of the scope of the current review.
- Some of the included studies were of considerable duration (2.5–4 years), which might have introduced confounding factors.

## 5 | CONCLUSIONS

The present review found direct evidence, with moderate certainty, for the efficacy of HVMPC to treat pressure ulcers albeit its safety needs to be confirmed. Moreover, the positive observations on many of the skin lesions with pathophysiologic factors potentially contributing to diabetic ulcers might indirectly suggest that its efficacy may not be limited to pressure ulcers. What is also noteworthy is that the definition and pathophysiology of pressure ulcers might overlap with diabetic ulcers. These findings and observations highlight the potential of HVMPC and the need for further investigation.

### FUNDING INFORMATION

This work was supported by an FCT grant—Foundation for Science and Technology (Fundação para Ciência e Tecnologia)—Portugal, with reference number SFRH/BD/144090/2019. The funding source had no involvement in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### DATA AVAILABILITY STATEMENT

Data available in article supplementary material.

### ORCID

*Beshoy Girgis* https://orcid.org/0000-0003-3202-8609
*Davide Carvalho* https://orcid.org/0000-0002-3156-3741
*José Alberto Duarte* https://orcid.org/0000-0003-4756-5917

### REFERENCES

1. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. *Sci Transl Med*. 2014; 6(265):265sr6. doi:10.1126/scitranslmed.3009337
2. Mast BA, Schultz GS. Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. *Wound Repair Regen*. 1996; 4(4):411-420. doi:10.1046/j.1524-475X.1996.40404.x
3. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol*. 2007;127(3): 514-525. doi:10.1038/sj.jid.5700701
4. Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. *Open Biol*. 2020;10(9):200223. doi:10.1098/rsob.200223
5. Lazarus GS, Cooper DM, Knighton DR, et al. Definitions and guidelines for assessment of wounds and evaluation of healing. *Wound Repair Regen*. 1994;2(3):165-170. doi:10.1046/j.1524-475X.1994.20305.x
6. Eriksson E, Liu PY, Schultz GS, et al. Chronic wounds: treatment consensus. *Wound Repair Regen*. 2022;30(2):156-171. doi:10.1111/wrr.12994
7. Eaglstein WH, Falanga V. Chronic wounds. *Surg Clin*. 1997;77(3): 689-700. doi:10.1016/S0039-6109(05)70575-2
8. Andrews KL, Derby KM, Jacobson TM, Sievers BA, Kiemele LJ. Prevention and management of chronic wounds. *Braddom's Physical Medicine and Rehabilitation*. Elsevier; 2021:469-484. e4. doi:10.1016/B978-0-323-62539-5.00024-2
9. Edsberg LE, Black JM, Goldberg M, McNichol L, Moore L, Sieggreen M. Revised national pressure ulcer advisory panel pressure injury staging system: revised pressure injury staging system. *J Wound Ostomy Continence Nurs*. 2016;43(6):585-597. doi:10.1097/WON.0000000000000281
10. Schaper NC, van Netten JJ, Apelqvist J, et al. Practical guidelines on the prevention and management of diabetic foot disease (IWGDF 2019 update). *Diabetes Metab Res Rev*. 2020;36:e3266. doi:10.1002/dmrr.3266
11. Lepäntalo M, Apelqvist J, Setacci C, et al. Chapter V: diabetic foot. *Eur J Vasc Endovasc Surg*. 2011;42:S60-S74. doi:10.1016/S1078-5884(11)60012-9
12. Reiber GE, Vileikyte L, Ed B, et al. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diabetes Care*. 1999;22(1):157-162. doi:10.2337/diacare.22.1.157
13. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J Clin Invest*. 2007;117(5):1219-1222. doi:10.1172/JCI32169
14. Cho H, Blatchley MR, Duh EJ, Gerecht S. Acellular and cellular approaches to improve diabetic wound healing. *Adv Drug Deliv Rev*. 2019;146:267-288. doi:10.1016/j.addr.2018.07.019
15. Blakytny R, Jude E. The molecular biology of chronic wounds and delayed healing in diabetes. *Diabet Med*. 2006;23(6):594-608. doi:10.1111/j.1464-5491.2006.01773.x



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

16. Falanga V. Wound healing and its impairment in the diabetic foot. *Lancet*. 2005;366(9498):1736-1743. doi:10.1016/S0140-6736(05)67700-8
17. Kloth L, Polak A, Watson T. *Electrical stimulation for wounds*. Electro Physical Agents E-Book: Evidence-Based Practice; 2020:359.
18. Houghton PE. Electrical stimulation therapy to promote healing of chronic wounds: a review of reviews. *Chronic Wound Care Manag Res*. 2017;4:25-44. doi:10.2147/CWCMR.S101323
19. Chen Z, Chen Z-Y, Liu W-H, Li G-S. Electric stimulation as an effective adjunctive therapy for diabetic foot ulcer: a meta-analysis of randomized controlled trials. *Adv Skin Wound Care*. 2020;33(11):608-612. doi:10.1097/01.ASW.0000695784.82605.1e
20. Eleftheriadou I, Samakidou G, Tentolouris A, Papanas N, Tentolouris N. Nonpharmacological management of diabetic foot ulcers: an update. *Int J Low Extrem Wounds*. 2021;20(3):188-197. doi:10.1177/1534734620963561
21. Zulbaran-Rojas A, Park C, El-Refaei N, Lepow B, Najafi B. Home-based electrical stimulation to accelerate wound healing—a double-blinded randomized control trial. *J Diabetes Sci Technol*. 2021;0(0):1-10. doi:10.1177/19322968211035128
22. Rayman G, Vas P, Dhatariya K, et al. Guidelines on use of interventions to enhance healing of chronic foot ulcers in diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev*. 2020;36:e3283. doi:10.1002/dmrr.3283
23. Polak A, Franek A, Taradaj J. High-voltage pulsed current electrical stimulation in wound treatment. *Adv Wound Care*. 2014;3(2):104-117. doi:10.1089/wound.2013.0445
24. Khouri C, Kotzki S, Roustit M, Blaise S, Gueyffier F, Cracowski J-L. Hierarchical evaluation of electrical stimulation protocols for chronic wound healing: an effect size meta-analysis. *Wound Repair Regen*. 2017;25(5):883-891. doi:10.1111/wrr.12594
25. Brem H, Jacobs T, Vileikyte L, et al. Wound-healing protocols for diabetic foot and pressure ulcers. *Surg Technol Int*. 2003;11:85-92.
26. Ousey K, Chadwick P, Jawień A, et al. Identifying and treating foot ulcers in patients with diabetes: saving feet, legs and lives. *J Wound Care*. 2018;27(Sup5):S1-S52. doi:10.12968/jowc.2018.27.Sup5.S1
27. van Netten JJ, Bus SA, Apelqvist J, et al. Definitions and criteria for diabetic foot disease. *Diabetes Metab Res Rev*. 2020;36:e3268. doi:10.1002/dmrr.3268
28. Bus SA. The role of pressure offloading on diabetic foot ulcer healing and prevention of recurrence. *Plast Reconstr Surg*. 2016;138(3 S):179S-187S. doi:10.1097/PRS.0000000000002686
29. Alavi A, Sibbald RG, Mayer D, et al. Diabetic foot ulcers: part I. pathophysiology and prevention. *J Am Acad Dermatol*. 2014;70:e1-e18. doi:10.1016/j.jaad.2013.06.055
30. Bus S, Ulbrecht J. Biomechanics of the diabetic foot for the uninitiated. *The Foot in Diabetes*. 2020;125-137. doi:10.1002/9781119445821.ch7
31. Wensley F, Kerry C, Rayman G. Increased risk of hospital-acquired foot ulcers in people with diabetes: large prospective study and implications for practice. *BMJ Open Diabetes Res Care*. 2018;6(1):e000510. doi:10.1136/bmjdrc-2018-000510
32. Slowikowski GC, Funk M. Factors associated with pressure ulcers in patients in a surgical intensive care unit. *J Wound Ostomy Cont Nurs*. 2010;37(6):619-626. doi:10.1097/WON.0b013e3181f90a34
33. Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. Impaired wound healing. *Clin Dermatol*. 2007;25(1):19-25. doi:10.1016/j.clindermatol.2006.12.005
34. Lazaro J, Izzo V, Meaume S, Davies A, Lobmann R, Uccioli L. Elevated levels of matrix metalloproteinases and chronic wound healing: an updated review of clinical evidence. *J Wound Care*. 2016;25(5):277-287. doi:10.12968/jowc.2016.25.5.277
35. Lavery LA, Davis KE, Berriman SJ, et al. WHS guidelines update: diabetic foot ulcer treatment guidelines. Wound repair and regeneration: official publication of the wound healing society [and] the European tissue repair. *Society*. 2016;24(1):112-126. doi:10.1111/wrr.12391
36. Griffiths C, Barker J, Bleiker TO, Chalmers R, Creamer D. *Rook's Textbook of Dermatology*. 9th ed. John Wiley & Sons, Ltd; 2016. p. 85.4-85.5.
37. System CM. Revisions to Appendix PP, State Operations Manual (SOM):Guidance to Surveyors for Long Term Care Facilities (LTC) for Minimum Data Set (MDS) 3.0 Implementation October 1; 2010. https://www.hhs.gov/guidance/sites/default/files/hhs-guidance-documents/scletter10_33.pdf.
38. Kottner J, Cuddigan J, Carville K, et al. Pressure ulcer/injury classification today: an international perspective. *J Tissue Viability*. 2020;29:197-203. doi:10.1016/j.jtv.2020.04.003
39. Lee CK, Hansen SL. Management of acute wounds. *Surg Clin N Am*. 2009;89(3):659-676. doi:10.1016/j.suc.2009.03.005
40. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. *Clin Dermatol*. 2007;25(1):9-18. doi:10.1016/j.clindermatol.2006.09.007
41. Enoch S, Leaper DJ. Basic science of wound healing. *Surgery (Oxford)*. 2008;26(2):31-37. doi:10.1383/surg.23.2.37.60352
42. Gould L, Li WW. Defining complete wound closure: closing the gap in clinical trials and practice. *Wound Repair Regen*. 2019;27(3):201-224. doi:10.1111/wrr.12707
43. U.S. Food and Drug Administration. Guidance for industry: chronic cutaneous ulcer and burn wounds—developing products for treatment. *Wound Repair Regen*. 2001;9(4):258-268. doi:10.1046/j.1524-475X.2001.00258.x
44. Driver VR, Gould LJ, Dotson P, et al. Identification and content validation of wound therapy clinical endpoints relevant to clinical practice and patient values for FDA approval. Part 1. Survey of the wound care community. *Wound Repair Regen*. 2017;25(3):454-465. doi:10.1111/wrr.12533
45. Driver VR, Gould LJ, Dotson P, Allen LL, Carter MJ, Bolton LL. Evidence supporting wound care end points relevant to clinical practice and patients' lives. Part 2. Literature survey. *Wound Repair Regen*. 2019;27(1):80-89. doi:10.1111/wrr.12676
46. Gould LJ, Liu J, Wan R, Carter MJ, Dotson M, Driver VR. Evidence supporting wound care end points relevant to clinical practice and patients' lives. Part 3: the patient survey. *Wound Repair Regen*. 2021;29(1):60-69. doi:10.1111/wrr.12872
47. Sterne JA, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019;366:l4898. doi:10.1136/bmj.l4898
48. Sterne JA, Savović J, Page MJ, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions . *BMJ*. 2016;355:i4919. doi:10.1136/bmj.i4919
49. Munn Z, Barker TH, Moola S, et al. Methodological quality of case series studies: an introduction to the JBI critical appraisal tool. *JBI Evidence Synthesis*. 2020;18(10):2127-2133. doi:10.11124/JBISRIR-D-19-00099
50. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *Br Med J*. 2003;327(7414):557-560. doi:10.1136/bmj.327.7414.557
51. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol*. 2005;5(1):13. doi:10.1186/1471-2288-5-13
52. Higgins JP, Thomas J, Chandler J, et al. *Cochrane Handbook for Systematic Reviews of Interventions*. 2nd ed. John Wiley & Sons; 2019.
53. Group GW. Grading quality of evidence and strength of recommendations. *BMJ*. 2004;328(7454):1490. doi:10.1136/bmj.328.7454.1490
54. Schünemann H, Brożek J, Guyatt G, Oxman A. GRADE handbook for grading quality of evidence and strength of recommendations. Updated October 2013. The GRADE Working Group; 2013.
55. McMaster University and Evidence Prime. GRADEpro GDT: GRADEpro Guideline Development Tool [Software]. gradepro.org.



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

56. Ahmad ET. High-voltage pulsed galvanic stimulation: effect of treatment duration on healing of chronic pressure ulcers. Annals of burns and fire. *Disasters*. 2008;21(3):124-128.
57. Akers TK, Gabrielson AL. The effect of high voltage galvanic stimulation on the rate of healing of decubitus ulcers. *Biomed Sci Instrum*. 1984;20:99-100.
58. Fitzgerald GK, Newsome D. Treatment of a large infected thoracic spine wound using high voltage pulsed monophasic current. *Phys Ther*. 1993;73(6):355-360. doi:10.1093/ptj/73.6.355
59. Franek A, Kostur R, Polak A, et al. Using high-voltage electrical stimulation in the treatment of recalcitrant pressure ulcers: results of a randomized, controlled clinical study. *Ostomy Wound Manage*. 2012;58(3):30-44.
60. Franek A, Kostur R, Taradaj J, et al. Effect of high voltage monophasic stimulation on pressure ulcer healing: results from a randomized controlled trial. *Wounds*. 2011;23(1):15-23.
61. Gilcreast DM, Stotts NA, Froelicher ES, Baker LL, Moss KM. Effect of electrical stimulation on foot skin perfusion in persons with or at risk for diabetic foot ulcers. *Wound Repair Regen*. 1998;6(5):434-441. doi:10.1046/j.1524-475X.1998.60505.x
62. Goldman R, Brewley B, Zhou L, Golden M. Electrotherapy reverses inframalleolar ischemia: a retrospective, observational study. *Adv Skin Wound Care*. 2003;16(2):79-89. doi:10.1097/00129334-200303000-00009
63. Goldman R, Rosen M, Brewley B, Golden M. Electrotherapy promotes healing and microcirculation of infrapopliteal ischemic wounds: a prospective pilot study. *Adv Skin Wound Care*. 2004;17(6):284-294. doi:10.1097/00129334-200407000-00010
64. Goldman RJ, Brewley BI, Cohen R, Rudnick M. Use of electrotherapy to reverse expanding cutaneous gangrene in end-stage renal disease. 2003a. *Adv Skin Wound Care*. 2003;16(7):363-366. doi:10.1097/00129334-200312000-00013
65. Goldman RJ, Brewley BI, Golden MA. Electrotherapy reoxygenates inframalleolar ischemic wounds on diabetic patients: a case series. *Adv Skin Wound Care*. 2002;15(3):112-120. doi:10.1097/00129334-200205000-00006
66. Gomes RC, Guirro EC, Gonçalves AC, Junior JAF, Junior LOM, Guirro RR. High-voltage electric stimulation of the donor site of skin grafts accelerates the healing process. A randomized blinded clinical trial. *Burns*. 2018;44(3):636-645. doi:10.1016/j.burns.2017.10.006
67. Griffin JW, Tooms RE, Mendius RA, Clifft JK, Vander Zwaag R, el-Zeky F. Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. *Phys Ther*. 1991;71(6):433-442. doi:10.1093/ptj/71.6.433
68. Gui MS, Guirro RRJ, Sakabe DI, Sakabe FF. High voltage pulsed stimulation increases cicatrization of chronic cutaneous ulcers: analysis of six cases. *Fisioterapia e Pesquisa*. 2013;20:286-292. doi:10.1590/S1809-29502013000300014
69. Houghton PE, Campbell KE, Fraser CH, et al. Electrical stimulation therapy increases rate of healing of pressure ulcers in community-dwelling people with spinal cord injury. *Arch Phys Med Rehabil*. 2010;91(5):669-678. doi:10.1016/j.apmr.2009.12.026
70. Houghton PE, Kincaid CB, Lovell M, et al. Effect of electrical stimulation on chronic leg ulcer size and appearance. *Phys Ther*. 2003;83(1):17-28. doi:10.1093/ptj/83.1.17
71. Karsli PB, Gurcay E, Karaahmet OZ, Cakci A. High-voltage electrical stimulation versus ultrasound in the treatment of pressure ulcers. *Adv Skin Wound Care*. 2017;30(12):565-570. doi:10.1097/01.ASW.0000526606.72489.99
72. Kloth LC, Feedar JA. Acceleration of wound healing with high voltage, monophasic, pulsed current. *Phys Ther*. 1988;68(4):503-508. doi:10.1093/ptj/68.4.503
73. Lasko J, Kochik J, Serena T. Combining acoustic pressure wound therapy with electrical stimulation for treatment of chronic lower-extremity ulcers: a case series. *Adv Skin Wound Care*. 2010;23(10):446-449. doi:10.1097/01.ASW.0000383223.56900.13
74. Omar MT, El-Badawy AM, Borhan W, Nossier A. Improvement of edema and hand function in superficial second degree hand burn using electrical stimulation. *Egypt J Plast Reconstr Surg*. 2004;28(2):141-146.
75. Ortiz MCS, Villabona EH, Lemos DMC, Castellanos R. Effects of low level laser therapy and high voltage stimulation on diabetic wound healing. *Rev Univ Ind Santander Salud*. 2014;46(2):107-117.
76. Peters EJ, Lavery LA, Armstrong DG, Fleischli JG. Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial. *Arch Phys Med Rehabil*. 2001;82(6):721-725. doi:10.1053/apmr.2001.23780
77. Polak A, Kloth LC, Blaszczak E, et al. The efficacy of pressure ulcer treatment with cathodal and cathodal-anodal high-voltage monophasic pulsed current: a prospective, randomized, controlled clinical trial. *Phys Ther*. 2017;97(8):777-789. doi:10.1093/ptj/pzx052
78. Polak A, Kloth LC, Blaszczak E, et al. Evaluation of the healing progress of pressure ulcers treated with cathodal high-voltage monophasic pulsed current: results of a prospective, double-blind, randomized clinical trial. *Adv Skin Wound Care*. 2016;29(10):447-459. doi:10.1097/01.ASW.0000493164.75337.de
79. Polak A, Kucio C, Kloth LC, et al. A randomized, controlled clinical study to assess the effect of anodal and cathodal electrical stimulation on Periwound skin blood flow and pressure ulcer size reduction in persons with neurological injuries. *Ostomy Wound Manage*. 2018;64(2):10-29. doi:10.25270/owm.2018.2.1029
80. Polak A, Taradaj J, Nawrat-Szoltysik A, et al. Reduction of pressure ulcer size with high-voltage pulsed current and high-frequency ultrasound: a randomised trial. 2016a. *J Wound Care*. 2016;25(12):742-754. doi:10.12968/jowc.2016.25.12.742
81. Recio AC, Felter CE, Schneider AC, McDonald JW. High-voltage electrical stimulation for the management of stage III and IV pressure ulcers among adults with spinal cord injury: demonstration of its utility for recalcitrant wounds below the level of injury. *J Spinal Cord Med*. 2012;35(1):58-63. doi:10.1179/2045772311Y.0000000044
82. Sakabe FF, Sakabe DI, Marracini Fernandes FR, Reges G. Estimulação pulsada de alta voltagem reduz a área de úlceras de diferentes etiologias: um estudo piloto. *Fisioterapia*. 2018;19(1):72-79. doi:10.33233/fb.v19i1.2124
83. Santana de Souza AC, Lomasso Costa MM, Vieira Costa PH, Souza da Silva F. Efeito da estimulação elétrica de alta voltagem para o tratamento de úlceras por pressão: um estudo experimental de caso único. *Fisioterapia*. 2017;18(6):676-685. doi:10.33233/fb.v18i6.2046
84. Silva Ede F, Martins CC, Guirro EC, Guirro RR. High voltage electrical stimulation as an alternative treatment for chronic ulcers of the lower limbs. *An Bras Dermatol*. 2010;85(4):567-569. doi:10.1590/s0365-05962010000400025
85. Zhou K, Krug K, Stachura J, et al. Silver-collagen dressing and high-voltage, pulsed-current therapy for the treatment of chronic full-thickness wounds: a case series. 2016b. *Ostomy Wound Manage*. 2016;62(3):36-44.
86. Zhou K, Schenk R, Brogan MS. The wound healing trajectory and predictors with combined electric stimulation and conventional care: one outpatient wound care clinic's experience. 2016a. *Eur J Clin Invest*. 2016;46(12):1017-1023. doi:10.1111/eci.12685
87. Elshazly F, Hassan A. Influence of high voltage pulsed galvanic stimulation on wound healing in children with burn. *Bull Fac Phys Ther Cairo Univ*. 1998;3(1):61-68.
88. Arora M, Harvey LA, Glinsky JV, et al. Electrical stimulation for treating pressure ulcers. *Cochrane Database Syst Rev*. 2020;1:CD012196. doi:10.1002/14651858.CD012196.pub2



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

89. Chen L, Ruan Y, Ma Y, Ge L, Han L. Effectiveness and safety of electrical stimulation for treating pressure ulcers: a systematic review and meta-analysis. *Int J Nurs Pract*. 2022;e13041. doi:10.1111/ijn.13041
90. Girgis B, Duarte JA. High voltage monophasic pulsed current (HVMPC) for stage II-IV pressure ulcer healing. A systematic review and meta-analysis. *J Tissue Viability*. 2018;27(4):274-284. doi:10.1016/j.jtv.2018.08.003
91. Gorin DR, Cordts PR, LaMorte WW, Menzoian JO. The influence of wound geometry on the measurement of wound healing rates in clinical trials. *J Vasc Surg*. 1996;23(3):524-528. doi:10.1016/S0741-5214(96)80021-8
92. Jessup RL. What is the best method for assessing the rate of wound healing?: a comparison of 3 mathematical formulas. *Adv Skin Wound Care*. 2006;19(3):138-147. doi:10.1097/00129334-200604000-00009
93. Bull RH, Staines KL, Collarte AJ, Bain DS, Ivins NM, Harding KG. Measuring progress to healing: a challenge and an opportunity. *Int Wound J*. 2021;19:734-740. doi:10.1111/iwj.13669
94. Gottrup F, Apelqvist J, Price P. Outcomes in controlled and comparative studies on non-healing wounds: recommendations to improve the quality of evidence in wound management. *J Wound Care*. 2010;19(6):237-268. doi:10.12968/jowc.2010.19.6.48471
95. Nussbaum EL, Houghton P, Anthony J, Rennie S, Shay BL, Hoens AM. Neuromuscular electrical stimulation for treatment of muscle impairment: critical review and recommendations for clinical practice. *Physiother Can*. 2017;69(5):1-76. doi:10.3138/ptc.2015-88
96. Bourguignon GJ, Jy W, Bourguignon LY. Electric stimulation of human fibroblasts causes an increase in $Ca^{2+}$ influx and the exposure of additional insulin receptors. *J Cell Physiol*. 1989;140(2):379-385. doi:10.1002/jcp.1041400224
97. Jy W, Fregien N, Bourguignon G, Bourguignon LY. Role of Ca2+ in the regulation of hormone receptor exposure during lymphocyte activation. *Biochim Biophys Acta*. 1989;983(2):153-160. doi:10.1016/0005-2736(89)90228-9
98. Bourguignon LY, Jy W, Majercik MH, Bourguignon GJ. Lymphocyte activation and capping of hormone receptors. *J Cell Biochem*. 1988;37(2):131-150. doi:10.1002/jcb.240370202
99. Berridge MJ, Bootman MD, Lipp P. Calcium-a life and death signal. *Nature*. 1998;395(6703):645-648. doi:10.1038/27094
100. Tsien RW, Tsien RY. Calcium channels, stores, and oscillations. *Annu Rev Cell Biol*. 1990;6(1):715-760. doi:10.1146/annurev.cb.06.110190.003435
101. Cho MR, Marler JP, Thatte HS, Golan DE. Control of calcium entry in human fibroblasts by frequency-dependent electrical stimulation. *Front Biosci*. 2002;7(1):1-8. doi:10.2741/A733
102. Brosenitsch TA, Katz DM. Physiological patterns of electrical stimulation can induce neuronal gene expression by activating N-type calcium channels. *J Neurosci*. 2001;21(8):2571-2579. doi:10.1523/JNEUROSCI.21-08-02571.2001
103. Xu J, Wang W, Clark C, Brighton C. Signal transduction in electrically stimulated articular chondrocytes involves translocation of extracellular calcium through voltage-gated channels. *Osteoarthr Cartil*. 2009;17(3):397-405. doi:10.1016/j.joca.2008.07.001
104. Novak ML, Koh TJ. Macrophage phenotypes during tissue repair. *J Leukoc Biol*. 2013;93(6):875-881. doi:10.1189/jlb.1012512
105. Appay V, van Lier RA, Sallusto F, Roederer M. Phenotype and function of human T lymphocyte subsets: consensus and issues. *Cytometry A*. 2008;73(11):975-983. doi:10.1002/cyto.a.20643
106. Patel GK, Wilson CH, Harding KG, Finlay AY, Bowden PE. Numerous keratinocyte subtypes involved in wound re-epithelialization. *J Investig Dermatol*. 2006;126(2):497-502. doi:10.1038/sj.jid.5700101
107. Morasso MI, Tomic-Canic M. Epidermal stem cells: the cradle of epidermal determination, differentiation and wound healing. *Biol Cell*. 2005;97(3):173-183. doi:10.1042/BC20040098
108. Barman PK, Koh TJ. Macrophage dysregulation and impaired skin wound healing in diabetes. *Front Cell Dev Biol*. 2020;8:528. doi:10.3389/fcell.2020.00528
109. Cole MA, Quan T, Voorhees JJ, Fisher GJ. Extracellular matrix regulation of fibroblast function: redefining our perspective on skin aging. *J Cell Commun Signal*. 2018;12(1):35-43. doi:10.1007/s12079-018-0459-1
110. Mascharak S, Longaker MT. Fibroblast heterogeneity in wound healing: hurdles to clinical translation. *Trends Mol Med*. 2020;26:1101-1106. doi:10.1016/j.molmed.2020.07.008


## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Girgis B, Carvalho D, Duarte JA. The effect of high-voltage monophasic pulsed current on diabetic ulcers and their potential pathophysiologic factors: A systematic review and meta-analysis. *Wound Rep Reg*. 2023;31(2):171-186. doi:10.1111/wrr.13063



1524475x, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/wrr.13063 by Cochrane Portugal, Wiley Online Library on [08/02/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

**Summary of findings:**

## High-voltage monophasic pulsed current plus standard wound care compared to sham/no stimulation plus standard wound care for the treatment of pressure ulcers

**Patient or population:** Pressure ulcers
**Setting:** Inpatient and home care settings
**Intervention:** High-voltage monophasic pulsed current plus standard wound care
**Comparison:** Sham/no stimulation plus standard wound care

| Outcomes | Anticipated absolute effects* (95% CI) | | Relative effect (95% CI) | № of participants (studies) | Certainty of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| | **Risk with Sham/no stimulation plus standard wound care** | **Risk with High-voltage monophasic pulsed current plus standard wound care** | | | | |
| Incidence of complete healing (ICH) assessed with: proportion of completely healed ulcers follow-up: range 20 days to 12 weeks | **Study population** | | **RR 2.08** (1.42 to 3.04) | 358 (8 RCTs) | ⨁⨁⨁◯ Moderate[a,b,c,d] | Likely increased the probability of complete healing |
| | 171 per 1,000 | **355 per 1,000** (243 to 519) | | | | |
| | **Low** | | | | | |
| | 100 per 1,000 | **208 per 1,000** (142 to 304) | | | | |
| | **High** | | | | | |
| | 300 per 1,000 | **624 per 1,000** (426 to 912) | | | | |
| Wound-healing status (WHS) assessed with: percent wound surface area reduction follow-up: range 4 weeks to 8 weeks | The mean wound-healing status was **38.88** percent | MD **33.76 percent higher** (21.38 higher to 46.15 higher) | - | 401 (7 RCTs) | ⨁◯◯◯ Very low[c,d,e,f,g] | May have increased percent wound surface area reduction but the evidence is very uncertain |
| Incidence of worsening of healing (IWH) assessed with: proportion of worsened ulcers follow-up: range 4 weeks to 12 weeks | 140 per 1,000 | **25 per 1,000** (8 to 84) | **RR 0.18** (0.06 to 0.60) | 222 (5 RCTs) | ⨁⨁⨁◯ Moderate[d,h,i,j] | Likely reduced the probability of worsening of healing |
| Incidence of complete healing - not measured | Not estimable [k] | | | - | - | |
| Wound-related pain follow-up: range 7 days to 16 weeks | Not estimable [l] | | | 57 (3 RCTs) | ⨁◯◯◯ Very low[j,l,m] | It is very uncertain whether there were differences in wound-related pain |
| Adverse reactions follow-up: range 20 days to 12 weeks | Most common adverse reaction was red, raised, itchy skin beneath large dispersive electrode. One patient had a persistent (>24h) red area or burn under active electrode | | | 374 (8 RCTs) | ⨁⨁◯◯ Low[d,m,n] | It is uncertain whether all possible adverse reactions were identified |

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

**CI:** confidence interval; **MD:** mean difference; **RR:** risk ratio

**GRADE Working Group grades of evidence**
**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.
**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

**Explanations**

a. No serious risk of bias despite including two studies with some concerns of bias due to loss to follow-up and failure to adhere to the intention-to-treat principle; the number of patients lost to follow-up in the treatment groups were either equivalent or higher than the control groups. Two studies were at high risk of bias arising from randomization process and due to deviations from intended interventions; excluding both studies from meta-analysis did not significantly affect the results; RR 2.02; 95% CI: [1.35, 3.01], P=0.0006; $I^2$=0%, P=0.71.
b. Optimal information size was calculated, assuming α of 0.01, and β of 0.1, and was found to be (n=97); confidence intervals do not include the possibility of no effect. Control risks were calculated from studies included in the meta-analysis.
c. Publication bias strongly suspected due to small studies, and positive tests for funnel plot asymmetry; Egger's test, Begg's test, and non-parametric trim-and-fill analysis of publication bias.
d. The number of participants is slightly inflated by (n=8); one study included participants with more than one ulcer.
e. Serious risk of bias due to selective outcome reporting and deviations from intended interventions in three studies; excluding these studies

improved precision and reduced the estimate; MD 30.15; 95% CI: [22.29, 38.02], P=<0.00001; $I^2$=0%, P=0.79.
f. Optimal information size was calculated, assuming α of 0.01, and β of 0.1, and was found to be (n=39); common standard deviation (sigma=36.865); confidence intervals do not include the possibility of no effect. Control risk was calculated from studies included in the meta-analysis (mean).
g. Serious inconsistency; considerable overall heterogeneity $I^2$=76%, P<0.00001, points estimates and confidence intervals vary considerably; heterogeneity was explored in subgroup analyses by intervention protocol and duration; both analyses did not explain heterogeneity; although excluding a group with intervention duration of 45 minutes reduces value to $I^2$=18%, P=0.28, it does not explain heterogeneity as other studies showed inconsistent results.
h. No serious risk of bias despite including two studies with some concerns of bias due to loss to follow-up and failure to adhere to the intention-to-treat principle; the number of patients lost to follow-up in the treatment groups were either equivalent or higher than the control groups. One study was at high risk of bias arising from randomization process and due to deviations from intended interventions; excluding this study from meta-analysis did not significantly affect the results; RR 0.22; 95% CI: [0.06, 0.79], P=0.02; $I^2$=0%, P=0.96.
i. Optimal information size was calculated, assuming α of 0.01, and β of 0.1, and was found to be (n=116); confidence intervals do not include the possibility of no effect.
j. Publication bias strongly suspected due to small studies.
k. Time to complete healing was inappropriately measured; study reported mean time to complete healing instead of hazard ratio.
l. Conflicting evidence based on improperly reported results; statistical between-group comparisons were only performed in one study, which reported no significant differences between groups in wound-related pain.
m. Serious risk of bias due to selective outcome reporting and loss to follow-up and failure to adhere to the intention to treat principle.
n. Publication bias strongly suspected due to small studies, and selective availability of data from published studies; reports of other adverse reactions were identified in other studies.

# Supporting information

## 2. Methods

### 2.2 Information sources

#### 2.2.1 Search strategies

**United States National Library of Medicine (NLM) and National Institutes of Health (NIH) PubMed:**

Diabetic Ulcer

Search: **((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) AND (Diabetic Ulcer)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("diabete"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes"[All Fields] AND "insipidus"[All Fields]) OR "diabetes insipidus"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabets"[All Fields]) AND ("ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR

"ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer s"[All Fields] OR "ulcerous"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]

**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Diabetic:** "diabete"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes"[All Fields] AND "insipidus"[All Fields]) OR "diabetes insipidus"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabets"[All Fields]

**Ulcer:** "ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer's"[All Fields] OR "ulcerous"[All Fields]

**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Diabetic Wound

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Diabetic Wound)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("diabete"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes"[All Fields] AND "insipidus"[All Fields]) OR "diabetes insipidus"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabets"[All Fields]) AND ("injuries"[MeSH Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound s"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]))) NOT ("narration"[MeSH Terms] OR

"narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Diabetic:** "diabete"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes"[All Fields] AND "insipidus"[All Fields]) OR "diabetes insipidus"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabets"[All Fields]
**Wound:** "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound's"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]

**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Ischemic Ulcer

Search: **((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Ischemic Ulcer) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("ischaemics"[All Fields] OR "ischemia"[MeSH Terms] OR "ischemia"[All Fields] OR "ischaemic"[All Fields] OR "ischemic"[All Fields] OR "ischemical"[All Fields] OR "ischemically"[All Fields] OR "ischemics"[All Fields] OR "ischemized"[All Fields]) AND ("ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer s"[All Fields] OR "ulcerous"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Ischemic:** "ischaemics"[All Fields] OR "ischemia"[MeSH Terms] OR "ischemia"[All Fields] OR "ischaemic"[All Fields] OR "ischemic"[All Fields] OR "ischemical"[All Fields] OR "ischemically"[All Fields] OR "ischemics"[All Fields] OR "ischemized"[All Fields]
**Ulcer:** "ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer's"[All Fields] OR "ulcerous"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Ischemic Wound

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic**

**Pulsed Current OR Electrical Stimulation ) ) AND (Ischemic Wound)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("ischaemics"[All Fields] OR "ischemia"[MeSH Terms] OR "ischemia"[All Fields] OR "ischaemic"[All Fields] OR "ischemic"[All Fields] OR "ischemical"[All Fields] OR "ischemically"[All Fields] OR "ischemics"[All Fields] OR "ischemized"[All Fields]) AND ("injuries"[MeSH Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound s"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR

"pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Ischemic:** "ischaemics"[All Fields] OR "ischemia"[MeSH Terms] OR "ischemia"[All Fields] OR "ischaemic"[All Fields] OR "ischemic"[All Fields] OR "ischemical"[All Fields] OR "ischemically"[All Fields] OR "ischemics"[All Fields] OR "ischemized"[All Fields]
**Wound:** "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound's"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

**Arterial Ulcer**

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Arterial Ulcer)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("arterialization"[All Fields] OR "arterializations"[All Fields] OR "arterialize"[All Fields] OR "arterialized"[All Fields] OR "arterializing"[All Fields] OR "arterially"[All Fields] OR "arterials"[All Fields] OR "arterie"[All Fields] OR "arteries"[MeSH Terms] OR "arteries"[All Fields] OR "arterial"[All Fields] OR "arteris"[All Fields] OR "artery"[All Fields] OR "arterious"[All Fields] OR "artery s"[All Fields] OR "arterys"[All Fields]) AND ("ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer s"[All Fields] OR "ulcerous"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Arterial:** "arterialization"[All Fields] OR "arterializations"[All Fields] OR "arterialize"[All Fields] OR "arterialized"[All Fields] OR "arterializing"[All Fields] OR "arterially"[All Fields] OR "arterials"[All Fields] OR "arterie"[All Fields] OR "arteries"[MeSH Terms] OR "arteries"[All Fields] OR "arterial"[All Fields] OR "arteris"[All Fields] OR "artery"[All Fields] OR "arterious"[All Fields] OR "artery's"[All Fields] OR "arterys"[All Fields]
**Ulcer:** "ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer's"[All Fields] OR "ulcerous"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Pressure Ulcer

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Pressure Ulcer)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All

Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All

Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pressure Ulcer:** "pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Pressure Sore

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Pressure Sore)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All

Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR ("pressure"[All Fields] AND "sore"[All Fields]) OR "pressure sore"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]

**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pressure Sore:** "pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR ("pressure"[All Fields] AND "sore"[All Fields]) OR "pressure sore"[All Fields]

**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]

**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Pressure Wound

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Pressure Wound)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("pressure"[MeSH Terms] OR "pressure"[All Fields] OR "pressures"[All Fields] OR "pressure s"[All Fields] OR "pressurisation"[All Fields] OR "pressurised"[All Fields] OR "pressuriser"[All Fields] OR "pressurization"[All Fields] OR "pressurizations"[All Fields] OR "pressurize"[All Fields] OR "pressurized"[All Fields] OR "pressurizer"[All Fields] OR "pressurizes"[All Fields] OR "pressurizing"[All Fields]) AND ("injuries"[MeSH Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound s"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pressure:** "pressure"[MeSH Terms] OR "pressure"[All Fields] OR "pressures"[All Fields] OR "pressure's"[All Fields] OR "pressurisation"[All Fields] OR "pressurised"[All Fields] OR "pressuriser"[All Fields] OR "pressurization"[All Fields] OR "pressurizations"[All Fields] OR "pressurize"[All Fields] OR "pressurized"[All Fields] OR "pressurizer"[All Fields] OR "pressurizes"[All Fields] OR "pressurizing"[All Fields]
**Wound:** "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound's"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Decubitus Ulcer

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Decubitus Ulcer)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR ("decubitus"[All Fields] AND "ulcer"[All Fields]) OR "decubitus ulcer"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Decubitus Ulcer:** "pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR ("decubitus"[All Fields] AND "ulcer"[All Fields]) OR "decubitus ulcer"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Bedsore

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Bedsore)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR

"pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR "bedsore"[All Fields] OR "bedsores"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]

**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Bedsore:** "pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR "bedsore"[All Fields] OR "bedsores"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Skin Wounds

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Skin Wounds)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("skin"[MeSH Terms] OR "skin"[All Fields]) AND ("injuries"[MeSH Subheading] OR

"injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound s"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Skin:** "skin"[MeSH Terms] OR "skin"[All Fields]
**Wounds:** "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound's"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]

**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Skin Ulcers

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Skin Ulcers)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("skin ulcer"[MeSH Terms] OR ("skin"[All Fields] AND "ulcer"[All Fields]) OR "skin ulcer"[All Fields] OR ("skin"[All Fields] AND "ulcers"[All Fields]) OR "skin ulcers"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Skin Ulcers:** "skin ulcer"[MeSH Terms] OR ("skin"[All Fields] AND "ulcer"[All Fields]) OR "skin ulcer"[All Fields] OR ("skin"[All Fields] AND "ulcers"[All Fields]) OR "skin ulcers"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Skin Injuries

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Skin Injuries)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All

Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("skin"[MeSH Terms] OR "skin"[All Fields]) AND ("injurie"[All Fields] OR "injuried"[All Fields] OR "injuries"[MeSH Subheading] OR "injuries"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "injurious"[All Fields] OR "injury s"[All Fields] OR "injuryed"[All Fields] OR "injurys"[All Fields] OR "injury"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Skin:** "skin"[MeSH Terms] OR "skin"[All Fields]
**Injuries:** "injurie"[All Fields] OR "injuried"[All Fields] OR "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "injurious"[All Fields] OR "injury's"[All Fields] OR "injuryed"[All Fields] OR "injurys"[All Fields] OR "injury"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Skin Lesions

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Skin Lesions)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR

"galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("skin"[MeSH Terms] OR "skin"[All Fields]) AND ("lesion"[All Fields] OR "lesion s"[All Fields] OR "lesional"[All Fields] OR "lesions"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]

**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Skin:** "skin"[MeSH Terms] OR "skin"[All Fields]
**Lesions:** "lesion"[All Fields] OR "lesion's"[All Fields] OR "lesional"[All Fields] OR "lesions"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Burns

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Burns)) NOT (narrative) NOT (animal)**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("burning"[All Fields] OR "burns"[MeSH Terms] OR "burns"[All Fields] OR "burned"[All Fields] OR "burnings"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR

"narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (english[Filter] OR portuguese[Filter])

**Translations**
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Burns:** "burning"[All Fields] OR "burns"[MeSH Terms] OR "burns"[All Fields] OR "burned"[All Fields] OR "burnings"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

**Scopus:**

TITLE-ABS-KEY ( high-voltage AND pulsed AND current ) OR TITLE-ABS-KEY ( high-voltage AND electrical AND stimulation ) OR TITLE-ABS-KEY ( high-voltage AND pulsed AND galvanic AND stimulation ) OR TITLE-ABS-KEY ( high-voltage

AND monophasic AND pulsed AND current ) OR TITLE-ABS-KEY ( electrical AND stimulation ) AND TITLE-ABS-KEY ( diabetic AND ulcer ) OR TITLE-ABS-KEY ( diabetic AND wound ) OR TITLE-ABS-KEY ( ischemic AND ulcer ) OR TITLE-ABS-KEY ( ischemic AND wound ) OR TITLE-ABS-KEY ( arterial AND ulcer ) OR TITLE-ABS-KEY ( pressure AND ulcer ) OR TITLE-ABS-KEY ( pressure AND sore ) OR TITLE-ABS-KEY ( pressure AND wound ) OR TITLE-ABS-KEY ( decubitus AND ulcer ) OR TITLE-ABS-KEY ( bedsore ) OR TITLE-ABS-KEY ( skin AND wounds ) OR TITLE-ABS-KEY ( skin AND ulcers ) OR TITLE-ABS-KEY ( skin AND injuries ) OR TITLE-ABS-KEY ( skin AND lesions ) OR TITLE-ABS-KEY ( burns ) AND NOT ( narrative ) AND NOT ( animal ) AND ( LIMIT-TO ( DOCTYPE , "ar" ) OR LIMIT-TO ( DOCTYPE , "re" ) ) AND ( LIMIT-TO ( LANGUAGE , "English" )

**Physiotherapy Evidence Database PEDro:**

Abstract & Title: “High-Voltage Pulsed Current” AND Problem: “skin lesion, wound, burn”.

Abstract & Title: High-Voltage Electrical Stimulation AND Problem: “skin lesion, wound, burn”.

Abstract & Title: High-Voltage Pulsed Galvanic Stimulation AND Problem: “skin lesion, wound, burn”.

Abstract & Title: High-Voltage Monophasic Pulsed Current AND Problem: “skin lesion, wound, burn”.

Abstract & Title: Electrical Stimulation AND Problem: “skin lesion, wound, burn”.

**Google Scholar:**

High-Voltage Pulsed Current AND Diabetic Ulcer, Diabetic Wound, Ischemic Ulcer, Ischemic Wound, Arterial Ulcer, Pressure Ulcer, Pressure Sore, Pressure Wound, Decubitus Ulcer, Bedsore, Skin Ulcers, Skin Injuries, Skin Wounds, Wound Healing, Burns.

High-Voltage Electrical Stimulation AND Diabetic Ulcer, Diabetic Wound, Ischemic Ulcer, Ischemic Wound, Arterial Ulcer, Pressure Ulcer, Pressure Sore, Pressure Wound, Decubitus Ulcer, Bedsore, Skin Ulcers, Skin Injuries, Skin Wounds, Wound Healing, Burns.

High-Voltage Pulsed Galvanic Stimulation AND Diabetic Ulcer, Diabetic Wound, Ischemic Ulcer, Ischemic Wound, Arterial Ulcer, Pressure Ulcer, Pressure Sore, Pressure Wound, Decubitus Ulcer, Bedsore, Skin Wounds, Skin Ulcers, Skin Injuries, Skin Lesions, Wound Healing, Burns.

High-Voltage Monophasic Pulsed Current AND Diabetic Ulcer, Diabetic Wound, Ischemic Ulcer, Ischemic Wound, Arterial Ulcer, Pressure Ulcer, Pressure Sore, Pressure Wound, Decubitus Ulcer, Bedsore, Skin Wounds, Skin Ulcers, Skin Injuries, Skin Lesions, Wound Healing, Burns.

Electrical Stimulation AND Diabetic Ulcer, Diabetic Wound, Ischemic Ulcer, Ischemic Wound, Arterial Ulcer, Pressure Ulcer, Pressure Sore, Pressure Wound, Decubitus Ulcer, Bedsore, Skin Wounds, Skin Ulcers, Skin Injuries, Skin Lesions, Wound Healing, Burns.

**Search strategy; repeated PubMed search:**

**United States National Library of Medicine (NLM) and National Institutes of Health (NIH) PubMed:**

Diabetic Ulcer

Search: **((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) AND (Diabetic Ulcer)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("diabete"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes"[All Fields] AND "insipidus"[All Fields]) OR "diabetes insipidus"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabets"[All Fields]) AND ("ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer s"[All Fields] OR "ulcerous"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Diabetic:** "diabete"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes"[All Fields] AND "insipidus"[All Fields]) OR "diabetes insipidus"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabets"[All Fields]
**Ulcer:** "ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer's"[All Fields] OR "ulcerous"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Diabetic Wound

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Diabetic Wound)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("diabete"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes"[All Fields] AND "insipidus"[All Fields]) OR "diabetes insipidus"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabets"[All Fields]) AND ("injuries"[MeSH Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound s"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields])))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Diabetic:** "diabete"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes"[All Fields] AND "insipidus"[All Fields]) OR "diabetes insipidus"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabets"[All Fields]
**Wound:** "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound's"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Ischemic Ulcer

Search: **((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Ischemic Ulcer) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart

rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("ischaemics"[All Fields] OR "ischemia"[MeSH Terms] OR "ischemia"[All Fields] OR "ischaemic"[All Fields] OR "ischemic"[All Fields] OR "ischemical"[All Fields] OR "ischemically"[All Fields] OR "ischemics"[All Fields] OR "ischemized"[All Fields]) AND ("ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer s"[All Fields] OR "ulcerous"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Ischemic:** "ischaemics"[All Fields] OR "ischemia"[MeSH Terms] OR "ischemia"[All Fields] OR "ischaemic"[All Fields] OR "ischemic"[All Fields] OR "ischemical"[All Fields] OR "ischemically"[All Fields] OR "ischemics"[All Fields] OR "ischemized"[All Fields]
**Ulcer:** "ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer's"[All Fields] OR "ulcerous"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Ischemic Wound

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Ischemic Wound)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR

"galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("ischaemics"[All Fields] OR "ischemia"[MeSH Terms] OR "ischemia"[All Fields] OR "ischaemic"[All Fields] OR "ischemic"[All Fields] OR "ischemical"[All Fields] OR "ischemically"[All Fields] OR "ischemics"[All Fields] OR "ischemized"[All Fields]) AND ("injuries"[MeSH Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound s"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]

**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Ischemic:** "ischaemics"[All Fields] OR "ischemia"[MeSH Terms] OR "ischemia"[All Fields] OR "ischaemic"[All Fields] OR "ischemic"[All Fields] OR "ischemical"[All Fields] OR "ischemically"[All Fields] OR "ischemics"[All Fields] OR "ischemized"[All Fields]
**Wound:** "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound's"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

**Arterial Ulcer**

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Arterial Ulcer)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields]

AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("arterialization"[All Fields] OR "arterializations"[All Fields] OR "arterialize"[All Fields] OR "arterialized"[All Fields] OR "arterializing"[All Fields] OR "arterially"[All Fields] OR "arterials"[All Fields] OR "arterie"[All Fields] OR "arteries"[MeSH Terms] OR "arteries"[All Fields] OR "arterial"[All Fields] OR "arteris"[All Fields] OR "artery"[All Fields] OR "arterious"[All Fields] OR "artery s"[All Fields] OR "arterys"[All Fields]) AND ("ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer s"[All Fields] OR "ulcerous"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Arterial:** "arterialization"[All Fields] OR "arterializations"[All Fields] OR "arterialize"[All Fields] OR "arterialized"[All Fields] OR "arterializing"[All Fields] OR "arterially"[All Fields] OR "arterials"[All Fields] OR "arterie"[All Fields] OR "arteries"[MeSH Terms]

OR "arteries"[All Fields] OR "arterial"[All Fields] OR "arteris"[All Fields] OR "artery"[All Fields] OR "arterious"[All Fields] OR "artery's"[All Fields] OR "arterys"[All Fields]
**Ulcer:** "ulcer"[MeSH Terms] OR "ulcer"[All Fields] OR "ulcerate"[All Fields] OR "ulcerated"[All Fields] OR "ulcerates"[All Fields] OR "ulcerating"[All Fields] OR "ulceration"[All Fields] OR "ulcerations"[All Fields] OR "ulcerative"[All Fields] OR "ulcers"[All Fields] OR "ulcer's"[All Fields] OR "ulcerous"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Pressure Ulcer

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Pressure Ulcer)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pressure Ulcer:** "pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Pressure Sore

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Pressure Sore)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields]

AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR ("pressure"[All Fields] AND "sore"[All Fields]) OR "pressure sore"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]

**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pressure Sore:** "pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR ("pressure"[All Fields] AND "sore"[All Fields]) OR "pressure sore"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Pressure Wound

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Pressure Wound)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("pressure"[MeSH Terms] OR "pressure"[All Fields] OR "pressures"[All Fields] OR

"pressure s"[All Fields] OR "pressurisation"[All Fields] OR "pressurised"[All Fields] OR "pressuriser"[All Fields] OR "pressurization"[All Fields] OR "pressurizations"[All Fields] OR "pressurize"[All Fields] OR "pressurized"[All Fields] OR "pressurizer"[All Fields] OR "pressurizes"[All Fields] OR "pressurizing"[All Fields]) AND ("injuries"[MeSH Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound s"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pressure:** "pressure"[MeSH Terms] OR "pressure"[All Fields] OR "pressures"[All Fields] OR "pressure's"[All Fields] OR "pressurisation"[All Fields] OR "pressurised"[All Fields] OR "pressuriser"[All Fields] OR "pressurization"[All Fields] OR "pressurizations"[All Fields] OR "pressurize"[All Fields] OR "pressurized"[All Fields] OR "pressurizer"[All Fields] OR "pressurizes"[All Fields] OR "pressurizing"[All Fields]

**Wound:** "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound's"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Decubitus Ulcer

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Decubitus Ulcer)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR ("decubitus"[All Fields] AND "ulcer"[All Fields]) OR "decubitus ulcer"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Decubitus Ulcer:** "pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR ("decubitus"[All Fields] AND "ulcer"[All Fields]) OR "decubitus ulcer"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Bedsore

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Bedsore)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields]

AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR "bedsore"[All Fields] OR "bedsores"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]

**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Bedsore:** "pressure ulcer"[MeSH Terms] OR ("pressure"[All Fields] AND "ulcer"[All Fields]) OR "pressure ulcer"[All Fields] OR "bedsore"[All Fields] OR "bedsores"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Skin Wounds

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Skin Wounds)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("skin"[MeSH Terms] OR "skin"[All Fields]) AND ("injuries"[MeSH Subheading] OR

"injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound s"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Skin:** "skin"[MeSH Terms] OR "skin"[All Fields]
**Wounds:** "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "wound's"[All Fields] OR "wounded"[All Fields] OR "wounding"[All Fields] OR "woundings"[All Fields] OR "wound"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Skin Ulcers

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Skin Ulcers)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("skin ulcer"[MeSH Terms] OR ("skin"[All Fields] AND "ulcer"[All Fields]) OR "skin ulcer"[All Fields] OR ("skin"[All Fields] AND "ulcers"[All Fields]) OR "skin ulcers"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR

"pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Skin Ulcers:** "skin ulcer"[MeSH Terms] OR ("skin"[All Fields] AND "ulcer"[All Fields]) OR "skin ulcer"[All Fields] OR ("skin"[All Fields] AND "ulcers"[All Fields]) OR "skin ulcers"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Skin Injuries

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Skin Injuries)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR

"stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("skin"[MeSH Terms] OR "skin"[All Fields]) AND ("injurie"[All Fields] OR "injuried"[All Fields] OR "injuries"[MeSH Subheading] OR "injuries"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "injurious"[All Fields] OR "injury s"[All Fields] OR "injuryed"[All Fields] OR "injurys"[All Fields] OR "injury"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]

**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Skin:** "skin"[MeSH Terms] OR "skin"[All Fields]
**Injuries:** "injurie"[All Fields] OR "injuried"[All Fields] OR "injuries"[Subheading] OR "injuries"[All Fields] OR "wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "injurious"[All Fields] OR "injury's"[All Fields] OR "injuryed"[All Fields] OR "injurys"[All Fields] OR "injury"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Skin Lesions

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Skin Lesions)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND (("skin"[MeSH Terms] OR "skin"[All Fields]) AND ("lesion"[All Fields] OR "lesion s"[All Fields] OR "lesional"[All Fields] OR "lesions"[All Fields]))) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]
**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Skin:** "skin"[MeSH Terms] OR "skin"[All Fields]
**Lesions:** "lesion"[All Fields] OR "lesion's"[All Fields] OR "lesional"[All Fields] OR "lesions"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

Burns

Search: **(((High-Voltage Pulsed Current OR High-Voltage Electrical Stimulation OR High-Voltage Pulsed Galvanic Stimulation OR High-Voltage Monophasic Pulsed Current OR Electrical Stimulation ) ) AND (Burns)) NOT (narrative) NOT (animal)** Filters: **from 2021/7/8 - 2022/4/22**

(((((("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart

rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("High-Voltage"[All Fields] AND ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) OR ("High-Voltage"[All Fields] AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]) AND ("stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator s"[All Fields] OR "stimulators"[All Fields])) OR ("High-Voltage"[All Fields] AND ("monophasic"[All Fields] OR "monophasics"[All Fields]) AND ("pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse s"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]) AND ("current"[All Fields] OR "current s"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields])) OR ("electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields])) AND ("burning"[All Fields] OR "burns"[MeSH Terms] OR "burns"[All Fields] OR "burned"[All Fields] OR "burnings"[All Fields])) NOT ("narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative s"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields])) NOT ("animals"[MeSH Terms:noexp] OR "animal"[All Fields])) AND (2021/7/8:2022/4/22[pdat])

**Translations**

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]

**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]

**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]

**Galvanic:** "galvanic"[All Fields] OR "galvanically"[All Fields] OR "galvanism"[All Fields] OR "galvanization"[All Fields] OR "galvanized"[All Fields] OR "galvanizing"[All Fields]

**Stimulation:** "stimulate"[All Fields] OR "stimulated"[All Fields] OR "stimulates"[All Fields] OR "stimulating"[All Fields] OR "stimulation"[All Fields] OR "stimulations"[All

Fields] OR "stimulative"[All Fields] OR "stimulator"[All Fields] OR "stimulator's"[All Fields] OR "stimulators"[All Fields]
**Monophasic:** "monophasic"[All Fields] OR "monophasics"[All Fields]
**Pulsed:** "pulse"[MeSH Terms] OR "pulse"[All Fields] OR "heart rate"[MeSH Terms] OR ("heart"[All Fields] AND "rate"[All Fields]) OR "heart rate"[All Fields] OR "pulses"[All Fields] OR "pulse's"[All Fields] OR "pulsed"[All Fields] OR "pulsing"[All Fields]
**Current:** "current"[All Fields] OR "current's"[All Fields] OR "currently"[All Fields] OR "currents"[All Fields]
**Electrical Stimulation:** "electric stimulation"[MeSH Terms] OR ("electric"[All Fields] AND "stimulation"[All Fields]) OR "electric stimulation"[All Fields] OR ("electrical"[All Fields] AND "stimulation"[All Fields]) OR "electrical stimulation"[All Fields]
**Burns:** "burning"[All Fields] OR "burns"[MeSH Terms] OR "burns"[All Fields] OR "burned"[All Fields] OR "burnings"[All Fields]
**narrative:** "narration"[MeSH Terms] OR "narration"[All Fields] OR "narrative"[All Fields] OR "narratives"[All Fields] OR "narrative's"[All Fields] OR "narratively"[All Fields] OR "narrativity"[All Fields]
**animal:** "animals"[MeSH Terms:noexp] OR animal[All Fields]

## 3. Results

### 3.1 Results of the systematic review

#### 3.1.1 List of excluded studies

**Animal studies**


1. Wang, Xiao-Feng, Meng-Lu Li, Qing-Qing Fang, Wan-Yi Zhao, Dong Lou, Yan-Yan Hu, Jun Chen, Xiao-Zhi Wang, and Wei-Qiang Tan. "Flexible electrical stimulation device with Chitosan-Vaseline® dressing accelerates wound healing in diabetes." *Bioactive materials* 6, no. 1 (2021): 230-243.

2. Kim, Tae Hoon, Hwi-young Cho, and Suk Min Lee. "High-voltage pulsed current stimulation enhances wound healing in diabetic rats by restoring the expression of collagen, α-smooth muscle actin, and TGF-β1." *The Tohoku journal of experimental medicine* 234, no. 1 (2014): 1-6.

3. Lee, Jae-Hyoung, Chan-Eui Park, and Rae-Joon Park. "Electrical Stimulation Induces the Collagen Deposition and TGF- β1 mRNA Expression in Skin Wound of Rat." *The Journal of Korean Physical Therapy* 22, no. 3 (2010): 87-92.

4. Herdiawan, Rina Puspasari, Andri Rezano, Irma Ruslina, and Pritha Pitaloka. "Comparison of wound healing of skin incision on albino rat (Rattus norwegicus) by treatment of electrical stimulations." *Journal of the Medical Sciences (Berkala ilmu Kedokteran)* 51, no. 2 (2019): 98-105.

5. Souza, Ana Karina, Tamires Rodrigues Souza, Lais Mara Siqueira das Neves, Gabriela de Paula Marcondes Ferreira Leite, Sérgio Britto Garcia, Rinaldo Roberto de Jesus Guirro, Rafael Inácio Barbosa, and Elaine Caldeira de Oliveira Guirro. "Effect of High Voltage Pulsed Current on the integration of total skin grafts in rats submitted to nicotine action." *Journal of tissue viability* 28, no. 3 (2019): 161-166.

6. Lee, Jae-hyoung, In-young Song, and Kim Jong-Gyu. "Acceleration of wound healing and collagen deposition in rat skin by high voltage pulsed current stimulation." *Journal of Korean Physical Therapy* 15, no. 4 (2003): 1-12.

7. Lee, Jae-Hyoung, Jong-Sook Lee, and Eyn-Young Kil. "Keratinocyte proliferation in aged rat skin by high voltage pulsed current stimulation." *Biomedical Science Letters* 10, no. 4 (2004): 361-366.

8. Lee, Jae-Hyoung, Jong-Sook Lee, Myung-A. Jeong, Seung-Joo Jekal, Eyn-Young Kil, Seung-Teack Park, and Chan-Eui Park. "Electrical Stimulation Promotes Healing Accompanied by NOR in Keratinocytes and IGF-1 mRNA Expression in Skin Wound of Rat." *Biomedical Science Letters* 13, no. 1 (2007): 25-32.

9. Cruz, Norma I., Fernando E. Bayrón, and Albert J. Suárez. "Accelerated healing of full-thickness burns by the use of high-voltage pulsed galvanic stimulation in the pig." *Annals of plastic surgery* 23, no. 1 (1989): 49-55.

10. Choi, Eun-Young, Jae-Hyoung Lee, and Seung-Joo JeKal. "Effect of High Voltage Pulsed Current Stimulation on Surgical Wound of Rat." *Journal of Korean Physical Therapy Science* 2, no. 2 (1995): 495-508.

11. Jeon, Jae-Keun, Seung-Kyu Park, and Joon-Hee Lee. "Effects of high voltage pulsed current stimulation with a visible contraction intensity on expression of TGF-β1 and synthesis of type I collagen in wound-induced white rats." *Journal of physical therapy science* 27, no. 5 (2015): 1485-1490.

12. Brown, Marybeth, Mary Kate McDonnell, and David N. Menton. "Polarity effects on wound healing using electric stimulation in rabbits." *Archives of Physical Medicine and Rehabilitation* 70, no. 8 (1989): 624-627.

13. Brown, Marybeth, Prem P. Gogia, David R. Sinacore, and David N. Menton. "High-voltage galvanic stimulation on wound healing in guinea pigs: longer-term effects." *Archives of physical medicine and rehabilitation* 76, no. 12 (1995): 1134-1137.

14. das Neves, Lais Mara Siqueira, Elaine Caldeira de Oliveira Guirro, Fernanda Luiza de Almeida Albuquerque, and Alexandre Marcio Marcolino. "Effects of high-voltage electrical stimulation in improving the viability of musculocutaneous flaps in rats." *Annals of plastic surgery* 77, no. 4 (2016): e50-e54.

15. Ahmed, Emad T., and Mohamed M. Abdel-khalek Khalaf. "Comparison of the Effect of High Voltage Pulsed Galvanic Stimulation versus Ultrasonic on the Acceleration of Burn Wound Healing." *Bull. Fac. Ph. Th. Cairo Univ*: Vol,11 no. 2 (2006): 135-144.

16. Radwan, Ghada, and Khodor Haidar Hassan. "EFFECT OF PULSED GALVANIC ELECTRO STIMULATION AND ULTRASUONBD ON BURN HEALING. A RANDOMIZED CLINICAL TRIALS." *European Scientific Journal* (2015) /SPECIAL/ edition, Vol. 2: 167-175.

**Reviews**

1. Girgis, Beshoy, and José Alberto Duarte. "High Voltage Monophasic Pulsed Current (HVMPC) for stage II-IV pressure ulcer healing. A systematic review and meta-analysis." *Journal of tissue viability* 27.4 (2018): 274-284.
2. Polak, Anna, Andrzej Franek, and Jakub Taradaj. "High-voltage pulsed current electrical stimulation in wound treatment." *Advances in wound care* 3.2 (2014): 104-117.

3. Khouri, Charles, Sylvain Kotzki, Matthieu Roustit, Sophie Blaise, Francois Gueyffier, and Jean-Luc Cracowski. "Hierarchical evaluation of electrical stimulation protocols for chronic wound healing: An effect size meta-analysis." *Wound Repair and Regeneration* 25, no. 5 (2017): 883-891.

**Non-peer reviewed**

1. Van Gils, Carl. “It’s electrifying” *OrthoKineticReview* (2001): 18-21.

2. Matos, Lílian Ramine Ramos de Souza, Naiara Kássia Macêdo da Silva Bezerra, Pollianna Tavares de Barros, Karoliny Teixeira Santos, and Márcia Bento Moreira. “ELETROESTIMULAÇÃO DE ALTA VOLTAGEM NO REPARO TECIDUAL DE LESÃO POR PRESSÃO: UM RELATO DE EXPERIÊNCIA”. *Conbracis*.

3. Polak, Anna, Agnieszka Nawrat-Szołtysik, Tomasz Ickowicz, Ewa Kucio, Kamila Kasprzak, Aleksandra Chlebek, Beata Etfer, Małgorzata Pniowska, Jolanta Rajca, Sandra Łagodzic, Wojciech Kania. “Pressure injury treatment with anodal and cathodal electrical stimulation in persons with central nervous system injuries. A prospective, randomized, clinical study. Preliminary report” *Medical Rehabilitation (Med Rehabil)* 2017; 21 (3): 23-34. DOI: 10.5604/01.3001.0011.6824.

**Duplicate data**

1. Polak, Anna, Luther C. Kloth, Malgorzata Paczula, Agnieszka Nawrat-Szoltysik, Ewa Kucio, Ahmed Manasar, Edward Blaszczak et al. "Pressure Injuries Treated With Anodal and Cathodal High-voltage Electrical Stimulation: the Effect on Blood Serum Concentration of Cytokines and Growth Factors in Patients With Neurological Injuries. A Randomized Clinical Study." *Wound Management & Prevention* 65, no. 11 (2019): 19-32.

**Other ES currents**

1. Adunsky, Abraham, and Avi Ohry. "Decubitus direct current treatment (DDCT) of pressure ulcers: results of a randomized double-blinded placebo controlled study." *Archives of gerontology and geriatrics* 41, no. 3 (2005): 261-269.

2. Asadi, Mohammad Reza, Giti Torkaman, Mehdi Hedayati, Mohammad Reza Mohajeri-Tehrani, Mousa Ahmadi, and Roghieh Fathi Gohardani. "Angiogenic effects of low-intensity cathodal direct current on ischemic diabetic foot ulcers: A randomized controlled trial." *diabetes research and clinical practice* 127 (2017): 147-155.

3. Bosanquet, David C., Nicky Ivins, Nia Jones, and Keith G. Harding. "Microcirculatory Flux and Pulsatility in Arterial Leg Ulcers is Increased by

Intermittent Neuromuscular Electrostimulation of the Common Peroneal Nerve." *Annals of Vascular Surgery* 71 (2021): 308-314.

4. Liani, Mario, Ernesto Trabassi, Claudio Cusaro, Elisabetta Zoppis, Elisabetta Maduli, Roberto Pezzato, Paola Piccoli et al. "Effects of a pulsatile electrostatic field on ischemic injury to the diabetic foot: evaluation of refractory ulcers." *Primary care diabetes* 8, no. 3 (2014): 244-249.

5. Sosa, Ivan J., Onix Reyes, and Damien P. Kuffler. "Elimination of a pressure ulcer with electrical stimulation-a case study." *Puerto Rico health sciences journal* 27, no. 2 (2008).

6. Fakhri, Omar, and Mudher A. Amin. "The effect of low-voltage electric therapy on the healing of resistant skin burns." (1987): 15-18.

7. WOLCOTT, LESTER, PAUL WHEELER, HENRY HARDWICKE, and BLAIR ROWLEY. "Accelerated healing of skin ulcers by electrotherapy: preliminary clinical results." *Southern medical journal* 62, no. 7 (1969): 795-801.

8. Petrofsky, Jerrold, Ernie Schwab, Taiken Lo, Maria Cúneo, James George, Jina Kim, and Abdul Al-Malty. "Effects of electrical stimulation on skin blood flow in controls and in and around stage III and IV wounds in hairy and non hairy skin." *Medical science monitor* 11, no. 7 (2005): CR309-CR316.

9. Lawson, Daryl, and Jerrold S. Petrofsky. "A randomized control study on the effect of biphasic electrical stimulation in a warm room on skin blood flow and healing rates in chronic wounds of patients with and without diabetes." *Medical science monitor* 13, no. 6 (2007): CR258-CR263.

10. Gentzkow, Gary D., Sheldon V. Pollack, Luther C. Kloth, and Harrison A. Stubbs. "Improved healing of pressure ulcers using Dermapulse, a new electrical stimulation device." *Wounds* (1991).

11. Castana, Ourania, Aekaterini Dimitrouli, Theodoros Argyrakos, Emilia Theodorakopoulou, Nektarios Stampolidis, Emmanouil Papadopoulos, Athanasios Pallantzas, Ioannis Stasinopoulos, and Konstantinos Poulas. "Wireless electrical stimulation: an innovative powerful tool for the treatment of a complicated chronic ulcer." *The international journal of lower extremity wounds* 12, no. 1 (2013): 18-21.

12. Cosmo, Henry Svensson, Siv Bornmyr, Sven-Olof Wikström, Peter P. "Effects of transcutaneous nerve stimulation on the microcirculation in chronic leg ulcers." *Scandinavian journal of plastic and reconstructive surgery and hand surgery* 34, no. 1 (2000): 61-64.

13. Wikström, Sven Olof, Paul Svedman, Henry Svensson, and A. Syed Tanweer. "Effect of transcutaneous nerve stimulation on microcirculation in intact skin and

blister wounds in healthy volunteers." *Scandinavian journal of plastic and reconstructive surgery and hand surgery* 33, no. 2 (1999): 195-201.

14. Adegoke, B. O. A., and K. A. Badmos. "Acceleration of pressure ulcer healing in spinal cord injured patients using interrupted direct current." *African journal of medicine and medical sciences* 30, no. 3 (2001): 195-197.

15. Malin, Edward W., Chaya M. Galin, Kimberley F. Lairet, Todd F. Huzar, James F. Williams, Evan M. Renz, Steven E. Wolf, and Leopoldo C. Cancio. "Silver-coated nylon dressing plus active DC microcurrent for healing of autogenous skin donor sites." *Annals of plastic surgery* 71, no. 5 (2013): 481-484.

16. Suh, HyeJin, Jerrold Petrofsky, Anne Fish, Vivian Hernandez, Enrique Mendoza, Kelly Collins, Tienning Yang, Armia Abdul, Jennifer Batt, and Daryl Lawson. "A new electrode design to improve outcomes in the treatment of chronic non-healing wounds in diabetes." *Diabetes technology & therapeutics* 11, no. 5 (2009): 315-322.

17. Lundeberg, Thomas CM, Sven V. Eriksson, and Mats Malm. "Electrical nerve stimulation improves healing of diabetic ulcers." *Annals of Plastic Surgery* 29, no. 4 (1992): 328-331.

18. Hampton, Sylvie, and Lynn King. "Healing an intractable wound using bio-electrical stimulation therapy." *British journal of nursing* 14, no. Sup3 (2005): S30-S32.

19. Hampton, Sylvie, and Fiona Collins. "Treating a pressure ulcer with bio-electric stimulation therapy." *British journal of nursing* 15, no. Sup1 (2006): S14-S18.

20. Jones, Nia J., Nicola Ivins, Vicky Ebdon, Susan Hagelstein, and Keith G. Harding. "Neuromuscular electrostimulation on lower limb wounds." *British Journal of Nursing* 27, no. 20 (2018): S16-S21.

21. García-Pérez, Sara, Ma Carmen García-Ríos, José Manuel Pérez-Mármol, Rosa María Tapia-Haro, Manuel Albornoz-Cabello, Marie Carmen Valenza, and María Encarnación Aguilar-Ferrándiz. "Effectiveness of transcutaneous electrical nerve stimulation energy in older adults: a pilot clinical trial." *Advances in skin & wound care* 31, no. 10 (2018): 462-469.

22. Feedar, Jeffrey A., Luther C. Kloth, and Gary D. Gentzkow. "Chronic dermal ulcer healing enhanced with monophasic pulsed electrical stimulation." *Physical Therapy* 71, no. 9 (1991): 639-649.

23. Suh, HyeJin, Jerrold S. Petrofsky, Takkin Lo, Daryl Lawson, Tina Yu, Tiffany M. Pfeifer, and Theresa Morawski. "The combined effect of a three-channel electrode delivery system with local heat on the healing of chronic wounds." *Diabetes technology & therapeutics* 11, no. 10 (2009): 681-688.

24. Baker, Lucinda L., Richard Chambers, Sharon K. DeMuth, and Fernando Villar. "Effects of electrical stimulation on wound healing in patients with diabetic ulcers." *Diabetes care* 20, no. 3 (1997): 405-412.

25. Lundeberg, T., J. Kjartansson, and U. Samuelsson. "Effect of electrical nerve stimulation on healing of ischaemic skin flaps." *The lancet* 332, no. 8613 (1988): 712-714.

26. Lessiani, G., V. Galati, G. Franzone, and P. Iodice. "Efficacy of modulated microcurrent stimulation in pressure ulcers treatment: A monocentric, prospective, double-blind, randomized study." *J Nov Physiother* 4, no. 224 (2014): 2.

27. Ud-Din, Sara, Donna Perry, Pamela Giddings, James Colthurst, Karen Zaman, Symon Cotton, Sigrid Whiteside, Julie Morris, and Ardeshir Bayat. "Electrical stimulation increases blood flow and haemoglobin levels in acute cutaneous wounds without affecting wound closure time: evidenced by non-invasive assessment of temporal biopsy wounds in human volunteers." *Experimental dermatology* 21, no. 10 (2012): 758-764.

28. Huckfeldt, Roger, A. Bart Flick, Debbie Mikkelson, Cindy Lowe, and Phillip J. Finley. "Wound closure after split-thickness skin grafting is accelerated with the use of continuous direct anodal microcurrent applied to silver nylon wound contact dressings." *Journal of burn care & research* 28, no. 5 (2007): 703-707.

29. Jercinovic, Aleksandra, Renata Karba, Lojze Vodovnik, Aneta Stefanovska, Peter Kroselj, Rajko Turk, Ivan Dzidic, Helena Benko, and Rajmond Savrin. "Low frequency pulsed current and pressure ulcer healing." *IEEE transactions on rehabilitation engineering* 2, no. 4 (1994): 225-233.

30. Shahrokhi, Akram, Azam Ghorbani, and Atefeh Aminianfar. "Impact of interferential current on recovery of pressure ulcers grade 1 and 2." *Iranian journal of nursing and midwifery research* 19, no. 7 Suppl1 (2014): S91.

31. Leloup, Pauline, Pascal Toussaint, Jean-Paul Lembelembe, Philippe Célérier, and Hervé Maillard. "The analgesic effect of electrostimulation (WoundEL®) in the treatment of leg ulcers." *International wound journal* 12, no. 6 (2015): 706-709.

32. Petrofsky, Jerrold Scott, Daryl Lawson, Lee Berk, and Hyejin Suh. "Enhanced healing of diabetic foot ulcers using local heat and electrical stimulation for 30 min three times per week." *Journal of diabetes* 2, no. 1 (2010): 41-46.

33. Janković, Aleksandar, and Ivana Binić. "Frequency rhythmic electrical modulation system in the treatment of chronic painful leg ulcers." *Archives of dermatological research* 300, no. 7 (2008): 377.

34. O'connor, T., Z. Moore, D. Patton, P. Wilson, C. Gillen, M. Hughes, and A. Reilly. "Combined use of modulated ultrasound and electric current stimulation for

diabetic foot ulcers: a case series." *Journal of wound care* 26, no. 11 (2017): 632-640.

35. Nair, Harikrishna KR. "Microcurrent as an adjunct therapy to accelerate chronic wound healing and reduce patient pain." *Journal of wound care* 27, no. 5 (2018): 296-306.

36. Karba, R., H. Benko, R. Savrin, and L. Vodovnik. "Combination of occlusive dressings and electrical stimulation in pressure ulcer treatment." *Medical science research* 23, no. 10 (1995): 671-673.

37. Ibrahim, Zizi M., Instar S. Waked, and Olfat Ibrahim. "Negative pressure wound therapy versus microcurrent electrical stimulation in wound healing in burns." *Journal of wound care* 28, no. 4 (2019): 214-219.

38. Yarboro, Douglas D., and Robert Smith. "Transcutaenous electrical nerve stimulation to manage a lower extremity wound complicated by peripheral arterial disease: a case report."

39. Petrofsky, J., D. Lawson, M. Prowse, and H. J. Suh. "Effects of a 2-, 3-and 4-electrode stimulator design on current dispersion on the surface and into the limb during electrical stimulation in controls and patients with wounds." *Journal of medical engineering & technology* 32, no. 6 (2008): 485-497.

40. Mulder, Gary D. "Treatment of open-skin wounds with electric stimulation." *Archives of physical medicine and rehabilitation* 72, no. 6 (1991): 375-377.

41. Gault, Walter R., and Paul F. Gatens Jr. "Use of low intensity direct current in management of ischemic skin ulcers." *Physical Therapy* 56, no. 3 (1976): 265-269.

42. Ramadhinara, Adisaputra, and Konstantinos Poulas. "Use of wireless microcurrent stimulation for the treatment of diabetes-related wounds: 2 case reports." *Advances in Skin & Wound Care* 26, no. 1 (2013): 1-4.

43. Magnoni, Cristina, Elena Rossi, Chiara Fiorentini, Andrea Baggio, Barbara Ferrari, and G. Alberto. "Electrical stimulation as adjuvant treatment for chronic leg ulcers of different aetiology: an RCT." *Journal of wound care* 22, no. 10 (2013): 525-533.

44. Young, S., S. Hampton, and M. Tadej. "Study to evaluate the effect of low-intensity pulsed electrical currents on levels of oedema in chronic non-healing wounds." *Journal of wound care* 20, no. 8 (2011): 368-373.

45. Lawson, Daryl, and Jerrold Petrofsky. "The Effect of Monophasic Vs. Biphasic Current on Healing Rate and Blood Flow in People With Pressure and

Neuropathic Ulcers." *Journal of Acute Care Physical Therapy* 4, no. 1 (2013): 26-33.

46. Asbjornsen, G., B. Hernaes, and G. Molvaer. "THE EFFECT OF TRANSCUTANEOUS ELECTRICAL NERVE-STIMULATION ON PRESSURE SORES IN GERIATRIC-PATIENTS." *Journal of Clinical and Experimental Gerontology* 12, no. 4 (1990): 209-214.

47. Kaada, B. "Promoted healing of chronic ulceration by transcutaneous nerve stimulation (TNS)." *Vasa* 12, no. 3 (1983): 262-269.

48. Allen, J., and P. E. Houghton. "A case study for electrical stimulation on a stage III pressure ulcer." *Wound Care Canada* 2, no. 1 (2004): 34-36.

49. Asadi, M. R., G. Torkaman, M. R. Mohajeri-Tehrani, M. Hedayati, Gh Mehdizadeh, A. A. Manouchehri, A. Zarghami et al. "XML Effects of Electrical Stimulation on the Management of Ischemic Diabetic Foot Ulcers." (2015).

**Other ulcer types**

1. Blaszczak, E., A. Franek, J. Taradaj, and P. Dolibog. "Evaluation of healing process dynamics of the leg venous ulcers treated by means of selected physical methods." *Fizjoterapia* 15, no. 1 (2007): 3-16.

2. Franek, Andrzej, Anna Polak, and Marek Kucharzewski. "Modern application of high voltage stimulation for enhanced healing of venous crural ulceration." *Medical engineering & physics* 22, no. 9 (2000): 647-655.

3. Franek, A., J. Taradaj, L. Cierpka, and E. Blaszczak. "High voltage stimulation for healing acceleration of venous leg ulcers." *Phlebologie* 34, no. 05 (2005): 255-260.
4. Franek, A., J. Taradaj, A. Polak, L. Cierpka, and E. Blaszczak. "Efficacy of high voltage stimulation for healing of venous leg ulcers in surgically and conservatively treated patients." *Phlebologie* 35, no. 03 (2006): 127-133.

5. Santos, Rittche Pires, Carolina Almeida Nascimento, and Everaldo Nery De Andrade. "Uso da eletroestimulação de alta voltagem na cicatrização de úlceras venosas." *Fisioterapia em Movimento* 22, no. 4 (2017).

**Ineligible language**

1. Bäuerle, J., & Neander, K. D. (1996). Use of pulsed electrical stimulation in the therapy of decubitus ulcers. *Krankenpflege Journal*, *34*(6), 270-275.
2. ShKh, Gantsev, P. Waymak, Gantseva KhKh, Zagidullin ShZ, and V. G. Makats. "The efficacy of bioelectrostimulation in treating patients with burn injuries." *Klinicheskaia meditsina* 70, no. 1 (1992): 86-88.

3. Anisimov, V. N., D. A. Podushkin, and Iu V. Velichko. "Electric stimulation of postoperative skin wounds in patients with diabetes mellitus." *Vestnik khirurgii imeni II Grekova* 140, no. 4 (1988): 66-67.

4. Razvozova, E. P., and L. N. Sinitsyn. "Stimulation of the regeneration of skin wounds by microcurrents." *Voprosy kurortologii, fizioterapii, i lechebnoi fizicheskoi kultury* 6 (1985): 63-65.

5. Kaada, B. "Treatment of peripheral circulatory disorders and chronic ulceration by transcutaneous nerve stimulation." *Tidsskrift for den Norske laegeforening: tidsskrift for praktisk medicin, ny raekke* 102, no. 30 (1982): 1563-1570.

6. Edel, H., P. Fücker, and K. Liebscher. "Direct-current electrotherapy of chronic skin ulcers using a newly designed instrument." *Dermatologische Monatschrift* 164, no. 3 (1978): 200-202.

7. Duma-Drzewińska, A., and A. Z. Buczyński. "High-frequency pulse currents in the treatment of bedsores." *Polski tygodnik lekarski (Warsaw, Poland: 1960)* 33, no. 22 (1978): 885-887.

8. Materniak, Krzysztof, Anna Nowak-Wróżyna, Marek Kawecki, and Mariusz Nowak. "Elektrostymulacja wysokonapięciowa w leczeniu trudno gojących się ran i obrzęków." *Leczenie Ran* 9, no. 1 (2012).

9. Polak, A., A. Franek, W. Huńka-Zurawińska, W. Bendkowski, M. Kucharzewski, and D. Swist. "High voltage electrical stimulation in leg ulcer's treatment." *Wiadomosci Lekarskie (Warsaw, Poland: 1960)* 53, no. 7-8 (2000): 417-426.

10. Polak, A., A. Walczak, and J. Taradaj. "High-voltage pulsed current in pressure ulcer healing: results of a randomized, controlled trial—preliminary study." *Leczenie Ran* 10, no. 1 (2013): 13-22.

## 3.3 Risk of bias assessment results

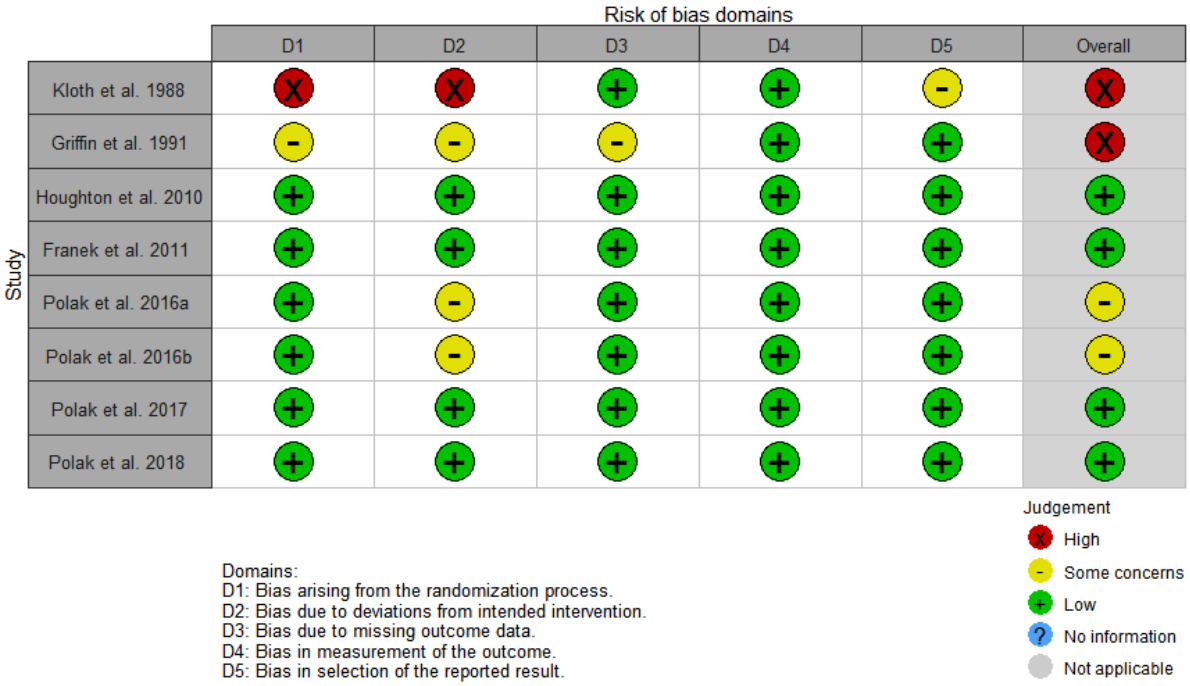



**Figure S1. Risk-of-bias plot of incidence of complete healing** in RCTs including pressure ulcers

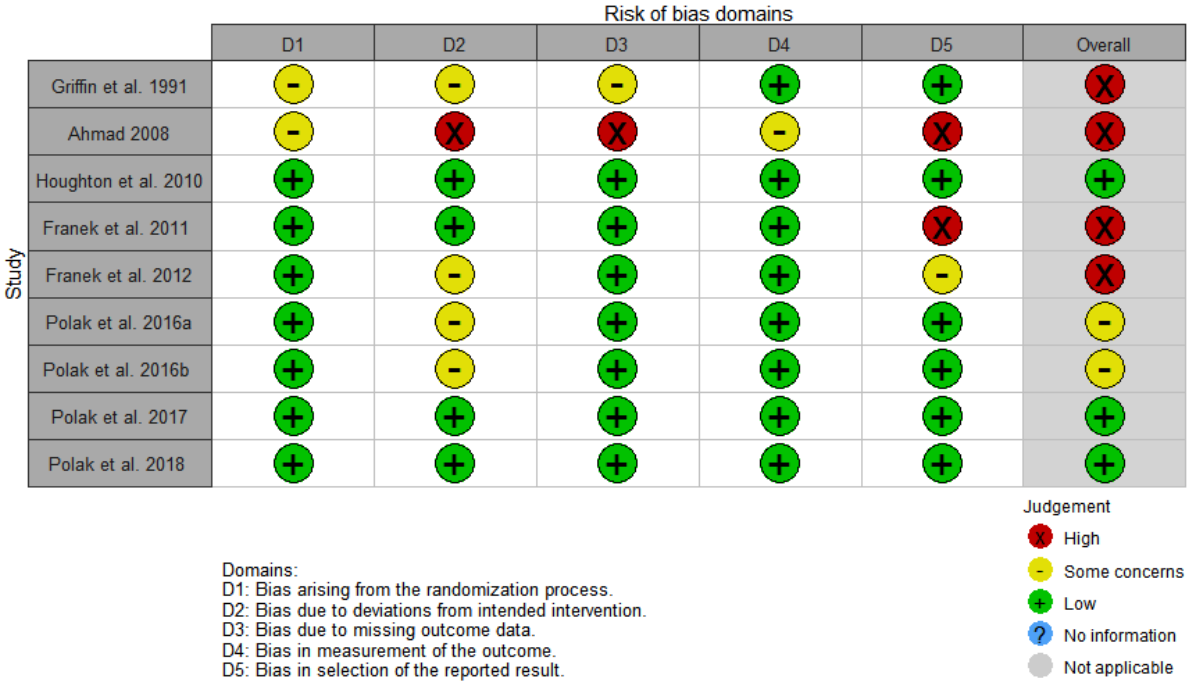



**Figure S2. Risk-of-bias plot of wound-healing status** in RCTs assessing pressure ulcers

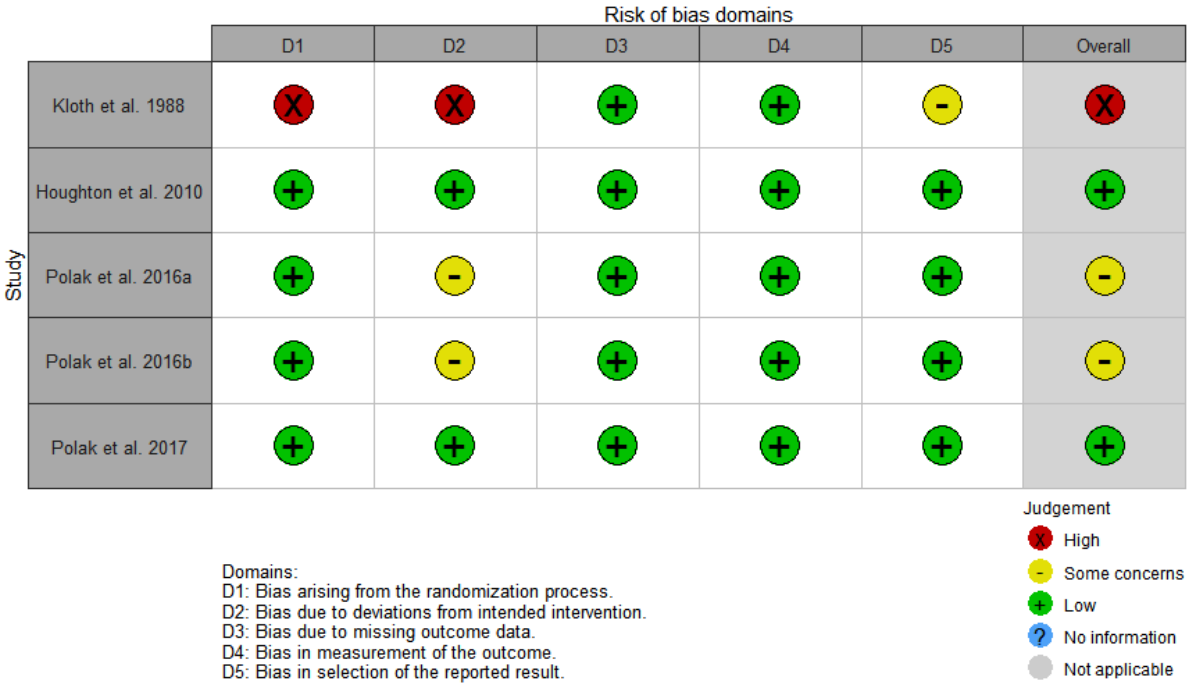



**Figure S3. Risk-of-bias plot of incidence of worsening of healing** in RCTs evaluating pressure ulcers

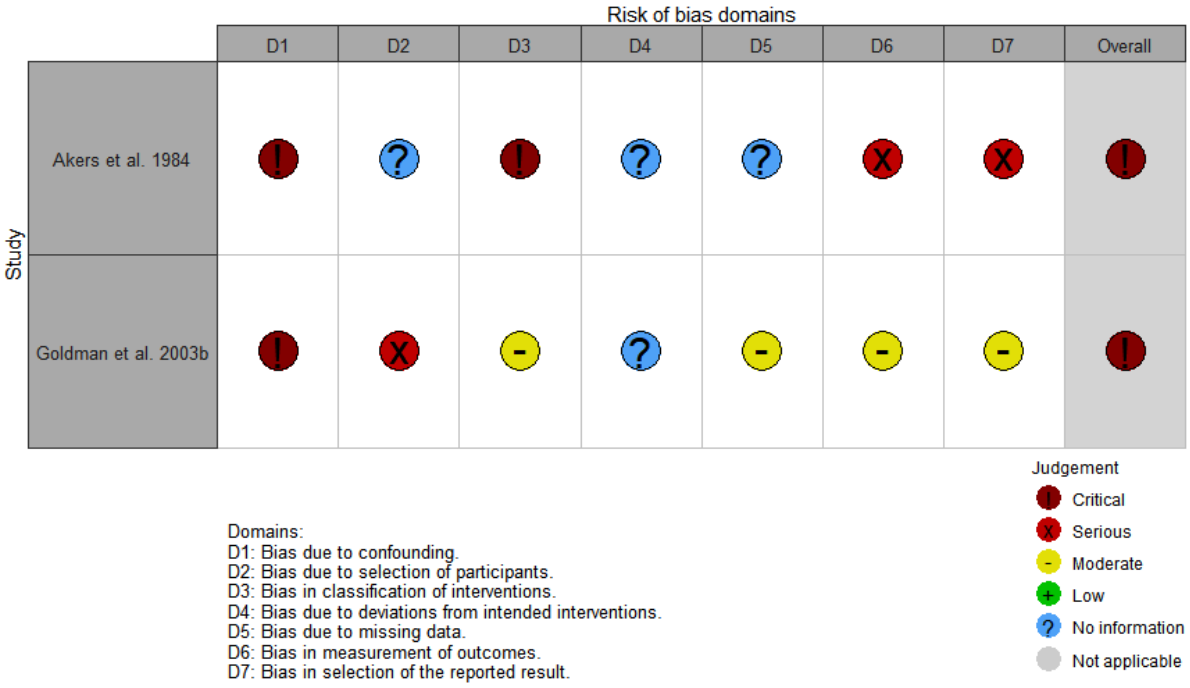



**Figure S4. Risk-of-bias plot of wound-healing status** in NRCTs assessing pressure ulcers

| Study | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Overall |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Gilcreast et al. 1998 (Gilcreast et al., 1998) | Yes | Yes | Yes | Unclear | Unclear | Yes | Yes | Yes | Yes | Yes | High quality |
| Goldman et al. 2002 (Goldman et al., 2002) | Yes | Yes | Yes | Yes | Unclear | Yes | Yes | Yes | Yes | Yes | High quality |
| Silva et al. 2010 (Silva Ede et al., 2010) | No | Yes | Yes | Unclear | Unclear | Yes | Yes | Yes | No | Unclear | Low quality |
| Lasko et al. 2010 (Lasko et al., 2010) | No | Unclear | Unclear | No | Unclear | Yes | Yes | No | No | Unclear | Low quality |
| Recio et al. 2012 (Recio et al., 2012) | No | Unclear | Unclear | Unclear | Unclear | Yes | Yes | Yes | No | Unclear | Low quality |
| Gui et al. 2013 (Gui et al., 2013) | No | Unclear | Yes | No | No | No | No | No | Yes | No | Low quality |
| Zhou et al. 2016a (Zhou, Schenk, et al., 2016) | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes | Yes | Yes | Yes | Moderate quality |
| Zhou et al. 2016b (Zhou, Krug, et al., 2016) | Yes | Yes | Yes | Yes | Unclear | Yes | Yes | Yes | Yes | Yes | High quality |
| Karsli et al. 2017 (Karsli et al., 2017) | Yes | Unclear | Unclear | Unclear | Unclear | Yes | No | No | Yes | No | Low quality |
| Sakabe et al. 2018 (Sakabe et al., 2018) | Yes | Yes | Yes | Unclear | Unclear | Yes | Yes | Yes | Yes | Unclear | Moderate quality |

**Table S1. Methodological quality assessment results of case series studies.** JBI critical appraisal checklist for case series; overall appraisal of studies was based on question responses, as follows; high quality (1 "No" or 2 "Unclear"), moderate quality (2 "No" or 2-4 "Unclear"), low quality (>2 "No" or >4 "Unclear"). JBI checklist items are: 1. Were there clear criteria for inclusion in the case series? 2. Was the condition measured in a standard, reliable way for all participants included in the case series? 3. Were valid methods used for identification of the condition for all participants included in the case series? 4. Did the case series have consecutive inclusion of participants? 5. Did the case series have complete inclusion of participants? 6. Was there clear reporting of the demographics of the participants in the study? 7. Was there clear reporting of clinical information of the participants? 8. Were the outcomes or follow up results of cases clearly reported? 9. Was there clear reporting of the presenting site(s)/clinic(s) demographic information? 10. Was statistical analysis appropriate?

| Study | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Overall |
|---|---|---|---|---|---|---|---|---|---|
| Fitzgerald et al. 1993 (Fitzgerald & Newsome, 1993) | Yes | Yes | Yes | No | Yes | Yes | Yes | Yes | High quality |
| Goldman et al. 2003a (R. J. Goldman et al., 2003) | Yes | Yes | Yes | No | Yes | Yes | No | Yes | Moderate quality |
| Santana de Souza et al. 2017 (Santana de Souza et al., 2017) | Yes | Yes | No | Yes | No | Unclear | No | Yes | Low quality |

**Table S2. Methodological quality assessment results of case report studies.** JBI critical appraisal checklist for case reports; overall appraisal of studies was based on question responses, as follows; high quality (1 "No" or 2 "Unclear"), moderate quality (2 "No" or 2-4 "Unclear"), low quality (>2 "No" or >4 "Unclear"). JBI checklist items are: 1. Were patient's demographic characteristics clearly described? 2. Was the patient's history clearly described and presented as a timeline? 3. Was the current clinical condition of the patient on presentation clearly described? 4. Were diagnostic tests or assessment methods and the results clearly described? 5. Was the intervention(s) or treatment procedure(s) clearly described? 6. Was the post-intervention clinical condition clearly described? 7. Were adverse events (harms) or unanticipated events identified and described? 8. Does the case report provide takeaway lessons?

### 3.4 Results of individual studies

#### 3.4.1 Diabetic ulcers

The proportion of complete wound healing was higher in the treatment group (n=13/20; 65%) compared with the control group (n=7/20; 35%) [$X^2$=3.60, P=0.058]; some concerns of bias (Peters et al., 2001). Nonetheless, no significant differences in the rate of wound healing (86.2% versus 71.4%, over the 12-week duration of the study); high risk of bias, and the average time to complete healing (6.8 ± 3.4 weeks, and 6.9 ± 2.8 weeks); some concerns of bias, were found between treatment and control groups, respectively (Peters et al., 2001).

While healing (defined as closure of at least 75% or more of the sum of areas of all ulcers per patient) was reached by 80% (n=8/10) of patients in the treatment group and 66% (n=6/9) of patients in the control group during the 16 weeks of intervention, a statistically significant difference was not found (P=0.87); high risk of bias (Ortiz et al., 2014). Moreover, the same study reported no statistically significant differences between groups in percent wound surface area reduction (P=0.36); high risk of bias, nerve conduction study (P>0.11); high risk of bias, protective sensation test (P>0.52); high risk of bias, or quality of life (P>0.18); high risk of bias (Ortiz et al., 2014). A case series study stated that $TcPO_2$, measured at 30- and 60-minute time intervals following treatment, was increased (mean 14.8 mm Hg) in a subset of patients 27% (n=35/132); with a total of 24 patients with foot ulcers (18% of the sample), while 73% (n=97/132) experienced a decreased transcutaneous oxygen reading (mean 12.2 mm Hg); high quality (Gilcreast et al., 1998). Patients who demonstrated increased $TcPO_2$ in response to HVMPC were older, had greater neuropathy, adequate blood flow (toe blood pressure > 70 mm Hg), and higher blood glucose levels; high quality (Gilcreast et al., 1998).

### 3.4.2 Ischemic/Neuropathic ulcers in diabetic patients

Ischemic wounds in the treatment group decreased in surface area in comparison with the control group after 4 weeks of treatment (n=4 each group; P<0.05); high risk of bias (Goldman et al., 2004). A significant improvement in periwound microcirculation was reported at weeks 8 (P<0.05, $TcPO_2$; high risk of bias, P<0.01, laser doppler; some concerns of bias) and 12 (P<0.05, laser doppler; some concerns of bias) (Goldman et al., 2004). Pain improvement was similar between groups at week 14 (VAS score of treatment group, at week 0 = 4.7 ± 0.3, and at week 14 = 2.4 ± 2.9, delta = 2.3; control group, week 0 = 6.5 ± 3.1, week 14 = 4.4 ± 0.7, delta = 2.1); high risk of bias (Goldman et al., 2004). A case series study reported an increase in periwound $TcPO_2$ from 2 ± 2 mm Hg (mean ± SD) before treatment to 33 ± 18 mm Hg following 157 ± 114 days from the start of treatment (n=6; P<0.05); high quality (Goldman et al., 2002). A case report mentioned that both left posterior calf and left heel wounds completely healed by 250, and 234 days, respectively, after HVMPC, and that periwound $TcPO_2$ increased from 20 mm Hg to 50 mm Hg during the healing phase; moderate quality (R. J. Goldman et al., 2003).

The treatment group (n=10) showed smaller wound surface areas compared with the control group (n=7), from weeks 20 through 52 following treatment (P<0.05); critical risk of bias (R. Goldman et al., 2003). After one year of treatment, 90% of wounds in the treatment group healed completely compared with 29% of wounds in the control group (P<0.05); critical risk of bias (R. Goldman et al., 2003). Maximum periwound $TcPO_2$ improved from a mean (SD) of 6 ± 8 mm Hg at baseline, to 26 ± 20 mm Hg during HVMPC treatment (P<0.05); critical risk of bias (R. Goldman et al., 2003).

### 3.4.3 Pressure ulcers

Ulcers in the treatment group (n=9) healed at a rate of 44.8 ± 22.6% (mean ± SD) a week, whereas ulcers in the control group (n=7) increased in area at a mean rate of 11.59 ± 18.6% (mean ± SD), over a period of 7.4 weeks (mean); high risk of bias (Kloth & Feedar, 1988). Furthermore, ulcers in the treatment group healed completely, while ulcers in the control group increased a mean of 28.9 ± 89.8% (mean ± SD) in surface area, over a period of 7.4 weeks; high risk of bias (Kloth & Feedar, 1988). Percentage of wound surface area reduction was significantly greater in the treatment group (TG; n=8) compared with the control group (CG; n=9) at 5 (median (min, max): TG: -32 (-12,-100), CG: -14 (+17,-74); P=0.03), 15 (median (min, max): TG: -66 (-42,-100) ; CG: -44 (+22,-100), P=0.05), and 20 (median (min, max): TG: -80 (-52,-100), CG: -52 (-14,-100), P=0.05) days following treatment; high risk of bias (Griffin et al., 1991). Three ulcers healed completely in the treatment group compared with two ulcers in the control group; high risk of bias (Griffin et al., 1991).

A significant reduction in wound surface area between the three treatment groups (n=15 each), which received HVMPC for 45, 60 and 120 minutes, and the control group (n=15) was reported after three and five weeks of treatment (P<0.001); high risk of bias (Ahmad, 2008). Wound surface area was significantly reduced in the treatment group (n=16) 70 ± 25% (mean ± SD) compared with the control group (n=18) 36 ± 61% (mean ± SD); (P=0.048), after 3 month of treatment; low risk of bias (Houghton et al., 2010). Complete healing occurred in 6/16 ulcers in the treatment group compared with 5/18 ulcers in the control group; low risk of bias (Houghton et al., 2010). However, worsening of healing occurred in 4/18 ulcers in the control group in comparison with 0/16 ulcers in the treatment group; low risk of bias (Houghton et al., 2010).

The treatment group (n=29) showed a relative change in wound surface area of 85.38% compared with 40.08% in the control group (n=29) after 6 weeks of treatment; high risk of bias (Franek et al., 2011). 8/29 ulcers in the treatment group achieved complete healing, while 4/29 ulcers healed completely in the control group; low risk of bias (Franek et al., 2011). Following 6 weeks of treatment, wound surface area was significantly reduced in the treatment group (n=26) 88.9 ± 14% (mean ± SD) compared with the control group (n=24) 44.4 ± 63.1% (mean ± SD) (P=0.00003); high risk of bias (Franek et al., 2012). A significantly greater reduction in the wound surface area was found in the treatment group (n=24, 29 ulcers) 76.19 ± 32.83% (mean ± SD) in comparison with the control group (n=28, 31 ulcers) 48.97 ± 53.42% (mean ± SD), following 6 weeks of treatment (P=0.030); some concerns of bias (Polak, Taradaj, et al., 2016). Moreover, a significantly higher proportion of ulcers healed completely in the treatment group (15/29) versus the control group (7/31) (P=0.031); some concerns of bias (Polak, Taradaj, et al., 2016). There was no statistically significant difference, however, between the proportion of ulcers that increased in surface area in the treatment (0/29) and control (2/31) groups (P=0.49); some concerns of bias (Polak, Taradaj, et al., 2016).

The percentage of wound surface area reduction in the treatment group (n=25) was 80.31 ± 29.02% (mean ± SD) compared with 54.65 ± 42.65% (mean ± SD) in the control group (n=24), after 6 weeks of treatment (P=0.046); some concerns of bias (Polak, Kloth, et al., 2016). A higher percentage of complete healing (stage II and III ulcers) was achieved in the treatment group (9/11, and 3/14, respectively) than in the control group (6/11, and 1/13, respectively), nonetheless, the difference was not statistically significant (P=0.74, and P=0.6, respectively); some concerns of bias

(Polak, Kloth, et al., 2016). 2 stage III ulcers increased in surface area in the control group (2/24) while none of the treatment group ulcers (0/25) increased in surface area (P=0.22); some concerns of bias (Polak, Kloth, et al., 2016).

A significant reduction in wound surface area was found in both the cathodal HVMPC (n=23) 82.34%; 95% CI: [70.06, 94.63] and cathodal-anodal HVMPC (n=20) 70.77%; 95% CI: [53.51, 88.04] groups in comparison with the control group (n=20) 40.53%; 95% CI: [23.60, 57.46] (P=0.0006, and P=0.0124, respectively), after 6 weeks of treatment; low risk of bias (Polak et al., 2017). The proportion of complete healing was higher in the cathodal HVMPC (11/23) and cathodal-anodal HVMPC (9/20) groups than in the control group (0/20) (P=0.013, and P=0.045, respectively); low risk of bias (Polak et al., 2017). Increase in wound surface area was reported in 2 ulcers in the control group, 1 ulcer in the cathodal-anodal HVMPC group, while no ulcers increased in surface area in the cathodal HVMPC group (P>0.05); low risk of bias (Polak et al., 2017).

The cumulative change in periwound skin blood flow was significantly higher in the anodal HVMPC (n=20) 109.52%; 95% CI: [44.59, 174.45], and cathodal HVMPC (n=21) 131.54%; 95% CI: [60.28, 202.80] groups than the control group (n=20) 35.83%; 95% CI: [14.63, 57.02] after 2 weeks of treatment (P=0.0470, and P=0.0152, respectively), nevertheless, the difference was not statistically significant after 4 weeks of treatment (P=0.74); low risk of bias (Polak et al., 2018). Following 8 weeks of treatment, the percentage of wound surface area reduction was 64.10%; 95% CI: [50.42, 77.78] for the anodal HVMPC group, 74.06%; 95% CI: [63.48, 84.63] for the cathodal HVMPC group, and 41.42%; 95% CI: [28.37, 54.47] for the control group; there were statistically significant differences between the treatment groups and the

control group (P=0.0391, and P=0.0024, respectively); low risk of bias (Polak et al., 2018). Complete healing was reached in 4/20 ulcer in the anodal HVMPC group, in 7/21 ulcers in the cathodal HVMPC group, and in 2/20 ulcers in the control group, however, the differences between the treatment groups and the control group were not statistically significant (P=0.3721, and P=0.0645, respectively); low risk of bias (Polak et al., 2018). Time to complete healing was 6.7 weeks; 95% CI: [5.04, 8.36] in the anodal HVMPC group, 7.38 weeks; 95% CI: [5.72, 9.04] in the cathodal HVMPC group, and 6.05 weeks; 95% CI: [4.92, 7.18] in the control group, nonetheless, differences among groups were not statistically significant (P=0.5111); some concerns of bias (Polak et al., 2018). The greatest reduction in wound surface area occurred in the group that received HVMPC alone, although there were no statistically significant differences between groups; critical risk of bias (Akers & Gabrielson, 1984).

A case series study reported that ulcers healed at a rate of 43% (mean) in the HVMPC group (n=25) versus and 63% (mean) in the ultrasound therapy group (n=22); however, the difference between both groups was not statistically significant after 4-12 weeks of treatment; low quality (Karsli et al., 2017). A case series study (n=3) found that chronic pressure ulcers (11-14 months) completely healed following 7-22 weeks of treatment; low quality (Recio et al., 2012). A case series study (n=4) stated that HVMPC improved the healing of 6 ulcers as it led to a reduction in wound surface area of more than 78%, and complete healing of 3 ulcers; low quality (Gui et al., 2013). A case report concluded that a reduction in wound surface area of 17% was achieved after 4 weeks of HVMPC treatment, in addition to better wound appearance; low quality (Santana de Souza et al., 2017).

### 3.4.4 Acute wounds

a. Postoperative wounds

A significant reduction in pain was reported following HVMPC electrical stimulation of the donor site of skin grafts; pain was absent on the third day in the treatment group (n=15) and on the sixth day in the control group (n=15); high risk of bias (Gomes et al., 2018). Time to wound epithelialization, when considering spontaneous dressing detachment, was significantly shorter in the treatment group (4.7 ± 0.2 days, average) compared with the control group (7.0 ± 1.3 days, average) ($P<0.05$); low risk of bias (Gomes et al., 2018). A significant difference (pre- and immediately post, respectively) in skin temperature was not found in both experimental (34.84 ± 1.19 and 35.22 ± 1.17) and control groups (34.68 ± 0.76 and 34.73 ± 1.60); high risk of bias (Gomes et al., 2018). Donor site wound healing characteristics such as pigmentation, vascularization, irregularities, and the quality of healing were better in the treatment group; low risk of bias (Gomes et al., 2018). A case report stated that complete healing occurred in a large postoperative infected thoracic spine wound after 10 weeks of treatment; high quality (Fitzgerald & Newsome, 1993).

b. Burns

The healing rate of burn wounds in children was 18.9%, 46.66%, and 81.67% in the treatment group (n=7) after the first, second, and third week of treatment, respectively, while the control group (n=7) had healing rates of 7.56%, 19.0%, and 34.3%; high risk of bias (Elshazly & Hassan, 1998). The percentage of improvement in edema reduction in the treatment group (n=15, 17 wounds) was 5.95%, 13.4%, and 24.46% compared with 5.4%, 7.3%, and 12.2% in the control group (n=15, 18 wounds), after 3, 7, and 15 days of treatment, respectively; high risk of bias (Omar et al., 2004). The percentage of improvement in range of motion in the treatment group was 11.44%, 20.48%, and

38.5% compared with 10.42%, 16.56%, and 26.9% in the control group (n=15, 18 wounds), after 3, 7, and 15 days of treatment, respectively; high risk of bias (Omar et al., 2004). Normal hand function was restored in 90% of patients in the treatment group versus 80% of patients in the control group, after 12 weeks; high risk of bias (Omar et al., 2004).

### 3.4.5 Multiple etiology wounds and ulcers

HVMPC intervention for chronic leg ulcers in the treatment group (n=14) reduced wound surface area by 44.3 ± 8.8% (mean ± SD), which was more than 2 times greater than the control group (n=13) 16.0 ± 8.9% (mean ± SD), over a 4-week treatment period; low risk of bias (Houghton et al., 2003). A better improvement in wound appearance was reported in the treatment group as reflected by the decrease in the photographic wound assessment tool score, after 4 weeks of treatment ($P<0.05$); low risk of bias (Houghton et al., 2003). Nonetheless, no statistically significant differences were found in wound appearance at the 1-month follow-up assessment; low risk of bias (Houghton et al., 2003).

A case series study reported complete healing in a mean of 17.6 weeks (range, 8-36 weeks) of 10 lower extremity ulcers following a concomitant treatment with acoustic pressure wound therapy (mean, 18.3 weeks) and HVMPC (mean, 13.5 weeks); low quality (Lasko et al., 2010). A mean reduction of 36% in wound surface area was found in a case series study conducted on chronic lower limb wounds of three participants, after 15 weeks of treatment; low quality (Silva Ede et al., 2010). Pain was reduced by 55% and 43% in two participants after 15 weeks of treatment; low quality (Silva Ede et al., 2010).

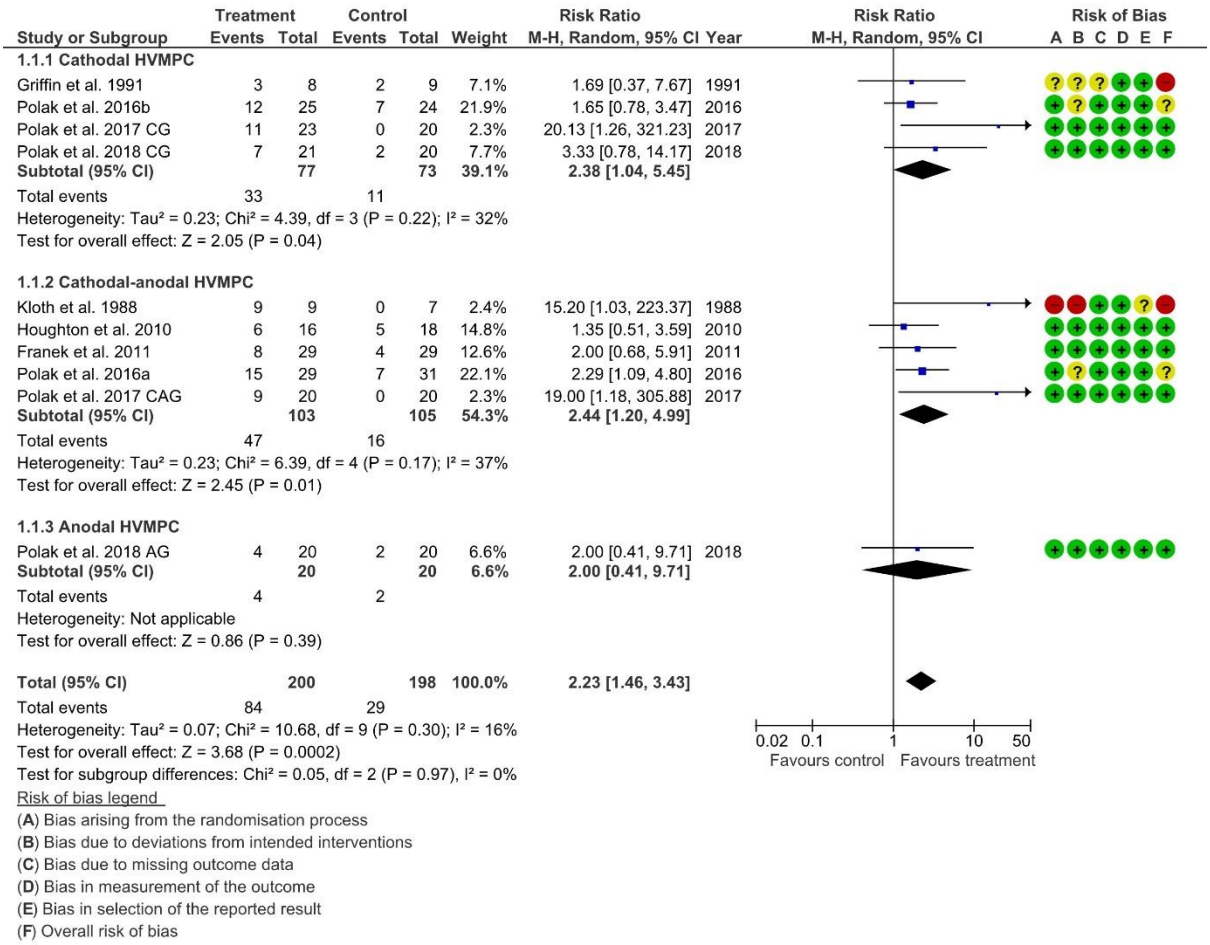
A case series study (n=159) stated that the percentage of patients with complete wound closure was 59.12%, 61.01%, and 65.41% following 100, 112, and 140 days of treatment, respectively; moderate quality (Zhou, Schenk, et al., 2016). A case series study (n=10, 14 wounds) found a reduction in wound surface area of 42.52 ± 34.16% (mean ± SD; P=0.002) and wound volume of 66.84 ± 25.07% (mean ± SD; P=0.001), after 2 weeks of treatment; high quality (Zhou, Krug, et al., 2016). One patient was discharged with complete wound closure; high quality (Zhou, Krug, et al., 2016). A case series study (n=6, 10 ulcers) concluded that HVMPC promoted complete healing of 4 ulcers, and reduced the surface area of 6 ulcers; moderate quality (Sakabe et al., 2018). Trochanteric and medial malleolus ulcers reached complete healing after 10 sessions, while neuropathic ulcers closed after 20 sessions; moderate quality (Sakabe et al., 2018).

## 3.8 Subgroup analyses results

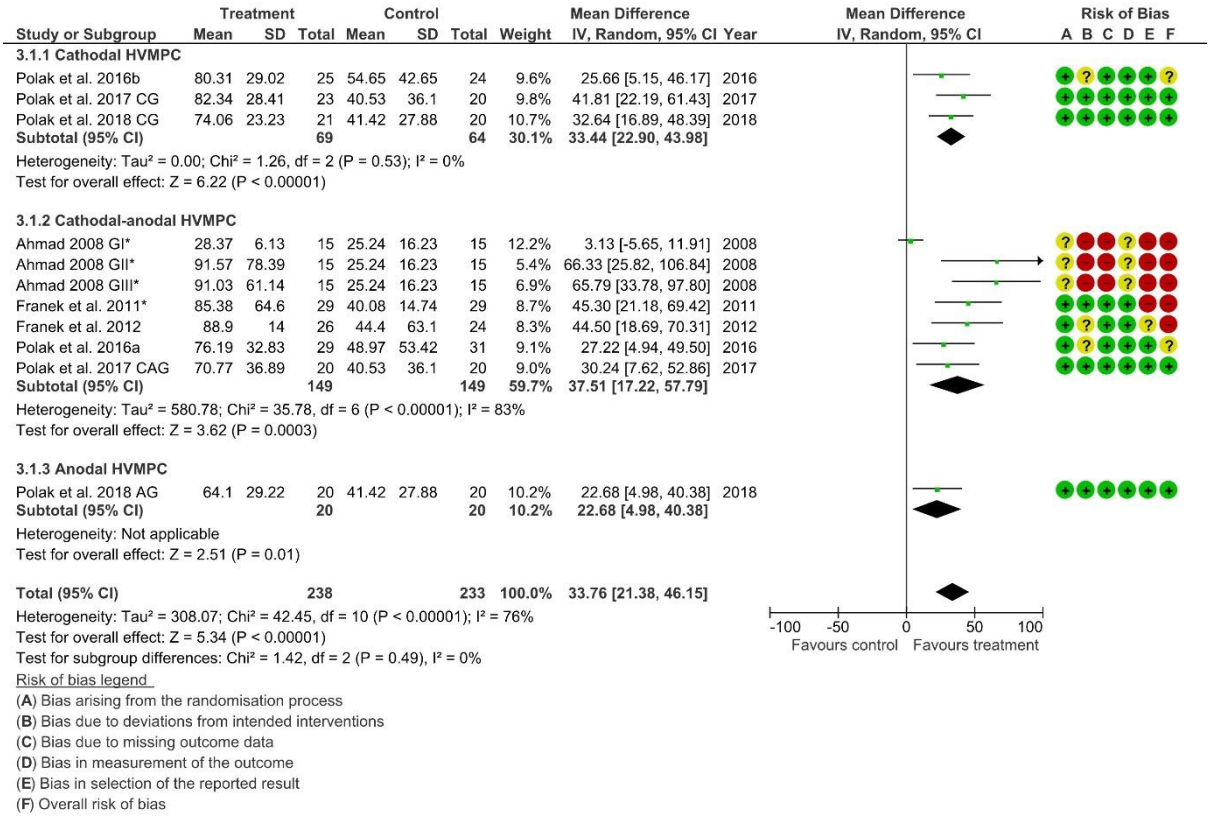
| Subgroup | Outcome | № U | № S | Effect size | 95% CI | P value | Heterogeneity | Test for subgroup differences |
|---|---|---|---|---|---|---|---|---|
| Cathodal-anodal | ICH | 208 | 5 | RR 2.44 | 1.20, 4.99 | **0.01** | $I^2$=37%, P=0.17 | $I^2$=0%, P=0.97 |
| | WHS | 268 | 5 | MD 37.51 | 17.22, 57.79 | **0.0003** | $I^2$=83%, P<0.00001 | $I^2$=0%, P=0.49 |
| | IWH | 150 | 4 | RR 0.20 | 0.05, 0.79 | **0.02** | $I^2$=0%, P=0.79 | $I^2$=0%, P=0.93 |
| Cathodal | ICH | 150 | 4 | RR 2.38 | 1.04, 5.45 | **0.04** | $I^2$=32%, P=0.22 | $I^2$=0%, P=0.97 |
| | WHS | 133 | 3 | MD 33.44 | 22.90, 43.98 | **<0.00001** | $I^2$=0%, P=0.53 | $I^2$=0%, P=0.49 |
| | IWH | 92 | 2 | RR 0.18 | 0.02, 1.51 | 0.12 | $I^2$=0%, P=0.97 | $I^2$=0%, P=0.93 |
| Anodal | ICH | 40 | 1 | RR 2.00 | 0.41, 9.71 | 0.39 | N/A | $I^2$=0%, P=0.97 |
| | WHS | 40 | 1 | MD 22.68 | 4.98, 40.38 | **0.01** | N/A | $I^2$=0%, P=0.49 |
| | IWH | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Duration ≥ 50 min | ICH | 342 | 7 | RR 1.99 | 1.36, 2.93 | **0.0005** | $I^2$=0%, P=0.80 | $I^2$=53.5%, P=0.14 |
| | WHS | 386 | 7 | MD 35.71 | 27.97, 43.45 | **<0.00001** | $I^2$=18%, P=0.28 | $I^2$=96.6%, P<0.00001 |
| | IWH | 206 | 4 | RR 0.22 | 0.06, 0.79 | **0.02** | $I^2$=0%, P=0.96 | $I^2$=0%, P=0.57 |
| Duration < 50 min | ICH | 16 | 1 | RR 15.20 | 1.03, 223.37 | **0.05** | N/A | $I^2$=53.5%, P=0.14 |
| | WHS | 30 | 1 | MD 3.13 | -5.65, 11.91 | 0.48 | N/A | $I^2$=96.6%, P<0.00001 |
| | IWH | 16 | 1 | RR 0.09 | 0.01, 1.42 | 0.09 | N/A | $I^2$=0%, P=0.57 |

**Table S3. Subgroup analyses summary;** protocols and durations of HVMPC intervention were compared; ICH, incidence of complete healing; WHS, wound-healing status; IWH, incidence of worsening of healing; № U, number of ulcers; № S, number of studies; 95% CI, 95% confidence interval; RR, relative risk; MD, mean difference; N/A, not available or not applicable

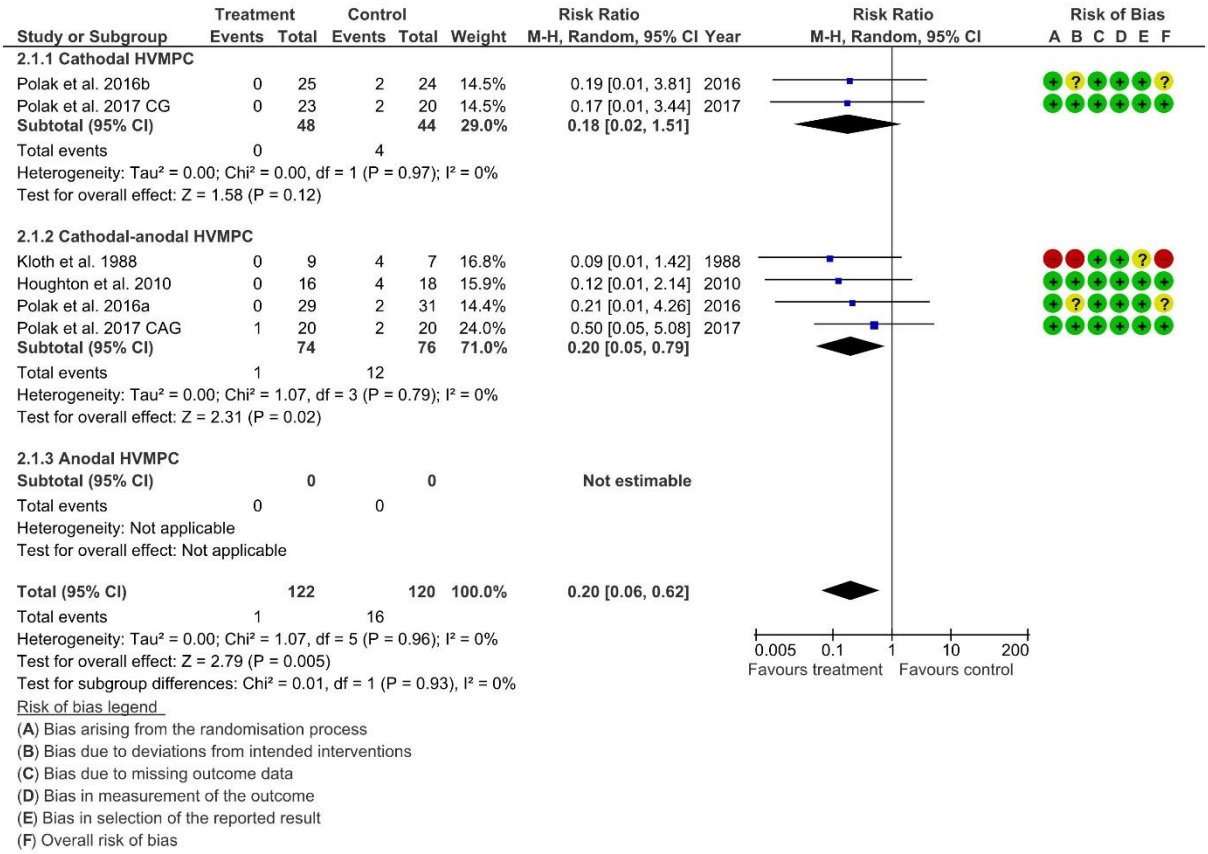
## 3.8.1 Protocols




**Forest plot of relative risk (RR) analysis of proportion of completely healed pressure ulcers;** RR>1, probability of complete healing is higher in treatment arm; RR=1, no difference in probability of complete healing between treatment and control arms; RR<1, probability of complete healing is lower in treatment arm. The number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group; AG, anodal group

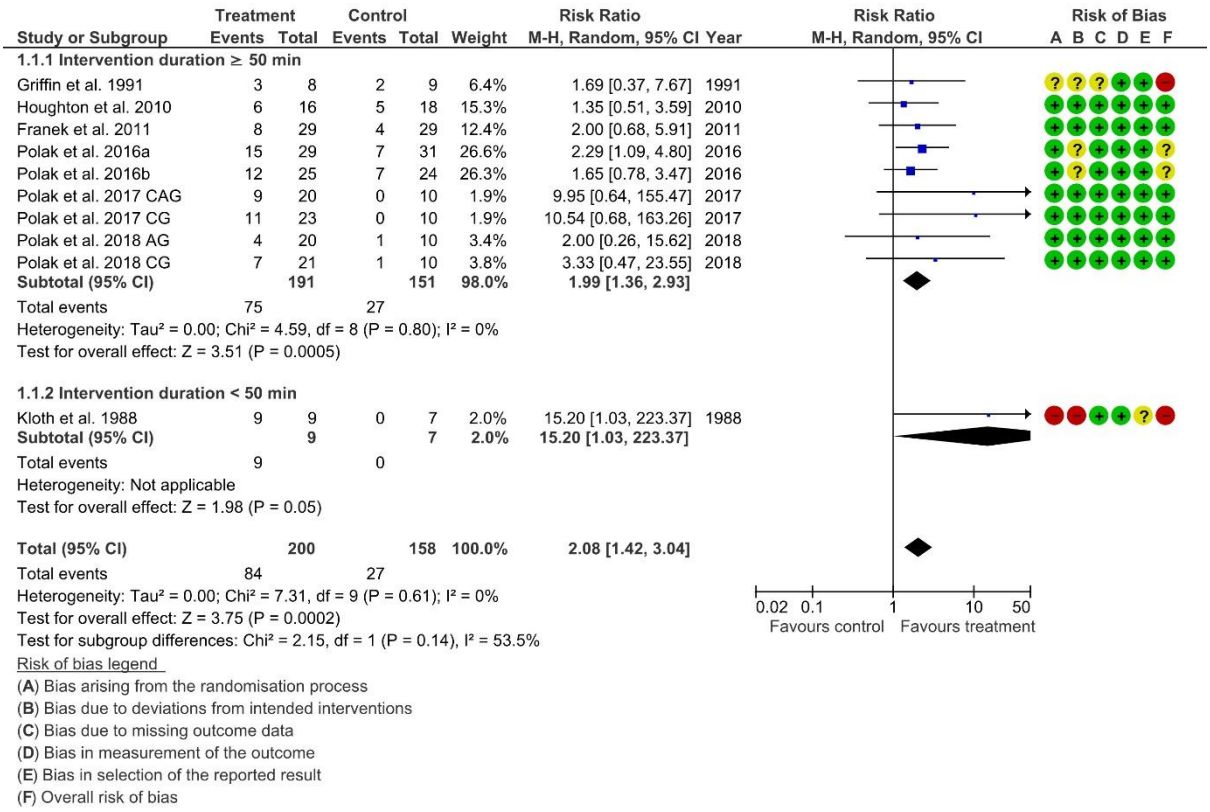



**Forest plot of mean difference (MD) analysis of percent wound surface area reduction of pressure ulcers at 4-8 weeks;** values smaller than zero favors control arm, whereas values greater than zero favors treatment arm. The biological sample size (n) is 401 participants; experimental arm (n=238), and control arm (n=163); the number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group; AG, anodal group; *, mean and/or standard deviation values were calculated and/or imputed for this sample

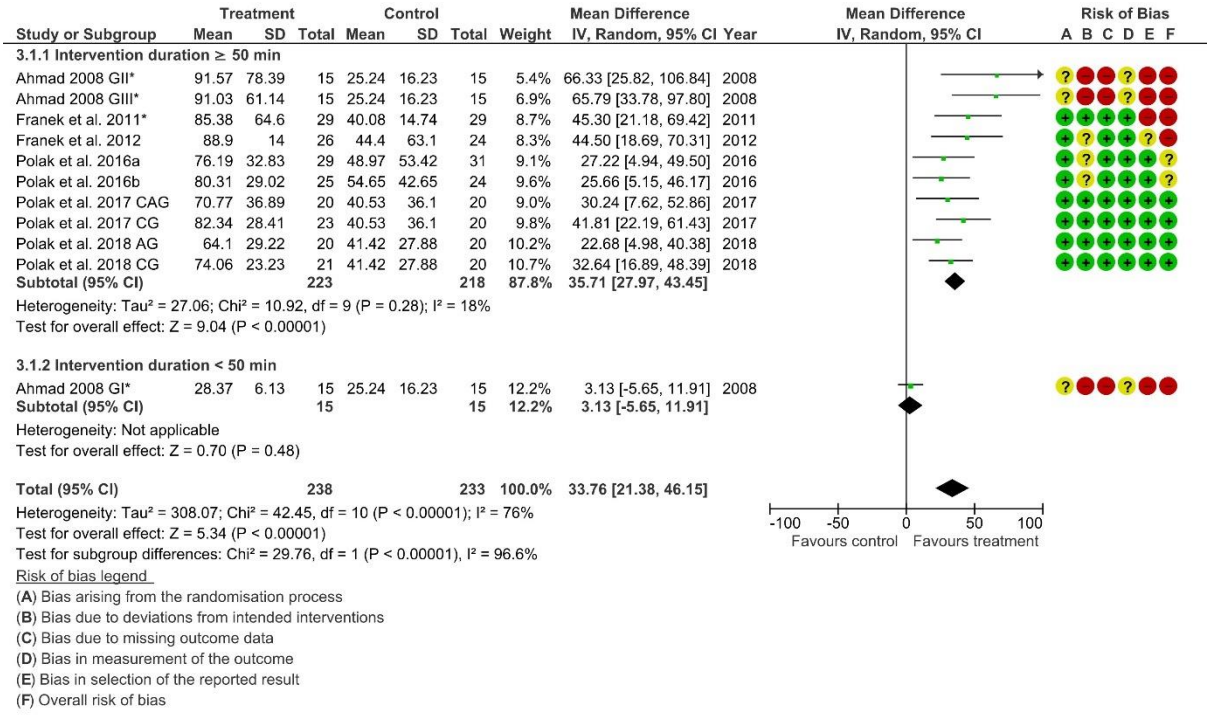



**Forest plot of relative risk (RR) analysis of proportion of worsened pressure ulcers;** RR>1, probability of worsening of healing is higher in control arm; RR=1, no difference in probability of worsening of healing between treatment and control arms; RR<1, probability of worsening of healing is lower in treatment arm. The number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group

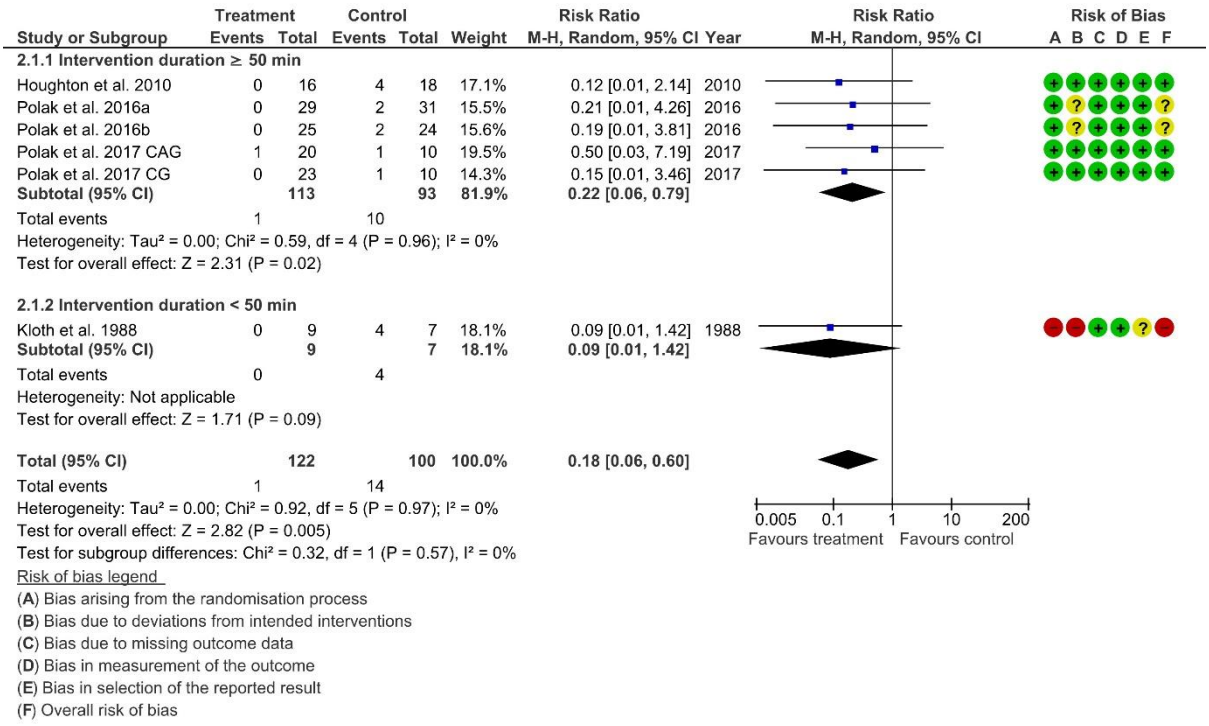
## 3.8.2 Intervention duration




**Forest plot of relative risk (RR) analysis of proportion of completely healed pressure ulcers;** RR>1, probability of complete healing is higher in treatment arm; RR=1, no difference in probability of complete healing between treatment and control arms; RR<1, probability of complete healing is lower in treatment arm. The number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group; AG, anodal group




**Forest plot of mean difference (MD) analysis of percent wound surface area reduction of pressure ulcers at 4-8 weeks;** values smaller than zero favors control arm, whereas values greater than zero favors treatment arm. The biological sample size (n) is 401 participants; experimental arm (n=238), and control arm (n=163); the number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group; AG, anodal group; *, mean and/or standard deviation values were calculated and/or imputed for this sample




**Forest plot of relative risk (RR) analysis of proportion of worsened pressure ulcers;** RR>1, probability of worsening of healing is higher in control arm; RR=1, no difference in probability of worsening of healing between treatment and control arms; RR<1, probability of worsening of healing is lower in treatment arm. The number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group

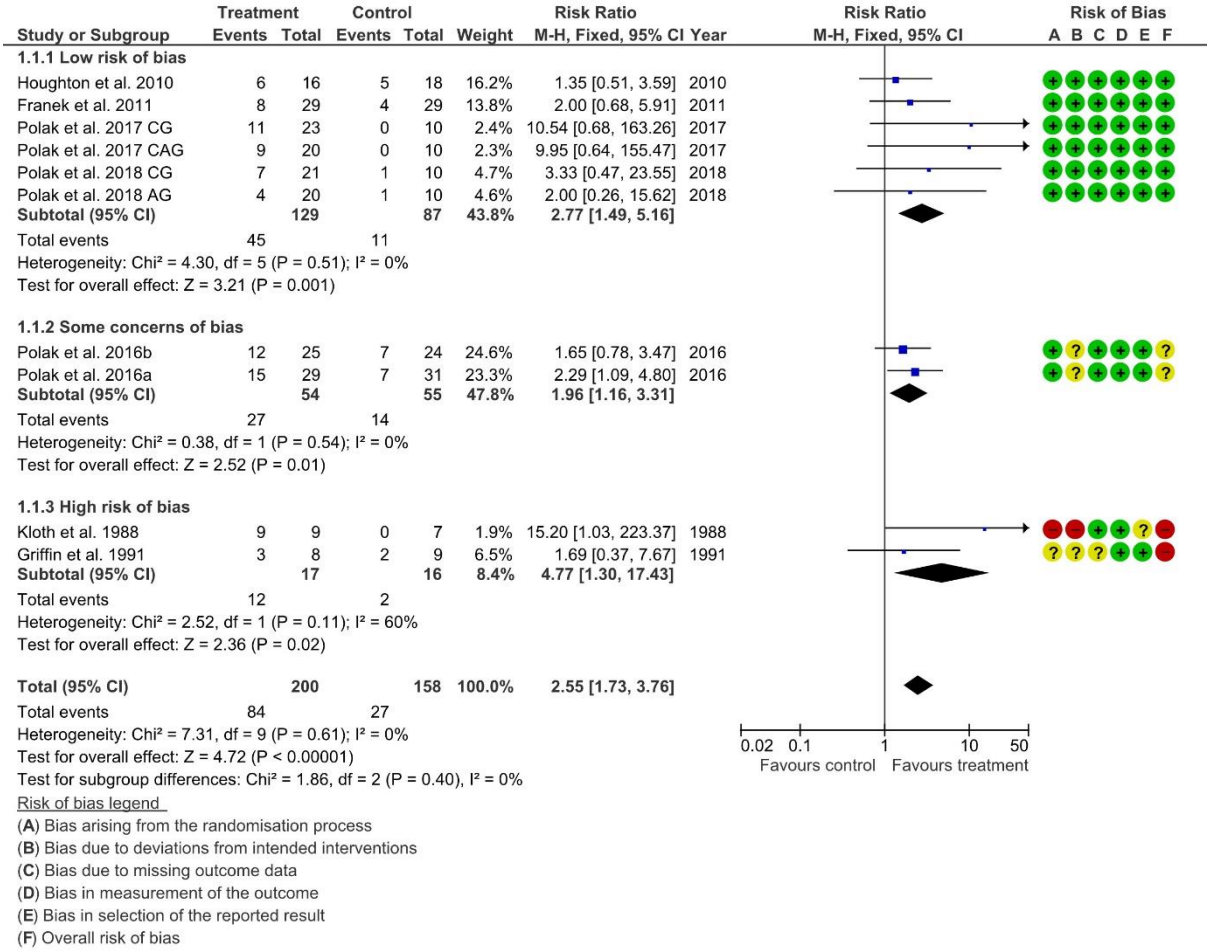
## 3.9 Sensitivity analyses results

| Outcome | Analysis | № U | № S | Effect size | 95% CI | P value | Heterogeneity | Test for subgroup differences |
|---|---|---|---|---|---|---|---|---|
| ICH DUs | Fixed | 59 | 2 | RR 1.55 | 0.98, 2.44 | 0.06 | $I^2$=7%, P=0.30 | N/A |
| | Random | 59 | 2 | RR 1.43 | 0.92, 2.24 | 0.11 | $I^2$=7%, P=0.30 | N/A |
| ICH PrUs | Fixed | 358 | 8 | RR 2.55 | 1.73, 3.76 | **<0.00001** | $I^2$=0%, P=0.61 | $I^2$=0%, P=0.40 |
| | Random | 358 | 8 | RR 2.08 | 1.42, 3.04 | **0.0002** | $I^2$=0%, P=0.61 | $I^2$=0%, P=0.84 |
| WHS PrUs | Fixed | 401 | 7 | MD 22.90 | 17.47, 28.33 | **<0.00001** | $I^2$=76%, P<0.00001 | $I^2$=69.6%, P=0.04 |
| | Random | 401 | 7 | MD 33.76 | 21.38, 46.15 | **<0.00001** | $I^2$=76%, P<0.00001 | $I^2$=0%, P=0.62 |
| IWH PrUs | Fixed | 222 | 5 | RR 0.17 | 0.05, 0.54 | **0.003** | $I^2$=0%, P=0.97 | $I^2$=0%, P=0.87 |
| | Random | 222 | 5 | RR 0.18 | 0.06, 0.60 | **0.005** | $I^2$=0%, P=0.97 | $I^2$=0%, P=0.85 |
| ICH PrUs | Included | 358 | 8 | RR 2.08 | 1.42, 3.04 | **0.0002** | $I^2$=0%, P=0.61 | $I^2$=0%, P=0.84 |
| | Excluded | 325 | 6 | RR 2.02 | 1.35, 3.01 | **0.0006** | $I^2$=0%, P=0.71 | $I^2$=0%, P=0.83 |
| WHS PrUs | Included | 401 | 7 | MD 33.76 | 21.38, 46.15 | **<0.00001** | $I^2$=76%, P<0.00001 | $I^2$=0%, P=0.62 |
| | Excluded | 233 | 4 | MD 30.15 | 22.29, 38.02 | **<0.00001** | $I^2$=0%, P=0.79 | $I^2$=0%, P=0.57 |
| IWH PrUs | Included | 222 | 5 | RR 0.18 | 0.06, 0.60 | **0.005** | $I^2$=0%, P=0.97 | $I^2$=0%, P=0.85 |
| | Excluded | 206 | 4 | RR 0.22 | 0.06, 0.79 | **0.02** | $I^2$=0%, P=0.96 | $I^2$=0%, P=0.94 |

**Table S4. Summary of sensitivity analyses results;** fixed- vs random-effects models, and inclusion vs exclusion of high risk of bias studies analyses were performed; PrUs, pressure ulcers; DUs, diabetic ulcers; ICH, incidence of complete healing; WHS, wound-healing status; IWH, incidence of worsening of healing; № U, number of ulcers; № S, number of studies; 95% CI, 95% confidence interval; RR, relative risk; MD, mean difference; N/A, not available or not applicable

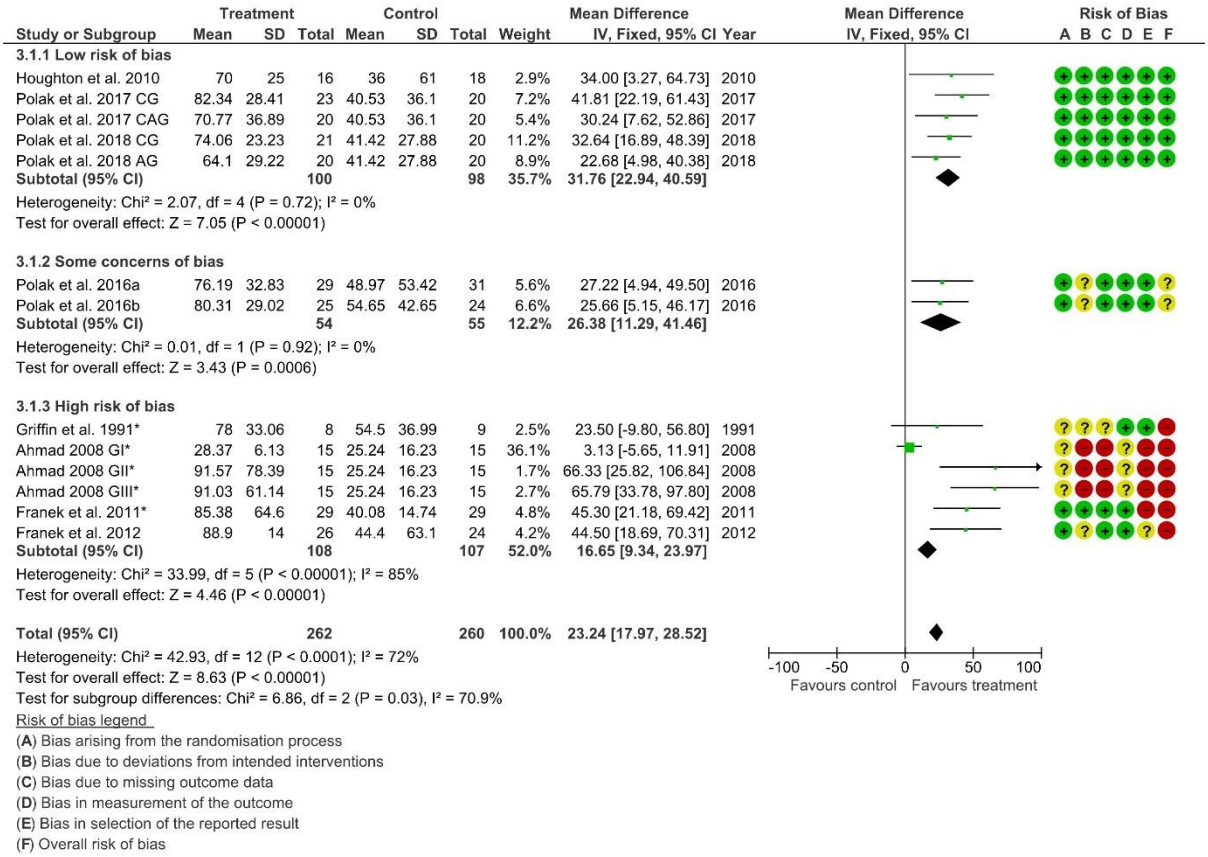
## 3.9.1 Fixed-effects model

### 3.9.1.1 Incidence of complete healing of pressure ulcers



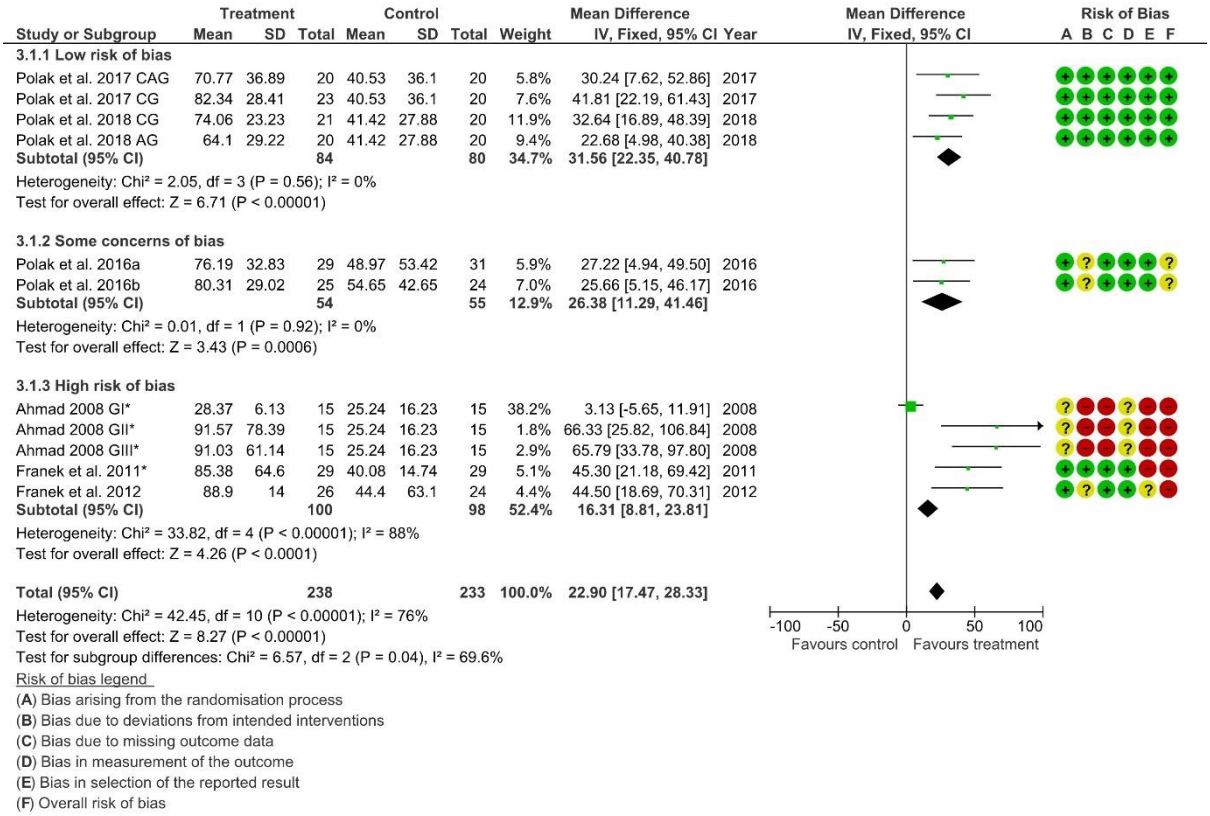

**Forest plot of relative risk (RR) analysis of proportion of completely healed pressure ulcers;** RR>1, probability of complete healing is higher in treatment arm; RR=1, no difference in probability of complete healing between treatment and control arms; RR<1, probability of complete healing is lower in treatment arm. The number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group; AG, anodal group

## 3.9.1.2 Percent wound surface area reduction of pressure ulcers (all studies)



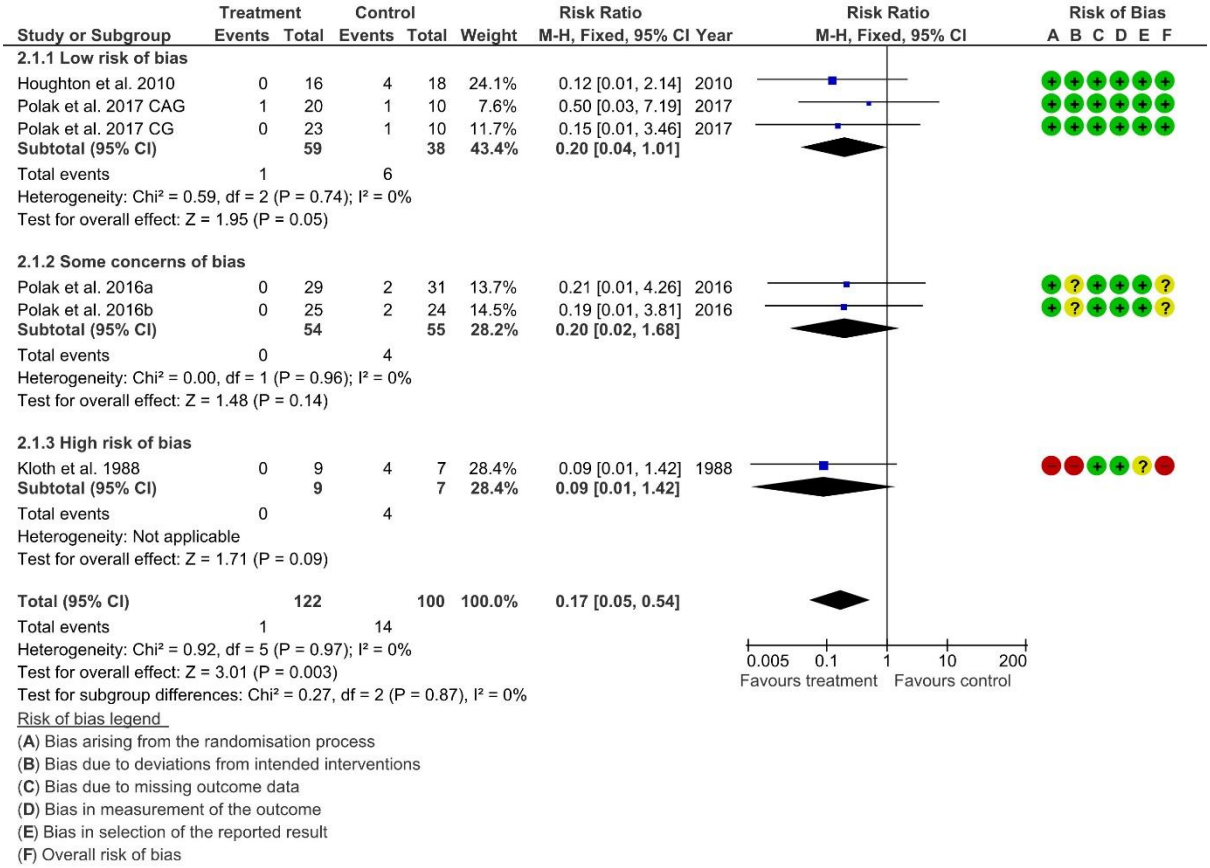

**Forest plot of mean difference (MD) analysis of percent wound surface area reduction of pressure ulcers at 20 days-12 weeks;** values smaller than zero favors control arm, whereas values greater than zero favors treatment arm. The biological sample size (n) is 452 participants; experimental arm (n=262), and control arm (n=190); the number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group; AG, anodal group; *, mean and/or standard deviation values were calculated and/or imputed for this sample

## 3.9.1.3 Percent wound surface area reduction of pressure ulcers at 4-8 weeks



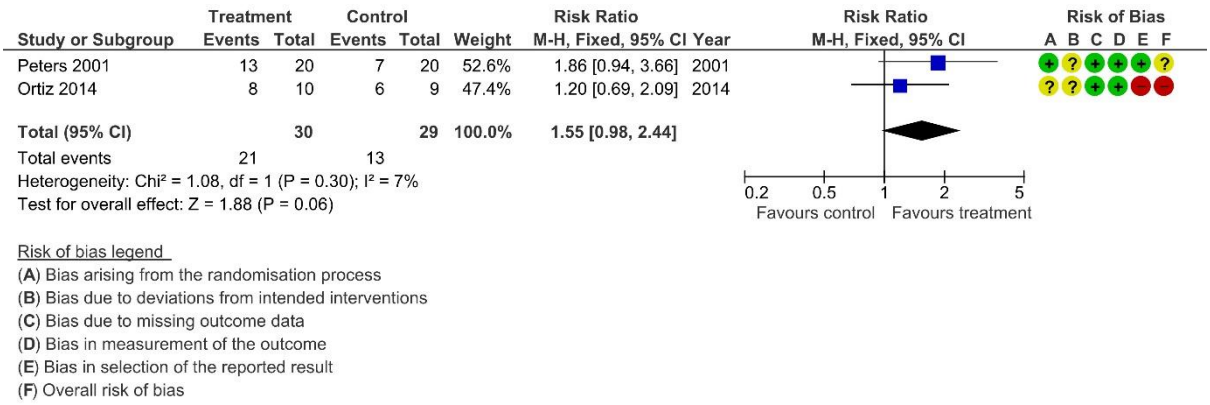

**Forest plot of mean difference (MD) analysis of percent wound surface area reduction of pressure ulcers at 4-8 weeks;** values smaller than zero favors control arm, whereas values greater than zero favors treatment arm. The biological sample size (n) is 401 participants; experimental arm (n=238), and control arm (n=163); the number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group; AG, anodal group; *, mean and/or standard deviation values were calculated and/or imputed for this sample

## 3.9.1.4 Incidence of worsening of healing of pressure ulcers




**Forest plot of relative risk (RR) analysis of proportion of worsened pressure ulcers;** RR>1, probability of worsening of healing is higher in control arm; RR=1, no difference in probability of worsening of healing between treatment and control arms; RR<1, probability of worsening of healing is lower in treatment arm. The number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group

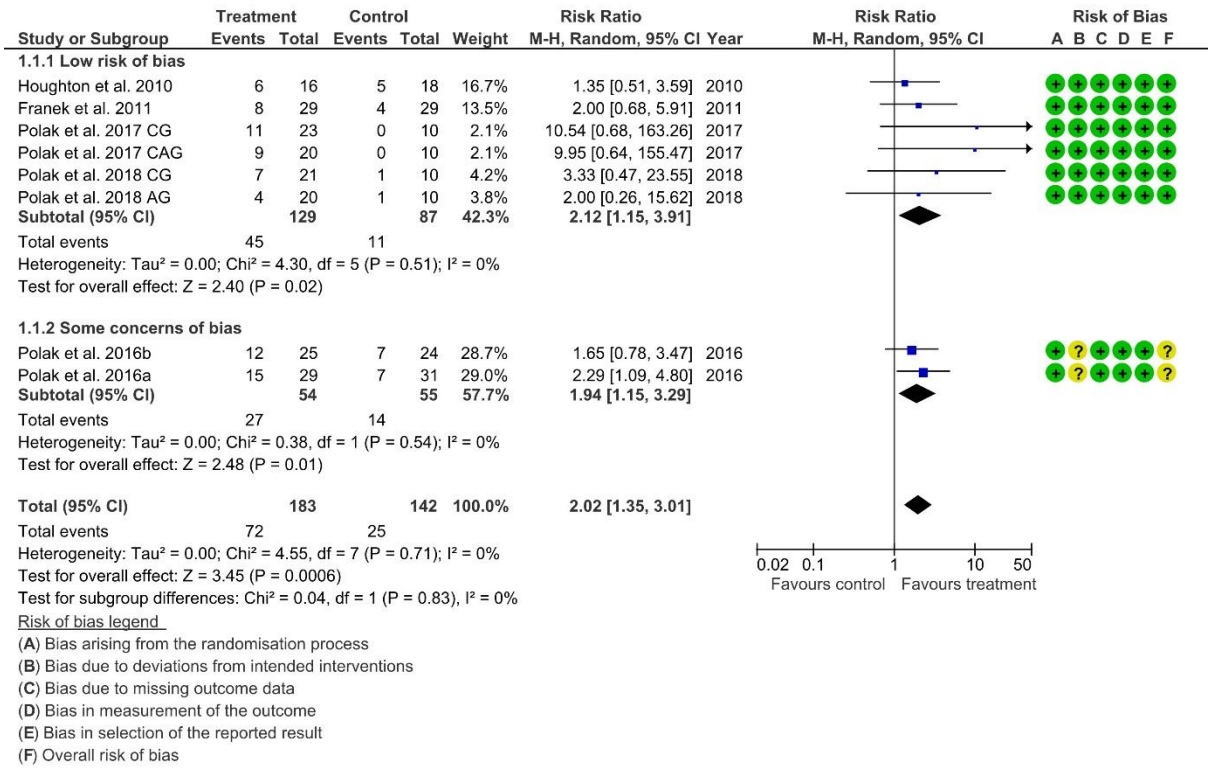
## 3.9.1.5 Incidence of complete healing of diabetic ulcers




**Forest plot of relative risk (RR) analysis of proportion of completely healed diabetic ulcers;** RR>1, probability of complete healing is higher in treatment arm; RR=1, no difference in probability of complete healing between treatment and control arms; RR<1, probability of complete healing is lower in treatment arm

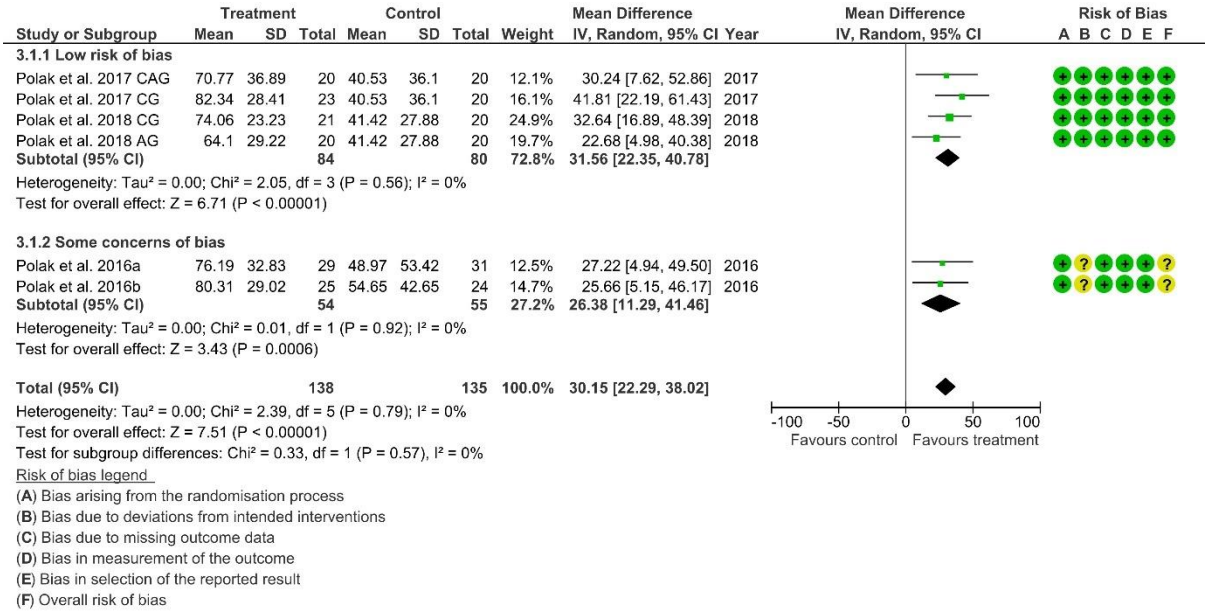
## 3.9.2 Exclusion of high risk of bias studies

### 3.9.2.1 Incidence of complete healing of pressure ulcers



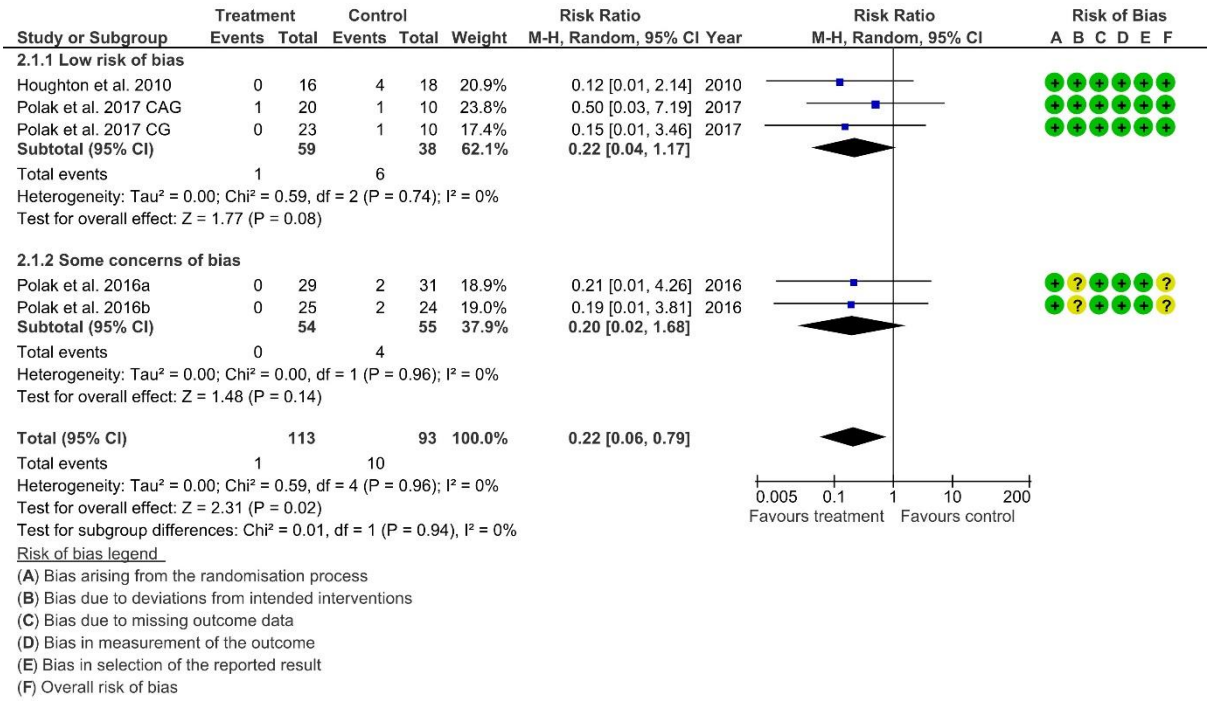

**Forest plot of relative risk (RR) analysis of proportion of completely healed pressure ulcers;** RR>1, probability of complete healing is higher in treatment arm; RR=1, no difference in probability of complete healing between treatment and control arms; RR<1, probability of complete healing is lower in treatment arm. The number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group; AG, anodal group

## 3.9.2.2 Percent wound surface area reduction of pressure ulcers at 4-8 weeks




**Forest plot of mean difference (MD) analysis of percent wound surface area reduction of pressure ulcers at 4-8 weeks;** values smaller than zero favors control arm, whereas values greater than zero favors treatment arm. The biological sample size (n) is 401 participants; experimental arm (n=238), and control arm (n=163); the number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group; AG, anodal group

## 3.9.2.3 Incidence of worsening of healing of pressure ulcers




**Forest plot of relative risk (RR) analysis of proportion of worsened pressure ulcers;** RR>1, probability of worsening of healing is higher in control arm; RR=1, no difference in probability of worsening of healing between treatment and control arms; RR<1, probability of worsening of healing is lower in treatment arm. The number of participants is slightly inflated by (n=8) as one study included participants with more than one ulcer. CG, cathodal group; CAG, cathodal-anodal group

## 3.10 Risk of reporting biases

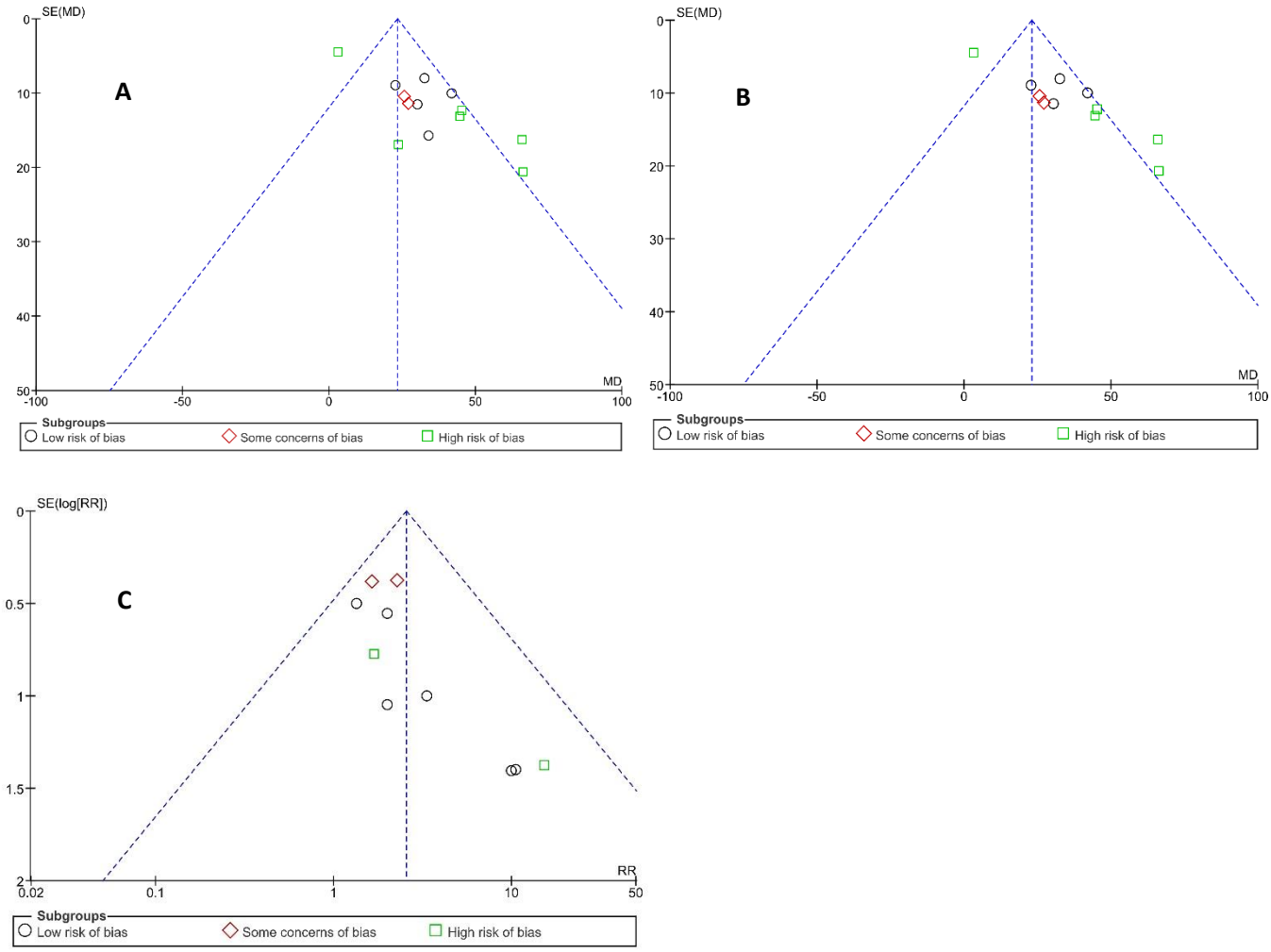



**Figure S5. Funnel plots to investigate publication bias; asymmetry may suggest publication bias in synthesis;** A, funnel plot of all studies reporting on percent wound surface area reduction; B, funnel plot of studies reporting percent wound surface area reduction in 4-8 weeks; C, funnel plot of studies reporting on incidence of complete healing

### 3.10.1 Percent wound surface area reduction in all studies assessing pressure ulcers (20 days to 12 weeks)

```
Regression-based Egger test for small-study effects
Random-effects model
Method: REML

H0: beta1 = 0; no small-study effects
            beta1 =      3.45
      SE of beta1 =     0.799
                z =      4.32
       Prob > |z| =    0.0000
Begg's test for small-study effects

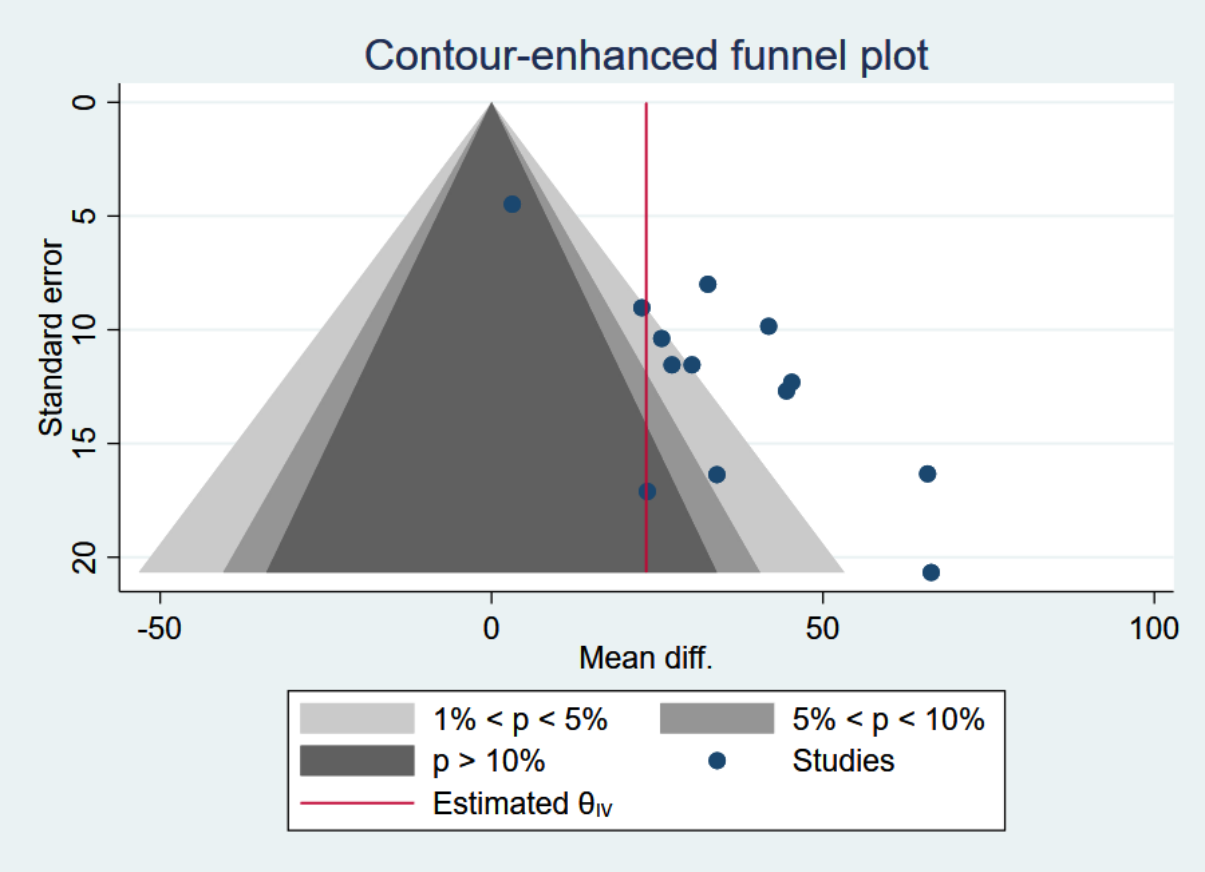
Kendall's score =     26.00
    SE of score =    16.391
              z =      1.53
     Prob > |z| =    0.1272
Nonparametric trim-and-fill analysis of publication bias
Linear estimator, imputing on the left

Iteration                           Number of studies =     19
  Model: Random-effects                      observed =     13
 Method: REML                                 imputed =      6

Pooling
  Model: Random-effects
 Method: REML
```

| Studies | Mean Diff. | [95% Conf. | Interval] |
|---|---|---|---|
| Observed | 32.218 | 22.555 | 41.881 |
| Observed + Imputed | 22.244 | 12.208 | 32.280 |

3.10.2 Percent wound surface area reduction in all studies assessing pressure ulcers (20 days to 12 weeks)



### 3.10.3 Percent wound surface area reduction in 4-8 weeks in studies assessing pressure ulcers

```
Regression-based Egger test for small-study effects
Random-effects model
Method: REML

H0: beta1 = 0; no small-study effects
            beta1 =      4.53
      SE of beta1 =     0.749
                z =      6.05
       Prob > |z| =    0.0000
Begg's test for small-study effects

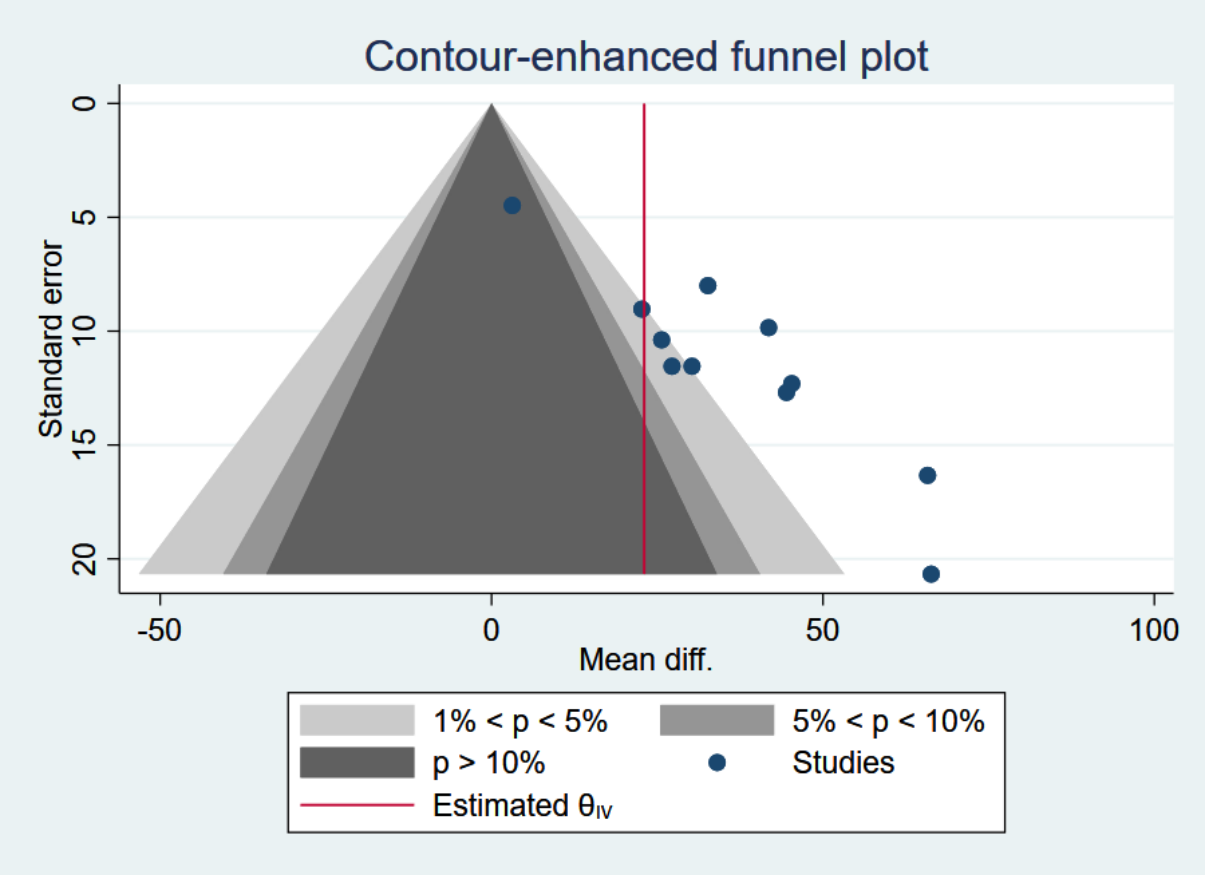
Kendall's score =     31.00
    SE of score =    12.845
              z =      2.34
     Prob > |z| =    0.0195
Nonparametric trim-and-fill analysis of publication bias
Linear estimator, imputing on the left

Iteration                              Number of studies =     14
  Model: Random-effects                         observed =     11
 Method: REML                                    imputed =      3

Pooling
  Model: Random-effects
 Method: REML
```

| Studies | Mean Diff. | [95% Conf. | Interval] |
|---|---|---|---|
| Observed | 32.987 | 22.199 | 43.775 |
| Observed + Imputed | 27.156 | 16.232 | 38.081 |

3.10.4 Percent wound surface area reduction in 4-8 weeks in studies assessing pressure ulcer



### 3.10.5 Proportion of complete healing in studies assessing pressure ulcers

```
Regression-based Egger test for small-study effects
Random-effects model
Method: REML

H0: beta1 = 0; no small-study effects
            beta1 =      1.79
      SE of beta1 =     0.751
                z =      2.38
       Prob > |z| =    0.0172
Begg's test for small-study effects

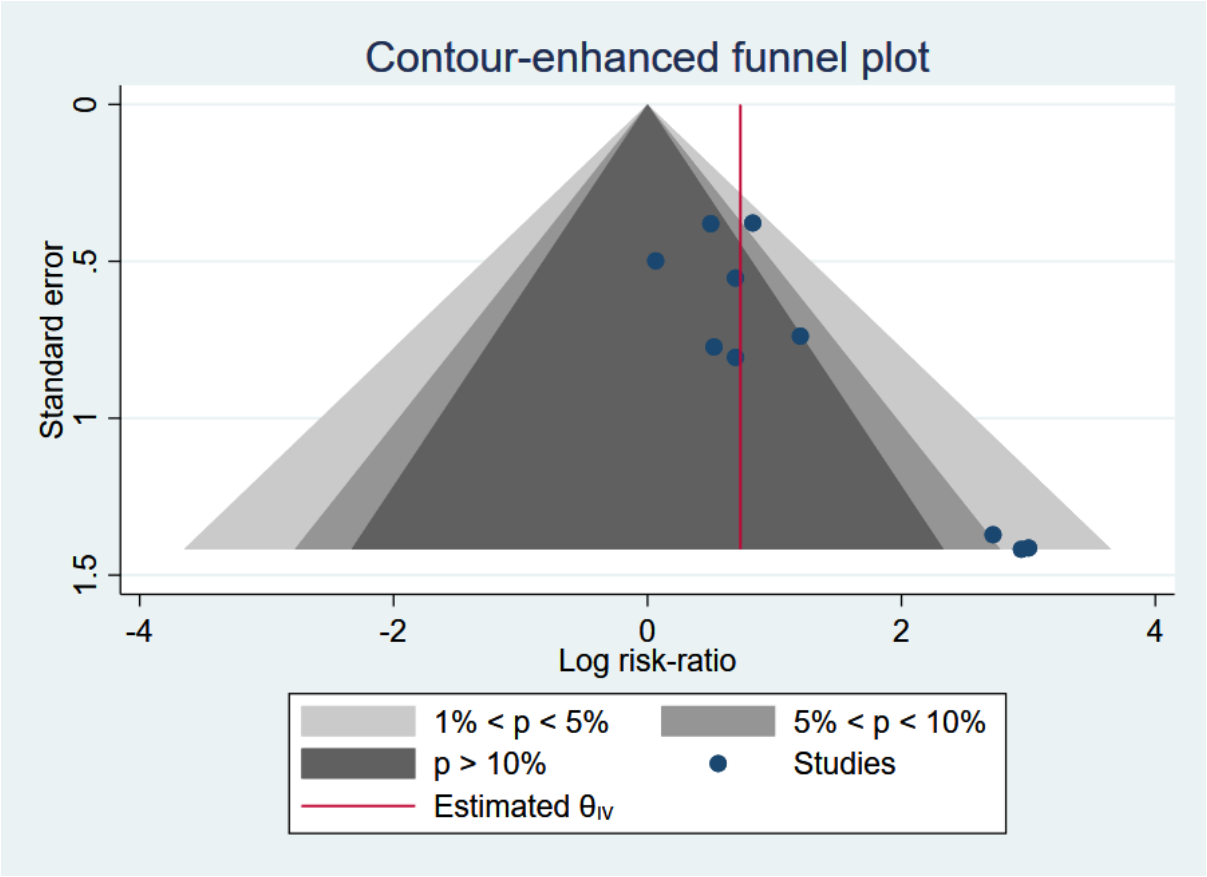
Kendall's score =     25.00
    SE of score =    11.180
              z =      2.15
     Prob > |z| =    0.0318
Nonparametric trim-and-fill analysis of publication bias
Linear estimator, imputing on the left

Iteration                             Number of studies =     13
  Model: Random-effects                        observed =     10
 Method: REML                                   imputed =      3

Pooling
  Model: Random-effects
 Method: REML
```

| Studies | Log Risk-Ratio | [95% Conf. | Interval] |
|---|---|---|---|
| Observed | 0.732 | 0.360 | 1.104 |
| Observed + Imputed | 0.607 | 0.245 | 0.968 |

3.10.6 Proportion of complete healing in studies assessing pressure ulcers



### 3.10.7 Proportion of worsening of healing in studies assessing pressure ulcers

```
Regression-based Egger test for small-study effects
Random-effects model
Method: REML

H0: beta1 = 0; no small-study effects
            beta1 =      0.48
      SE of beta1 =     2.845
                z =      0.17
       Prob > |z| =    0.8666

Begg's test for small-study effects

Kendall's score =     11.00
    SE of score =     6.658
              z =      1.50
     Prob > |z| =    0.1331

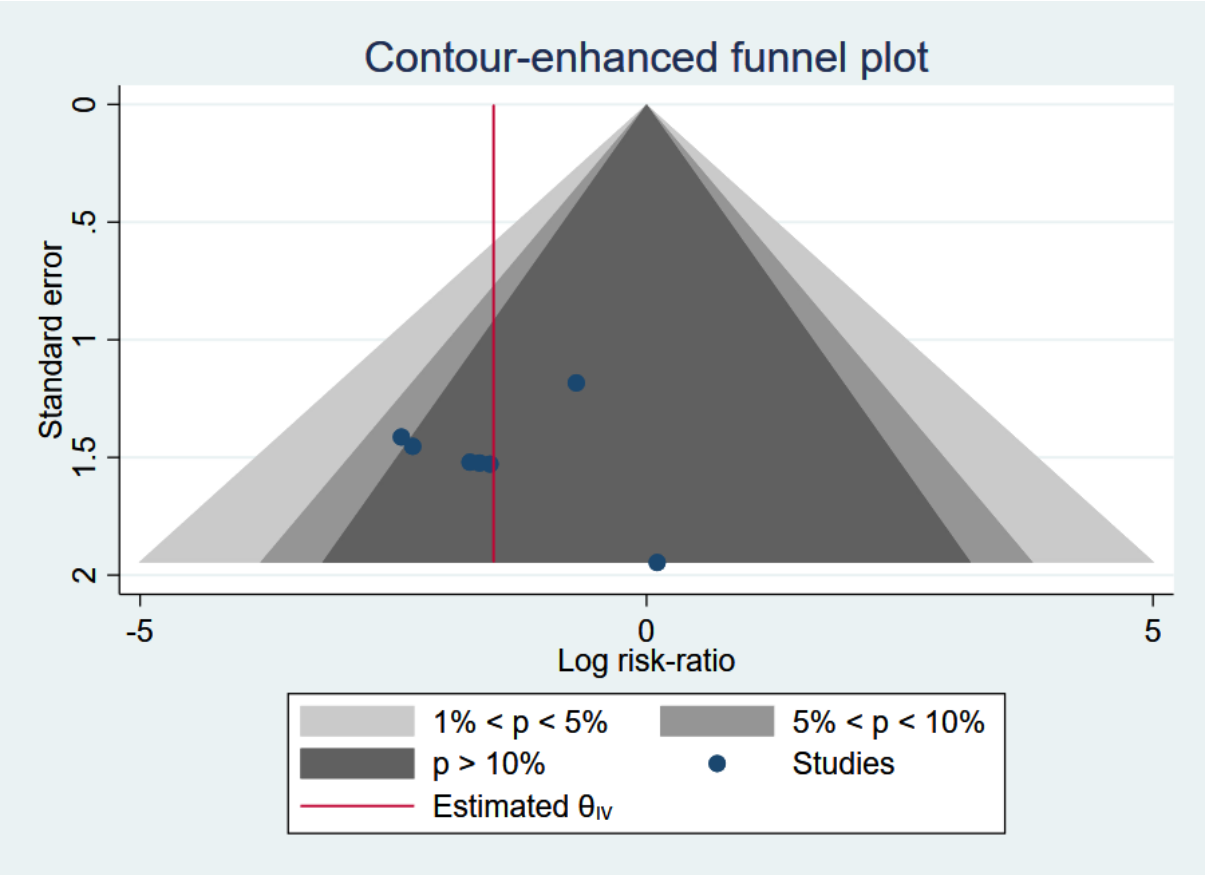
Nonparametric trim-and-fill analysis of publication bias
Linear estimator, imputing on the left

Iteration                             Number of studies =      7
  Model: Random-effects                        observed =      7
 Method: REML                                  imputed =      0

Pooling
  Model: Random-effects
 Method: REML
```

| Studies | Log Risk-Ratio | [95% Conf. | Interval] |
|---|---|---|---|
| Observed | -1.510 | -2.598 | -0.422 |
| Observed + Imputed | -1.510 | -2.598 | -0.422 |

3.10.8 Proportion of worsening of healing in studies assessing pressure ulcers



## 4. Discussion

Positive effects of HVMPC were observed in a number of *in vivo* animal studies, which largely mirrored the findings in human studies. These studies included wounds of variable etiologies; diabetic wounds (Kim et al., 2014; X.-F. Wang et al., 2021), burn wounds (Ahmed & Khalaf, 2006; Cruz et al., 1989) and surgical wounds (Brown et al., 1995; Brown et al., 1989; Choi et al., 1995; Jeon et al., 2015). Potential explanations to these effects may be provided by studies which concluded that HVMPC may increase fibroblast (G. J. Bourguignon & L. Y. Bourguignon, 1987; G. J. Bourguignon et al., 1989; Cruz et al., 1989) and keratinocyte proliferation (Lee et al., 2004), enhance collagen synthesis (G. J. Bourguignon & L. Y. Bourguignon, 1987; Falanga, 1987; Jeon et al., 2015; Kim et al., 2014; Lee et al., 2010; Lee et al., 2003), augment blood flow (Heath & GIBBS, 1992; Hecker et al., 1985; Mohr et al., 1987), and have antibacterial effects (Gomes et al., 2015; Herminawaty et al., 2014; Kang et al., 1996; Kincaid & Lavoie, 1989; Kuykendall et al., 2004; Merriman et al., 2004; Szuminsky et al., 1994; Wu et al., 2021).

The mechanisms behind the reported effects of HVMPC may be further explained by several *in vitro* studies. An increase in the influx of calcium ions was observed within the first min, which reached a maximum level by 3 min of HVMPC ES of human dermal fibroblasts (G. J. Bourguignon et al., 1989). Moreover, a sharp increase in insulin binding to fibroblasts began during the second min, and reached close to a maximum level after 3 min of stimulation (G. J. Bourguignon et al., 1989). The same phenomena were reported following HVMPC ES of mouse splenic T-lymphocytes (Bourguignon et al., 1988; Jy et al., 1989). Additionally, exposure to electric fields was shown to induce calcium influx in fibroblasts, keratinocytes (Cho et al., 2002; Dubé et al., 2012; Onuma

& Hui, 1988), and other cell types (Beebe et al., 2003; Cho et al., 1999; Thompson et al., 2014; Titushkin & Cho, 2009; White et al., 2004; Zhang et al., 2008). Calcium signaling pathways are well-known regulators of cell differentiation and proliferation (Berridge, 1995; Berridge et al., 2000; Roderick & Cook, 2008). The messenger role of calcium may be carried through a number of calcium-binding proteins which function as buffers to modulate cellular responses (Lansdown, 2002).

Indeed, a calcium and calmodulin-mediated contractile system was suggested to be involved in the exposure of interleukin-2 (IL-2) receptors, and the subsequent increase in protein and DNA synthesis in response to HVMPC (G. Bourguignon & L. Bourguignon, 1987; G. J. Bourguignon et al., 1989; Bourguignon et al., 1988; Jy et al., 1989). The exposure of IL-2 receptors might be directly upregulated by IL-2 (Depper et al., 1985; Kotturi et al., 2003), which might be produced through the calcium–calcineurin–NFAT signaling pathway (Chow et al., 1999; Hogan et al., 2003). IL-2 receptors were identified on the membrane of human fibroblasts and keratinocytes (Plaisance et al., 1994; Plaisance et al., 1992; Rocha-Zavaleta et al., 2004; Yasumura et al., 1994). It was reported that IL-2 may promote growth and stimulate DNA synthesis of fibroblasts (Abdullah et al., 1991; Kang et al., 2013; Ozawa et al., 2003). Moreover, T-lymphocytes, in particular, may have a modulatory role in wound healing (Martin & Muir, 1990; Schäffer & Barbul, 1998), and their growth and proliferation may be regulated by IL-2 (Leonard et al., 1985; Neckers & Cossman, 1983; Reem & Yeh, 1984). Decreased synthesis of IL-2 in T-lymphocytes, possibly due to an intrinsic defect, was reported in insulin-dependent diabetes mellitus (IDDM) (Luger et al., 1988; Roncarolo et al., 1988; Tomoda et al., 1994; Zier et al., 1984). Nevertheless, the exact

function of IL-2 in T-lymphocytes remains unknown since a paradoxical immunosuppressive effect was suggested (Ross & Cantrell, 2018).

The rise in insulin binding following HVMPC was attributed to an increase in the number of insulin receptors rather than an increase in receptor binding affinity (G. J. Bourguignon et al., 1989; Bourguignon et al., 1988; Jy et al., 1989). The exposure of insulin receptors might occur through the calcium-calmodulin-dependent protein kinases types II/IV-CREB (Liu et al., 2012; Sun et al., 1994), and the calcium-calcineurin-NFAT signaling pathways (Demozay et al., 2011; Soleimanpour et al., 2010). Human dermal fibroblasts and keratinocytes were found to have insulin receptors (Berhanu & Olefsky, 1981; Dunaif et al., 1995; Rechler & Podskalny, 1976; Verrando & Ortonne, 1985). Insulin may impact glucose entry, growth, migration and proliferation of these cells (Benoliel et al., 1997; Conover et al., 1989; Fujimoto & Williams, 1974; Hidaka et al., 1980; Howard et al., 1979; Liu et al., 2009; Saltiel & Kahn, 2001; Schüppel et al., 2008; Shen et al., 2001). High-glucose levels in the microenvironment of fibroblasts and keratinocytes, nonetheless, may negatively influence various cellular functions (Hehenberger & Hansson, 1997; Hehenberger, Heilborn, et al., 1998; Hu & Lan, 2016; Lan et al., 2008; Li et al., 2019; Mendoza-Naranjo et al., 2012; Spravchikov et al., 2001; Xuan et al., 2014). Fibroblasts derived from diabetic ulcers were shown to have reduced growth, migration and proliferation (Blakytny et al., 2000; Hehenberger, Heilborn, et al., 1998; Hehenberger, Kratz, et al., 1998; Lerman et al., 2003; Liang et al., 2016; Loots et al., 1999; Mendoza-Naranjo et al., 2012; Rowe et al., 1977). Furthermore, hyperproliferation with inhibited migration of keratinocytes, obtained from the edges of chronic diabetic wounds, were reported (Stojadinovic et al., 2005; Usui et al., 2008; Wang et al., 2020).

## References


Abdullah, A., McCauley, R. L., & Herndon, D. N. (1991). Stimulation of human dermal fibroblasts with interleukin 2. *J Burn Care Rehabil, 12*(1), 23-25. doi:http://dx.doi.org/10.1097/00004630-199101000-00006

Ahmad, E. T. (2008). High-voltage pulsed galvanic stimulation: effect of treatment duration on healing of chronic pressure ulcers. *Annals of Burns and Fire Disasters 2008 Sep;21(3):124-128*.

Ahmed, E. T., & Khalaf, M. M. A.-k. (2006). Comparison of the Effect of High Voltage Pulsed Galvanic Stimulation versus Ultrasonic on the Acceleration of Burn Wound Healing.

Akers, T. K., & Gabrielson, A. L. (1984). The effect of high voltage galvanic stimulation on the rate of healing of decubitus ulcers. *Biomed Sci Instrum, 20*, 99-100. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6608962

Beebe, S. J., White, J., Blackmore, P. F., Deng, Y., Somers, K., & Schoenbach, K. H. (2003). Diverse effects of nanosecond pulsed electric fields on cells and tissues. *DNA and cell biology, 22*(12), 785-796. doi:http://dx.doi.org/10.1089/104454903322624993

Benoliel, A.-M., Kahn-Peries, B., Imbert, J., & Verrando, P. (1997). Insulin stimulates haptotactic migration of human epidermal keratinocytes through activation of NF-kappa B transcription factor. *Journal of Cell Science, 110*(17), 2089-2097. doi:http://dx.doi.org/10.1242/jcs.110.17.2089

Berhanu, P., & Olefsky, J. M. (1981). Effects of insulin and insulin-like agents on the glucose transport system of cultured human fibroblasts. *Diabetes, 30*(6), 523-529. doi:http://dx.doi.org/10.2337/diab.30.6.523

Berridge, M. J. (1995). Calcium signalling and cell proliferation. *Bioessays, 17*(6), 491-500. doi:http://dx.doi.org/10.1002/bies.950170605

Berridge, M. J., Lipp, P., & Bootman, M. D. (2000). The versatility and universality of calcium signalling. *Nature reviews Molecular cell biology, 1*(1), 11-21. doi:http://dx.doi.org/10.1038/35036035

Blakytny, R., Jude, E. B., Martin Gibson, J., Boulton, A. J., & Ferguson, M. W. (2000). Lack of insulin-like growth factor 1 (IGF1) in the basal keratinocyte layer of diabetic skin and diabetic foot ulcers. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 190*(5), 589-594. doi:http://dx.doi.org/10.1002/(SICI)1096-9896(200004)190:5<589::AID-PATH553>3.0.CO;2-T

Bourguignon, G., & Bourguignon, L. (1987). Electric stimulation of protein and DNA synthesis in human fibroblasts. *The FASEB Journal, 1*(5), 398-402. doi:http://dx.doi.org/10.1096/fasebj.1.5.3678699

Bourguignon, G. J., & Bourguignon, L. Y. (1987). Electric stimulation of protein and DNA synthesis in human fibroblasts. *FASEB J, 1*(5), 398-402. doi:http://dx.doi.org/10.1096/fasebj.1.5.3678699

Bourguignon, G. J., Jy, W., & Bourguignon, L. Y. (1989). Electric stimulation of human fibroblasts causes an increase in Ca2+ influx and the exposure of additional insulin receptors. *J Cell Physiol, 140*(2), 379-385. doi:http://dx.doi.org/10.1002/jcp.1041400224

Bourguignon, L. Y., Jy, W., Majercik, M. H., & Bourguignon, G. J. (1988). Lymphocyte activation and capping of hormone receptors. *J Cell Biochem, 37*(2), 131-150. doi:http://dx.doi.org/10.1002/jcb.240370202

Brown, M., Gogia, P. P., Sinacore, D. R., & Menton, D. N. (1995). High-voltage galvanic stimulation on wound healing in guinea pigs: longer-term effects. *Archives of physical medicine and rehabilitation, 76*(12), 1134-1137. doi:http://dx.doi.org/10.1016/S0003-9993(95)80122-7

Brown, M., McDonnell, M. K., & Menton, D. N. (1989). Polarity effects on wound healing using electric stimulation in rabbits. *Archives of physical medicine and rehabilitation, 70*(8), 624-627. doi:http://dx.doi.org/10.5555/uri:pii:0003999389900919

Cho, M. R., Marler, J. P., Thatte, H. S., & Golan, D. E. (2002). Control of calcium entry in human fibroblasts by frequency-dependent electrical stimulation. *Front Biosci, 7*(1), 1-8. doi:http://dx.doi.org/10.2741/A733

Cho, M. R., Thatte, H. S., Silvia, M. T., & Golan, D. E. (1999). Transmembrane calcium influx induced by ac electric fields. *The FASEB Journal, 13*(6), 677-683. doi:http://dx.doi.org/10.1096/fasebj.13.6.677

Choi, E.-Y., Lee, J.-H., & JeKal, S.-J. (1995). Effect of High Voltage Pulsed Current Stimulation on Surgical Wound of Rat. *Journal of Korean Physical Therapy Science, 2*(2), 495-508. doi:http://dx.doi.org/10.1589/jpts.27.1485

Chow, C.-W., Rincón, M., & Davis, R. J. (1999). Requirement for transcription factor NFAT in interleukin-2 expression. *Molecular and cellular biology, 19*(3), 2300-2307. doi:http://dx.doi.org/10.1128/MCB.19.3.2300

Conover, A., Hintz, R., & Rosenfeld, R. (1989). Direct evidence that the insulin receptor mediates a mitogenic response in cultured human fibroblasts. *Hormone and metabolic research, 21*(02), 59-63. doi:http://dx.doi.org/10.1055/s-2007-1009151

Cruz, N. I., Bayrón, F. E., & Suárez, A. J. (1989). Accelerated healing of full-thickness burns by the use of high-voltage pulsed galvanic stimulation in the pig. *Annals of Plastic Surgery, 23*(1), 49-55. doi:http://dx.doi.org/10.1097/00000637-198907000-00009

Demozay, D., Tsunekawa, S., Briaud, I., Shah, R., & Rhodes, C. J. (2011). Specific glucose-induced control of insulin receptor substrate-2 expression is mediated via Ca2+-dependent calcineurin/NFAT signaling in primary pancreatic islet β-cells. *Diabetes, 60*(11), 2892-2902. doi:http://dx.doi.org/10.2337/db11-0341

Depper, J. M., Leonard, W. J., Drogula, C., Krönke, M., Waldmann, T. A., & Greene, W. C. (1985). Interleukin 2 (IL-2) augments transcription of the IL-2 receptor gene. *Proceedings of the National Academy of Sciences, 82*(12), 4230-4234. doi:http://dx.doi.org/10.1073/pnas.82.12.4230

Dubé, J., Rochette-Drouin, O., Lévesque, P., Gauvin, R., Roberge, C. J., Auger, F. A., . . . Moulin, V. J. (2012). Human keratinocytes respond to direct current stimulation by increasing intracellular calcium: preferential response of poorly differentiated cells. *Journal of cellular physiology, 227*(6), 2660-2667. doi:http://dx.doi.org/10.1002/jcp.23008

Dunaif, A., Xia, J., Book, C.-B., Schenker, E., & Tang, Z. (1995). Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. *The Journal of clinical investigation, 96*(2), 801-810. doi:http://dx.doi.org/10.1172/JCI118126

Elshazly, F., & Hassan, A. (1998). Influence of High Voltage Pulsed Galvanic Stimulation on Wound Healing in Children with Burn. *Bull. Fac. Ph. Th. Cairo Univ., Vol 3*(1), 61-68.

Falanga, V. (1987). Electrical stimulation increases the expression of fibroblasts receptors for transforming growth factor-beta. *J Invest Dermatol, 88*, A.
Fitzgerald, G. K., & Newsome, D. (1993). Treatment of a large infected thoracic spine wound using high voltage pulsed monophasic current. *Physical Therapy, 73*(6), 355-360. doi:http://dx.doi.org/10.1093/ptj/73.6.355
Franek, A., Kostur, R., Polak, A., Taradaj, J., Szlachta, Z., Blaszczak, E., . . . Kucio, C. (2012). Using high-voltage electrical stimulation in the treatment of recalcitrant pressure ulcers: results of a randomized, controlled clinical study. *Ostomy-Wound Management, 58*(3), 30.
Franek, A., Kostur, R., Taradaj, J., Blaszczak, E., Szlachta, Z., Dolibog, P., . . . Polak, A. (2011). Effect of High Voltage Monophasic Stimulation on Pressure Ulcer Healing: Results From a Randomized Controlled Trial. *WOUNDS-A COMPENDIUM OF CLINICAL RESEARCH AND PRACTICE, 23*(1), 15-23.
Fujimoto, W. Y., & Williams, R. H. (1974). Insulin action on the cultured human fibroblast: glucose uptake, protein synthesis, RNA synthesis. *Diabetes, 23*(5), 443-448. doi:http://dx.doi.org/10.2337/diab.23.5.443
Gilcreast, D. M., Stotts, N. A., Froelicher, E. S., Baker, L. L., & Moss, K. M. (1998). Effect of electrical stimulation on foot skin perfusion in persons with or at risk for diabetic foot ulcers. *Wound Repair and Regeneration, 6*(5), 434-441. doi:http://dx.doi.org/10.1046/j.1524-475X.1998.60505.x
Goldman, R., Brewley, B., Zhou, L., & Golden, M. (2003). Electrotherapy reverses inframalleolar ischemia: a retrospective, observational study. 2003b. *Adv Skin Wound Care, 16*(2), 79-89. doi:http://dx.doi.org/10.1097/00129334-200303000-00009
Goldman, R., Rosen, M., Brewley, B., & Golden, M. (2004). Electrotherapy promotes healing and microcirculation of infrapopliteal ischemic wounds: a prospective pilot study. *Adv Skin Wound Care, 17*(6), 284-294. doi:http://dx.doi.org/10.1097/00129334-200407000-00010
Goldman, R. J., Brewley, B. I., Cohen, R., & Rudnick, M. (2003). Use of electrotherapy to reverse expanding cutaneous gangrene in end-stage renal disease. 2003a. *Adv Skin Wound Care, 16*(7), 363-366. doi:http://dx.doi.org/10.1097/00129334-200312000-00013
Goldman, R. J., Brewley, B. I., & Golden, M. A. (2002). Electrotherapy reoxygenates inframalleolar ischemic wounds on diabetic patients: a case series. *Adv Skin Wound Care, 15*(3), 112-120. doi:http://dx.doi.org/10.1097/00129334-200205000-00006
Gomes, R. C., Brandino, H. E., de Sousa, N. T., Santos, M. F., Martinez, R., & Guirro, R. R. (2015). Polarized currents inhibit in vitro growth of bacteria colonizing cutaneous ulcers. *Wound Repair Regen, 23*(3), 403-411. doi:http://dx.doi.org/10.1111/wrr.12296
Gomes, R. C., Guirro, E. C., Gonçalves, A. C., Junior, J. A. F., Junior, L. O. M., & Guirro, R. R. (2018). High-voltage electric stimulation of the donor site of skin grafts accelerates the healing process. A randomized blinded clinical trial. *BURNS, 44*(3), 636-645. doi:http://dx.doi.org/10.1016/j.burns.2017.10.006
Griffin, J. W., Tooms, R. E., Mendius, R. A., Clifft, J. K., Vander Zwaag, R., & el-Zeky, F. (1991). Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. *Physical Therapy 1991 Jun;71(6):433-442*. doi:http://dx.doi.org/10.1093/ptj/71.6.433
Gui, M. S., Guirro, R. R. d. J., Sakabe, D. I., & Sakabe, F. F. (2013). High voltage pulsed stimulation increases cicatrization of chronic cutaneous ulcers: analysis

of six cases. *Fisioterapia e Pesquisa, 20*, 286-292. doi:http://dx.doi.org/10.1590/S1809-29502013000300014
Heath, M. E., & GIBBS, S. B. (1992). High-voltage pulsed galvanic stimulation: effects of frequency of current on blood flow in the human calf muscle. *Clinical Science, 82*(6), 607-613. doi:http://dx.doi.org/10.1042/cs0820607
Hecker, B., Carron, H., & Schwartz, D. P. (1985). Pulsed galvanic stimulation: effects of current frequency and polarity on blood flow in healthy subjects. *Arch Phys Med Rehabil, 66*(6), 369-371. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/4004533
Hehenberger, K., & Hansson, A. (1997). High glucose-induced growth factor resistance in human fibroblasts can be reversed by antioxidants and protein kinase C-inhibitors. *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease, 15*(3), 197-201. doi:http://dx.doi.org/10.1002/(SICI)1099-0844(199709)15:3<197::AID-CBF740>3.0.CO;2-7
Hehenberger, K., Heilborn, J. D., Brismar, K., & Hansson, A. (1998). Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal dermal fibroblasts treated with high glucose is associated with increased formation of L-lactate. *Wound Repair and Regeneration, 6*(2), 135-141. doi:http://dx.doi.org/10.1046/j.1524-475X.1998.60207.x
Hehenberger, K., Kratz, G., Hansson, A., & Brismar, K. (1998). Fibroblasts derived from human chronic diabetic wounds have a decreased proliferation rate, which is recovered by the addition of heparin. *Journal of Dermatological Science, 16*(2), 144-151. doi:http://dx.doi.org/10.1016/S0923-1811(97)00042-X
Herminawaty, D., Defi, I., Probowo, T., & Parwati, T. (2014). Promising treatment for pressure ulcers using high voltage pulsed current stimulation. *Gerontechnology, 13*(2), 202. doi:http://dx.doi.org/10.4017/gt.2014.13.02.266.00
Hidaka, H., Howard, B. V., Kosmakos, F. C., Fields, R. M., Craig, J. W., Bennett, P. H., & Larner, J. (1980). Insulin stimulation of glycogen synthase in cultured human diploid fibroblasts. *Diabetes, 29*(10), 806-810. doi:http://dx.doi.org/10.2337/diacare.20.10.806
Hogan, P. G., Chen, L., Nardone, J., & Rao, A. (2003). Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes & development, 17*(18), 2205-2232. doi:http://dx.doi.org/10.1101/gad.1102703
Houghton, P. E., Campbell, K. E., Fraser, C. H., Harris, C., Keast, D. H., Potter, P. J., . . . Woodbury, M. G. (2010). Electrical stimulation therapy increases rate of healing of pressure ulcers in community-dwelling people with spinal cord injury. *Archives of Physical Medicine and Rehabilitation 2010 May;91(5):669-678*. doi:http://dx.doi.org/10.1016/j.apmr.2009.12.026
Houghton, P. E., Kincaid, C. B., Lovell, M., Campbell, K. E., Keast, D. H., Woodbury, M. G., & Harris, K. A. (2003). Effect of electrical stimulation on chronic leg ulcer size and appearance. *Physical Therapy, 83*(1), 17-28. doi:http://dx.doi.org/10.1093/ptj/83.1.17
Howard, B. V., Mott, D. M., Fields, R. M., & Bennett, P. H. (1979). Insulin stimulation of glucose entry in cultured human fibroblasts. *Journal of cellular physiology, 101*(1), 129-138. doi:http://dx.doi.org/10.1002/jcp.1041010115
Hu, S. C.-S., & Lan, C.-C. E. (2016). High-glucose environment disturbs the physiologic functions of keratinocytes: focusing on diabetic wound healing.

*Journal of Dermatological Science, 84*(2), 121-127. doi:http://dx.doi.org/10.1016/j.jdermsci.2016.07.008
Jeon, J.-K., Park, S.-K., & Lee, J.-H. (2015). Effects of high voltage pulsed current stimulation with a visible contraction intensity on expression of TGF-β1 and synthesis of type I collagen in wound-induced white rats. *Journal of physical therapy science, 27*(5), 1485-1490. doi:http://dx.doi.org/10.1589/jpts.27.1485
Jy, W., Fregien, N., Bourguignon, G., & Bourguignon, L. Y. (1989). Role of Ca2+ in the regulation of hormone receptor exposure during lymphocyte activation. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 983*(2), 153-160. doi:http://dx.doi.org/10.1016/0005-2736(89)90228-9
Kang, E.-J., Roh, J.-S., Lee, J.-S., Yi, C.-H., & Kim, T.-H. (1996). A Comparison of the Inhibitive Effect of High Voltage Pulsed Current Stimulation and Microcurrent Electrical Neuromuscular Stimulation on Bacterial Growth. *Physical Therapy Korea, 3*(1), 12-23.
Kang, R., Tang, D., Lotze, M. T., & Zeh III, H. J. (2013). Autophagy is required for IL-2-mediated fibroblast growth. *Experimental cell research, 319*(4), 556-565. doi:http://dx.doi.org/10.1016/j.yexcr.2012.11.012
Karsli, P. B., Gurcay, E., Karaahmet, O. Z., & Cakci, A. (2017). High-Voltage electrical stimulation versus ultrasound in the treatment of pressure ulcers. *Advances in skin & wound care, 30*(12), 565-570. doi:http://dx.doi.org/10.1097/01.ASW.0000526606.72489.99
Kim, T. H., Cho, H.-y., & Lee, S. M. (2014). High-voltage pulsed current stimulation enhances wound healing in diabetic rats by restoring the expression of collagen, α-smooth muscle actin, and TGF-β1. *The Tohoku journal of experimental medicine, 234*(1), 1-6. doi:http://dx.doi.org/10.1620/tjem.234.1
Kincaid, C. B., & Lavoie, K. H. (1989). Inhibition of bacterial growth in vitro following stimulation with high voltage, monophasic, pulsed current. *Phys Ther, 69*(8), 651-655. doi:http://dx.doi.org/10.1093/ptj/69.8.651
Kloth, L. C., & Feedar, J. A. (1988). Acceleration of wound healing with high voltage, monophasic, pulsed current. *Phys Ther, 68*(4), 503-508. doi:http://dx.doi.org/10.1093/ptj/68.4.503
Kotturi, M. F., Carlow, D. A., Lee, J. C., Ziltener, H. J., & Jefferies, W. A. (2003). Identification and functional characterization of voltage-dependent calcium channels in T lymphocytes. *Journal of Biological Chemistry, 278*(47), 46949-46960. doi:http://dx.doi.org/10.1074/jbc.M309268200
Kuykendall, C. A., Hutchinson, T. L., & Bloedaum, A. P. (2004). 112 Effects of High Voltage Pulsed Current on Bacterial Viability: An in Vitro Study. *Wound Repair and Regeneration, 12*(2). doi:http://dx.doi.org/10.1111/j.1067-1927.2004.0abstractdf.x
Lan, C. C., Liu, I. H., Fang, A. H., Wen, C. H., & Wu, C. S. (2008). Hyperglycaemic conditions decrease cultured keratinocyte mobility: implications for impaired wound healing in patients with diabetes. *British Journal of Dermatology, 159*(5), 1103-1115. doi:http://dx.doi.org/10.1111/j.1365-2133.2008.08789.x
Lansdown, A. B. (2002). Calcium: a potential central regulator in wound healing in the skin. *Wound Repair and Regeneration, 10*(5), 271-285. doi:http://dx.doi.org/10.1046/j.1524-475X.2002.10502.x
Lasko, J., Kochik, J., & Serena, T. (2010). Combining acoustic pressure wound therapy with electrical stimulation for treatment of chronic lower-extremity ulcers: A case series. *Advances in skin & wound care, 23*(10), 446-449. doi:http://dx.doi.org/10.1097/01.ASW.0000383223.56900.13

Lee, J.-H., Lee, J.-S., & Kil, E.-Y. (2004). Keratinocyte proliferation in aged rat skin by high voltage pulsed current stimulation. *대한의생명과학회지, 10*(4), 361-366.
Lee, J.-H., Park, C.-E., & Park, R.-J. (2010). Electrical Stimulation Induces the Collagen Deposition and TGF-${\beta} $1 mRNA Expression in Skin Wound of Rat. *The Journal of Korean Physical Therapy, 22*(3), 87-92.
Lee, J.-h., Song, I.-y., & Jong-Gyu, K. (2003). Acceleration of wound healing and collagen deposition in rat skin by high voltage pulsed current stimulation. *Journal of Korean Physical Therapy, 15*(4), 1-12.
Leonard, W. J., Krönke, M., Peffer, N. J., Depper, J. M., & Greene, W. C. (1985). Interleukin 2 receptor gene expression in normal human T lymphocytes. *Proceedings of the National Academy of Sciences, 82*(18), 6281-6285. doi:http://dx.doi.org/10.1073/pnas.82.18.6281
Lerman, O. Z., Galiano, R. D., Armour, M., Levine, J. P., & Gurtner, G. C. (2003). Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. *The American journal of pathology, 162*(1), 303-312. doi:http://dx.doi.org/10.1016/S0002-9440(10)63821-7
Li, L., Zhang, J., Zhang, Q., Zhang, D., Xiang, F., Jia, J., . . . Huang, Y. (2019). High glucose suppresses keratinocyte migration through the inhibition of p38 MAPK/autophagy pathway. *Frontiers in Physiology, 10*, 24. doi:http://dx.doi.org/10.3389/fphys.2019.00024
Liang, L., Stone, R. C., Stojadinovic, O., Ramirez, H., Pastar, I., Maione, A. G., . . . Kirsner, R. S. (2016). Integrative analysis of miRNA and mRNA paired expression profiling of primary fibroblast derived from diabetic foot ulcers reveals multiple impaired cellular functions. *Wound Repair and Regeneration, 24*(6), 943-953. doi:http://dx.doi.org/10.1111/wrr.12470
Liu, B., Barbosa-Sampaio, H., Jones, P. M., Persaud, S. J., & Muller, D. S. (2012). The CaMK4/CREB/IRS-2 cascade stimulates proliferation and inhibits apoptosis of β-cells. doi:http://dx.doi.org/10.1371/journal.pone.0045711
Liu, Y., Petreaca, M., Yao, M., & Martins-Green, M. (2009). Cell and molecular mechanisms of keratinocyte function stimulated by insulin during wound healing. *BMC cell biology, 10*(1), 1-15. doi:http://dx.doi.org/10.1186/1471-2121-10-1
Loots, M. A., Lamme, E. N., Mekkes, J. R., Bos, J. D., & Middelkoop, E. (1999). Cultured fibroblasts from chronic diabetic wounds on the lower extremity (non-insulin-dependent diabetes mellitus) show disturbed proliferation. *Archives of dermatological research, 291*(2), 93-99. doi:http://dx.doi.org/10.1007/s004030050389
Luger, A., Schernthaner, G., URBANSKI, A., & Luger, T. (1988). Cytokine production in patients with newly diagnosed insulin-dependent (type I) diabetes mellitus. *European journal of clinical investigation, 18*(3), 233-236. doi:http://dx.doi.org/10.1111/j.1365-2362.1988.tb01251.x
Martin, C., & Muir, I. (1990). The role of lymphocytes in wound healing. *British Journal of Plastic Surgery, 43*(6), 655-662. doi:http://dx.doi.org/10.1016/0007-1226(90)90185-3
Mendoza-Naranjo, A., Cormie, P., Serrano, A. E., Wang, C. M., Thrasivoulou, C., Sutcliffe, J. E., . . . Phillips, A. R. (2012). Overexpression of the gap junction protein Cx43 as found in diabetic foot ulcers can retard fibroblast migration. *Cell biology international, 36*(7), 661-667. doi:http://dx.doi.org/10.1042/CBI20110628

Merriman, H. L., Hegyi, C. A., Albright-Overton, C. R., Carlos, J., Jr., Putnam, R. W., & Mulcare, J. A. (2004). A comparison of four electrical stimulation types on Staphylococcus aureus growth in vitro. *J Rehabil Res Dev, 41*(2), 139-146. doi:http://dx.doi.org/10.1682/JRRD.2004.02.0139

Mohr, T., Akers, T. K., & Wessman, H. C. (1987). Effect of high voltage stimulation on blood flow in the rat hind limb. *Phys Ther, 67*(4), 526-533. doi:http://dx.doi.org/10.1093/ptj/67.4.526

Neckers, L. M., & Cossman, J. (1983). Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin 2. *Proceedings of the National Academy of Sciences, 80*(11), 3494-3498. doi:http://dx.doi.org/10.1073/pnas.80.11.3494

Omar, M. T., El-Badawy, A. M., Borhan, W., & Nossier, A. (2004). Improvement of edema and hand function in superficial second degree hand burn using electrical stimulation. *Egypt J Plast Reconstr Surg, 28*(2), 141-146.

Onuma, E. K., & Hui, S.-W. (1988). Electric field-directed cell shape changes, displacement, and cytoskeletal reorganization are calcium dependent. *The Journal of cell biology, 106*(6), 2067-2075. doi:http://dx.doi.org/10.1083/jcb.106.6.2067

Ortiz, M. C. S., Villabona, E. H., Lemos, D. M. C., & Castellanos, R. (2014). Effects of low level laser therapy and high voltage stimulation on diabetic wound healing. *Revista de la Universidad Industrial de Santander Salud 2014 May-Aug;46(2):107-117*.

Ozawa, A., Tada, H., Tamai, R., Uehara, A., Watanabe, K., Yamaguchi, T., . . . Sugawara, S. (2003). Expression of IL-2 receptor β and γ chains by human gingival fibroblasts and up-regulation of adhesion to neutrophils in response to IL-2. *Journal of leukocyte biology, 74*(3), 352-359. doi:http://dx.doi.org/10.1189/jlb.0103044

Peters, E. J., Lavery, L. A., Armstrong, D. G., & Fleischli, J. G. (2001). Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial. *Archives of physical medicine and rehabilitation, 82*(6), 721-725. doi:http://dx.doi.org/10.1053/apmr.2001.23780

Plaisance, S., Alileche, A., Han, D., Rubinstein, E., Sahraoui, Y., Jasmin, C., & Azzarone, B. (1994). How interleukin-2 can affect human fibroblasts behaviour. *Pathology-Research and Practice, 190*(9-10), 942-949. doi:http://dx.doi.org/10.1016/S0344-0338(11)81000-9

Plaisance, S., Rubinstein, E., Allleche, A., Sahraoui, Y., Krief, P., Augery-Bourget, Y., . . . Azzarone, B. (1992). Expression of the interleukin-2 receptor on human fibroblasts and its biological significance. *International immunology, 4*(7), 739-746. doi:http://dx.doi.org/10.1093/intimm/4.7.739

Polak, A., Kloth, L. C., Blaszczak, E., Taradaj, J., Nawrat-Szoltysik, A., Ickowicz, T., . . . Kucio, C. (2017). The Efficacy of Pressure Ulcer Treatment With Cathodal and Cathodal-Anodal High-Voltage Monophasic Pulsed Current: A Prospective, Randomized, Controlled Clinical Trial. *Phys Ther, 97*(8), 777-789. doi:http://dx.doi.org/10.1093/ptj/pzx052

Polak, A., Kloth, L. C., Blaszczak, E., Taradaj, J., Nawrat-Szoltysik, A., Walczak, A., . . . Kucio, C. (2016). Evaluation of the Healing Progress of Pressure Ulcers Treated with Cathodal High-Voltage Monophasic Pulsed Current: Results of a Prospective, Double-blind, Randomized Clinical Trial. 2016b. *Adv Skin Wound*

*Care, 29*(10), 447-459. doi:http://dx.doi.org/10.1097/01.ASW.0000493164.75337.de

Polak, A., Kucio, C., Kloth, L. C., Paczula, M., Hordynska, E., Ickowicz, T., . . . Franek, A. (2018). A Randomized, Controlled Clinical Study to Assess the Effect of Anodal and Cathodal Electrical Stimulation on Periwound Skin Blood Flow and Pressure Ulcer Size Reduction in Persons with Neurological Injuries. *Ostomy Wound Manage, 64*(2), 10-29. doi:http://dx.doi.org/10.25270/owm.2018.2.1029

Polak, A., Taradaj, J., Nawrat-Szoltysik, A., Stania, M., Dolibog, P., Blaszczak, E., . . . Kucio, C. (2016). Reduction of pressure ulcer size with high-voltage pulsed current and high-frequency ultrasound: a randomised trial. 2016a. *J Wound Care, 25*(12), 742-754. doi:http://dx.doi.org/10.12968/jowc.2016.25.12.742

Rechler, M. M., & Podskalny, J. M. (1976). Insulin receptors in cultured human fibroblasts. *Diabetes, 25*(4), 250-255. doi:http://dx.doi.org/10.2337/diab.25.4.250

Recio, A. C., Felter, C. E., Schneider, A. C., & McDonald, J. W. (2012). High-voltage electrical stimulation for the management of Stage III and IV pressure ulcers among adults with spinal cord injury: Demonstration of its utility for recalcitrant wounds below the level of injury. *The journal of spinal cord medicine, 35*(1), 58-63. doi:http://dx.doi.org/10.1179/2045772311Y.0000000044

Reem, G. H., & Yeh, N.-H. (1984). Interleukin 2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. *Science, 225*(4660), 429-430. doi:http://dx.doi.org/10.1126/science.6429853

Rocha-Zavaleta, L., Huitron, C., Cacéres-Cortés, J. R., Alvarado-Moreno, J. A., Valle-Mendiola, A., Soto-Cruz, I., . . . Rangel-Corona, R. (2004). Interleukin-2 (IL-2) receptor-βγ signalling is activated by c-Kit in the absence of IL-2, or by exogenous IL-2 via JAK3/STAT5 in human papillomavirus-associated cervical cancer. *Cellular signalling, 16*(11), 1239-1247. doi:http://dx.doi.org/10.1016/j.cellsig.2004.03.011

Roderick, H. L., & Cook, S. J. (2008). Ca 2+ signalling checkpoints in cancer: remodelling Ca 2+ for cancer cell proliferation and survival. *Nature Reviews Cancer, 8*(5), 361-375. doi:http://dx.doi.org/10.1038/nrc2374

Roncarolo, M., Zoppo, M., Bacchetta, R., Gabiano, C., Sacchetti, C., Cerutti, F., & Tovo, P. A. (1988). Interleukin-2 production and interleukin-2 receptor expression in children with newly diagnosed diabetes. *Clinical immunology and immunopathology, 49*(1), 53-62. doi:http://dx.doi.org/10.1016/0090-1229(88)90094-3

Ross, S. H., & Cantrell, D. A. (2018). Signaling and function of interleukin-2 in T lymphocytes. *Annual review of immunology, 36*, 411-433. doi:http://dx.doi.org/10.1146/annurev-immunol-042617-053352

Rowe, D. W., Starman, B. J., Fujimoto, W. Y., & Williams, R. H. (1977). Abnormalities in proliferation and protein synthesis in skin fibroblast cultures from patients with diabetes mellitus. *Diabetes, 26*(4), 284-290. doi:http://dx.doi.org/10.2337/diab.26.4.284

Sakabe, F. F., Sakabe, D. I., Marracini Fernandes, F. R., & Reges, G. (2018). Estimulação pulsada de alta voltagem reduz a área de úlceras de diferentes etiologias: um estudo piloto. *Fisioterapia Brasil, 19*(1). doi:http://dx.doi.org/10.33233/fb.v19i1.2124

Saltiel, A. R., & Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature, 414*(6865), 799-806. doi:http://dx.doi.org/10.1038/414799a
Santana de Souza, A. C., Lomasso Costa, M. M., Vieira Costa, P. H., & Souza da Silva, F. (2017). Efeito da estimulação elétrica de alta voltagem para o tratamento de úlceras por pressão: um estudo experimental de caso único. *Fisioterapia Brasil, 18*(6). doi:http://dx.doi.org/10.33233/fb.v18i6.2046
Schäffer, M., & Barbul, A. (1998). Lymphocyte function in wound healing and following injury. *Journal of British Surgery, 85*(4), 444-460. doi:http://dx.doi.org/10.1046/j.1365-2168.1998.00734.x
Schüppel, M., Kürschner, U., Kleuser, U., Schäfer-Korting, M., & Kleuser, B. (2008). Sphingosine 1-phosphate restrains insulin-mediated keratinocyte proliferation via inhibition of Akt through the S1P2 receptor subtype. *Journal of Investigative Dermatology, 128*(7), 1747-1756. doi:http://dx.doi.org/10.1038/sj.jid.5701259
Shen, S., Alt, A., Wertheimer, E., Gartsbein, M., Kuroki, T., Ohba, M., . . . Tennenbaum, T. (2001). PKCδ Activation: A Divergence Point in the Signaling of Insulin and IGF-1—Induced Proliferation of Skin Keratinocytes. *Diabetes, 50*(2), 255-264. doi:http://dx.doi.org/10.2337/diabetes.50.2.255
Silva Ede, F., Martins, C. C., Guirro, E. C., & Guirro, R. R. (2010). High voltage electrical stimulation as an alternative treatment for chronic ulcers of the lower limbs. *An Bras Dermatol, 85*(4), 567-569. doi:http://dx.doi.org/10.1590/s0365-05962010000400025
Soleimanpour, S. A., Crutchlow, M. F., Ferrari, A. M., Raum, J. C., Groff, D. N., Rankin, M. M., . . . Kushner, J. A. (2010). Calcineurin signaling regulates human islet β-cell survival. *Journal of Biological Chemistry, 285*(51), 40050-40059. doi:http://dx.doi.org/10.1074/jbc.M110.154955
Spravchikov, N., Sizyakov, G., Gartsbein, M., Accili, D., Tennenbaum, T., & Wertheimer, E. (2001). Glucose effects on skin keratinocytes: implications for diabetes skin complications. *Diabetes, 50*(7), 1627-1635. doi:http://dx.doi.org/10.2337/diabetes.50.7.1627
Stojadinovic, O., Brem, H., Vouthounis, C., Lee, B., Fallon, J., Stallcup, M., . . . Tomic-Canic, M. (2005). Molecular pathogenesis of chronic wounds: the role of β-catenin and c-myc in the inhibition of epithelialization and wound healing. *The American journal of pathology, 167*(1), 59-69. doi:http://dx.doi.org/10.1016/S0002-9440(10)62953-7
Sun, P., Enslen, H., Myung, P. S., & Maurer, R. A. (1994). Differential activation of CREB by Ca2+/calmodulin-dependent protein kinases type II and type IV involves phosphorylation of a site that negatively regulates activity. *Genes & development, 8*(21), 2527-2539. doi:http://dx.doi.org/10.1101/gad.8.21.2527
Szuminsky, N. J., Albers, A. C., Unger, P., & Eddy, J. G. (1994). Effect of narrow, pulsed high voltages on bacterial viability. *Phys Ther, 74*(7), 660-667. doi:http://dx.doi.org/10.1093/ptj/74.7.660
Thompson, G. L., Roth, C. C., Dalzell, D. R., Kuipers, M. A., & Ibey, B. L. (2014). Calcium influx affects intracellular transport and membrane repair following nanosecond pulsed electric field exposure. *Journal of Biomedical Optics, 19*(5), 055005. doi:http://dx.doi.org/10.1117/1.JBO.19.5.055005
Titushkin, I., & Cho, M. (2009). Regulation of cell cytoskeleton and membrane mechanics by electric field: role of linker proteins. *Biophysical journal, 96*(2), 717-728. doi:http://dx.doi.org/10.1016/j.bpj.2008.09.035

Tomoda, T., Kurashige, T., & Taniguchi, T. (1994). Imbalance of the interleukin 2 system in children with IDDM. *Diabetologia, 37*(5), 476-482. doi:http://dx.doi.org/10.1007/s001250050135
Usui, M. L., Mansbridge, J. N., Carter, W. G., Fujita, M., & Olerud, J. E. (2008). Keratinocyte migration, proliferation, and differentiation in chronic ulcers from patients with diabetes and normal wounds. *Journal of Histochemistry & Cytochemistry, 56*(7), 687-696. doi:http://dx.doi.org/10.1369/jhc.2008.951194
Verrando, P., & Ortonne, J.-P. (1985). Insulin binding properties of normal and transformed human epidermal cultured keratinocytes. *Journal of Investigative Dermatology, 85*(4), 328-332. doi:http://dx.doi.org/10.1111/1523-1747.ep12276922
Wang, A., Toma, M. A., Ma, J., Li, D., Vij, M., Chu, T., . . . Xu Landén, N. (2020). Circular RNA hsa_circ_0084443 is upregulated in diabetic foot ulcer and modulates keratinocyte migration and proliferation. *Advances in Wound Care, 9*(4), 145-160. doi:http://dx.doi.org/10.1089/wound.2019.0956
Wang, X.-F., Li, M.-L., Fang, Q.-Q., Zhao, W.-Y., Lou, D., Hu, Y.-Y., . . . Tan, W.-Q. (2021). Flexible electrical stimulation device with Chitosan-Vaseline® dressing accelerates wound healing in diabetes. *BIOACTIVE MATERIALS, 6*(1), 230-243. doi:http://dx.doi.org/10.1016/j.bioactmat.2020.08.003
White, J. A., Blackmore, P. F., Schoenbach, K. H., & Beebe, S. J. (2004). Stimulation of capacitative calcium entry in HL-60 cells by nanosecond pulsed electric fields. *Journal of Biological Chemistry, 279*(22), 22964-22972. doi:http://dx.doi.org/10.1074/jbc.M311135200
Wu, M., Rubin, A. E., Dai, T., Schloss, R., Usta, O. B., Golberg, A., & Yarmush, M. (2021). High-Voltage, Pulsed Electric Fields Eliminate Pseudomonas aeruginosa Stable Infection in a Mouse Burn Model. *Advances in Wound Care, 10*(9), 477-489. doi:http://dx.doi.org/10.1089/wound.2019.1147
Xuan, Y. H., Huang, B. B., Tian, H. S., Chi, L. S., Duan, Y. M., Wang, X., . . . Wei, T. M. (2014). High-glucose inhibits human fibroblast cell migration in wound healing via repression of bFGF-regulating JNK phosphorylation. *PLoS One, 9*(9), e108182. doi:http://dx.doi.org/10.1371/journal.pone.0108182
Yasumura, S., Lin, W. C., Weidmann, E., Hebda, P., & Whiteside, T. L. (1994). Expression of interleukin 2 receptors on human carcinoma cell lines and tumor growth inhibition by interleukin 2. *International journal of cancer, 59*(2), 225-234. doi:http://dx.doi.org/10.1002/ijc.2910590215
Zhang, J., Blackmore, P. F., Hargrave, B. Y., Xiao, S., Beebe, S. J., & Schoenbach, K. H. (2008). Nanosecond pulse electric field (nanopulse): a novel non-ligand agonist for platelet activation. *Archives of biochemistry and biophysics, 471*(2), 240-248. doi:http://dx.doi.org/10.1016/j.abb.2007.12.009
Zhou, K., Krug, K., Stachura, J., Niewczyk, P., Ross, M., Tutuska, J., & Ford, G. (2016). Silver-Collagen Dressing and High-voltage, Pulsed-current Therapy for the Treatment of Chronic Full-thickness Wounds: A Case Series. 2016b. *Ostomy Wound Manage, 62*(3), 36-44.
Zhou, K., Schenk, R., & Brogan, M. S. (2016). The wound healing trajectory and predictors with combined electric stimulation and conventional care: one outpatient wound care clinic's experience. 2016a. *Eur J Clin Invest, 46*(12), 1017-1023. doi:http://dx.doi.org/10.1111/eci.12685
Zier, K. S., Leo, M. M., Spielman, R. S., & Baker, L. (1984). Decreased synthesis of interleukin-2 (IL-2) in insulin-dependent diabetes mellitus. *Diabetes, 33*(6), 552-555. doi:http://dx.doi.org/10.2337/diab.33.6.552

# VI

## Induction and potential treatment of overuse tendon injury


### Authors

Beshoy Girgis[1], Ana F. Almeida[2,3], Marcia T. Rodrigues[2,3], Manuela E. Gomes[2,3], José Alberto Duarte[1]

1. CIAFEL, Faculty of Sports, University of Porto, Portugal
2. 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, Guimarães 4805-017, Portugal;
3. ICVS/3B's−PT Government Associate Laboratory, Guimarães 4710-057, Portugal



### Abstract

An international consensus defined tendinopathy as persistent tendon pain and loss of function related to mechanical loading. Nonetheless, neither the exact pathophysiology of tendinopathy nor its treatment guidelines are agreed upon. The objectives of this study were to develop a model for overuse-induced injury of Achilles tendons in rats, and subsequently, assess the effect of high-voltage monophasic pulsed current electrical (HVMPC) stimulation as a possible treatment. Nineteen 13-months-old, and twenty-eight 15-months-old male Wistar rats, weighing 499 ± 42 grams, and 514 ± 50 grams, respectively, were included in the study. The rats were randomly assigned to (I) sedentary control (SC, n=4) and (II) experimental groups (n=15); and (III) sham stimulation (n=14) and (IV) stimulation groups (n=14), respectively. Rats in the experimental group were sacrificed at the end of running protocol (EP; n=5), after 2 weeks of rest (2R; n=5), and after 4 weeks of rest (4R; n=5), whereas for the sham stimulation and stimulation groups; rats were sacrificed after 8 sessions of intervention (30 days of rest; n=4 per group; ES 4W), 12 sessions of intervention (45 days of rest; n=5 per group; ES 6W), and 16 sessions of intervention (60 days of rest; n=5 per group; ES 8W). Statistically significant reduction in Alcian-blue staining and cellular densities, and calcification were found at the EP, 2R, but only cellular density reduction was statistically significant at 4R compared with SC. A statistically significant increase in Alcian-blue staining density was found following 8 (30 days of rest) and 12 (45 days of rest) sessions of HVMPC, but not after 16 (60 days of rest) sessions of HVMPC. Moreover, calcification was detected after 8 and 12 sessions of HVMPC. The results

of the present study might indicate that tendon injury did occur although with incomplete recovery. Signs of tenocyte injury were detected, on cellular and genetic levels, long after the absence of significant histopathological findings. The results, moreover, showed reduction in enthesis proteoglycan content and cellularity which are novel findings that were not previously reported in response to treadmill running models and that are reproducible. The results of the present study, furthermore, may suggest that HVMPC electrical stimulation was effective in restoring proteoglycan expression. Nonetheless, whether spontaneous recovery occurred at later timepoints or that injury still exists but on a cellular level remain to be confirmed.

**Key Points**

The results may suggest a reproducible cellular injury of Achilles tendons was sustained for at least 15 days following the end of protocol and up to 60 days with administration of dexmedetomidine injections.

Reduction in cellular function and/or number, due to senescence and/or apoptosis, might explain reduced ground substance synthesis, particularly of carboxylated and sulfated glycosaminoglycans.

These changes might correlate with the “lag phase” of tensile strength recovery. Moreover, the “rebound phenomenon”, which might correlate with reactive hyperemia, was not observed throughout the entire study period; this might suggest that complete recovery did not occur.

Calcification was reproducibly induced by overuse tendon injury, and its resorption may occur spontaneously with rest.

The results might provide insight into the changes that characterize overuse-induced tendon injury, and that possibly precede tendinopathy, while the classic features described by the Bonar score may represent repair rather than injury.

HVMPC might improve cell activity and function, and might enhance calcification under certain conditions. These responses, moreover, occurred despite slower healing, possibly due to restraint and/or injections, which might correlate with lower body weight and dietary intake.

## Introduction

An international consensus defined tendinopathy as persistent tendon pain and loss of function related to mechanical loading (Scott et al., 2020). Nonetheless, neither the exact pathophysiology of tendinopathy nor its treatment guidelines are agreed upon (Abat et al., 2018; Scott et al., 2020; Vicenzino et al., 2020). The etiology of tendinopathy may include an array of intrinsic and extrinsic factors such as genetic, gender, aging, joint malalignment, impingement, muscle weakness, nutrition, hyperthermia, ischemia-reperfusion, excessive force and overuse (Fredberg & Stengaard-Pedersen, 2008). Tendinopathy might be traditionally viewed as a tendon injury correlated with repetitive mechanical load, with the mechanical strain theory being the most accepted to explain tendon overload injury (Fredberg & Stengaard-Pedersen, 2008). Histologic assessment of tendons may show a broad range of findings in tendinopathy including hypoxic, mucoid or myxoid and fatty degeneration, tenocyte necrosis, insertional focal degeneration, hyalin degeneration and fibrocartilaginous metaplasia, and calcific deposits (Fredberg & Stengaard-Pedersen, 2008). The characteristic features of tendinopathy are collagen disruption, augmented ground substance, increased number of tenocytes with rounded nuclei, and neovascularization (Fearon et al., 2014; Fredberg & Stengaard-Pedersen, 2008).

Animal models may provide a better understanding of the mechanisms underlying tendinopathy, which may permit development of an effective treatment (Lake et al., 2008). Many models in the literature attempted to induce tendon overuse injury in rats, nevertheless, choice of the appropriate model for each purpose may be needed (Dirks & Warden, 2011; Lake et al., 2008; Lui et al., 2011; Luo et al., 2023; Warden, 2007). These models included naturally occurring, treadmill, subacromial impingement and metabolic models, among others (Dirks & Warden, 2011; Lake et al., 2008; Lui et al., 2011; Luo et al., 2023; Warden, 2007). Treadmill running models may have the advantage of crudely resembling tendinopathy, but might be associated with chronic stress (Moraska et al., 2000).

Moderate treadmill running was shown to upregulate collagen type I and tenomodulin genes but did not alter non-tenocyte-related genes, whereas intensive treadmill running upregulated both (Zhang & Wang, 2013). Features of tendon disease including glycosaminoglycan accumulation, tenocyte proliferation and roundedness, and

collagen disorganization were reported following treadmill running (Glazebrook et al., 2008; Scott et al., 2007; Soslowsky et al., 2000). Only one study, however, examined the effect of rest on gene expression following supraspinatus tendon overuse injury (Jelinsky et al., 2008). It was concluded that recovery occurred in as little as two weeks of rest (Jelinsky et al., 2008). These controversial findings hinder the choice of the successful protocol; therefore, the development of a different protocol may be warranted.

Loading within physiological range may lead to increased collagen synthesis in tendon tissues (T. Wang et al., 2018). Both mechanical underload and overload, nonetheless, might have negative effects including upregulated matrix degradation enzymes and collagen type III production, respectively (T. Wang et al., 2018). Moreover, an increase in cell apoptosis was reported in either state (T. Wang et al., 2018). Chronic underloading might reduce fibrocartilage area, proteoglycan content and proliferation of chondrocytes at the enthesis (Roffino et al., 2021). Underloading, furthermore, was shown to induce heterotopic ossification in ex vivo cultured tendons (T. Wang et al., 2021). In fact, overuse-induced damage was hypothesized to disrupt contact between cells and pericellular matrix resulting in cellular understimulation and catabolic signaling (Chatterjee et al., 2022). Overuse tendon injury may be defined as “a structural mechanical tendinous lesion as a consequence of a failed adaptation of the cells and extracellular matrix” (Selvanetti et al., 1997). Several reviews have identified significant factors that facilitate the occurrence of tendon overuse injury (Archambault et al., 1995; Maganaris et al., 2004; Selvanetti et al., 1997). These factors may include old age, eccentric muscle contraction, failed adaptation and repetitive submaximal load with insufficient rest.

Different types of electrical currents, with diverse parameters and either alone or combined with other interventions, have been evaluated as possible treatments for musculoskeletal injuries (Akai & Hayashi, 2002; Bax et al., 2005; Imoto et al., 2011; Michlovitz, 2005). Most of the available treatments for tendinopathy, nonetheless, are not supported by sufficient evidence (Dingemanse et al., 2013; Irby et al., 2020; Van Der Vlist et al., 2020). Lack of standardization in treatment protocols and treating different degrees of tendinopathic conditions with a single approach may hinder reaching robust conclusions regarding the efficacy and reproducibility of results

(LaCourse et al., 2023; Vicenzino et al., 2020). In fact, treatment guidelines for major tendinopathies have not been agreed upon by the clinical and research community (Vicenzino et al., 2020). Significant healthcare consumption may result due to prescription of multiple treatments with inconclusive evidence (de Vos et al., 2021).

To our knowledge, the first report of high-voltage monophasic pulsed current (HVMPC) use for the treatment of tendinopathy dates back to year 1974 (Nirschl & Sobel, 1981). Dr. Robert P. Nirschl stated that this current may stimulate healing in addition to its analgesic effect (Nirschl & Sobel, 1981). Dr. Nirschl, moreover, mentioned that it replaced ultrasound as the primary treatment modality for lateral elbow tendinopathy (LET) (Nirschl & Sobel, 1981). The treatment protocol was included 20 minutes of cathodal HVMPC stimulation at 60 – 80 HZ, with the voltage adjusted to patients' tolerance (Nirschl & Sobel, 1981). The treatment was routinely followed by Whirlpool (Nirschl & Sobel, 1981).

A case report and a pilot study published in year 2009 assessed the efficacy of HVMPC, in combination with other interventions, for the treatment of LET (Radpasand, 2009; Radpasand & Owens, 2009). These studies found possible beneficial effects of the multimodal treatments and recommended further larger studies that evaluate individual treatments instead (Radpasand, 2009; Radpasand & Owens, 2009). A recent report with 180-day follow-up concluded that HVMPC reduced pain and improved function in patients with LET (Dolibog et al., 2022). The intervention was consequently regarded as effective and safe (Dolibog et al., 2022). The influence of HVMPC on Achilles tendon partial rupture was evaluated in Wistar rats (Rampazo et al., 2016). No significant differences were identified between groups regarding collagen synthesis and alignment, and angiogenesis (Rampazo et al., 2016). It may be argued, however, that the pathophysiology of chronic tendinopathy may vary from tendon partial rupture. Furthermore, an HVMPC protocol with longer application time and that is applied more frequently may offer a better representation of the actual effect.

Tenocytes may have a crucial role in the homeostasis, regulation of extracellular matrix and repair of tendon tissues following injury (Grinstein et al., 2019). Heterogeneity in the population of tenocytes and tendon stem progenitor cells was identified in normal

and pathological conditions (Bi et al., 2007; Kendal et al., 2020). Aging and tendinopathy are clearly linked as evidenced by the association between increasing age and tendon damage (Birch et al., 2016). A reduction in cell number per unit area and an increase in cell senescence markers were detected in tenocytes and tendon-derived cells, respectively, with aging (Grinstein et al., 2019). Higher expression of p16, p21, p53 levels and senescence-associated β-galactosidase compared with normal controls was found in patients with tendinopathy (Chen et al., 2022). Moreover, markers of apoptosis were identified in degenerative tendons, tendon tears and in patients with rotator cuff impingement (Benson et al., 2010; Chen et al., 2010; Lundgreen et al., 2013; Tuoheti et al., 2005; Wu et al., 2011; Yuan et al., 2002).

*In vitro* studies indicated that HVMPC may increase calcium influx, collagen synthesis and proliferation (Gerard J Bourguignon & Lilly YW Bourguignon, 1987; Gerard J Bourguignon et al., 1989; Lee et al., 2002; Lee et al., 2003). Consequently, confirming these findings *in vivo* and identifying the mechanisms through which HVMPC may influence cellular function may be significant. Voltage-gated calcium channels and voltage-gating of PIEZO1 mechanosensitive channels are potential candidates for explaining calcium influx (Catterall, 2011; Moroni et al., 2018). Hypoxia inducible factor-1 alpha (HIF-1α) may be highly expressed in tendinopathy, and its inhibition was found to reduce tenocyte migration and proliferation (Benson et al., 2010; Jiao et al., 2022; Mosca et al., 2017). Hypoxic cell injury was proposed as a critical pathophysiological mechanism in early tendinopathy (Millar et al., 2012).

The objectives of this study were to develop a model for overuse-induced injury of Achilles tendons in rats, and subsequently, assess the effect of high-voltage monophasic pulsed current electrical stimulation as a possible treatment. A treadmill running protocol that combines factors potentially contributing to tendon injury was therefore developed. The hypotheses were based on the Bonar score (Fearon et al., 2014); an increase in ground substance, cellularity, and calcification compared with sedentary controls. Moreover, no difference between experimental groups was hypothesized, which may indicate a sustained injury. The literature indicate that HVMPC may increase cellularity, reduce ground substance, and reduce calcification. The effect is hypothesized to be maintained throughout the study period, suggesting no difference between stimulation and sham stimulation groups.

**Outcomes**

The tendon healing process is known to include the proliferation of fibroblasts, synthesis of collagen and formation of new blood vessels, known as the "angiofibroblastic response" (Docheva et al., 2015; Fenwick et al., 2002; James et al., 2008; Sharma & Maffulli, 2005; Sharma & Maffulli, 2006). Therefore, the following outcomes were chosen to determine and compare the status of the healing process between groups:

**Primary outcome:**

▪ Ground substance: proteoglycan content density.

**Secondary outcomes:**

▪ Cellularity and circularity (density of tenocyte based on roundedness).

▪ Collagen fiber alignment (dispersion of fiber directionality).

▪ Calcification (surface area).

**Tertiary outcomes:**

▪ Gene expression for cellular proliferation (Ki-67), senescence (p16, p21, p53), collagen (Col1 and Col3), ground substance (decorin) and repair (tenomodulin, scleraxis and mohawk).

## Material and Methods

The ethical committee of the Faculty of Sports, University of Porto approved all animal procedures.

### Study design

Nineteen 13-months-old, and twenty-eight 15-months-old male Wistar rats, weighing 499 ± 42 grams, and 514 ± 50 grams, respectively, were included in the study. The rats were randomly assigned to sedentary control (n=4) and experimental groups (n=15); and sham stimulation (n=14) and stimulation groups (n=14), respectively. Rats in the experimental group were sacrificed at the end of running protocol (EP; n=5), after 2 weeks of rest (2R; n=5), and after 4 weeks of rest (4R; n=5), whereas for the stimulation/ sham stimulation groups; rats were sacrificed after 8 sessions of intervention (30 days of rest; n=4 per group; ES 4W), 12 sessions of intervention (45 days of rest; n=5 per group; ES 6W), and 16 sessions of intervention (60 days of rest; n=5 per group; ES 8W). Overuse injury was induced in both Achilles tendons of rats in the experimental, stimulation and sham stimulation groups, whereas the sedentary control group maintained free cage movement alone. Rats in the sedentary control group (n=4) were euthanized together with the euthanasia of the last experimental group. The Achilles tendons, of both hind limbs, in all groups were excised for histological analysis.

The purchased rats were 35-41-days old (Charles River Laboratories, Moulin Berger, 69130 Ecully, France; strain code: 273), and were nurtured until the desired age. Rats were sheltered in individual cages (1-2 rats/cage) and allowed normal cage activity, in a room with 12-hour inverted light-dark cycle, and room temperature maintained at 25°C. The running protocol was implemented during the dark cycle, and therefore, the treadmill transparent Perspex lid was covered to limit light-transmission thus maintaining the dark cycle during running. Care was taken not to block air inlets/outlets of the lid. Standard rat chow (Diete Standard; Mucedola srl, Via Galileo Galilei 4, 20019 Settimo Milanese (MI), Italy) and municipal tap water were provided *ad libitum* until 9 months of age, then a gradual (5% increments bi-weekly) dietary restriction protocol was implemented, reaching 20% reduction of maximum consumption per day for the 13-months-old rats (maximum consumption of 60 grams per rat at baseline). Nonetheless, a gradual (5% increments bi-weekly until 30%, then lastly 3%) dietary

restriction protocol was implemented, reaching 33% reduction of maximum consumption per day for the 15-months-old rats. Leftovers were carefully monitored throughout the restriction period, and restriction was only increased when weight gain was noticed. The dietary restriction was lifted two days before the start of the running protocol.

Alterations in body weight and chow consumption were, moreover, monitored at baseline and throughout the entire duration of the study. A digital scale (HR2391/00; Philips Electronics Nederland B.V. High Tech Campus 52, Eindhoven, 5656 AG, NOORD-BRABANT, NETHERLANDS) was used to perform both measurements. Standard rat feed pellets, with a fixed amount of 250 grams per rat, were weekly placed at the designated cage compartment.

**Overuse tendon injury protocol**

Overuse injury was induced in both Achilles tendons of all rats in the experimental group using a newly developed protocol. Two animal treadmills for rats, 5 lane each, with treadmill control LE8710 unit (Harvard Apparatus; 84 October Hill Road, Holliston, MA 01746, USA) were used to implement the running protocol. A computer software (SEDACOM 2.0; Panlab Laboratory equipment supplier, Cornellà de Llobregat, 08940 Barcelona, Spain) was used to control both treadmills simultaneously. The use of treadmills was alternated to overcome possible calibration differences.

Habituation to the treadmill, but not to running, was allowed for one week before the start of the protocol (5 min per day at a speed of 15 cm/sec). The parameters of the protocol were as follows: for the first week, rats ran at a speed of 20 cm/sec (12 m/min) for 70 minutes, on -20° treadmill decline (downhill), twice daily (three hours of rest in-between). Subsequently, in attempt to overcome adaptation, the running speed was increased each week by 5 cm/sec (3 m/min), so that the final speed reached 45 cm/sec (27 m/min) on the sixth week. The protocol was implemented five days a week, for six weeks. Warm-up and cool-down periods were administered for 10 minutes each (already included in the 70 minutes running time), with warm-up and cool-down speeds (starting at 10 cm/sec) being incrementally increased each week by 10% of the designated running speed (Figure 1).

In attempt to standardize the imposed load in order to reduce the variability in induced overuse injury, a period of free running were introduced into the protocol. The actual running duration (50 min) was further divided into enforced (shocks administered) and free (no shocks) running durations. During the first five weeks of the protocol, the enforced running duration was 40 min, while the free running duration was 10 min. During the last week of running (the sixth week), the enforced running duration was reduced to 30 min, whereas the free running duration was increased to 20 min. Electric shocks were only administered when necessary, and its intensity was kept at minimum (0.1-0.2 mA) when used, in order to avoid inducing undue stress of the rats. Humane endpoints of rapid or progressive body weight loss of 20% of baseline body weight and severe chromodacryorrhoea were considered.



Figure 1. An actual photograph of the running protocol being implemented; note the treadmill downhill decline and the covered Perspex lid.

**Electrical stimulation intervention**

HVMPC intervention was applied to both hindlimbs of the experimental group, whereas sham HVMPC (electrodes attached but no current) was administered to both hindlimbs of the control group. HVMPC was applied for 60 min per day, two days per week for 30 days (8 sessions), 45 days (12 sessions), and 60 days (16 sessions). The adopted protocol was based on positive results of in vitro and animal studies. Intelect® Mobile Stimulator (Chattanooga Group, 4717 Adams Road, Hixson, TN 37343, USA) was used to deliver HVMPC. The device was connected to the AC power, using the AC adapter provided with the device, to maintain constant output. HVMPC was determined to enhance fibroblast proliferation (near cathode) (G. J. Bourguignon & L. Y. Bourguignon, 1987; G. J. Bourguignon et al., 1989; Cruz et al., 1989), increase collagen synthesis (G. J. Bourguignon & L. Y. Bourguignon, 1987; Falanga, 1987; Kim et al., 2014). The parameters were set to a voltage of 25-35 V (usually 25 V; below that producing muscular contraction), a pulse frequency of 100 HZ, and a pulse interval (pulse spacing) of 154 µs. Flicker contraction of hindlimbs was used as an indication of good conductance. The negative electrode (cathode) was used throughout the intervention period as it has demonstrated positive effects (Assimacopoulos, 1968a, 1968b; Bigelow et al., 1979; Konikoff, 1976). The sedative dose was increased over the first two sessions from 0.3 to 0.33 to 0.35 mg/kg. Restraint of hindlimbs was sometimes necessary, for the first few seconds of stimulation, as momentary restoration of consciousness occasionally occurred.

Rats were laid prone on a custom raised grid platform in Trendelenburg position so that urine is displaced forward with gravity and away from the hindlimb electrodes. Wrapping towels were used to prevent hypothermia during sedation. Ophthalmic ointments (Recugel® ophthalmic gel 10 g; Bausch & Lomb GmbH, Brunsbütteler Damm 165-173, D-13581 Berlin, Germany, and Genteal® Gel ophthalmic gel 10 g; Alcon® laboratories, inc., a Novartis company, Fort Worth, Texas 76134, USA) were applied soon after the onset of sedation to avoid corneal lesions. The same ointment was used for rats in both groups. Moreover, the eyes were covered using a custom head cover in order to avoid retinal damage caused by light.

Intraperitoneal (IP) administration of Dexdomitor® 0.5 mg/ml (dexmedetomidine hydrochloride), at a dose of 0.35 mg/kg, achieved adequate sedation, and was

implemented to allow for the application of electrical stimulation. The drug was administered into the lower abdominal quadrants (left/right) and the injection site was alternated each injection day in order to reduce pain, local tissue trauma, and stress associated with injections. To reverse sedation, intramuscular (IM) administration of Antisedan® 5 mg/ml (atipamezole hydrochloride), at a dose of 0.3 mg/kg, was used at the end of intervention session. The quadriceps muscle, was chosen as the largest muscle in the hindlimbs, devoid of large nerves, in order to reduce pain and injury risk. Moreover, the injection site was alternated (left/right) so that pain, local tissue trauma, and stress are reduced. Braun Omnifix® syringes (B. Braun Melsungen AG, 34212 Melsungen, Hessen, Germany) with detached needles, along with 25G hypodermic needles (0.5 mm x 16 mm) were used to administer both the sedative and the reversing agents. Negative pressure was applied before each IP injection to ensure safe and appropriate administration. To avoid variability due to circadian rhythm responses to intervention and sedation/ reversal agents, the sequence of rats receiving the intervention was reversed each session. Nonetheless, rats from both groups were injected and received the intervention at the same time. Fasting was not implemented before injections since rats do not possess vomit reflex.

To reduce psychological stress related to restraint and injections, following recovery from sedation, each rat was provided with a small piece (one quarter) of biscuit (Froiz Marie Gold Biscuits 800g, Galletas Gull**o**n, S.A, 34800 Aguilar de Campoo, Palencia, Spain); weighing about ≈1.75 gm. Unconsumed and partially consumed biscuit pieces were placed alongside the chow in the dietary compartment, for later ingestion.

The monopolar technique was used as it presumably allows for deeper penetration of the current as the distance between electrodes is increased with this method (Denegar et al., 2015). A small active electrode Zimmertrode self-adhesive small single use electrodes 32 mm x 40 mm (Zimmer MedizinSysteme GmbH, 89231 Neu-Ulm, Germany) were custom fit into (15 mm × 13 mm) was placed on the both hindlimbs right above the Achilles tendon, whereas a large dispersive electrode Chattanooga® Dura-Stick® Plus self-adhesive electrodes (5.0 cm × 5.0 cm) was placed on the back of the rat (about at least 7.5 cm) away from the active electrode. The location of the dispersive electrode was alternated (above/below) to compensate for possible differences related to the depth of penetration. An additional adhesive tape

(Hansaplast® classic fixation tape; Beiersdorf AG, 20245, Hamburg, Germany) was used to secure the active electrodes in place, in order to further improve contact and conduction. Velcro® straps were also used to provide additional support to the large dispersive electrodes (Figure 2).

The HVMPC intervention was applied to the experimental group for 8, 12, and 16 sessions. The fur on the back and both hindlimbs (tendon length) of all rats were shaved using a hair trimmer. Remnants of fur were removed using a commercial hair depilator cream (Veet® hair removal cream for normal skin for men, and normal and sensitive skin and women; Veet®, Slough SL1 0NS UK) before the start of the intervention, and application was repeated as necessary, in order to further improve conduction. For shaving, the dose administered of Dexdomitor® was 0.2 mg/kg, whereas Antisedan® was diluted in sterile injectable distilled water and administrated through the intraperitoneal (IP) route at a dose of 0.25 mg/kg. Signs of acute dermal irritation were assessed for using the Draize dermal irritation scoring system (Administration et al., 1959). Animal condition, as detailed by Morton and Griffiths, was monitored throughout the study period (Morton & Griffiths, 1985). Humane endpoints of rapid or progressive body weight loss of 20% of baseline body weight and severe chromodacryorrhoea were considered.

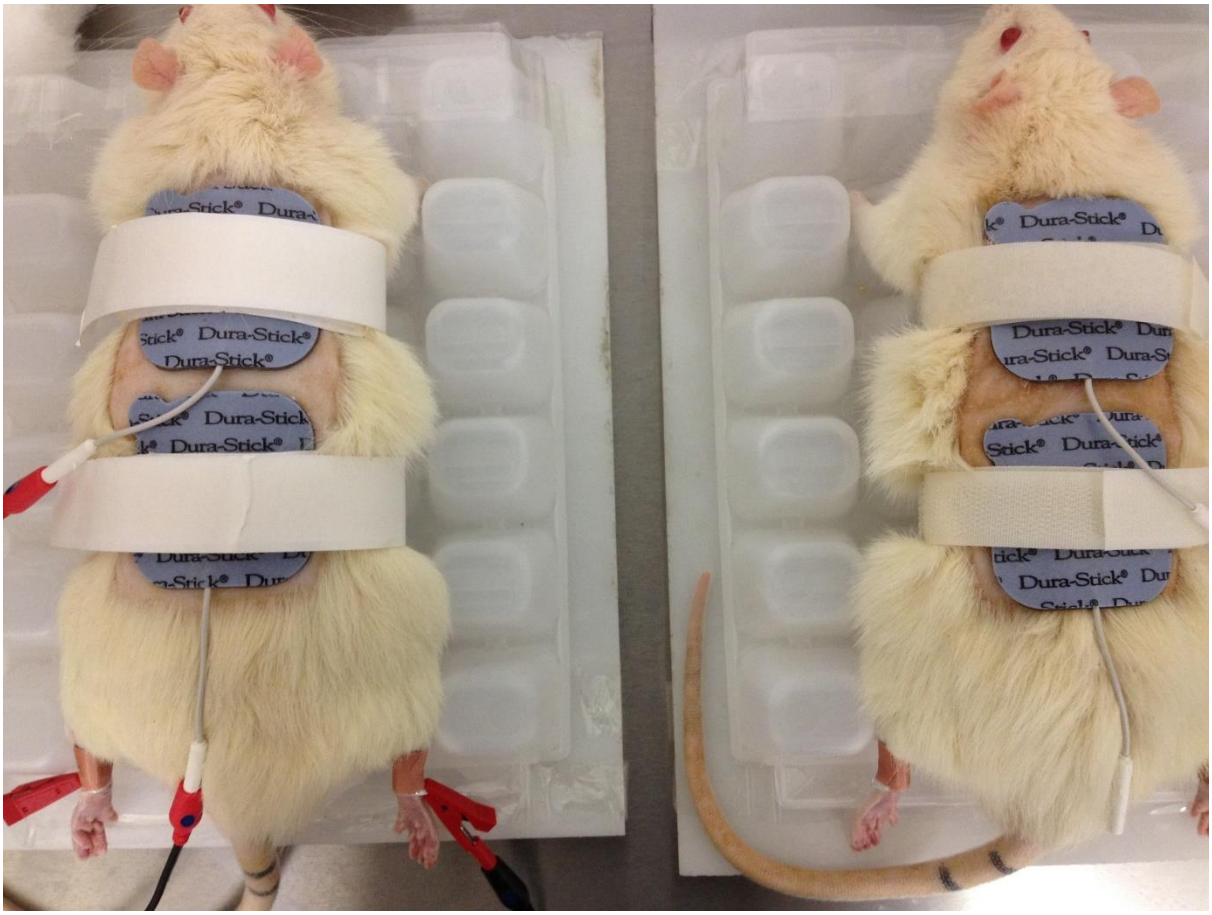


Figure 2. An actual photograph of the electrical stimulation protocol being implemented; note that rats from both stimulation (left) and sham (right) groups were concomitantly sedated.

**Euthanasia and Histological assessment**

Euthanasia was performed through anesthesia followed by harvesting vital organs (cardiopulmonary excision). Rats were anesthetized using ketamine (100 mg/kg) and xylazine (15 mg/kg) IP injection. Additional anesthetic doses were administered, as necessary, until absence of pedal withdrawal reflex was achieved. Both Achilles tendons were marked using a tissue marking dye (Epredia™ Mark-It™ Tissue-Marking Dye (Black), Thermo Fisher Scientific 168 Third Avenue, Waltham, MA 02451, USA), in order to identify and standardize the orientation of tendons for embedding. The marking dye, moreover, was used to determine the beginning and end of block trimming, and subsequently the start of sectioning and sample collection.

Following the excision of Achilles tendons of both hind limbs in all groups for histological analysis, the collected samples were coded so that subsequent analyses are blinded. Immediately after excision, samples were placed for fixation in formaldehyde 3.7-4.0% w/v buffered to pH=7 and stabilized with methanol (AppliChem GmbH, Ottoweg 4, DE-64291 Darmstadt, Germany), which were kept at 4°C for 1 week, and then put in individual cassettes (Tissue-Tek® Uni-Cassette® Biopsy Cassette Systems). Fixed samples were then placed in the tissue processor for dehydration in a progressive ethanol (C2H6O, EtOH, Fisher BioReagents™; Fisher Scientific UK Ltd., Bishop Meadow Road Loughborough Leicestershire, LE11 5RG Loughborough, United Kingdom) sequence as follows: 70%, 90%, and six stages of 100% for one hour each; followed by alcohol-xylene (1:1) for one hour and two stages of absolute xylene (C8H10, Fisher BioReagents™; Fisher Scientific UK Ltd., Bishop Meadow Road Loughborough Leicestershire, LE11 5RG Loughborough, United Kingdom) (one hour each), and lastly samples were kept in liquid paraffin at 60°C in preparation for embedding. Spin Tissue Processor STP 120 (Thermo Scientific™; 168 Third Avenue, Waltham, MA 0245, USA) was used to process samples. Subsequently, HistoStar™ Embedding Workstation (Thermo Scientific™; 168 Third Avenue, Waltham, MA 0245, USA) was used for paraffin embedding.

Paraffin-embedded samples were sectioned into 5 µm sections and secured on Menzel-Glaser (MENZEL GLASBEARBEITUNGSWERK GMBH & CO. KG. Saarbrückener Str. 248. D-38116 Braunschweig – Deutschland) Superfrost® Corners grounded, 45°, white and Polysine® adhesion glass slides. Leica RM2125 Rotary

Microtome (Leica Microsystems GmbH; Wetzlar, 35578, Germany) was used for sample sectioning. MX35 Premier+ microtome blades (Thermo Fisher Scientific 168 Third Avenue, Waltham, MA 02451, USA) were used for the sectioning. Samples were placed in an ice-cooled Baker's solution (Baker, 1941) to facilitate sectioning, which was done one section at a time, and the flotation-bath distilled water was kept at room temperature. The slides were numbered during sectioning, in order to unify sample analysis as much as possible, given that Achilles tendons are known to be composed of subtendons that may not follow a single orientation (Gains et al., 2020; Handsfield et al., 2020).

Hematoxylin (Hematoxylin solution acc. to Harris, 500 ml, glass; Carl Roth GmbH + Co. KG Schoemperlenstraße 3-5, 76185, Karlsruhe, Germany) and eosin (Eosin Y Solution, Alcoholic; Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA) (H&E) staining was used to identify nuclei and compare their number and activation between groups. Slides with number 1, 4, 7, 10, 13 and 16, from all groups, were stained with H&E. A modified regressive protocol was used for H&E staining (Cardiff et al., 2014). Moreover, Alcian-blue (pH 2.5; Alcian blue 8 GS (C.I. 74240), 5 g; Carl Roth GmbH + Co. KG Schoemperlenstraße 3-5, 76185, Karlsruhe, Germany; A stock solution of Alcian-blue stain pH 2.5 was prepared ((1.0 g Alcian blue 8GX + 97 ml distilled water (heat to dissolve), + 3 ml glacial acetic acid (Fluka 45726.)) and von Kossa stains (Silver plating kit acc. to von Kossa; Merck KGaA Headquarters of the Merck Group. Frankfurter Strasse 250. Darmstadt, 64293, Germany) were used to identify glycosaminoglycan content and microcalcification, respectively (Ippolito et al., 1983; Karlsson & Björnsson, 2001; Meloan & Puchtler, 1985).

Nuclear fast red-aluminum sulfate solution 0.1% (Kernechtrot C0482; Diapath S.p.A. via Pietro Savoldini, 71 - 24057 Martinengo (BG), Italy) was used as a counter-stain to von Kossa and Alcian-blue stained samples. Slides with number 3, 6, 9, 12, 15 and 18, from all groups, were stained with von Kossa and Alcian-blue. All solutions were filtered using nylon syringe filters (OlimPeak Syringe Filters, 0.2 µm, 13 mm Ø; Teknokroma, Camí de Can Calders,14, 08173 Sant Cugat del Vallés, Barcelona, Spain) at each time before use. For the sedentary control and experimental groups; 228 samples were stained with H&E, and additionally, 243 were stained with Alcian-blue and von Kossa stains (separately on the same slide). However, for the sham stimulation and

stimulation groups; 336 samples were stained with H&E, and moreover, 319 were stained with Alcian-blue and von Kossa stains (separately on the same slide). Samples were mounted using ROTI®Histokitt mounting medium (500 ml; Carl Roth GmbH + Co. KG Schoemperlenstraße 3-5, 76185, Karlsruhe, Germany), and covered with coverslips (24x60x0.16 mm; cod. 061061; Diapath S.p.A. via Pietro Savoldini, 71 - 24057 Martinengo (BG), Italy).

Images were acquired using Carl Zeiss Axio Imager AX10 A1M Microscope with Zeiss Axiocam 503 color (Carl Zeiss Microscopy GmbH; Carl Zeiss Promenade 10, 07745 Jena, Germany). Images of the Alcian-blue stained samples were acquired using the 10x objective and then stitched to form whole-tendon section images using Leica Application Suite Version 4.13.0 [Build:310] (Leica Microsystems GmbH, Ernst-Leitz-Strasse 17-37. 35578 Wetzlar, Germany). Then paratenon was cropped from stitched tendon images using Adobe Photoshop software (Adobe 345 Park Avenue San Jose, CA 95110-2704, United States of America) (Figure 3).

Images were then divided horizontally into three parts: proximal, middle and distal, similar to other studies (Disser et al., 2022; Heinemeier et al., 2012). The middle and distal parts may represent mid-substance and insertional tendon, respectively. The entire three parts were then analyzed using CellProfiler software (Broad Institute of MIT and Harvard) (Stirling et al., 2021). The area with Alcian-blue staining was determined once with two-class Otsu thresholding method and another with the three-class Otsu thresholding method (with considering middle intensity class pixels as background). The measured Alcian-blue areas were then divided by the sample surface area, in order to get the density of glycosaminoglycans in each sample.

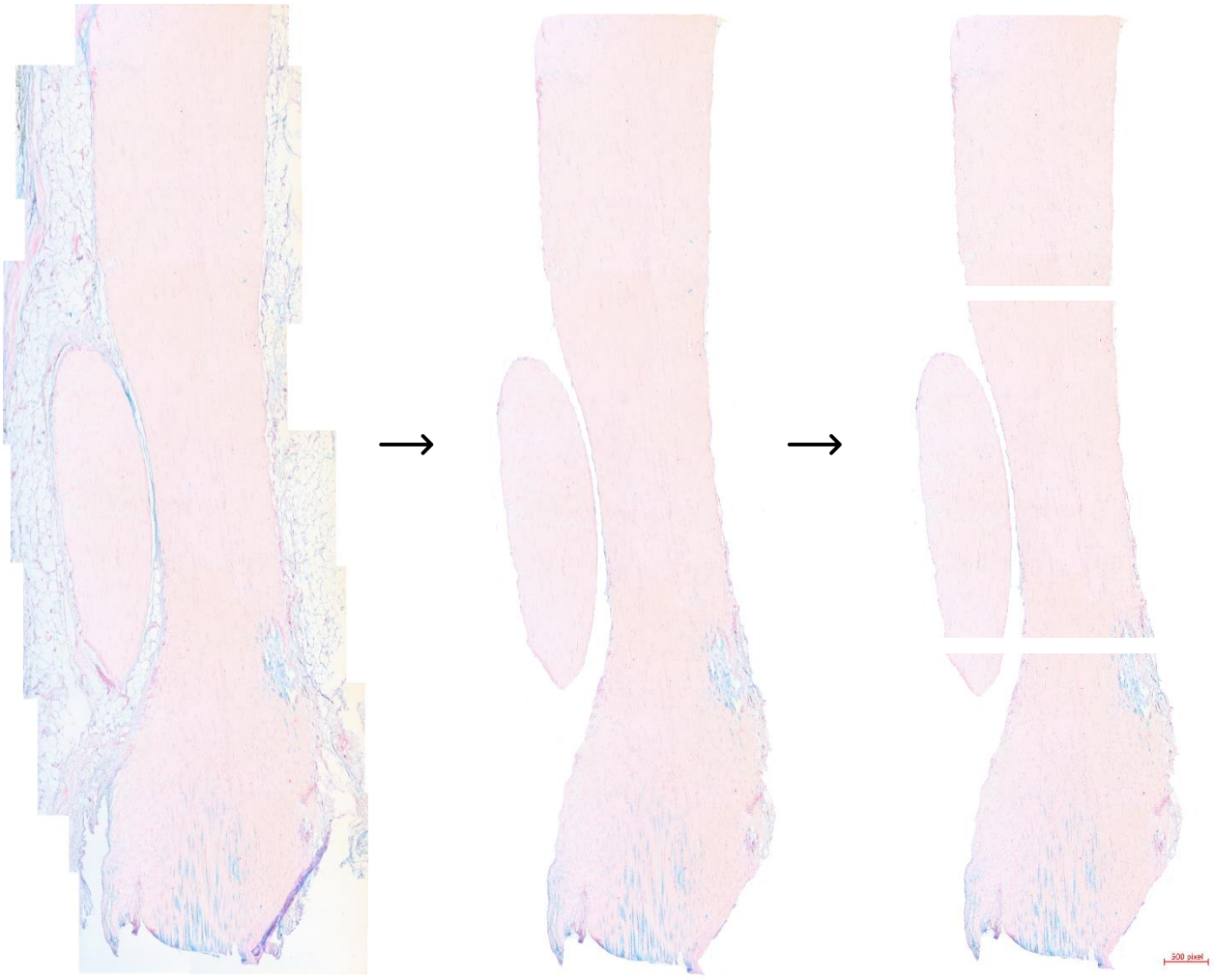


Figure 3. Image of the analytic process and division of the tendon in three parts representing: top, mid, and low.

Furthermore, a secondary analysis of glycosaminoglycan content was done, through excluding other structures that stains blue with Alcian-blue, such as sesamoid fibrocartilage and may act as confounding factors. Subsequently the identified objects were removed from images using the Object Selection tool and the resultant images were fed into CellProfiler. The three-class Otsu thresholding method was used to conduct this analysis.

Images with the minimum levels of Alcian-blue staining were identified in each sample based on descriptive statistical analysis, since a reduction in Alcian blue density was observed in the primary analysis. H&E-stained samples with the closest number to those with minimum levels of Alcian-blue staining were then selected and images were acquired using the 10x objective with manual extended depth of focus (quality: highest, registration method: translation, and interpolation: linear) of the Zeiss Zen 2.3 (lite edition) software (Carl Zeiss Microscopy GmbH; Carl Zeiss Promenade 10, 07745 Jena, Germany). Subsequently, images were given a code using the Gillmeister Rename Expert software 5.31.0 (M. & R. Gillmeister GbR, Pfingstanger 20, 38667 Bad Harzburg, Germany). Then, three regions of interest, following the previously described three-part tendon designation, with standard dimensions of 700 × 700 pixels were acquired at the sites with highest levels of ground substance staining (Figure 4).

CellProfiler software was used to identify the number of nuclei stained with H&E. Moreover, the circularity of the nuclei were assessed using ilastik (interactive image classification, segmentation and analysis) and FIJI (a distribution of ImageJ) software (Berg et al., 2019; Schindelin et al., 2012). Ilastik software was used to identify the nuclei through the object classification workflow [Inputs: Raw Data, Segmentation] and the results were exported as a binary image which was fed into FIJI software; the Otsu threshold was first applied then the circularity option of Analyze Particles module was run thrice with the following values; 0.00-0.33, 0.33-0.66, 0.66-1 (Chatterjee et al., 2023). Collagen fiber alignment was assessed through calculating dispersion values of the Directionality module (Chatterjee et al., 2023).

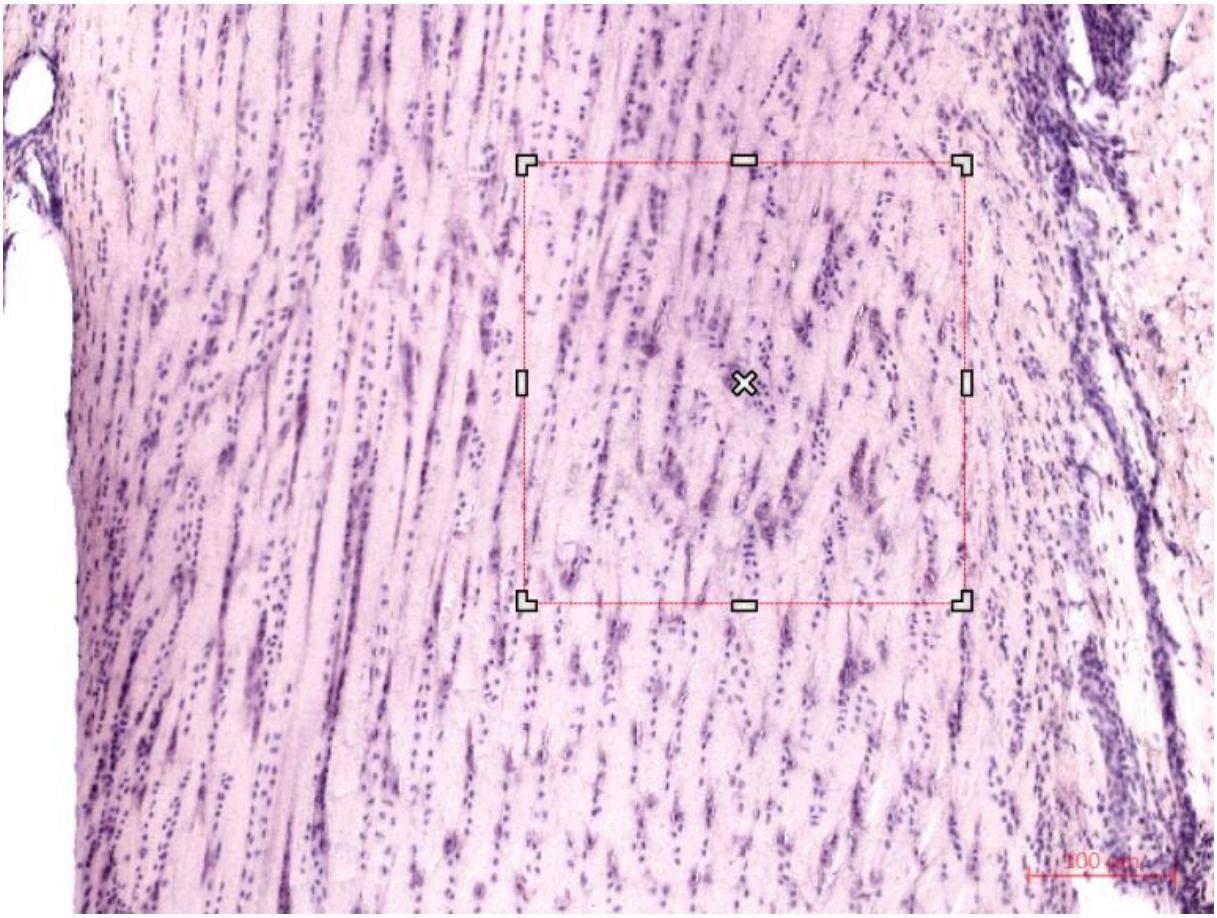


Figure 4. Microscope image showing the selection of a region of interest in H&E-stained samples using Zeiss Zen software; dimensions 700 × 700 pixels; magnification = 10x; scale bar= 100 µm

Samples with the minimum levels of Alcian-blue staining were used to identify the von Kossa stained-sample images to be acquired. Images were acquired, following the previously described three-part tendon designation, using the 10x objective with Zeiss Zen 3.3 (blue edition) software (Carl Zeiss Microscopy GmbH; Carl Zeiss Promenade 10, 07745 Jena, Germany) and were subsequently coded using the Gillmeister Rename Expert software 5.31.0 (M. & R. Gillmeister GbR, Pfingstanger 20, 38667 Bad Harzburg, Germany). Then, areas with mineral deposits were identified as regions of interest with 700 × 700 pixels. CellProfiler software was used to count the surface area of calcification (Figure 5).

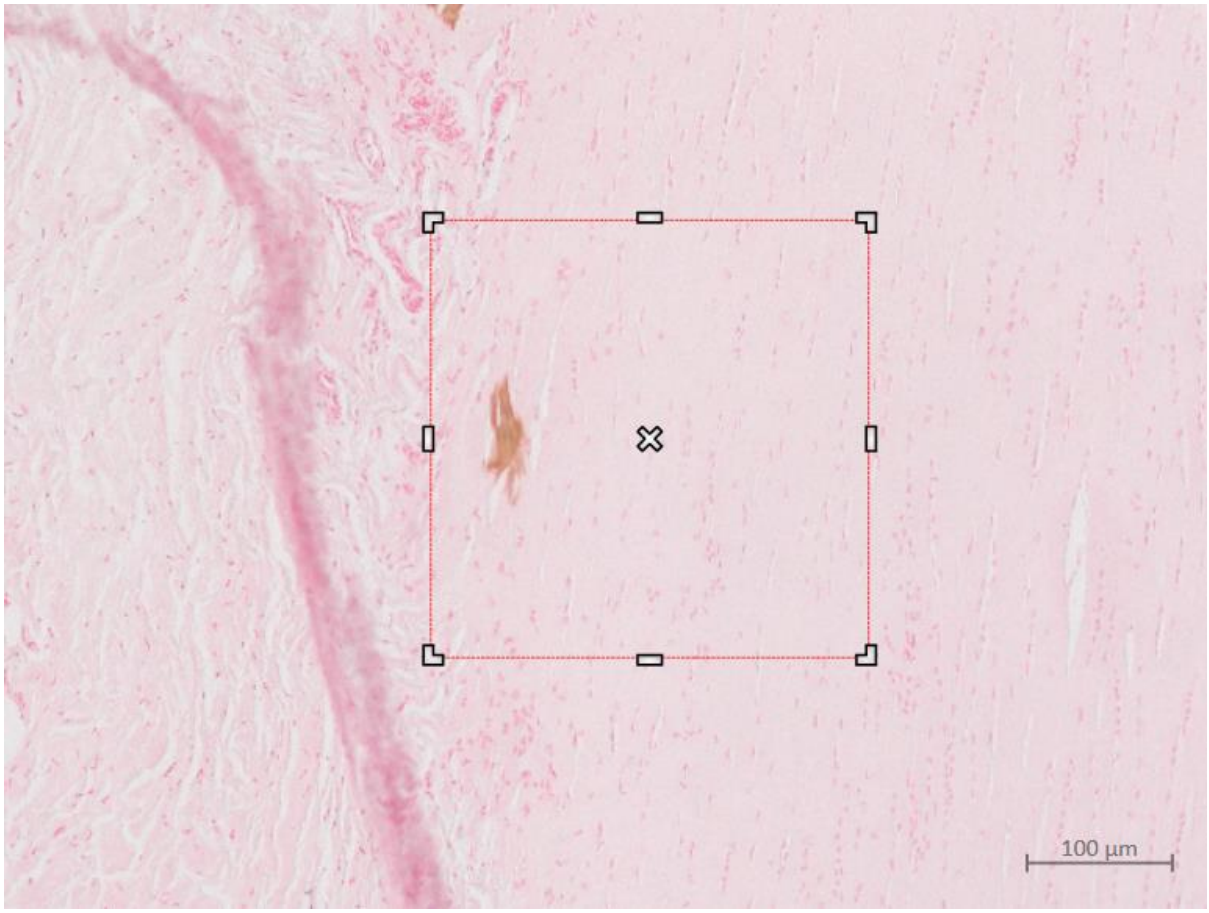


Figure 5. Microscope image showing the selection of a region of interest in von Kossa-stained samples using Zeiss Zen software; dimensions 700 × 700 pixels; magnification = 10x; scale bar= 100 µm

**Real-time polymerase chain reaction analysis**

RT-qPCR tests were performed for samples from the sedentary control and experimental groups only. RNeasy FFPE Kit (QIAGEN AG, Garstligweg 8, CH-8634 Hombrechtikon, Switzerland) was used, as per the manufacturer protocol, for total RNA extraction and purification from formalin-fixed and paraffin-embedded tendon tissue samples. In order to maintain consistency and comparability between groups; four sections (two slides; two samples per slide) bearing the same section numbers were used from each tendon. Slides with number 4 and 8, from all groups, were allocated for PCR tests. Subsequently, samples from both Achilles tendons of the same rat were combined to be processed. A NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific, 3411 Silverside Road, Bancroft Building, Suite 100, Wilmington, DE 19810, U.S.A.) was used to quantify the extracted RNA at 260/280 nm.

From each sample, one microgram of RNA, in a 20 µL reaction, was utilized to synthesize first-strand complementary DNA; qScript® cDNA Synthesis Kit (Quanta Biosciences, LLC. 100 Cummings Center Suite 407J Beverly, MA 01915, USA) using a Mastercycler® ep realplex Gradient S thermal cycler (Eppendorf SE, Barkhausenweg 1, 22339 Hamburg, Germany). PerfeCTa SYBR® Green FastMix kit (Quanta Biosciences, LLC. 100 Cummings Center Suite 407J Beverly, MA 01915, USA) was the method of choice to quantify transcripts through quantitative polymerase chain reaction (qPCR) following the manufacturer's methodology in a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific, 3411 Silverside Road, Bancroft Building, Suite 100, Wilmington, DE 19810 U.S.A.). Primer 3 software (Untergasser et al., 2012) was used to design primer sequences. The relative expression level of target genes was calculated using the $2^{-\Delta\Delta C_T}$ Method (Livak & Schmittgen, 2001).

The endogenous housekeeping genes GAPDH and RPL13a were tested for stability, using RefFinder web-based tool (Xie et al., 2023), and the more stable gene expression was determined to be GAPDH's, which was then used to normalize the transcript expression of target genes (*Ki-67*, *p16*, *p21*, *p53*, tenomodulin (*TNMD*), decorin (*DCN*), collagen 1 (*COL1*), collagen 3 (*COL3*), scleraxis (*SCX*), mohawk (*MKX*)). Transcript expression of the sedentary control group target genes were

additionally used as a calibrator. The primer sequences of all genes are available in the supplementary data, in addition to, the raw and calculated data.

**Data analysis**

**Histological assessment**

For ground substance data; normality and homogeneity were assessed using Shapiro-wilk test and Levene's statistic, respectively, which showed that the data are non-normally distributed and non-homogeneous. Non-parametric tests were therefore conducted; Independent Samples Kruskal-Wallis test, with correction for multiple comparisons using statistical hypothesis testing with Dunn's test. Mann-Whitney test was done for selected comparisons; sedentary control results were compared with end of protocol, 2 weeks of rest and 4 weeks of rest results; sedentary control results were compared with Sham ES 4W, Sham ES 6W and Sham ES 8W results. Furthermore, ES 4W vs Sham ES 4W, ES 6W vs Sham ES 6W, and ES 8W vs Sham ES 8W comparisons were done.

Normalization of results was explored; the method of choice was "Two-Step Approach for Transforming Continuous Variables to Normal" by Templeton (Templeton, 2011). Data were then reanalyzed after normality and homogeneity were confirmed. Parametric tests conducted were: One-way ANOVA test for repeated measures (followed by the *post hoc* test of Tukey) test was then conducted. Independent T-test for selected comparisons; sedentary control results were compared with end of protocol, 2 weeks of rest and 4 weeks of rest results; sedentary control results were compared with Sham ES 4W, Sham ES 6W and Sham ES 8W results. Furthermore, ES 4W vs Sham ES 4W, ES 6W vs Sham ES 6W, and ES 8W vs Sham ES 8W comparisons were done. GraphPad Prism software; version 9.5.1 (733); 225 Franklin Street. Fl. 26. Boston, MA 02110 was used to conduct all statistical analyses and produce graphs. Moreover, IBM SPSS statistics software; version 28.0.1.1 (15) was used to confirm the accuracy of the results. Statistical significance level considered was $P < 0.05$.

Data for cellularity, circularity, fiber alignment and baseline body weight were normally distributed and homogeneous, and consequently, parametric tests were conducted; One-Way ANOVA (*post hoc*: Tukey) test was done for between group comparisons and independent T-test was done for comparisons with the control group. Baseline chow consumption data did not pass the Shapiro-Wilk normality test, and

consequently, Kruskal-Wallis test was performed. Data for calcification, body weight and chow consumption progress were not compared between groups.

**Real-time polymerase chain reaction analysis**

Kruskal-Wallis test was used to assess the PCR results, due to their small sample size. An outlier sample was found and the results were thus presented with the outlier included and excluded.

## Results

### Overuse-induced tendon injury: an animal model

Non-statistically significant differences were found between groups with regard to mean body weight and chow consumption at baseline. Mean differences in baseline body weight between the sedentary control (SC) group and end of protocol (EP), 2 weeks of rest (2R) and 4 weeks of rest (4R) groups were -23.60; 95% CI: [-131.0, 83.83]; P= 0.9990, -24.40; 95% CI: [-125.7, 76.89]; P= 0.9980, and -30.40; 95% CI: [-131.7, 70.89]; P= 0.9898, respectively. Mean rank differences in baseline chow consumption were non-statistically significant between groups; H= 1.590; P= 0.6616; the SC group vs EP, 2R and 4R groups results were -3.200; Z= 0.8507; P= >0.9999, -4.200; Z= 1.184; P= >0.9999, and -1.600; Z= 0.4511; P= >0.9999, respectively (Figures 6, 7 and 8).

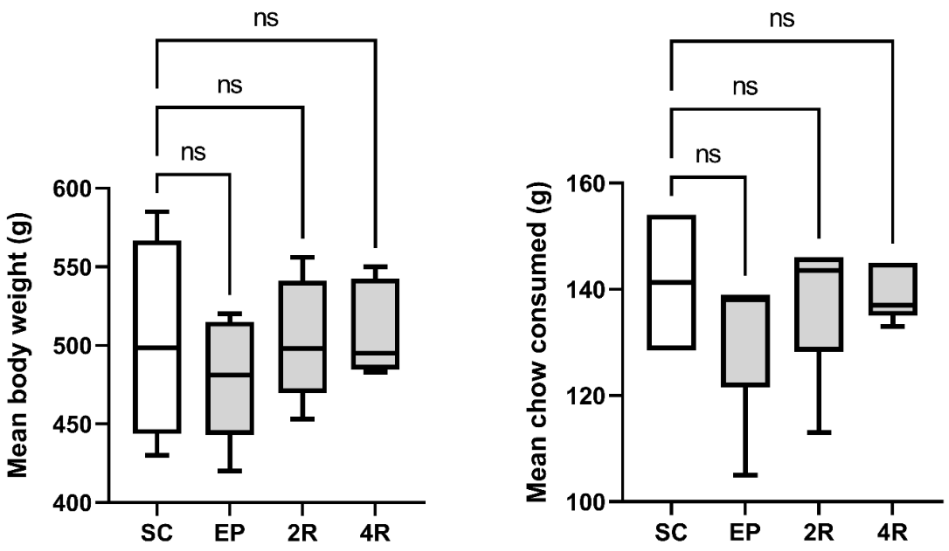



Figure 6. Mean body weight and chow consumption at baseline; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest; ns, non-statistically significant difference; g, grams; scale bars represent standard deviation

Mild chromodacryorrhoea according to scoring by Mason et al. (Mason et al., 2004) was observed only twice, and only in two rats, throughout the running protocol (during the last two weeks), which may indicate that the protocol was well tolerated. None of the humane endpoints were reached. All rats received the allocated protocol and were sacrificed in their due date.

## Body weight

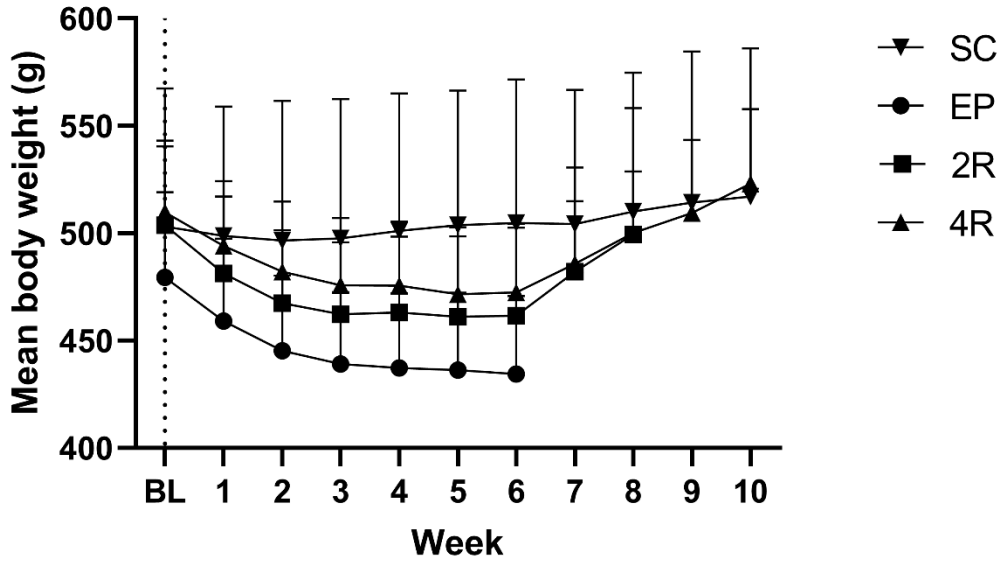



Figure 7. Change in mean body weight over the study span; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest; g, grams; note the gain in body weight following the end of running protocol at 6 weeks.

## Chow consumption

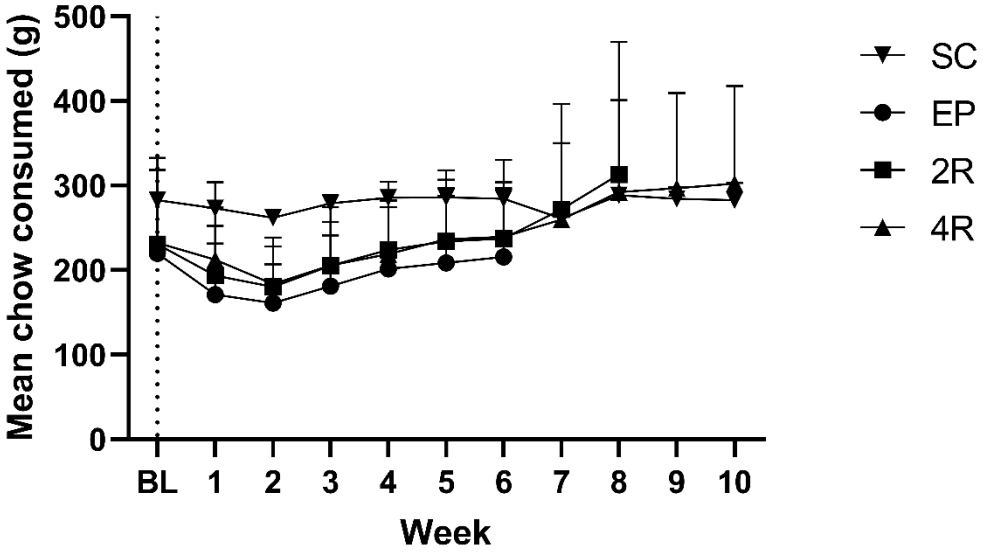



Figure 8. Change in mean chow consumption over the study span; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest; g, grams; note the increase in chow consumption following the end of running protocol at 6 weeks.

### Ground substance

#### Tendon middle third

Statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and EP groups in the middle-third of tendons. Moreover, a reduction in Alcian-blue density (two-class Otsu) was found between SC and 2R groups. Three-class Otsu Alcian-blue density analysis showed statistically significant differences between SC and EP groups. Comparing SC with 2R and 4R groups demonstrated non-statistically significant differences (three-class Otsu).

A statistically significant difference was found between EP and 4R groups in tendon middle-third using two-class Otsu but not three-class Otsu. Non-statistically significant differences, however, were identified between EP and 2R, and between 2R and 4R groups. Moreover, the differences between EP, 2R, and 4R groups were non-statistically significant in the three-class Otsu analysis.

### Ground substance with mineralized fibrocartilage excluded

Comparison between SC and EP groups, and SC and 2R groups using the three-class Otsu method and with areas of mineralized fibrocartilage excluded, revealed statistically significant differences. Non-statistically significant difference, however, was found between SC and 4R groups. Within-group comparison, using three-class Otsu method and including tendon middle-third but excluding areas of mineralized fibrocartilage, revealed non-statistically significant difference between EP, 2R and 4R groups.

#### Tendon lower third

The difference between SC and EP, 2R and 4R groups was non-statistically significant neither in two-class nor three-class Otsu assessments. Comparison between EP and 2R groups showed a statistically significant difference between groups, with both two-class and three-class Otsu analysis. Statistically significant differences were found between SC and EP, but not between SC and 2R, and SC and 4R groups in tendon lower-third, using two-class and three-class Otsu methods.

## Ground substance with mineralized fibrocartilage excluded

Comparing SC and EP groups revealed a statistically significant difference between the lower-third of tendons, excluding areas of mineralized fibrocartilage and using the three-class Otsu method. Both comparisons, nevertheless, of SC with 2R and 4R groups showed non-statistically significant results. Statistically significant differences were found both between EP and 2R, and EP and 4R groups, using the three-class Otsu method, excluding areas of mineralized fibrocartilage, and comparing tendon lower-third.

| | Comparison | Threshold | n | Median | Hodges-Lehmann | U | Difference between medians | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs.* EP | two-class Otsu | 47<br>59 | 5.930<br>2.460 | -2.460 | 830 | -3.470 | 0.0003 |
| | SC *vs.* EP | three-class Otsu | 47<br>59 | 2.190<br>0.7300 | 0.9400 | 901.5 | 1.460 | 0.0018 |
| | SC *vs.* EP | three-class Otsu MFC excluded | 47<br>59 | 1.740<br>0.6900 | 0.6500 | 938.5 | 1.050 | 0.0041 |
| | SC *vs.* 2R | two-class Otsu | 47<br>64 | 5.930<br>3.760 | -1.570 | 1172 | -2.170 | 0.0474 |
| | SC *vs.* 2R | three-class Otsu | 47<br>64 | 2.190<br>1.460 | 0.6100 | 1213 | 0.7300 | 0.0827 |
| | SC *vs.* 2R | three-class Otsu MFC excluded | 47<br>64 | 1.7400<br>1.000 | 0.5300 | 1157 | 0.7400 | 0.0381 |
| | SC *vs.* 4R | two-class Otsu | 47<br>67 | 5.930<br>4.870 | -1.040 | 1348 | -1.060 | 0.1936 |
| | SC *vs.* 4R | three-class Otsu | 47<br>67 | 2.190<br>1.570 | 0.4500 | 1332 | 0.6200 | 0.1638 |
| | SC *vs.* 4R | three-class Otsu MFC excluded | 47<br>67 | 1.740<br>1.070 | 0.3700 | 1304 | 0.6700 | 0.1201 |
| Tendon lower third | SC *vs.* EP | two-class Otsu | 47<br>59 | 13.20<br>9.600 | 3.510 | 1085 | 3.600 | 0.0551 |
| | SC *vs.* EP | three-class Otsu | 47<br>59 | 3.970<br>3.160 | 1.170 | 1093 | 0.8100 | 0.0620 |
| | SC *vs.* EP | three-class Otsu MFC excluded | 47<br>59 | 3.640<br>1.430 | 1.570 | 836 | 2.210 | 0.0004 |
| | SC *vs.* 2R | two-class Otsu | 47<br>59 | 2.190<br>0.7300 | -0.1900 | 901.5 | -0.3050 | 0.0018 |
| | SC *vs.* 2R | three-class Otsu | 47<br>64 | 3.970<br>4.530 | -0.6700 | 1361 | -0.5600 | 0.3941 |
| | SC *vs.* 2R | three-class Otsu MFC excluded | 47<br>64 | 3.640<br>2.795 | 0.6100 | 1292 | 0.8450 | 0.2061 |
| | SC *vs.* 4R | two-class Otsu | 47<br>67 | 13.20<br>12.18 | 0.7300 | 1507 | 1.020 | 0.7009 |
| | SC *vs.* 4R | three-class Otsu | 47<br>67 | 3.970<br>4.390 | 0.2200 | 1525 | -0.4200 | 0.7755 |
| | SC *vs.* 4R | three-class Otsu MFC excluded | 47<br>67 | 3.640<br>3.160 | 0.2700 | 1486 | 0.4800 | 0.6108 |

Table 1. Median difference in ground substance density ranks between sedentary control and experimental groups at the three timepoints, measured using two-class and three-class Otsu thresholding methods. Additionally, results of secondary analysis involving exclusion of mineralized fibrocartilage (MFC) are presented; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | Threshold | n | Mean Rank | Z | Mean Rank difference | H | df | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | EP *vs.* 2R | two-class Otsu | 59<br>64 | 79.25<br>97.90 | 1.879 | -18.65 | | | 0.1806 |
| | EP *vs.* 2R | three-class Otsu | 59<br>64 | 83.06<br>99.34 | 1.641 | -16.28 | | | 0.3025 |
| | EP *vs.* 2R | three-class Otsu MFC excluded | 59<br>64 | 83.70<br>98.45 | 1.486 | -14.75 | | | 0.4117 |
| | EP *vs.* 4R | two-class Otsu | 59<br>67 | 79.25<br>107.5 | 2.880 | -28.28 | | | 0.0119 |
| | EP *vs.* 4R | three-class Otsu | 59<br>67 | 83.06<br>102.8 | 2.009 | -19.72 | | | 0.1336 |
| | EP *vs.* 4R | three-class Otsu MFC excluded | 59<br>67 | 83.70<br>103.1 | 1.972 | -19.36 | | | 0.1457 |
| | 2R *vs.* 4R | two-class Otsu | 64<br>67 | 97.90<br>107.5 | 1.001 | -9.624 | | | 0.9501 |
| | 2R *vs.* 4R | three-class Otsu | 64<br>67 | 99.34<br>102.8 | 0.3579 | -3.440 | | | >0.9999 |
| | 2R *vs.* 4R | three-class Otsu MFC excluded | 64<br>67 | 98.45<br>103.1 | 0.4801 | -4.614 | | | >0.9999 |
| | Kruskal-Wallis | two-class Otsu | | | | | 8.479 | 2 | 0.0144 |
| | Kruskal-Wallis | three-class Otsu | | | | | 4.508 | 2 | 0.1050 |
| | Kruskal-Wallis | three-class Otsu MFC excluded | | | | | 4.168 | 2 | 0.1244 |
| Tendon lower third | EP *vs.* 2R | two-class Otsu | 59<br>64 | 80.94<br>105.0 | 2.426 | -24.07 | | | 0.0458 |
| | EP *vs.* 2R | three-class Otsu | 59<br>64 | 79.82<br>109.0 | 2.936 | -29.14 | | | 0.0100 |
| | EP *vs.* 2R | three-class Otsu MFC excluded | 59<br>64 | 74.89<br>100.9 | 2.624 | -26.04 | | | 0.0261 |
| | EP *vs.* 4R | two-class Otsu | 59<br>67 | 80.94<br>99.23 | 1.863 | -18.29 | | | 0.1874 |
| | EP *vs.* 4R | three-class Otsu | 59<br>67 | 79.82<br>96.45 | 1.693 | -16.63 | | | 0.2711 |
| | EP *vs.* 4R | three-class Otsu MFC excluded | 59<br>67 | 74.89<br>108.5 | 3.420 | -33.57 | | | 0.0019 |
| | 2R *vs.* 4R | two-class Otsu | 64<br>67 | 105.0<br>99.23 | 0.6018 | 5.784 | | | >0.9999 |
| | 2R *vs.* 4R | three-class Otsu | 64<br>67 | 109.0<br>96.45 | 1.302 | 12.51 | | | 0.5789 |
| | 2R *vs.* 4R | three-class Otsu MFC excluded | 64<br>67 | 100.9<br>108.5 | 0.7837 | -7.533 | | | >0.9999 |
| | Kruskal-Wallis | two-class Otsu | | | | | 6.360 | 2 | 0.0416 |
| | Kruskal-Wallis | three-class Otsu | | | | | 8.650 | 2 | 0.0132 |
| | Kruskal-Wallis | three-class Otsu MFC excluded | | | | | 12.63 | 2 | 0.0018 |

Table 2. Mean rank difference in ground substance density within experimental group, measured using two-class and three-class Otsu thresholding methods. Additionally, results of secondary analysis involving exclusion of mineralized fibrocartilage (MFC) are presented; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. Statistical significance level considered; P values less than or equal to 0.05 were considered statistically significant.

**Tendon middle-third ground substance - normalized data**

A statistically significant difference was found between SC and EP groups in the two-class Otsu analysis. The same comparison, moreover, revealed statistically significant differences using the three-class Otsu method. The comparisons between SC and 2R showed statistically significant differences in two-class Otsu but not three-class Otsu methods. Non-statistically significant differences, however, were found between SC and 4R groups in both two-class and three-class Otsu methods.

**Ground substance with mineralized fibrocartilage excluded - normalized data**

A statistically significant difference between SC and EP groups, using three-class Otsu method and analyzing tendon middle-third excluding areas of mineralized fibrocartilage. Nonetheless, non-statistically significant difference between SC and 2R groups was found. Non-statistically significant difference, furthermore, was identified between SC and 4R groups. Comparisons between EP, 2R and 4R groups, excluding areas of mineralized fibrocartilage and analyzing middle-third of tendons using three-class Otsu method, indicated non-statistically significant differences between groups.

**Tendon lower-third ground substance - normalized data**

Non-statistically significant differences were found between SC, and EP, 2R and 4R groups in tendon lower-third, neither using two-class nor three-class Otsu methods.

**Ground substance with mineralized fibrocartilage excluded - normalized data**

Following the normalization of data and excluding mineralized fibrocartilage areas, the analysis of tendon lower-third using three-class Otsu thresholding method revealed a statistically significant difference between SC and EP groups. However, comparing SC with 2R and 4R groups did not show statistically significant differences. Statistically significant differences were identified between EP and 2R, and EP and 4R, following the exclusion of mineralized fibrocartilage areas and analyzing tendon lower-third, using three-class Otsu method.

| | Comparison | Threshold | n | Mean | t | df | Difference between means (SEM) | [95% CI] | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs.* EP | two-class Otsu | 46<br>59 | 9.188<br>3.567 | 3.777 | 103 | 5.621 (1.488) | 2.669, 8.572 | 0.0003 |
| | SC *vs.* EP | three-class Otsu | 46<br>59 | 4.747<br>1.865 | 3.039 | 103 | 2.882 (0.9484) | 1.001, 4.763 | 0.0030 |
| | SC *vs.* EP | three-class Otsu MFC excluded | 46<br>59 | 2.703<br>1.249 | 2.960 | 103 | 1.453 (0.4911) | 0.4795, 2.427 | 0.0038 |
| | SC *vs.* 2R | two-class Otsu | 46<br>64 | 9.188<br>6.205 | 2.430 | 108 | 2.983 (1.227) | 0.5500, 5.416 | 0.0167 |
| | SC *vs.* 2R | three-class Otsu | 46<br>64 | 4.747<br>3.110 | 1.923 | 108 | 1.638 (0.8516) | -0.05014, 3.326 | 0.0571 |
| | SC *vs.* 2R | three-class Otsu MFC excluded | 46<br>64 | 2.703<br>1.855 | 1.970 | 108 | 0.8475 (0.4301) | -0.005161, 1.700 | 0.0514 |
| | SC *vs.* 4R | two-class Otsu | 46<br>67 | 9.188<br>7.466 | 1.328 | 111 | 1.722 (1.296) | -0.8471, 4.291 | 0.1869 |
| | SC *vs.* 4R | three-class Otsu | 46<br>67 | 4.747<br>3.432 | 1.448 | 111 | 1.315 (0.9084) | -0.4851, 3.115 | 0.1506 |
| | SC *vs.* 4R | three-class Otsu MFC excluded | 46<br>67 | 2.703<br>2.073 | 1.361 | 111 | 0.6295 (0.4626) | -0.2872, 1.546 | 0.1764 |
| Tendon lower third | SC *vs.* EP | two-class Otsu | 47<br>58 | 19.55<br>11.11 | 2.785 | 103 | 8.441 (3.031) | 2.430, 14.45 | 0.0064 |
| | SC *vs.* EP | three-class Otsu | 47<br>58 | 7.975<br>3.576 | 2.696 | 103 | 4.399 (1.632) | 1.163, 7.635 | 0.0082 |
| | SC *vs.* EP | three-class Otsu MFC excluded | 47<br>58 | 0.6700<br>0.3938 | 4.169 | 103 | 0.2762 (0.0662) | 0.1448, 0.4076 | <0.0001 |
| | SC *vs.* 2R | two-class Otsu | 47<br>64 | 19.55<br>18.77 | 0.2846 | 109 | 0.7725 (2.714) | -4.607, 6.153 | 0.7765 |
| | SC *vs.* 2R | three-class Otsu | 47<br>64 | 7.975<br>8.647 | 0.4899 | 109 | -0.6716 (1.371) | -3.389, 2.046 | 0.6252 |
| | SC *vs.* 2R | three-class Otsu MFC excluded | 47<br>64 | 0.6700<br>0.5884 | 1.519 | 109 | 0.08156 (0.0536) | -0.02485, 0.1880 | 0.1316 |
| | SC *vs.* 4R | two-class Otsu | 47<br>67 | 19.55<br>17.80 | 0.6758 | 112 | 1.746 ± 2.584 | -3.373, 6.865 | 0.5005 |
| | SC *vs.* 4R | three-class Otsu | 47<br>67 | 7.975<br>7.161 | 0.6116 | 112 | 0.8141 (1.331) | -1.823, 3.451 | 0.5420 |
| | SC *vs.* 4R | three-class Otsu MFC excluded | 47<br>67 | 0.6700<br>0.6334 | 0.6636 | 112 | 0.03657 (0.0551) | -0.07262, 0.1458 | 0.5083 |

Table 3. Mean difference in ground substance density between sedentary control and experimental groups at the three timepoints, measured using two-class and three-class Otsu thresholding methods. Additionally, results of secondary analysis involving exclusion of mineralized fibrocartilage (MFC) are presented; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | Threshold | n | Mean | df | Difference between means (SEM) | [95% CI] | F | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | EP *vs.* 2R | two-class Otsu | 59<br>64 | 3.567<br>6.205 | 187 | -2.638 (1.178) | -5.420, 0.1442 | | 0.0672 |
| | EP vs. 2R | three-class Otsu | 59<br>65 | 1.865<br>3.080 | 188 | -1.215 (0.8258) | -3.166, 0.7358 | | 0.3070 |
| | EP vs. 2R | three-class Otsu MFC excluded | 59<br>64 | 1.249<br>1.855 | 187 | -0.6060 (0.4071) | -1.568, 0.3558 | | 0.2987 |
| | EP vs. 4R | two-class Otsu | 59<br>67 | 3.567<br>7.466 | 187 | -3.899 (1.165) | -6.651, -1.147 | | 0.0028 |
| | EP vs. 4R | three-class Otsu | 59<br>67 | 1.865<br>3.432 | 188 | -1.567 (0.8199) | -3.504, 0.3697 | | 0.1382 |
| | EP *vs.* 4R | three-class Otsu MFC excluded | 59<br>67 | 1.249<br>2.073 | 187 | -0.8240 (0.4027) | -1.775, 0.1274 | | 0.1042 |
| | 2R vs. 4R | two-class Otsu | 64<br>67 | 6.205<br>7.466 | 187 | -1.261 (1.140) | -3.955, 1.433 | | 0.5118 |
| | 2R vs. 4R | three-class Otsu | 65<br>67 | 3.080<br>3.432 | 188 | -0.3522 (0.7995) | -2.241, 1.537 | | 0.8986 |
| | 2R *vs.* 4R | three-class Otsu MFC excluded | 64<br>67 | 1.855<br>2.073 | 187 | -0.2180 (0.3943) | -1.149, 0.7135 | | 0.8451 |
| | ANOVA | two-class Otsu | | | | | | 5.760 | 0.0037 |
| | ANOVA | three-class Otsu | | | | | | 1.975 | 0.1416 |
| | ANOVA | three-class Otsu MFC excluded | | | | | | 2.211 | 2.211 |
| Tendon lower third | EP *vs.* 2R | two-class Otsu | 58<br>64 | 11.11<br>18.77 | 186 | -7.668 (2.553) | -13.70, -1.637 | | 0.0085 |
| | EP vs. 2R | three-class Otsu | 58<br>64 | 3.576<br>8.647 | 186 | -5.071 (1.411) | -8.405, -1.736 | | 0.0012 |
| | EP vs. 2R | three-class Otsu MFC excluded | 58<br>64 | 0.3938<br>0.5884 | 186 | -0.1946 (0.05647) | -0.3281, -0.06122 | | 0.0020 |
| | EP vs. 4R | two-class Otsu | 58<br>67 | 11.11<br>17.80 | 186 | -6.695 (2.525) | -12.66, -0.7280 | | 0.0236 |
| | EP vs. 4R | three-class Otsu | 58<br>67 | 3.576<br>7.161 | 186 | -3.585 (1.396) | -6.884, -0.2859 | | 0.0296 |
| | EP *vs.* 4R | three-class Otsu MFC excluded | 58<br>67 | 0.3938<br>0.6334 | 186 | -0.2396 (0.05587) | -0.3716, -0.1076 | | <0.0001 |
| | 2R vs. 4R | two-class Otsu | 64<br>67 | 18.77<br>17.80 | 186 | 0.9735 (2.461) | -4.841, 6.788 | | 0.9174 |
| | 2R vs. 4R | three-class Otsu | 64<br>67 | 8.647<br>7.161 | 186 | 1.486 (1.361) | -1.729, 4.701 | | 0.5204 |
| | 2R *vs.* 4R | three-class Otsu MFC excluded | 64<br>67 | 0.5884<br>0.6334 | 186 | -0.04500 (0.05445) | -0.1736, 0.08364 | | 0.6871 |
| | ANOVA | two-class Otsu | | | | | | 5.290 | 0.0058 |
| | ANOVA | three-class Otsu | | | | | | 6.758 | 0.0015 |
| | ANOVA | three-class Otsu MFC excluded | | | | | | 10.16 | <0.0001 |

Table 4. Mean difference in ground substance density within experimental group at the three timepoints, measured using two-class and three-class Otsu thresholding methods. Additionally, results of secondary analysis involving exclusion of mineralized fibrocartilage (MFC) are presented; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P values less than or equal to 0.05 were considered statistically significant.

## Cellularity

Non-statistically significant differences were found in both the tendon middle-third and lower-third analyses of cellularity between EP, 2R and 4R groups. Non-statistically significant difference in cellularity in tendon middle-third was found when SC group was compared with EP, 2R and 4R groups. Data of the lower-third, nevertheless, indicated statistically significant differences between SC and 4R groups, but not EP and 2R groups.

| | Comparison | n | Mean Rank | Z | Mean Rank difference | H | df | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | EP *vs.* 2R | 10<br>10 | 13.95<br>15.45 | 0.3811 | -1.500 | | | >0.9999 |
| | EP *vs.* 4R | 10<br>10 | 13.95<br>17.10 | 0.8004 | -3.150 | | | >0.9999 |
| | 2R *vs.* 4R | 10<br>10 | 15.45<br>17.10 | 0.4192 | -1.650 | | | >0.9999 |
| | Kruskal-Wallis | | | | | 0.6411 | 2 | 0.7258 |
| Tendon lower third | EP *vs.* 2R | 10<br>10 | 15.20<br>16.10 | 0.2286 | -0.9000 | | | >0.9999 |
| | EP *vs.* 4R | 10<br>10 | 15.20<br>15.20 | 0.000 | 0.000 | | | >0.9999 |
| | 2R *vs.* 4R | 10<br>10 | 16.10<br>15.20 | 0.2286 | 0.9000 | | | >0.9999 |
| | Kruskal-Wallis | | | | | 0.06969 | 2 | 0.9658 |

Table 5. Mean rank difference in nuclei number between the three timepoints of the experimental group in tendon middle and lower-thirds analyses; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | n | Mean | t | df | Difference between means (SEM) | [95% CI] | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs.* EP | 8<br>10 | 383.3<br>301.7 | 1.272 | 16 | -81.55 (64.12) | -217.5, 54.38 | 0.2216 |
| | SC *vs.* 2R | 8<br>10 | 383.3<br>307.9 | 1.339 | 16 | -75.35 (56.29) | -194.7, 43.97 | 0.1994 |
| | SC *vs.* 4R | 8<br>10 | 383.3<br>307.5 | 1.597 | 16 | -75.75 (47.42) | -176.3, 24.78 | 0.1297 |
| Tendon lower third | SC vs. EP | 8<br>10 | 471.9<br>348.0 | 1.573 | 16 | -123.9 (78.75) | -290.8, 43.06 | 0.1353 |
| | SC *vs.* 2R | 8<br>10 | 471.9<br>356.1 | 1.606 | 16 | -115.8 (72.10) | -268.6, 37.06 | 0.1279 |
| | SC *vs.* 4R | 8<br>10 | 471.9<br>333.0 | 2.169 | 16 | -138.9 (64.02) | -274.6, -3.157 | 0.0455 |

Table 6. Mean difference in nuclei number between the sedentary control and experimental groups at the three timepoints in middle and lower-thirds analyses; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P values less than or equal to 0.05 were considered statistically significant.

## Circularity

| | Comparison | Threshold | n | Mean Rank | Z | Mean Rank difference | H | df | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | EP *vs.* 2R | Non-rounded | 10<br>10 | 14.80<br>16.30 | 0.3819 | -1.500 | | | >0.9999 |
| | EP *vs.* 4R | Non-rounded | 10<br>10 | 14.80<br>15.40 | 0.1527 | -0.6000 | | | >0.9999 |
| | 2R *vs.* 4R | Non-rounded | 10<br>10 | 16.30<br>15.40 | 0.2291 | 0.9000 | | | >0.9999 |
| | Kruskal-Wallis | Non-rounded | | | | | 0.1478 | 2 | 0.9288 |
| | EP *vs.* 2R | Semi-rounded | 10<br>10 | 14.70<br>14.40 | 0.07620 | 0.3000 | | | >0.9999 |
| | EP *vs.* 4R | Semi-rounded | 10<br>10 | 14.70<br>17.40 | 0.6858 | -2.700 | | | >0.9999 |
| | 2R *vs.* 4R | Semi-rounded | 10<br>10 | 14.40<br>17.40 | 0.7620 | -3.000 | | | >0.9999 |
| | Kruskal-Wallis | Semi-rounded | | | | | 0.7045 | 2 | 0.7031 |
| | EP *vs.* 2R | Fully-rounded | 10<br>10 | 16.30<br>16.20 | 0.02540 | 0.1000 | | | >0.9999 |
| | EP *vs.* 4R | Fully-rounded | 10<br>10 | 16.30<br>14.00 | 0.5842 | 2.300 | | | >0.9999 |
| | 2R *vs.* 4R | Fully-rounded | 10<br>10 | 16.20<br>14.00 | 0.5588 | 2.200 | | | >0.9999 |
| | Kruskal-Wallis | Fully-rounded | | | | | 0.4361 | 2 | 0.8041 |
| Tendon lower third | EP *vs.* 2R | Non-rounded | 10<br>10 | 16.85<br>14.50 | 0.6082 | 2.350 | | | >0.9999 |
| | EP *vs.* 4R | Non-rounded | 10<br>10 | 16.85<br>15.15 | 0.4400 | 1.700 | | | >0.9999 |
| | 2R *vs.* 4R | Non-rounded | 10<br>10 | 14.50<br>15.15 | 0.1682 | -0.6500 | | | >0.9999 |
| | Kruskal-Wallis | Non-rounded | | | | | 0.3945 | 2 | 0.8210 |
| | EP *vs.* 2R | Semi-rounded | 10<br>10 | 16.30<br>13.90 | 0.6096 | 2.400 | | | >0.9999 |
| | EP *vs.* 4R | Semi-rounded | 10<br>10 | 16.30<br>16.30 | 0.000 | 0.000 | | | >0.9999 |
| | 2R *vs.* 4R | Semi-rounded | 10<br>10 | 13.90<br>16.30 | 0.6096 | -2.400 | | | >0.9999 |
| | Kruskal-Wallis | Semi-rounded | | | | | 0.4955 | 2 | 0.7806 |
| | EP *vs.* 2R | Fully-rounded | 10<br>10 | 14.70<br>17.00 | 0.5842 | -2.300 | | | >0.9999 |
| | EP *vs.* 4R | Fully-rounded | 10<br>10 | 14.70<br>14.80 | 0.02540 | -0.1000 | | | >0.9999 |
| | 2R *vs.* 4R | Fully-rounded | 10<br>10 | 17.00<br>14.80 | 0.5588 | 2.200 | | | >0.9999 |
| | Kruskal-Wallis | Fully-rounded | | | | | 0.4361 | 2 | 0.8041 |

Table 7. Mean rank difference in nuclei roundedness between the three timepoints of the experimental group in tendon middle and lower-thirds analyses; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | Roundedness | n | Mean x̄ or Median x̃ | t or U | df | Mean (SEM) or Median difference | [95% CI] or Hodges-Lehmann | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs.* EP | Non-rounded | 8<br>10 | x̃ = 0.01317<br>x̃ = 0.01266 | 36 | 16 | -0.0005050 | -0.001927 | 0.7610 |
| | SC *vs.* 2R | Non-rounded | 8<br>10 | x̃ = 0.01317<br>x̃ = 0.01576 | 36 | 16 | 0.002588 | 0.001034 | 0.7618 |
| | SC *vs.* 4R | Non-rounded | 8<br>10 | x̃ = 0.01317<br>x̃ = 0.01236 | 40 | 16 | -0.0008120 | 0.000053 | >0.9999 |
| | SC *vs.* EP | Semi-rounded | 8<br>10 | x̄ = 0.3143<br>x̄ = 0.2386 | 1.024 | 16 | -0.07576 (0.07396) | -0.2325, 0.08102 | 0.3209 |
| | SC *vs.* 2R | Semi-rounded | 8<br>10 | x̄ = 0.3143<br>x̄ = 0.2384 | 1.129 | 16 | -0.07598 (0.06730) | -0.2187, 0.06668 | 0.2755 |
| | SC *vs.* 4R | Semi-rounded | 8<br>10 | x̄ = 0.3143<br>x̄ = 0.2952 | 0.3119 | 16 | -0.01911 (0.06127) | -0.1490, 0.1108 | 0.7592 |
| | SC *vs.* EP | Fully-rounded | 8<br>10 | x̄ = 0.6657<br>x̄ = 0.7436 | 0.9645 | 16 | 0.07790 (0.08077) | -0.09333, 0.2491 | 0.3492 |
| | SC *vs.* 2R | Fully-rounded | 8<br>10 | x̄ = 0.6657<br>x̄ = 0.7440 | 1.077 | 16 | 0.07833 (0.07274) | -0.07586, 0.2325 | 0.2975 |
| | SC *vs.* 4R | Fully-rounded | 8<br>10 | x̄ = 0.6657<br>x̄ = 0.6808 | 0.2111 | 16 | 0.01511 (0.07159) | -0.1367, 0.1669 | 0.8355 |
| Tendon lower third | SC *vs.* EP | Non-rounded | 8<br>10 | x̄ = 0.01805<br>x̄ = 0.01685 | 0.1279 | 16 | -0.001200 (0.00938) | -0.02109, 0.0186 | 0.8998 |
| | SC *vs.* 2R | Non-rounded | 8<br>10 | x̄ = 0.01805<br>x̄ = 0.00947 | 1.209 | 16 | -0.008577 (0.00709) | -0.02362, 0.0064 | 0.2443 |
| | SC *vs.* 4R | Non-rounded | 8<br>10 | x̃ = 0.01340<br>x̃ = 0.00400 | 33.50 | 16 | -0.009395 | -0.003295 | 0.5840 |
| | SC *vs.* EP | Semi-rounded | 8<br>10 | x̃ = 0.2776<br>x̃ = 0.2349 | 29 | 16 | -0.04274 | -0.04478 | 0.3599 |
| | SC *vs.* 2R | Semi-rounded | 8<br>10 | x̄ = 0.2747<br>x̄ = 0.1731 | 1.679 | 16 | -0.1016 (0.06054) | -0.2300, 0.02672 | 0.1127 |
| | SC *vs.* 4R | Semi-rounded | 8<br>10 | x̄ = 0.2747<br>x̄ = 0.2248 | 0.6904 | 16 | -0.04991 (0.07230) | -0.2032, 0.1034 | 0.4998 |
| | SC *vs.* EP | Fully-rounded | 8<br>10 | x̄ = 0.7073<br>x̄ = 0.7810 | 1.019 | 16 | 0.07376 (0.07239) | -0.07970, 0.2272 | 0.3234 |
| | SC *vs.* 2R | Fully-rounded | 8<br>10 | x̄ = 0.7073<br>x̄ = 0.8175 | 1.669 | 16 | 0.1103 (0.06606) | -0.02979, 0.2503 | 0.1146 |
| | SC *vs.* 4R | Fully-rounded | 8<br>10 | x̄ = 0.7073<br>x̄ = 0.7592 | 0.6359 | 16 | 0.05195 (0.08169) | -0.1212, 0.2251 | 0.5338 |

Table 8. Mean or median difference in nuclei roundedness between the three timepoints of the experimental group in tendon middle and lower-thirds analyses; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P values less than or equal to 0.05 were considered statistically significant.

## Fiber alignment

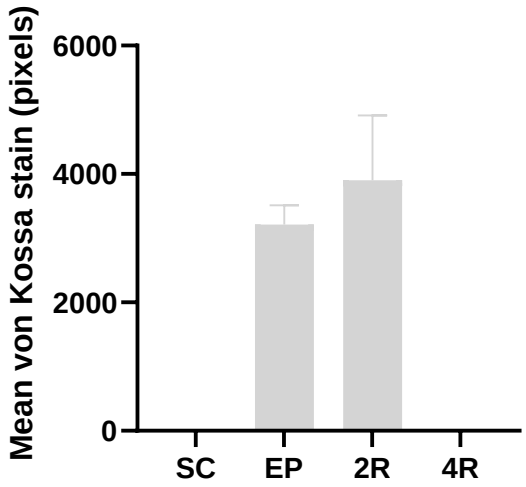
| | Comparison | n | Mean | t | df | Difference between means (SEM) | [95% CI] | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs.* EP | 8<br>10 | 4.869<br>6.279 | 1.827 | 16 | 1.410 (0.7717) | -0.2257, 3.046 | 0.0863 |
| | SC *vs.* 2R | 8<br>10 | 4.869<br>5.355 | 0.8005 | 16 | 0.4862 (0.6075) | -0.8015, 1.774 | 0.4352 |
| | SC *vs.* 4R | 8<br>10 | 4.869<br>6.097 | 1.434 | 16 | 1.228 (0.8564) | -0.5872, 3.044 | 0.1708 |
| Tendon lower third | SC *vs.* EP | 8<br>10 | 6.745<br>6.915 | 0.1186 | 16 | 0.1700 (1.433) | -2.868, 3.208 | 0.9070 |
| | SC *vs.* 2R | 8<br>10 | 6.745<br>6.406 | 0.2359 | 16 | -0.3390 (1.437) | -3.385, 2.707 | 0.8165 |
| | SC *vs.* 4R | 8<br>10 | 6.745<br>7.138 | 0.2537 | 16 | 0.3930 (1.549) | -2.891, 3.677 | 0.8030 |

Table 9. Mean difference in fiber dispersion between the sedentary control and experimental groups at the three timepoints in middle and lower-thirds analyses; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | n | Mean Rank | Z | Mean Rank difference | H | df | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | EP *vs.* 2R | 10<br>10 | 16.60<br>13.70 | 0.7367 | 2.900 | | | >0.9999 |
| | EP *vs.* 4R | 10<br>10 | 16.60<br>16.20 | 0.1016 | 0.4000 | | | >0.9999 |
| | 2R *vs.* 4R | 10<br>10 | 13.70<br>16.20 | 0.6351 | -2.500 | | | >0.9999 |
| | Kruskal-Wallis | | | | | 0.6376 | 2 | 0.7270 |
| Tendon lower third | EP *vs.* 2R | 10<br>10 | 16.20<br>14.00 | 0.5588 | 2.200 | | | >0.9999 |
| | EP *vs.* 4R | 10<br>10 | 16.20<br>16.30 | 0.02540 | -0.1000 | | | >0.9999 |
| | 2R *vs.* 4R | 10<br>10 | 14.00<br>16.30 | 0.5842 | -2.300 | | | >0.9999 |
| | Kruskal-Wallis | | | | | 0.4361 | 2 | 0.8041 |

Table 10. Mean rank difference in fiber dispersion between the three timepoints of the experimental group in tendon middle and lower-thirds analyses; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P values less than or equal to 0.05 were considered statistically significant.

**Calcification**



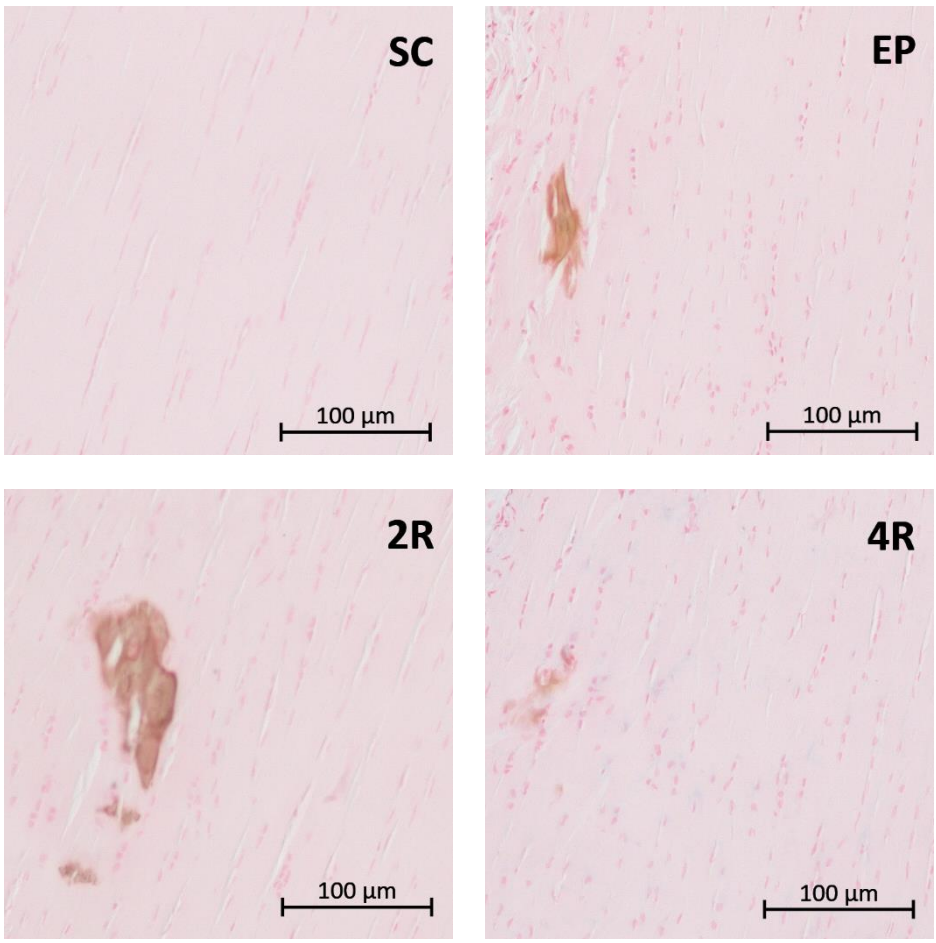




Figure 29. Mean pixels and representative images of von-Kossa-stained samples from sedentary control and experimental groups; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest; dimensions 700×700 pixels; magnification = 10x; scale bar= 100 µm

**Real-time polymerase chain reaction analysis**

Most of the transcript expressions of the *p16* and *p21* target genes were "undetermined", and consequently, these results were unobtainable. Nonetheless, the results of the few samples of these genes that were "determined" are available in the supplementary information. One of the samples, in the 2R group, was found to consistently have much higher values than all other samples, and across almost all genes. Therefore, this sample was considered an outlier, and was excluded in a secondary analysis. The differences between experimental groups were non-statistically significant for all target genes, with exception to *MKX* gene (Figure 30). The difference between 2R and 4R groups in *MKX* gene expression was found to be statistically significant; H= 6.785, df= 2, P= 0.0179; 2R vs. 4R: mean rank diff= 7.000; adjusted P= 0.0331 (Figure 31).

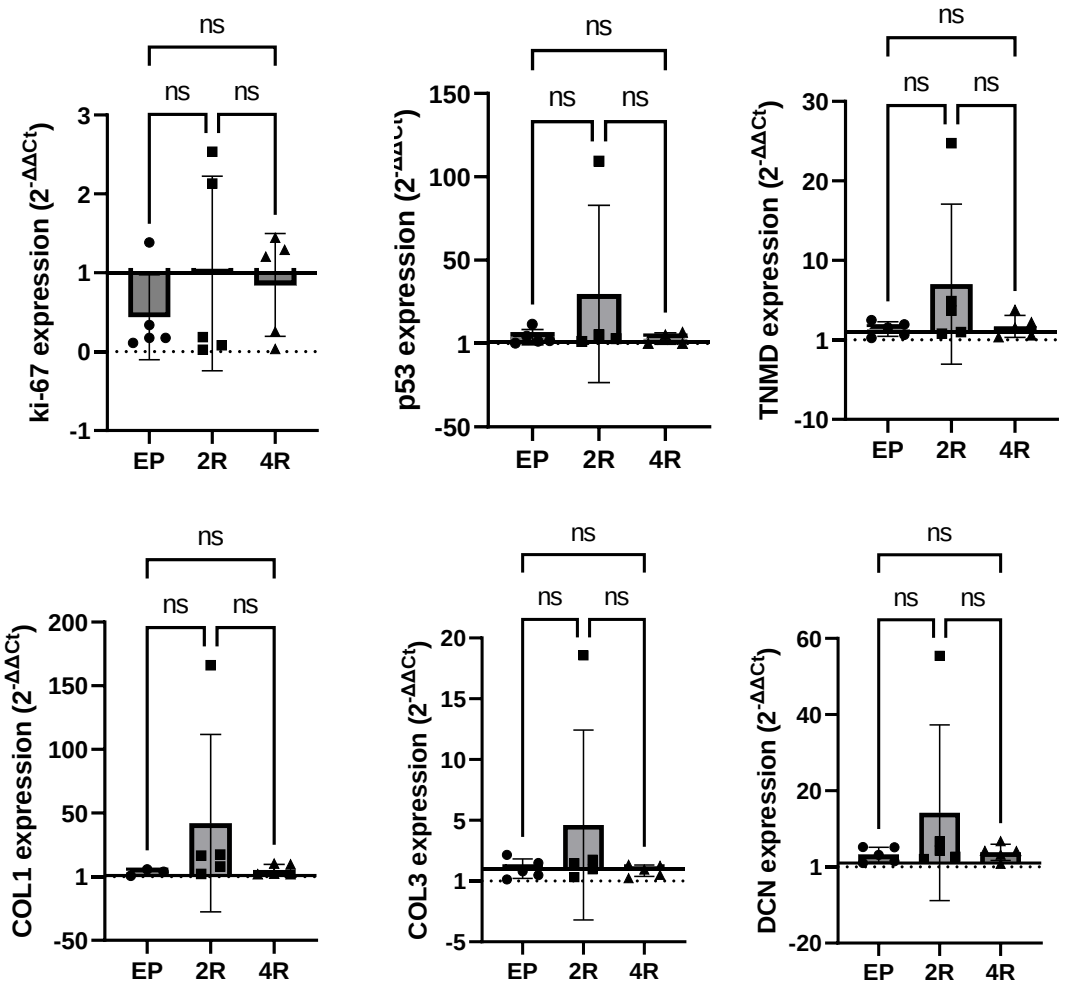



Figure 30. Relative gene expression results calculated using the $2^{-\Delta\Delta C}{}_{T}$ method; results are with the outlier included. Error bars represent mean ± standard error of the mean (SE) of three independent experiments.

At 4R, about 15% reduction in *Ki-67*, almost 3-fold increase in *p53*, about 70% increase in *TNMD*, about 5-fold increase in *Col1*, about 15% reduction in *Col3*, almost 4-fold increase in *DCN*, almost 1-fold reduction in *MKX*.

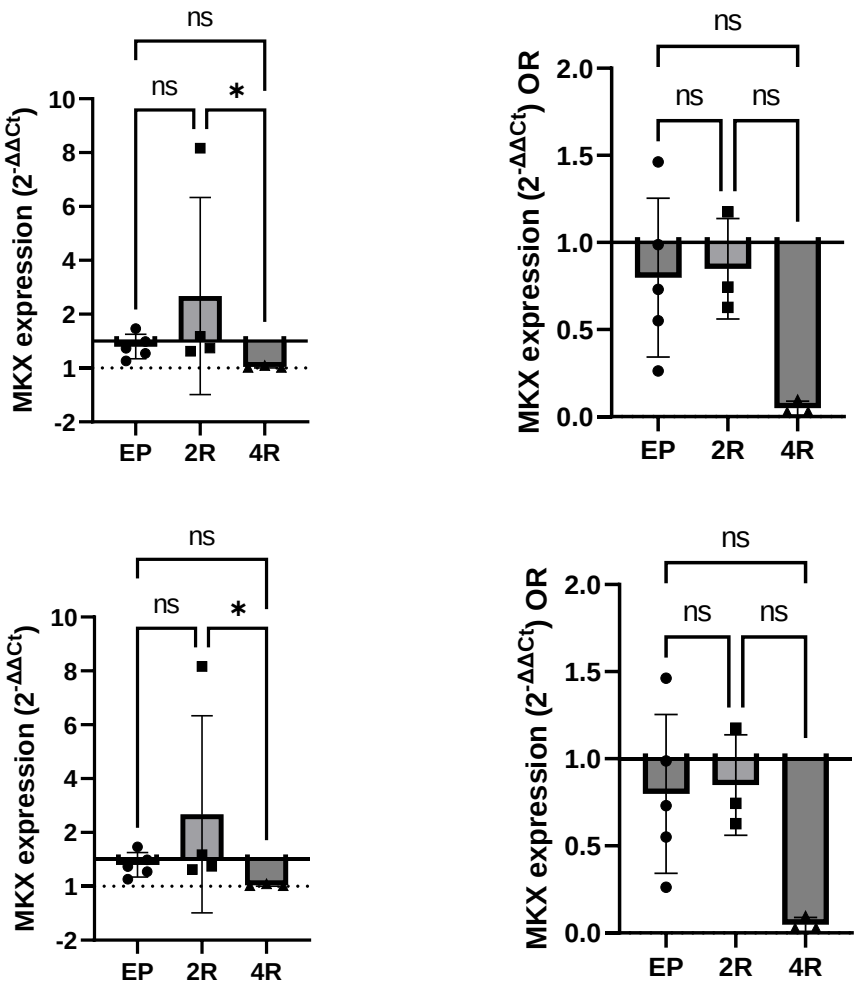



Figure 31. Relative gene expression results as calculated using the $2^{-\Delta\Delta C}{}_{T}$ method; results are with outlier included (left) and excluded (right). Error bars represent mean ± standard error of the mean (SE) of three independent experiments.

**Gene expression results – outlier removed**

At 4R, about 15% reduction in *Ki-67*, about 3-fold increase in *p53*, almost 70% increase in *TNMD*, about 5-fold increase in *Col1*, about 15% reduction in *Col3*, almost 4-fold increase in *DCN*, almost 1-fold reduction in *MKX*. Non-statistically significant differences were found between all groups, for all genes (Figures 31 and 32).

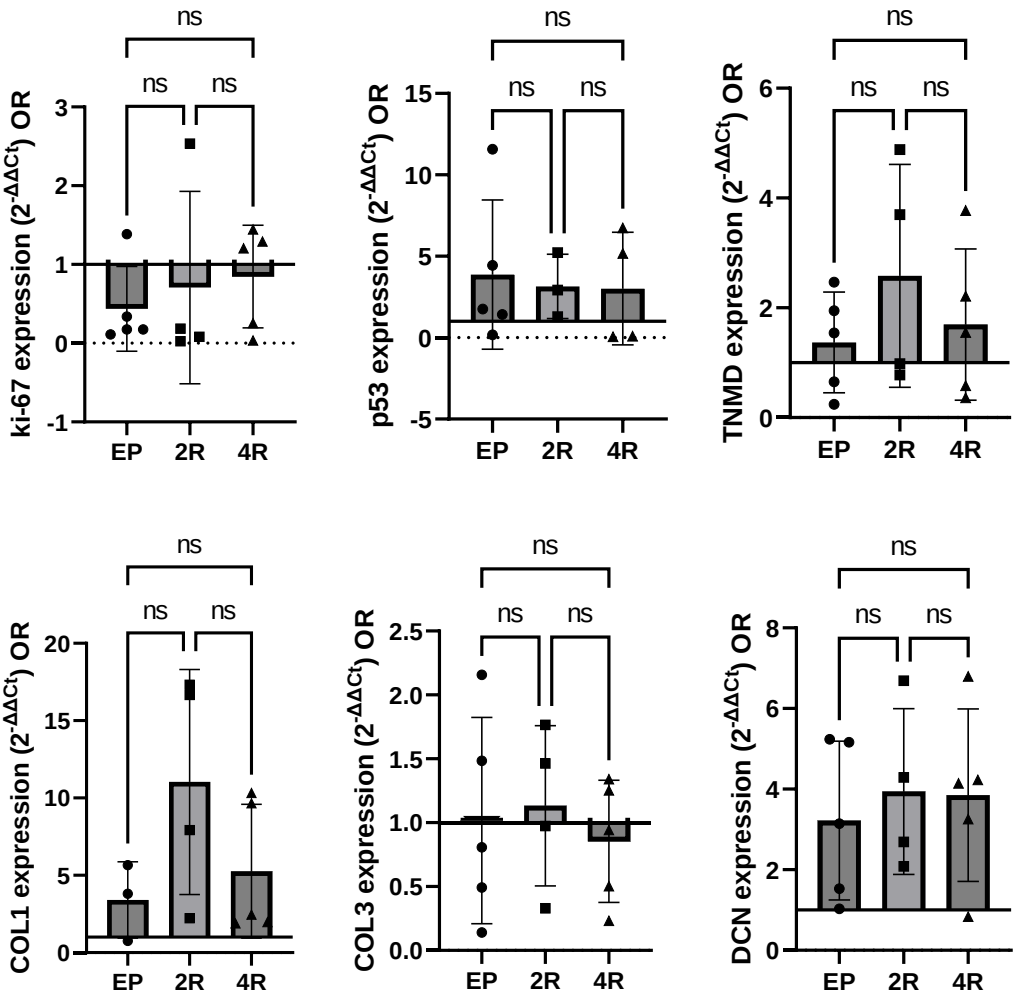



Figure 32. Relative gene expression results as calculated using the $2^{-\Delta\Delta C}{}_{T}$ method; results are with outlier removed. Error bars represent mean ± standard error of the mean (SE) of three independent experiments.

**The effect of HVMPC on Achilles tendon overuse injury in rats**

None of the humane endpoints were reached. Aggressive behavior to inmates was observed in 3 rats following the first application of depilatory cream. All rats received the designated interventions, with exception to one rat in the sham group, that died one week before its due euthanasia date. Autopsy of that animal did not reveal any signs of colitis or peritonitis.

Slight transient chromodacryorrhoea, scoring 1-2 on the scoring system of quantifying chromodacryorrhoea (Mason et al., 2004), was observed only six times (in three rats) throughout the running protocol (during the last two weeks), which may indicate that the protocol was well tolerated. No chromodacryorrhoea was observed during the application of HVMPC intervention or in response to injections. Acute dermal irritation due to depilator use was assessed using the Draize dermal irritation scoring system (Administration et al., 1959), and the grading was found to range between 1-2 indicating very slight to well-defined erythema. Examples of dermal irritation are provided in the supplementary data.

Five rats (three experimental, and two control) did not achieve adequate sedation during the study; compensatory sessions were administered to two rats (one experimental, and one control), additional sedative doses were administered to two rats (experimental group) in order to complete the stimulation session, and one rat in the experimental group was not administered additional doses (missed about 20 minutes of intervention).

Non-statistically significant differences were found between groups with regard to mean body weight and chow consumption at baseline. Baseline body weight and chow consumption were recorded in the sedentary control (SC) group and sham electrical stimulation (Sham ES) for 4 weeks (Sham ES 4W), for 6 weeks (Sham ES 6W), and for 8 weeks (Sham ES 8W), and electrical stimulation (ES) for 4 weeks (ES 4W), for 6 weeks (ES 6W), and for 8 weeks (ES 8W). Mean differences in baseline body weight were -32.25; 95% CI: [-145.5, 80.99]; P= 0.9930, -6.000; 95% CI: [-113.4, 101.4]; P= >0.9999, and 30.80; 95% CI: [-76.63, 138.2]; P= 0.9926 between SC and Sham ES 4W, Sham ES 6W and Sham ES 8W, respectively. Moreover, differences between Sham ES 4W and ES 4W, Sham ES 6W and ES 6W, and Sham ES 8W and ES 8W

were -3.250; 95% CI: [-110.0, 116.5]; P= >0.9999, 29.50; 95% CI: [-77.93, 136.9]; P= 0.9946; and 38.20; 95% CI: [-63.09, 139.5]; P= 0.9546, respectively (Figures 33 and 34).

Mean rank differences in baseline chow consumption were non-statistically significant between groups; H= 14.10, P= 0.0285; the SC group vs Sham ES 4W, Sham ES 6W and Sham ES 8W results were -8.500; Z= 1.284; P= >0.9999, 5.700; Z= 0.9077; P= >0.9999, and 10.90; Z= 1.736, P= >0.9999. Differences between Sham ES 4W and ES 4W, Sham ES 6W and ES 6W, and Sham ES 8W and ES 8W were -1.500; Z= 0.2266; P= >0.9999; -5.800; Z= 0.9797; P= >0.9999; and 8.600; Z= 1.453; P= >0.9999, respectively (Figures 33 and 35).

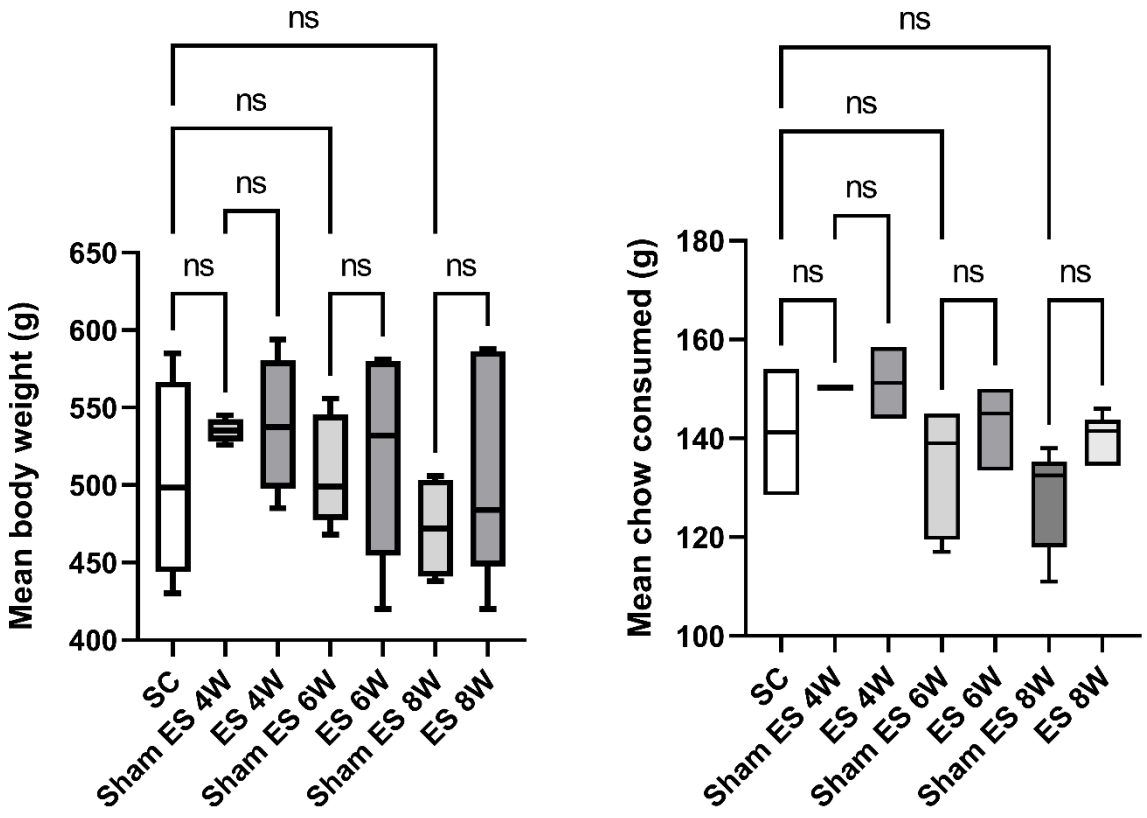



Figure 33. Mean body weight and chow consumption at baseline; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively; ns, non-statistically significant difference; g, grams; scale bars represent 95% confidence interval.

## Body weight

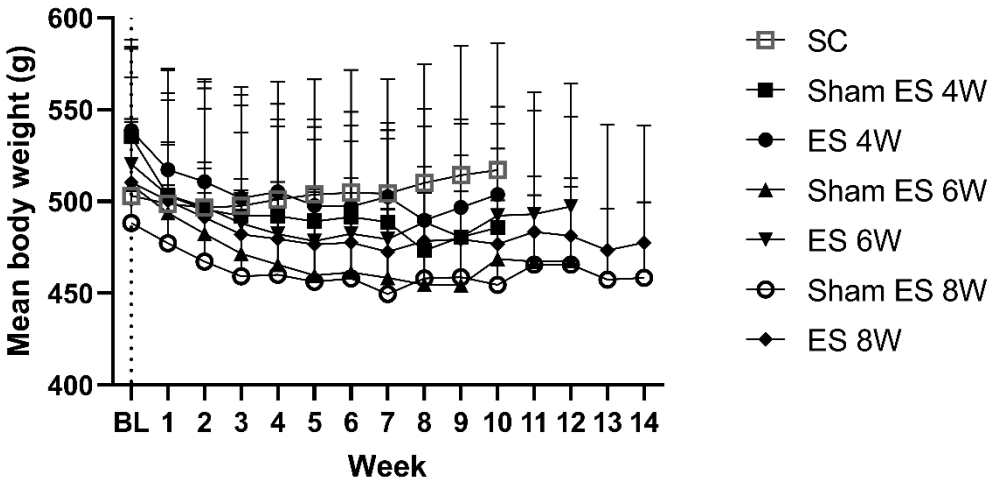



Figure 34. Change in mean body weight over the study span; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively; g, grams; note the minimal gain in body weight following the end of running protocol at 6 weeks.

## Chow consumption

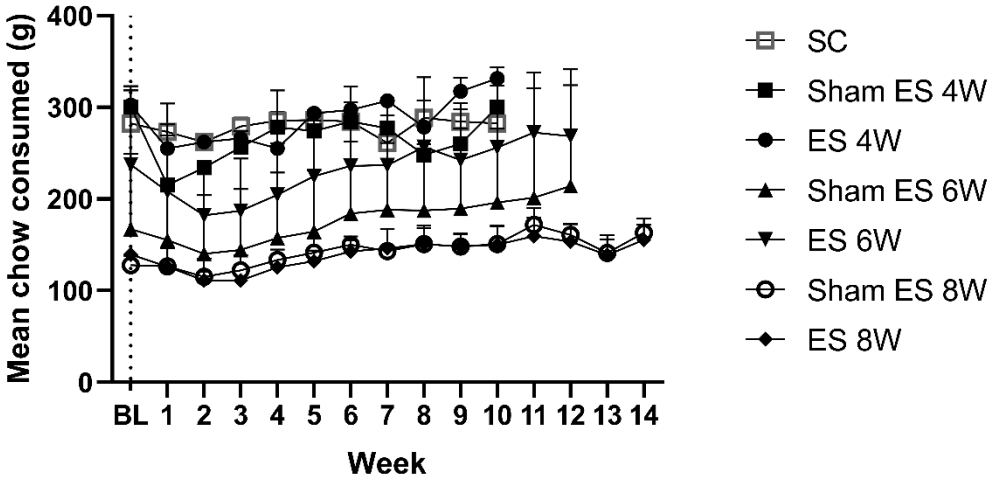



Figure 35. Change in mean chow consumption over the study span; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively; g, grams; note the minimal increase in chow consumption following the end of running protocol at 6 weeks.

### Ground substance

#### Tendon middle third

Statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and Sham ES 4W groups in the middle-third of tendons. Moreover, a statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and Sham ES 6W groups. Furthermore, a statistically significant difference in Alcian-blue density (two-class Otsu) between SC and Sham ES 8W groups was found in the middle-third of tendons.

Three-class Otsu Alcian-blue density analysis of middle-third of tendons showed statistically significant difference between SC and Sham ES 4W groups. Non-statistically significant difference in Alcian-blue density (three-class Otsu) was found between SC and Sham ES 6W groups. However, a statistically significant difference in Alcian-blue density (three-class Otsu) between SC and Sham ES 8W groups was found. Non-statistically significant difference was found between Sham ES 4W and ES 4W groups in Alcian-blue density analysis using two-class and three-class Otsu method. The difference between Sham ES 6W and ES 6W groups was non-statistically significant. Moreover, groups Sham ES 8W and ES 8W did not show statistically significant difference between their Alcian-blue density values.

### Ground substance with mineralized fibrocartilage excluded

Comparison between SC and Sham ES 4W groups, using the three-class Otsu method and with areas of mineralized fibrocartilage excluded, revealed a statistically significant difference. Additionally, SC and Sham ES 6W group difference was statistically significant. A statistically significant difference, furthermore, was found between SC and Sham ES 8W groups. Using three-class Otsu method and including tendon middle-third but excluding areas of mineralized fibrocartilage, between-group comparison revealed non-statistically significant difference between any of the groups.

**Tendon lower-third**

Statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and Sham ES 4W groups in the middle-third of tendons. Moreover, a statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and Sham ES 6W groups. Furthermore, a statistically significant difference in Alcian-blue density (two-class Otsu) between SC and Sham ES 8W groups was found.

Three-class Otsu Alcian-blue density analysis of lower-third of tendons showed statistically significant difference between SC and Sham ES 4W groups. Statistically significant difference in Alcian-blue density (three-class Otsu) was found between SC and Sham ES 6W groups. Moreover, a statistically significant difference in Alcian-blue density (three-class Otsu) between SC and Sham ES 8W groups was found. The differences between Sham ES 4W and ES 4W, Sham ES 6W and ES 6W, and Sham ES 8W and Sham ES 8W were all non-statistically significant.

**Ground substance with mineralized fibrocartilage excluded**

Comparison between SC and Sham ES 4W groups, using the three-class Otsu method and with areas of mineralized fibrocartilage excluded, showed a statistically significant difference. Additionally, SC and Sham ES 6W group difference was statistically significant. A statistically significant difference, furthermore, was found between SC and Sham ES 8W groups.

Using three-class Otsu method and including tendon lower-third but excluding areas of mineralized fibrocartilage, between-group comparison revealed non-statistically significant difference between any of the groups, except for between Sham ES 4W vs ES 4W groups.

| | Comparison | Threshold | n | Median | Hodges-Lehmann | U | Difference between medians | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs.* Sham ES 4W | two-class Otsu | 47<br>43 | 5.930<br>1.650 | -3.020 | 470 | -4.280 | <0.0001 |
| | SC *vs.* Sham ES 4W | three-class Otsu | 47<br>43 | 2.190<br>0.7700 | -1.330 | 515 | -1.420 | <0.0001 |
| | SC *vs.* Sham ES 4W | three-class Otsu MFC excluded | 47<br>43 | 1.740<br>0.5600 | -1.140 | 446 | -1.180 | <0.0001 |
| | SC *vs.* Sham ES 6W | two-class Otsu | 47<br>55 | 5.930<br>3.480 | -1.640 | 974.5 | -2.450 | 0.0325 |
| | SC *vs.* Sham ES 6W | three-class Otsu | 47<br>55 | 2.190<br>1.800 | -0.6200 | 1045 | -0.3900 | 0.0964 |
| | SC *vs.* Sham ES 6W | three-class Otsu MFC excluded | 47<br>55 | 1.740<br>0.8800 | -0.7800 | 857 | -0.8600 | 0.0032 |
| | SC *vs.* Sham ES 8W | two-class Otsu | 47<br>59 | 5.930<br>3.610 | -2.380 | 927.5 | -2.320 | 0.0033 |
| | SC *vs.* Sham ES 8W | three-class Otsu | 47<br>59 | 2.190<br>1.090 | -1.000 | 930.5 | -1.100 | 0.0035 |
| | SC *vs.* Sham ES 8W | three-class Otsu MFC excluded | 47<br>59 | 1.740<br>0.6400 | -0.8700 | 857 | -1.100 | 0.0007 |
| Tendon lower third | SC *vs.* Sham ES 4W | two-class Otsu | 47<br>43 | 13.20<br>3.810 | -8.440 | 298 | -9.390 | <0.0001 |
| | SC *vs.* Sham ES 4W | three-class Otsu | 47<br>43 | 3.970<br>1.140 | -2.930 | 299 | -2.830 | <0.0001 |
| | SC *vs.* Sham ES 4W | three-class Otsu MFC excluded | 47<br>43 | 3.640<br>0.7600 | -2.520 | 262.5 | -2.880 | <0.0001 |
| | SC *vs.* Sham ES 6W | two-class Otsu | 47<br>55 | 13.20<br>8.410 | -4.240 | 891.5 | -4.790 | 0.0068 |
| | SC *vs.* Sham ES 6W | three-class Otsu | 47<br>55 | 3.970<br>3.050 | -1.290 | 973 | -0.9200 | 0.0317 |
| | SC *vs.* Sham ES 6W | three-class Otsu MFC excluded | 47<br>55 | 3.640<br>1.520 | -1.690 | 691 | -2.120 | <0.0001 |
| | SC *vs.* Sham ES 8W | two-class Otsu | 47<br>59 | 13.20<br>5.980 | -5.980 | 753 | -7.280 | <0.0001 |
| | SC *vs.* Sham ES 8W | three-class Otsu | 47<br>59 | 3.970<br>1.830 | -2.130 | 767 | -2.140 | <0.0001 |
| | SC *vs.* Sham ES 8W | three-class Otsu MFC excluded | 47<br>59 | 3.640<br>1.630 | -1.730 | 727.5 | -2.010 | <0.0001 |

Table 11. Median difference in ground substance density ranks between sedentary control and sham stimulation groups at the three timepoints, measured using two-class and three-class Otsu thresholding methods. Additionally, results of secondary analysis involving exclusion of mineralized fibrocartilage (MFC) are presented; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | Threshold | n | Mean Rank | Z | Mean Rank difference | H | df | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | Sham ES 4W *vs.* ES 4W | two-class Otsu | 43<br>44 | 123.0<br>148.8 | 1.312 | -25.80 | | | >0.9999 |
| | Sham ES 4W *vs.* ES 4W | three-class Otsu | 43<br>44 | 123.3<br>134.0 | 0.5431 | 10.67 | | | >0.9999 |
| | Sham ES 4W *vs.* ES 4W | three-class Otsu MFC excluded | 43<br>44 | 122.2<br>145.6 | 1.191 | 23.41 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | two-class Otsu | 55<br>57 | 185.0<br>172.2 | 0.7383 | 12.79 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | three-class Otsu | 55<br>57 | 190.7<br>181.5 | 0.5315 | 9.207 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | three-class Otsu MFC excluded | 55<br>57 | 183.3<br>179.3 | 0.2294 | 3.974 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | two-class Otsu | 59<br>59 | 163.9<br>150.9 | 0.7734 | 13.05 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | three-class Otsu | 59<br>59 | 159.7<br>151.7 | 0.4716 | -7.958 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | three-class Otsu MFC excluded | 59<br>59 | 164.6<br>148.0 | 0.9884 | -16.68 | | | >0.9999 |
| | Kruskal-Wallis | two-class Otsu | | | | | 13.43 | 5 | 0.0196 |
| | Kruskal-Wallis | three-class Otsu | | | | | 20.14 | 5 | 0.0012 |
| | Kruskal-Wallis | three-class Otsu MFC excluded | | | | | 15.62 | 5 | 0.0080 |
| Tendon lower third | Sham ES 4W *vs.* ES 4W | two-class Otsu | 43<br>44 | 95.17<br>163.7 | 3.487 | 68.53 | | | 0.0073 |
| | Sham ES 4W *vs.* ES 4W | three-class Otsu | 43<br>44 | 95.41<br>161.0 | 3.339 | 65.63 | | | 0.0126 |
| | Sham ES 4W *vs.* ES 4W | three-class Otsu MFC excluded | 43<br>44 | 88.85<br>164.4 | 3.844 | 75.55 | | | 0.0018 |
| | Sham ES 6W *vs.* ES 6W | two-class Otsu | 55<br>57 | 176.4<br>184.9 | 0.4909 | -8.505 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | three-class Otsu | 55<br>57 | 180.4<br>181.8 | 0.07713 | -1.336 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | three-class Otsu MFC excluded | 55<br>57 | 156.8<br>191.7 | 2.015 | -34.90 | | | 0.6590 |
| | Sham ES 8W *vs.* ES 8W | two-class Otsu | 59<br>59 | 146.8<br>173.0 | 1.551 | 26.17 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | three-class Otsu | 59<br>59 | 145.2<br>175.6 | 1.801 | 30.39 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | three-class Otsu MFC excluded | 59<br>59 | 154.0<br>181.6 | 1.638 | 27.64 | | | >0.9999 |
| | Kruskal-Wallis | two-class Otsu | | | | | 29.90 | 5 | <0.0001 |
| | Kruskal-Wallis | three-class Otsu | | | | | 30.51 | 5 | <0.0001 |
| | Kruskal-Wallis | three-class Otsu MFC excluded | | | | | 36.40 | 5 | <0.0001 |

Table 12. Mean rank difference in ground substance density within sham stimulation, and within stimulation groups, at the three timepoints, measured using two-class and three-class Otsu thresholding methods. Additionally, results of secondary analysis involving exclusion of mineralized fibrocartilage (MFC) are presented; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. Statistical significance level considered; P values less than or equal to 0.05 were considered statistically significant.

**Tendon middle-third ground substance – normalized data**

A statistically significant difference was found between SC and Sham ES 4W groups in the two-class Otsu analysis. The same comparison, moreover, using the three-class Otsu method revealed statistically significant differences. The comparison between SC and Sham ES 6W groups was statistically significant using two-class but not three-class Otsu method. The difference between SC and Sham ES 8W groups was statistically significant using two-class Otsu method, and statistically significant using three-class Otsu.

**Ground substance with mineralized fibrocartilage excluded - normalized data**

A statistically significant difference between SC and Sham ES 4W groups, using three-class Otsu method and analyzing tendon middle-third excluding areas of mineralized fibrocartilage. A statistically significant difference between SC and Sham ES 6W groups was found. Furthermore, a statistically significant difference, was identified between SC and Sham ES 8W groups.

Comparisons between Sham ES 4W and ES 4W groups, Sham ES 6W and ES 6W, and Sham ES 8W and ES 8W groups, excluding areas of mineralized fibrocartilage and analyzing middle-third of tendons using three-class Otsu method, indicated non-statistically significant differences between groups.

**Tendon lower-third ground substance - normalized data**

A statistically significant difference was found between SC and Sham ES 4W groups in the two-class Otsu analysis. The same comparison, moreover, using the three-class Otsu method revealed statistically significant differences. The comparison between SC and Sham ES 6W groups showed statistically significant differences using both two-class and three-class Otsu methods. The difference between SC and Sham ES 8W groups was statistically significant using two-class Otsu method, and three-class Otsu.

**Ground substance with mineralized fibrocartilage excluded - normalized data**

A statistically significant difference was found between SC and Sham ES 4W groups in the two-class Otsu analysis. The comparison between SC and Sham ES 6W groups using three-class Otsu method was statistically significant. The difference between SC and Sham ES 8W groups was statistically significant using three-class Otsu method.

Comparisons between Sham ES 4W and ES 4W excluding areas of mineralized fibrocartilage and analyzing lower-third of tendons using three-class Otsu method, indicated statistically significant differences between groups. The other comparisons, between Sham ES 6W and ES 6W, and Sham ES 8W and ES 8W groups, did not show statistically significant differences.

| | Comparison | Threshold | n | Mean | t | df | Difference between means (SEM) | [95% CI] | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs.* Sham ES 4W | two-class Otsu | 46<br>43 | 9.188<br>2.242 | 4.903 | 87 | -6.946 (1.417) | -9.762, -4.130 | <0.0001 |
| | SC *vs.* Sham ES 4W | three-class Otsu | 46<br>43 | 4.747<br>0.5274 | 4.752 | 87 | -4.220 (0.8880) | -5.985, -2.455 | <0.0001 |
| | SC *vs.* Sham ES 4W | three-class Otsu MFC excluded | 46<br>43 | 2.703<br>0.2233 | 5.231 | 87 | -2.479 (0.4740) | -3.421, -1.537 | <0.0001 |
| | SC *vs.* Sham ES 6W | two-class Otsu | 46<br>55 | 9.188<br>6.039 | 2.382 | 99 | -3.149 (1.322) | -5.773, -0.5262 | 0.0191 |
| | SC *vs.* Sham ES 6W | three-class Otsu | 46<br>55 | 4.747<br>3.240 | 1.854 | 99 | -1.508 (0.8133) | -3.121, 0.1063 | 0.0668 |
| | SC *vs.* Sham ES 6W | three-class Otsu MFC excluded | 46<br>55 | 2.703<br>1.446 | 3.017 | 99 | -1.257 (0.4165) | -2.083, -0.4301 | 0.0032 |
| | SC *vs.* Sham ES 8W | two-class Otsu | 46<br>59 | 9.188<br>4.537 | 3.728 | 103 | -4.651 (1.248) | -7.126, -2.177 | 0.0003 |
| | SC *vs.* Sham ES 8W | three-class Otsu | 46<br>59 | 4.747<br>2.024 | 3.435 | 103 | -2.724 (0.7930) | -4.296, -1.151 | 0.0009 |
| | SC *vs.* Sham ES 8W | three-class Otsu MFC excluded | 46<br>59 | 2.703<br>1.171 | 3.664 | 103 | -1.532 (0.4181) | -2.361, -0.7025 | 0.0004 |
| Tendon lower third | SC *vs.* Sham ES 4W | two-class Otsu | 47<br>43 | 19.55<br>1.285 | 6.960 | 88 | -18.26 (2.624) | -23.48, -13.05 | <0.0001 |
| | SC *vs.* Sham ES 4W | three-class Otsu | 47<br>43 | 7.975<br>-0.7358 | 6.801 | 88 | -8.711 (1.281) | -11.26, -6.165 | <0.0001 |
| | SC *vs.* Sham ES 4W | three-class Otsu MFC excluded | 47<br>43 | 0.6700<br>0.2684 | 7.806 | 88 | -0.4016 (0.05145) | -0.5039, -0.2994 | <0.0001 |
| | SC *vs.* Sham ES 6W | two-class Otsu | 47<br>55 | 19.55<br>11.20 | 3.144 | 100 | -8.345 (2.654) | -13.61, -3.079 | 0.0022 |
| | SC *vs.* Sham ES 6W | three-class Otsu | 47<br>55 | 7.975<br>4.648 | 2.559 | 100 | -3.327 (1.300) | -5.907, -0.7477 | 0.0120 |
| | SC *vs.* Sham ES 6W | three-class Otsu MFC excluded | 47<br>55 | 0.6700<br>0.4371 | 4.745 | 100 | -0.2329 (0.04909) | -0.3303, -0.1355 | <0.0001 |
| | SC *vs.* Sham ES 8W | two-class Otsu | 47<br>59 | 19.55<br>7.399 | 4.780 | 104 | -12.15 (2.541) | -17.19, -7.109 | <0.0001 |
| | SC *vs.* Sham ES 8W | three-class Otsu | 47<br>59 | 7.975<br>2.458 | 4.657 | 104 | -5.517 (1.185) | -7.866, -3.168 | <0.0001 |
| | SC *vs.* Sham ES 8W | three-class Otsu MFC excluded | 47<br>59 | 0.6700<br>0.4329 | 4.773 | 104 | -0.2371 (0.04968) | -0.3356, -0.1386 | <0.0001 |

Table 13. Mean difference in ground substance density between sedentary control and sham stimulation groups at the three timepoints, measured using two-class and three-class Otsu thresholding methods. Additionally, results of secondary analysis involving exclusion of mineralized fibrocartilage (MFC) are presented; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | Threshold | n | Mean | df | Difference between means (SEM) | [95% CI] | F | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | Sham ES 4W *vs.* ES 4W | two-class Otsu | 43<br>44 | 2.242<br>3.992 | 311 | 1.750 (1.275) | -1.906, 5.405 | | 0.7434 |
| | Sham ES 4W *vs.* ES 4W | three-class Otsu | 43<br>44 | 0.5274<br>1.190 | 311 | 0.6628 (0.7549) | -1.502, 2.828 | | 0.9516 |
| | Sham ES 4W *vs.* ES 4W | three-class Otsu MFC excluded | 43<br>44 | 0.2233<br>0.8998 | 311 | 0.6765 (0.3761) | -0.4019, 1.755 | | 0.4680 |
| | Sham ES 6W *vs.* ES 6W | two-class Otsu | 55<br>57 | 6.039<br>4.921 | 311 | 1.118 (1.124) | -2.104, 4.340 | | 0.9194 |
| | Sham ES 6W *vs.* ES 6W | three-class Otsu | 55<br>57 | 3.240<br>2.944 | 311 | 0.2961 (0.6654) | -1.612, 2.204 | | 0.9978 |
| | Sham ES 6W *vs.* ES 6W | three-class Otsu MFC excluded | 55<br>57 | 1.446<br>1.483 | 311 | -0.03733 (0.3315) | -0.9879, 0.9133 | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | two-class Otsu | 59<br>59 | 4.537<br>3.924 | 311 | -0.6122 (1.094) | -3.751, 2.526 | | 0.9935 |
| | Sham ES 8W *vs.* ES 8W | three-class Otsu | 59<br>59 | 2.024<br>1.757 | 311 | -0.2668 (0.6482) | -2.126, 1.592 | | 0.9985 |
| | Sham ES 8W *vs.* ES 8W | three-class Otsu MFC excluded | 59<br>59 | 1.171<br>0.8385 | 311 | -0.3325 (0.3229) | -1.259, 0.5934 | | 0.9076 |
| | ANOVA | two-class Otsu | | | | | | 2.178 | 0.0564 |
| | ANOVA | three-class Otsu | | | | | | 4.205 | 0.0010 |
| | ANOVA | three-class Otsu MFC excluded | | | | | | 3.460 | 0.0047 |
| Tendon lower third | Sham ES 4W *vs.* ES 4W | two-class Otsu | 43<br>44 | 1.285<br>9.831 | 311 | 8.546 (2.412) | 1.629, 15.46 | | 0.0060 |
| | Sham ES 4W *vs.* ES 4W | three-class Otsu | 43<br>44 | -0.735<br>3.386 | 311 | 4.122 (1.237) | 0.5754, 7.668 | | 0.0123 |
| | Sham ES 4W *vs.* ES 4W | three-class Otsu MFC excluded | 43<br>44 | 0.2684<br>0.4577 | 311 | 0.1894 (0.04953) | 0.04732, 0.3314 | | 0.0022 |
| | Sham ES 6W *vs.* ES 6W | two-class Otsu | 55<br>57 | 11.20<br>13.05 | 311 | -1.852 (2.126) | -7.948, 4.245 | | 0.9532 |
| | Sham ES 6W *vs.* ES 6W | three-class Otsu | 55<br>57 | 4.648<br>5.125 | 311 | -0.4773 (1.090) | -3.603, 2.649 | | 0.9980 |
| | Sham ES 6W *vs.* ES 6W | three-class Otsu MFC excluded | 55<br>57 | 0.4371<br>0.5568 | 311 | -0.1198 (0.04366) | -0.2449, 0.005447 | | 0.0699 |
| | Sham ES 8W *vs.* ES 8W | two-class Otsu | 59<br>59 | 7.399<br>10.94 | 311 | -1.852 (2.126) | -2.395, 9.483 | | 0.5254 |
| | Sham ES 8W *vs.* ES 8W | three-class Otsu | 59<br>59 | 2.458<br>4.222 | 311 | 1.763 (1.062) | -1.282, 4.808 | | 0.5589 |
| | Sham ES 8W *vs.* ES 8W | three-class Otsu MFC excluded | 59<br>59 | 0.4329<br>0.5005 | 311 | 0.06763 (0.04253) | -0.05433, 0.1896 | | 0.6057 |
| | ANOVA | two-class Otsu | | | | | | 6.558 | <0.0001 |
| | ANOVA | three-class Otsu | | | | | | 6.506 | <0.0001 |
| | ANOVA | three-class Otsu MFC excluded | | | | | | 8.429 | <0.0001 |

Table 14. Mean difference in ground substance density between sham stimulation and stimulation groups, at the three timepoints, measured using three-class Otsu thresholding method with mineralized fibrocartilage (MFC) excluded; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P values less than or equal to 0.05 were considered statistically significant.

## Cellularity

| | Comparison | n | Mean Rank | Z | Mean Rank difference | H | df | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | Sham ES 4W *vs.* ES 4W | 8<br>8 | 30.25<br>37.25 | 0.8585 | -7.000 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | 10<br>10 | 22.00<br>31.80 | 1.344 | -9.800 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | 10<br>10 | 22.60<br>29.20 | 0.9049 | -6.600 | | | >0.9999 |
| | Kruskal-Wallis | | | | | 5.720 | 5 | 0.3344 |
| Tendon lower third | Sham ES 4W *vs.* ES 4W | 8<br>8 | 31.94<br>41.38 | 1.157 | -9.438 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | 10<br>10 | 19.65<br>29.70 | 1.378 | -10.05 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | 10<br>10 | 20.85<br>30.75 | 1.357 | -9.900 | | | >0.9999 |
| | Kruskal-Wallis | | | | | 10.73 | 5 | 0.0570 |

Table 15. Mean rank difference in nuclei number between sham stimulation and stimulation groups at the three timepoints in tendon middle and lower-thirds analyses; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | n | Mean $\bar{x}$ or Median $\tilde{x}$ | t or U | df | Mean (SEM) or Median difference | [95% CI] or Hodges-Lehmann | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs.* Sham ES 4W | 8<br>8 | $\tilde{x}$ = 410.5<br>$\tilde{x}$ = 396.0 | 28 | | -14.50 | -29.00 | 0.7209 |
| | SC *vs.* Sham ES 6W | 8<br>10 | $\bar{x}$ = 383.3<br>$\bar{x}$ = 330.7 | 0.7204 | 16 | -52.55 (72.94) | -207.2, 102.1 | 0.4817 |
| | SC *vs.* Sham ES 8W | 8<br>10 | $\bar{x}$ = 383.3<br>$\bar{x}$ = 338.3 | 0.7889 | 16 | -44.95 (56.98) | -165.7, 75.84 | 0.4417 |
| Tendon lower third | SC *vs.* Sham ES 4W | 8<br>8 | $\bar{x}$ = 471.9<br>$\bar{x}$ = 450.0 | 0.2413 | 14 | -21.88 (90.64) | -216.3, 172.5 | 0.8128 |
| | SC *vs.* Sham ES 6W | 8<br>10 | $\bar{x}$ = 471.9<br>$\bar{x}$ = 354.6 | 1.536 | 16 | -117.3 (76.35) | -279.1, 44.58 | 0.1441 |
| | SC *vs.* Sham ES 8W | 8<br>10 | $\bar{x}$ = 471.9<br>$\bar{x}$ = 384.3 | 1.354 | 16 | -87.58 (64.68) | -224.7, 49.54 | 0.1946 |

Table 16. Mean or median difference in nuclei number between the sedentary control, sham stimulation and stimulation groups at the three timepoints in tendon middle and lower-thirds analyses; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P values less than or equal to 0.05 were considered statistically significant.

## Circularity

| | Comparison | Roundedness | n | Mean Rank | Z | Mean Rank difference | H | df | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | Sham ES 4W *vs.* ES 4W | Non-rounded | 8<br>8 | 26.06<br>34.06 | 0.9887 | -8.000 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | Non-rounded | 10<br>10 | 28.45<br>24.80 | 0.5044 | 3.650 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | Non-rounded | 10<br>10 | 33.35<br>24.90 | 1.168 | 8.450 | | | >0.9999 |
| | Kruskal-Wallis | Non-rounded | | | | | 3.043 | 5 | 0.6934 |
| | Sham ES 4W *vs.* ES 4W | Semi-rounded | 8<br>8 | 28.00<br>24.88 | 0.3832 | 3.125 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | Semi-rounded | 10<br>10 | 31.90<br>27.70 | 0.5758 | 4.200 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | Semi-rounded | 10<br>10 | 29.80<br>27.90 | 0.2605 | 1.900 | | | >0.9999 |
| | Kruskal-Wallis | Semi-rounded | | | | | 0.9384 | 5 | 0.9674 |
| | Sham ES 4W *vs.* ES 4W | Fully-rounded | 8<br>8 | 29.63<br>30.50 | 0.1073 | -0.8750 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | Fully-rounded | 10<br>10 | 25.00<br>29.90 | 0.6718 | -4.900 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | Fully-rounded | 10<br>10 | 27.10<br>29.50 | 0.3290 | -2.400 | | | >0.9999 |
| | Kruskal-Wallis | Fully-rounded | | | | | 0.8039 | 5 | 0.9768 |
| Tendon lower third | Sham ES 4W *vs.* ES 4W | Non-rounded | 8<br>8 | 22.38<br>30.50 | 1.057 | -8.125 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | Non-rounded | 10<br>10 | 29.35<br>28.80 | 0.08002 | 0.5500 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | Non-rounded | 10<br>10 | 30.30<br>28.85 | 0.2110 | 1.450 | | | >0.9999 |
| | Kruskal-Wallis | Non-rounded | | | | | 1.583 | 5 | 0.9033 |
| | Sham ES 4W *vs.* ES 4W | Semi-rounded | 8<br>8 | 29.75<br>30.88 | 0.1380 | -1.125 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | Semi-rounded | 10<br>10 | 29.70<br>27.30 | 0.3290 | 2.400 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | Semi-rounded | 10<br>10 | 28.20<br>25.90 | 0.3153 | 2.300 | | | >0.9999 |
| | Kruskal-Wallis | Semi-rounded | | | | | 0.5824 | 5 | 0.9888 |
| | Sham ES 4W *vs.* ES 4W | Fully-rounded | 8<br>8 | 27.50<br>26.19 | 0.1610 | 1.313 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | Fully-rounded | 10<br>10 | 27.30<br>29.75 | 0.3359 | -2.450 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | Fully-rounded | 10<br>10 | 28.60<br>31.00 | 0.3291 | -2.400 | | | >0.9999 |
| | Kruskal-Wallis | Fully-rounded | | | | | 0.5393 | 5 | 0.9906 |

Table 17. Mean rank difference in nuclei roundedness between sham stimulation and stimulation groups at the three timepoints in tendon middle and lower-thirds analyses; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | Roundedness | n | Mean x̄ or Median x̃ | t or U | df | Mean (SEM) or Median difference | [95% CI] or Hodges-Lehmann | P |
|---|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs*. Sham ES 4W | Non-rounded | 8<br>8 | x̃ = 0.01317<br>x̃ = 0.009879 | 20 | | -0.003290 | -0.004682 | 0.2325 |
| | SC *vs*. Sham ES 6W | Non-rounded | 8<br>10 | x̃ = 0.01317<br>x̃ = 0.01175 | 31 | | -0.001417 | -0.001964 | 0.4587 |
| | SC *vs*. Sham ES 8W | Non-rounded | 8<br>10 | x̃ = 0.01317<br>x̃ = 0.01461 | 38 | | 0.001445 | 0.0008090 | 0.8968 |
| | SC *vs*. Sham ES 4W | Semi-rounded | 8<br>8 | x̄ = 0.3143<br>x̄ = 0.2526 | 0.9294 | 14 | -0.06173 (0.06641) | -0.2042, 0.08071 | 0.3684 |
| | SC *vs*. Sham ES 6W | Semi-rounded | 8<br>10 | x̃ = 0.3324<br>x̃ = 0.2833 | 27 | | -0.04917 | -0.05354 | 0.2743 |
| | SC *vs*. Sham ES 8W | Semi-rounded | 8<br>10 | x̃ = 0.3324<br>x̃ = 0.2693 | 30 | | -0.06310 | -0.06936 | 0.4082 |
| | SC *vs*. Sham ES 4W | Fully-rounded | 8<br>8 | x̄ = 0.6657<br>x̄ = 0.7372 | 0.9843 | 14 | 0.07155 (0.07269) | -0.08436, 0.2275 | 0.3417 |
| | SC *vs*. Sham ES 6W | Fully-rounded | 8<br>10 | x̄ = 0.6657<br>x̄ = 0.7022 | 0.6525 | 16 | 0.03649 (0.05593) | -0.08208, 0.1551 | 0.5234 |
| | SC vs. Sham ES 8W | Fully-rounded | 8<br>10 | x̃ = 0.6601<br>x̃ = 0.7202 | 31 | | 0.06010 | 0.06703 | 0.4598 |
| Tendon lower third | SC *vs*. Sham ES 4W | Non-rounded | 8<br>8 | x̃ = 0.01340<br>x̃ = 0.000 | 10.50 | | -0.01340 | -0.01221 | 0.0200 |
| | SC *vs*. Sham ES 6W | Non-rounded | 8<br>10 | x̃ = 0.01340<br>x̃ = 0.004286 | 22 | | -0.009112 | -0.007926 | 0.1177 |
| | SC *vs*. Sham ES 8W | Non-rounded | 8<br>10 | x̃ = 0.01340<br>x̃ = 0.002219 | 25 | | -0.01118 | -0.006580 | 0.1978 |
| | SC *vs*. Sham ES 4W | Semi-rounded | 8<br>8 | x̃ = 0.2776<br>x̃ = 0.09195 | 13 | | -0.1856 | -0.1821 | 0.0499 |
| | SC *vs*. Sham ES 6W | Semi-rounded | 8<br>10 | x̄ = 0.2747<br>x̄ = 0.1528 | 2.069 | 16 | -0.1219 (0.05892) | -0.2468, 0.002981 | 0.0551 |
| | SC *vs*. Sham ES 8W | Semi-rounded | 8<br>10 | x̃ = 0.2776<br>x̃ = 0.04883 | 21 | | -0.2288 | -0.1849 | 0.1011 |
| | SC *vs*. Sham ES 4W | Fully-rounded | 8<br>8 | x̃ = 0.7090<br>x̃ = 0.9070 | 13 | | 0.1980 | 0.1865 | 0.0499 |
| | SC *vs*. Sham ES 6W | Fully-rounded | 8<br>10 | x̄ = 0.7073<br>x̄ = 0.8417 | 2.098 | 16 | 0.1345 (0.06408) | -0.001392, 0.2703 | 0.0521 |
| | SC *vs*. Sham ES 8W | Fully-rounded | 8<br>10 | x̃ = 0.7090<br>x̃ = 0.9510 | 23 | | 0.2420 | 0.2000 | 0.1457 |

Table 18. Mean or median difference in nuclei roundedness between the sedentary control and sham stimulation groups at the three timepoints in tendon middle and lower-thirds analyses; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P values less than or equal to 0.05 were considered statistically significant.

## Fiber alignment

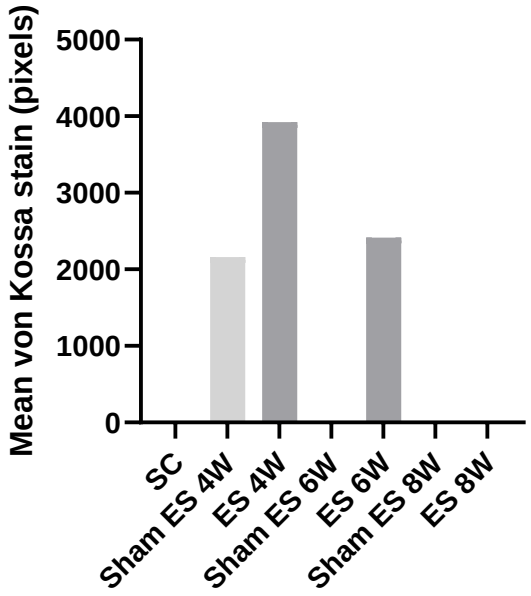
| | Comparison | n | Mean x̄ | t | df | Difference between means (SEM) | [95% CI] | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | SC *vs.* Sham ES 4W | 8<br>8 | 4.869<br>5.544 | 0.6758 | 14 | 0.6750 (0.9988) | -1.467, 2.817 | 0.5102 |
| | SC *vs.* Sham ES 6W | 8<br>10 | 4.869<br>5.154 | 0.5367 | 16 | 0.2853 (0.5315) | -0.8415, 1.412 | 0.5989 |
| | SC *vs.* Sham ES 8W | 8<br>10 | 4.869<br>4.958 | 0.08982 | 16 | 0.08925 (0.9936) | -2.017, 2.196 | 0.9295 |
| Tendon lower third | SC *vs.* Sham ES 4W | 8<br>8 | 6.745<br>6.220 | 0.3930 | 14 | -0.5250 (1.336) | -3.390, 2.340 | 0.7003 |
| | SC *vs.* Sham ES 6W | 8<br>10 | 6.745<br>6.789 | 0.03199 | 16 | 0.04400 (1.376) | -2.872, 2.960 | 0.9749 |
| | SC *vs.* Sham ES 8W | 8<br>10 | 6.745<br>6.239 | 0.3492 | 16 | -0.5060 (1.449) | -3.578, 2.566 | 0.7315 |

Table 19. Mean difference in fiber dispersion between the sedentary control and sham stimulation groups at the three timepoints in tendon middle and lower-thirds analyses; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P values less than or equal to 0.05 were considered statistically significant.

| | Comparison | n | Mean Rank | Z | Mean Rank difference | H | df | P |
|---|---|---|---|---|---|---|---|---|
| Tendon middle third | Sham ES 4W *vs.* ES 4W | 8<br>8 | 27.50<br>29.44 | 0.2376 | -1.938 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | 10<br>10 | 28.75<br>35.00 | 0.8569 | -6.250 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | 10<br>10 | 20.40<br>29.90 | 1.303 | -9.500 | | | >0.9999 |
| | Kruskal-Wallis | | | | | 4.188 | 5 | 0.5227 |
| Tendon lower third | Sham ES 4W *vs.* ES 4W | 8<br>8 | 28.06<br>26.31 | 0.2146 | 1.750 | | | >0.9999 |
| | Sham ES 6W *vs.* ES 6W | 10<br>10 | 30.20<br>36.50 | 0.8638 | -6.300 | | | >0.9999 |
| | Sham ES 8W *vs.* ES 8W | 10<br>10 | 24.90<br>24.50 | 0.05484 | 0.4000 | | | >0.9999 |
| | Kruskal-Wallis | | | | | 3.753 | 5 | 0.5854 |

Table 20. Mean rank difference in fiber dispersion between sham stimulation and stimulation groups at the three timepoints in tendon middle and lower-thirds analyses; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P values less than or equal to 0.05 were considered statistically significant

## Calcification



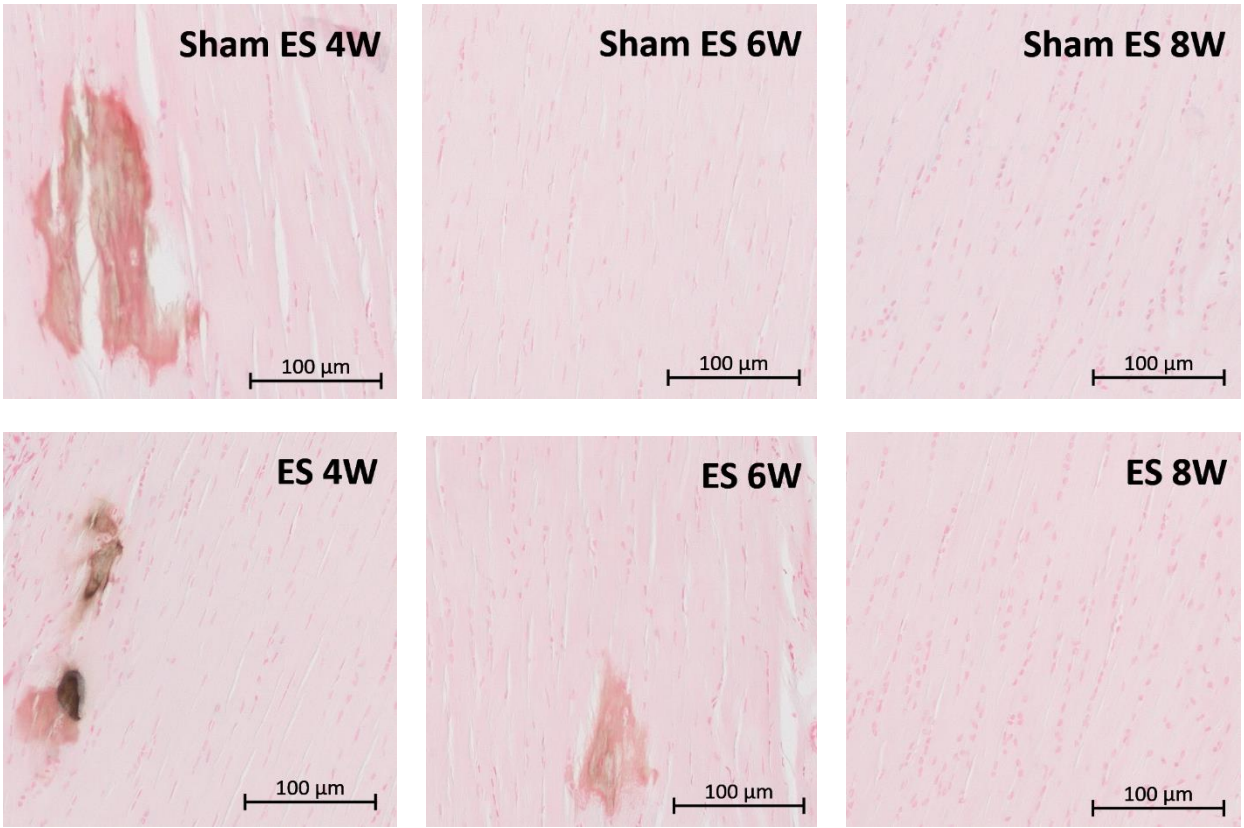




Figure 65. Mean pixels of von-Kossa-stained samples from sham and stimulation groups; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively; dimensions 700×700 pixels; magnification = 10x; scale bar= 100 µm

## Discussion

The results of the present study might indicate that tendon injury did occur although with incomplete recovery. Signs of tendon injury were detected, on cellular and genetic levels, long after the absence of significant histopathological findings. The results, moreover, showed reduction in enthesis proteoglycan content and cellularity which are novel findings that were not previously reported in response to treadmill running models and that are reproducible. Nonetheless, the Bonar score that is commonly used in classifying the severity of tendon injury indicate that increases but not decreases in proteoglycan content are considered pathologic (Fearon et al., 2014). The Bonar score, similarly, regards proliferation as the pathologic finding associated with tendon injuries (Fearon et al., 2014). These considerations, however, are mainly concerning mid-substance tendon injuries identified in human subjects. Therefore, the results might provide insight into the changes that characterize overuse-induced tendon injury, and that possibly precede tendinopathy, while the classic features described by the Bonar score may represent repair rather than injury.

Furthermore, the results of the present study may suggest that HVMPC electrical stimulation was effective to restore proteoglycan expression after 8 sessions of electrical stimulation (30 days of rest). Nonetheless, whether spontaneous recovery occurred at later timepoints or that injury still exists but on a cellular level remains to be confirmed. It is possible, however, that animals in the experimental group were less severely injured than the sham group. An additional explanation might be that electrical stimulation was indeed effective but only during the initial period of intervention, possibly during cellular senescence. i.e., during the proliferation but not the remodeling phase of healing.

Heterotopic ossification may be regarded as one of the pathologic features of tendinopathy and enthesopathy (D'Agostino & Olivieri, 2006; Järvinen et al., 1997; Schett et al., 2017). Calcification was found in the present study at the end of running protocol and after 15 days of rest. Calcification may be associated with cell death of chondrocyte-like cells (Benjamin et al., 2006) and its identification in histologic assessments following the treadmill running protocol may confirm the occurrence of an overuse-induced injury. Calcific tendinopathy entails the presence of calcium deposits in the extracellular matrix of tendons (Jozsa et al., 1980; Jozsa et al., 1990;

Kannus & Jozsa, 1991). Nonetheless, the severity of tendon injury may differ, between and within conditions, and either on functional or structural levels. Tenocytes showing signs of hypoxia, such as hypoxic vacuoles, mitochondrial, nuclear and endoplasmic reticulum alterations, and intramitochondrial and intracytoplasmic calcifications were identified in tendinopathy (Jozsa et al., 1982).

Moreover, the PCR results might suggest that, on a cellular level, complete recovery did not occur. Degenerated tendons might have significantly higher expression levels of *Col1* and Col3 mRNAs compared to normal controls (Ireland et al., 2001). Moreover, higher levels of Col1 and tenomodulin might suggest repair and/or remodeling of tendons following moderate and intense treadmill running (Zhang & Wang, 2013). Increased expression of Col1 (5-fold) and tenomodulin (almost 70%) genes, but with reduced Col3 (about 15%) were found in the current study after 4 weeks of rest. Significantly lower expression levels of mohawk might be found in human tendinopathy (Liu et al., 2015). Increased or decreased levels of decorin may be observed in tendinopathy in addition to increased large proteoglycans biglycan and aggrecan levels (Dean et al., 2012). . The present study showed an increase in decorin gene expression levels of almost 4-fold, and 1-fold reduction in mohawk expression at the latest timepoint.

Ki-67 is a marker of cell proliferation that was reported to correlate with the number of tenocytes (Disser et al., 2019). Chronic tendon degeneration may be characterized by increases in tenocyte numbers, since Ki-67 positive tenocytes were found in tendinopathic biopsy samples (Scott et al., 2007). Human tendinopathic tenocytes might express higher levels of p16, p21, p53 and senescence-associated β-galactosidase compared with normal controls, moreover, these levels might positively correlate with tendinopathy severity (Chen et al., 2022). In torn tendons, human tenocytes might express increased levels of cellular senescence markers (p16 and p53) and senescence-associated secretory phenotype mediators (IL-6 and IL-8) (Hawthorne et al., 2023; Lundgreen et al., 2011). Apoptotic cell death mediated through caspase-3 was identified in tendinopathic tendons in addition to increased cellularity (Chen et al., 2010; Gaida et al., 2012; Lian et al., 2007; Pearce et al., 2009). Degenerative tendons in patients with tendinopathy, rotator cuff impingement and tendon tears showed apoptosis (Benson et al., 2010; Chen et al., 2010; Lundgreen et

al., 2013; Tuoheti et al., 2005; Wu et al., 2011; Yuan et al., 2002). The results of the current study at 4 weeks of rest showed about 15% reduction in Ki-67 and 3-fold increase in p53 gene expression levels.

Fibrocartilaginous metaplasia, accompanied by proteoglycan synthesis, may be a precursor to calcification (Uhthoff & Loehr, 1997). Indeed, human tenocytes were reported to have the capacity to differentiate into hypertrophic chondrocyte-like cells and form calcium deposits *in vitro* (Darrieutort-Laffite et al., 2019). Cells with rounded nuclei and pericellular lacunae may surround the deposits, which suggest chondrocyte differentiation (Darrieutort-Laffite et al., 2019). Hypertrophic chondrocytes were found to express several chondrocyte markers including collagen type X, matrix metalloproteinase 13 and cartilage oligomeric matrix protein (Darrieutort-Laffite et al., 2019). Collagen type II, however, was not detected and aggrecan expression was reduced (Darrieutort-Laffite et al., 2019). Reduced endoplasmic reticulum, and abundant villous projections and dense granules may characterize fibrocartilaginous metaplasia, with cells acquiring the rounded shape of chondrocytes in contrast to the spindle shape of tenocytes (Rufai et al., 1996; Wong & Buck, 1972). The differences between groups in cellular circularity were non-statistically significant in the present study. Nonetheless, statistically-significant reductions in cellularity were found in all experimental groups when compared to the sedentary control group. A reduction followed by a rebound increase in cellularity was reported in response to fatigue-overload tendon injury by a recent study (Chainani et al., 2024), which might confirm that an injury did occur albeit without recovery.

Fibrocartilaginous entheses are transitional areas that attach fibrous tendon tissues to bone (Benjamin & Ralphs, 2001; Rufai et al., 1995). Aggrecan and type II collagen were reported to be present in tendon fibrocartilage and articular cartilage (Kiani et al., 2002; Rufai et al., 1995; Waggett et al., 1998). Compressive loading of tendons may lead to a range of fibrocartilaginous differentiation, with rounded cell morphology, gap-junction loss and slight to strong proteoglycan increase (Benjamin & Ralphs, 1998; Ralphs et al., 1998; Rufai et al., 1992). Fibrocartilage cells in tendons might share some features with chondrocytes (Rufai et al., 1996). Moreover, chondrocytes are known to dedifferentiate into a fibroblast-like phenotype when isolated from their matrix, and produce type I and III collagens (Charlier et al., 2019).

Proteoglycan synthesis may be enhanced in response to increased loading magnitude, nevertheless, extended loading duration may significantly reduce proteoglycan synthesis (Jørgensen et al., 2017; Koob et al., 1992; Steinmeyer & Knue, 1997). Proteoglycans may contribute to the viscoelastic properties of tendons (Puxkandl et al., 2002; Robinson et al., 2004). The expression of proteoglycan mRNA genes was found to increase after 30 minutes to 6 hours of chondrocyte loading (Holmvall et al., 1995; Huang et al., 2007; Thomas et al., 2011; Zheng et al., 2012). Loading of chondrocytes for 12 hours may not alter aggrecan mRNA levels compared to unloaded cells (Huang et al., 2007; Iimoto et al., 2005; Nishida et al., 2008; Shimizu et al., 2004; Zheng et al., 2012). Loading of more than 16 hours, nonetheless, may lead to reversal of cell responses and reduction in proteoglycan mRNA levels (Huang et al., 2007; Iimoto et al., 2005; Shimizu et al., 2004; Zheng et al., 2012). Reduction in proteoglycan synthesis was reported following loading of chondrocytes of longer than 24 hours (Gassner et al., 1999; Xu et al., 2000). Inconsistent results were reported by another study suggesting an increase in proteoglycan synthesis (Long et al., 2001).

Short-term loading of bovine articular cartilage explants was reported to stimulate glycosaminoglycan synthesis, whereas long duration loading may inhibit it (Bachrach et al., 1995; Guilak et al., 1994; Parkkinen et al., 1992). A dose-dependent inhibition of proteoglycan release was observed in another study that found suppressed proteoglycan synthesis rates in the first 4-5 hours following compression removal, followed by increased synthesis rates from 8-48 hours after removal of compression (Guilak et al., 1994). Proportionate reduction in proteoglycan breakdown, furthermore, might occur with increasing compressive pressure (JONES et al., 1982). Conversely, a decrease in tissue proteoglycan content might correlate with increased proteoglycan synthesis rates (Hardingham et al., 1972).

Overloading of chondrocytes at 1.0 MPa compression lead to severe inhibition of proteoglycan synthesis rates that did not completely recover for 24 hours, which might indicate cellular damage (Guilak et al., 1994; Parkkinen et al., 1992). Excessive loads at lower loading rates may reduce glycosaminoglycan content, cell viability and ability to recover, and increase overall damage over time (Nickien et al., 2018). High loading at a high rate, however, was shown to reduce proteoglycan gene expression during the first 6 hours that may partially recover, suggesting loss of cell viability (van Haaften

et al., 2017). Cyclic loading might enhance proteoglycan loss, and collagen damage might exacerbate this loss further (Henao-Murillo et al., 2021). The response of chondrocytes to loading, moreover, might be dependent on its parameters and duty cycle (Palmoski & Brandt, 1984; Sah et al., 1991; Schneiderman et al., 1986).

Chondrocyte senescence might be accompanied by increased p21 and p16 levels, decreased production and increased degradation of glycosaminoglycans (Yagi et al., 2023). Mechanotransduction in chondrocytes and tenocytes may involve calcium signaling which is mediated through PIEZO1 channels (Lee et al., 2014; Passini et al., 2021; Rocio Servin-Vences et al., 2017). Inhibition of PIEZO channels, nonetheless, might protect articular chondrocytes from mechanically induced cell death (Lee et al., 2014). Decreased tenocyte viability in response to tendon strains above 4% were reported, suggesting involvement of apoptotic pathways (Stauber et al., 2020). Short term, high strain mechanical loading of 20%, maintained for 6 hours, was found to induce apoptosis of tenocytes (Scott et al., 2005). Disruption of tendon collagen fibers might lead to additional reduction in cell viability that might even extend far from the injury site (Kondratko-Mittnacht et al., 2015).

Hypertrophic chondrocytes may be terminally differentiated cells that secrete collagen type X (Böhme et al., 1995; Kato et al., 1988; Pacifici et al., 1990). Hypertrophic chondrocytes may undergo apoptosis or transdifferentiate into osteoblasts (Ahmed et al., 2007; G. Yang et al., 2014; L. Yang et al., 2014). The apoptotic bodies that are formed during chondrocyte cell death may contribute to pathologic calcification of cartilage (Hashimoto et al., 1998). Facilitation of endochondral ossification may be preceded by type X collagen expression, which may regulate matrix mineralization and compartmentalization of matrix components (Aigner et al., 1993; Grant et al., 1987; Shen, 2005). Apoptosis of chondrocytes might occur in a process that differs from classical apoptosis, termed chondroptosis, which includes autophagy of cell organelles and cytoplasm with the formation of empty lacunae (Roach et al., 2004). The observed reduction in proteoglycan content in the present study that was accompanied by a reduction in cell number might suggest, therefore, that the running protocol led to overstimulation of chondrocyte-like cells leading to apoptosis.

A rebound increase after the initial reduction, in proteoglycan content, might have been observed if animal were sacrificed at a later timepoint, since an increase in synthesis rate of proteoglycans may follow initial suppression (Guilak et al., 1994). Nevertheless, absence of this phenomenon might suggest reduction in cell viability (Holmvall et al., 1995). Enthesopathy may involve the presence of apoptotic cells and the release of matrix metalloproteinases, which may reduce proteoglycan synthesis and augment its degradation, respectively (Saito et al., 2022; Sudoł-Szopińska et al., 2015). It may, therefore, be considered that the observed findings might suggest a form of enthesis injury. Variability in the severity of injury might explain the large standard deviations and might justify the non-statistically significant results observed in many of the outcome measures. Moreover, sample choice was independent of the presence or absence of injury, i.e., all samples were considered, analyzed, and their results combined regardless of the state and severity of injury.

Severity of injury may differ between and within animals, since it is not unexpected that despite the standardization of loading intensity and duration, whether tendon injury would occur and to what extent may not be identical. Furthermore, whether electrical stimulation would influence healing is obviously dependent on the extent of injury. Results of all samples were combined and analyzed altogether, and regardless of the degree of injury, which may explain the large standard deviations observed in many of the results of outcome measures.

Dexmedetomidine was reported to have antioxidative, anti-inflammatory and apoptosis regulatory effects (Li et al., 2015; Wang et al., 2019; Xiang et al., 2014). Reduction in inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and C-reactive protein were found following dexmedetomidine administration (Li et al., 2015; Wang et al., 2019; Xiang et al., 2014). Standardization of sedative doses administered was, therefore, implemented. Moreover, the administration of an antagonist reversal agent that was equally standardized may abolish these effects (Li et al., 2020; Liang et al., 2017). IL-6 may mediate downregulation cartilage-specific matrix genes such as type II collagen, aggrecan, and link proteins (Keller et al., 2023; Legendre et al., 2005; Legendre et al., 2003; Liao et al., 2022). A chondroprotective effect and promotion of osteochondral repair were found in response to TNF-α

inhibition (Chisari et al., 2020). Nevertheless, due to the prolonged sedation time, it remains possible that Dexmedetomidine administration influenced the results.

Electrical stimulation was found to induce or inhibit apoptosis and increase proliferation (Love et al., 2018). HVMPC electrical stimulation may enhance migration and proliferation of fibroblasts and tenocytes (Gerard J Bourguignon & Lilly YW Bourguignon, 1987; Gerard J Bourguignon et al., 1989; Lee et al., 2002; Lee et al., 2003). Augmented collagen synthesis was additionally reported in response to HVMPC electrical stimulation (Gerard J Bourguignon & Lilly YW Bourguignon, 1987; Gerard J Bourguignon et al., 1989; Lee et al., 2002; Lee et al., 2003). Calcium influx might occur within the first minute of HVMPC electrical stimulation and reaches a maximum level after 3 minutes (Gerard J Bourguignon et al., 1989). Calcium signaling may regulate proteoglycan synthesis and chondrocyte differentiation, proliferation and apoptosis (Matta et al., 2015; Parvizi et al., 2002; Wang et al., 2003).

One potential limitation of electrical stimulation, nevertheless, in its current form, may be the non-selective stimulation of cells. Single-cell RNA sequencing of enthesis showed cellular and molecular heterogeneity during fibrocartilage development (Zhang et al., 2023). Multi-omic single-cell analysis revealed at least five distinct type I collagen expressing tenocyte populations, endothelial cells, T-cells and monocytes (Kendal et al., 2020). Several subtypes of endothelial cells and macrophage populations were identified in healthy and tendinopathic tendons (Akbar et al., 2021). A unique resident T-cell population found at the enthesis might induce inflammation when activated (Lories & McInnes, 2012; Sherlock et al., 2012). Different tendon stem progenitor cell populations, moreover, may exist in peritenon compared with tendon proper (Cadby et al., 2014; Mienaltowski et al., 2013). It remains unknown, however, whether all electrically stimulated cells would respond, how and to what extent.

As HVMPC stimulation was found to induce calcium influx, augment collagen synthesis and increase cell proliferation, which may lead to increased metabolic demand (Berg & Kerr, 1992; Ichioka et al., 2008). It might be expected, therefore, that HVMPC electrical stimulation might create a state of transient relative ischemia. Hypoxia may promote fibrocartilaginous metaplasia and heterotopic ossification, and is one of the theories for the development of calcific tendinitis (Agarwal et al., 2016; Freeman et al.,

2010; Malghem et al., 2015; Wang et al., 2016). Signs of hypoxia were identified in tenocytes isolated from patients with calcific tendinopathy (Jozsa et al., 1982).

Growth plate chondrocytes were reported to release matrix vesicles, which are primary initiators of extracellular mineral deposition in endochondral calcification (Wuthier, 1993). Calcium ions might be actively acquired, concentration within chondrocytes and then shed as calcium rich matrix vesicles (Wuthier, 1993). An increase in intracellular calcium concentration, moreover, may be related to production of matrix vesicles (Buckwalter et al., 1987; Iannotti et al., 1994). Further increase in intracellular calcium might lead to cell damage (Iannotti et al., 1994). A voltage-dependent calcium channel might contribute to calcium influx necessary for intravesicular initiation of mineralization (Kirsch & Wuthier, 1994; Thouverey et al., 2011). TGF-β may be required for chondrocyte proliferation and differentiation, moreover, it might initiate and promote heterotopic ossification (Van der Kraan et al., 2009; X. Wang et al., 2018; Wildemann et al., 2003). HVMPC electrical stimulation might increase TGF-β1 expression in wounds (Jeon et al., 2015). The results of the present study showed the presence of calcification after 8 sessions (30 days) and 12 sessions (45 days) of HVMPC electrical stimulation. One of the pathologic features of tendinopathy and enthesopathy may be heterotopic ossification [78-80]. A progressive increase in heterotopic ossification deposits during healing was reported following tendon transection (Pierantoni et al., 2023). The deposits started near the calcaneal bone and, at the latest timepoint (20 weeks), were almost everywhere in the tendon (Pierantoni et al., 2023).

Several factors might have contributed to the excessive and prolonged reduction in proteoglycan content in animals in this study compared with the previous study. These may include older age (2 months older), psychological stress caused by physical restraint and intraperitoneal injection (Cloutier & Newberry, 2008), dehydration due to sedative diuretic effect, reduced chow consumption, systemic effect of eye ointment and treats consumption. The depilator cream was applied to the back and both hindlimbs of all rats. The mild-moderate irritation produced could have systemic effects. However, since dermal irritation was a random event in both groups, it is unlikely that this had a significant systematic impact on the results.

**Limitations**

• Differences in the running style and form, for example: uphill vs downhill, slow vs fast, bipedal vs quadrupedal were observed during the protocol which may lead to differences in the severity of the injury due to differences in the imposed load.

• The design of the treadmills does not permit selective administration of electric shocks i.e.; all rats receive shocks at the same time. It was observed that this may lead to undue stress in the rats already running, therefore electric shocks were only administered when necessary.

• Skin irritation as a result of depilator cream use was observed, however, it was a random event in rats of both groups. Although impractical, an on-demand approach with repeated use as appropriate was adopted. Despite reduction in exposure time (as short as 30 seconds), switching from normal male skin, to normal female skin, to sensitive skin creams and following a strict application timing protocol using a digital timer, skin irritation still occurred randomly.

• Variability in the level of tightness in electrodes' attachment may have occurred, since it was dependent on conduction state in the experimental group but not in the control group. Nonetheless, the same researcher implemented both interventions in attempt to standardize the application in both groups.

• Due to the small volume of administration of reversing agent, a large margin of error in filling the syringe may have led to dosing inaccuracies. Nevertheless, it is unlikely that these differences had a significant effect, since the dosing of sedative was appropriate

• One rat in the control group died one week before the due euthanasia date.

• The software (SEDACOM), on rare occasions, did not activate or deactivate the administration of shocks according the designated protocol.

## Acknowledgements

This research was partially funded by the European Research Council, Consolidator Grant Magtendon, grant number 772817 and Portuguese National Science Foundation (FCT), grants numbers SFRD/BD/144816/2019 and COVID/BD/153447/2023. The authors would also like to thank the contributions to this research from the project "TERM RES Hub – Scientific Infrastructure for Tissue Engineering and Regenerative Medicine", reference PINFRA/22190/2016 (Norte-01-0145-FEDER-022190), funded by the FCT in cooperation with the Northern Portugal Regional Coordination and Development Commission (CCDR-N), for providing relevant lab facilities, state-of-the art equipment and highly qualified human resources.

## References


Abat, F., Alfredson, H., Cucchiarini, M., Madry, H., Marmotti, A., Mouton, C., . . . Spang, C. (2018). Current trends in tendinopathy: consensus of the ESSKA basic science committee. Part II: treatment options. *Journal of experimental orthopaedics, 5*, 1-17. doi:http://dx.doi.org/10.1186/s40634-018-0145-5

Abdullah, A., McCauley, R. L., & Herndon, D. N. (1991). Stimulation of human dermal fibroblasts with interleukin 2. *J Burn Care Rehabil, 12*(1), 23-25. doi:http://dx.doi.org/10.1097/00004630-199101000-00006

Administration, D., Food, A. o., & States, D. O. o. t. U. (1959). *Appraisal of the safety of chemicals in foods, drugs, and cosmetics*: Association of Food & Drug Officials of the United States.

Agarwal, S., Loder, S., Brownley, C., Cholok, D., Mangiavini, L., Li, J., . . . Ranganathan, K. (2016). Inhibition of Hif1α prevents both trauma-induced and genetic heterotopic ossification. *Proceedings of the National Academy of Sciences, 113*(3), E338-E347. doi:http://dx.doi.org/10.1073/pnas.1515397113

Ahmad, E. T. (2008). High-voltage pulsed galvanic stimulation: effect of treatment duration on healing of chronic pressure ulcers. *Annals of Burns and Fire Disasters 2008 Sep;21(3):124-128*.

Ahmed, E. T., & Khalaf, M. M. A.-k. (2006). Comparison of the Effect of High Voltage Pulsed Galvanic Stimulation versus Ultrasonic on the Acceleration of Burn Wound Healing.

Ahmed, Y., Tatarczuch, L., Pagel, C., Davies, H., Mirams, M., & Mackie, E. (2007). Physiological death of hypertrophic chondrocytes. *Osteoarthritis and cartilage, 15*(5), 575-586. doi:http://dx.doi.org/10.1016/j.joca.2006.10.016

Aigner, T., Reichenberger, E., Bertling, W., Kirsch, T., Stöss, H., & Von der Mark, K. (1993). Type X collagen expression in osteoarthritic and rheumatoid articular cartilage. *Virchows Archiv B, 63*, 205-211. doi:http://dx.doi.org/10.1007/BF02899263

Akai, M., & Hayashi, K. (2002). Effect of electrical stimulation on musculoskeletal systems; a meta-analysis of controlled clinical trials. *Bioelectromagnetics: Journal of the Bioelectromagnetics Society, The Society for Physical Regulation in Biology and Medicine, The European Bioelectromagnetics Association, 23*(2), 132-143. doi:http://dx.doi.org/10.1002/bem.106

Akbar, M., MacDonald, L., Crowe, L. A., Carlberg, K., Kurowska-Stolarska, M., Ståhl, P. L., . . . Millar, N. L. (2021). Single cell and spatial transcriptomics in human tendon disease indicate dysregulated immune homeostasis. *Annals of the Rheumatic Diseases*. doi:http://dx.doi.org/10.1136/annrheumdis-2021-220256

Akers, T. K., & Gabrielson, A. L. (1984). The effect of high voltage galvanic stimulation on the rate of healing of decubitus ulcers. *Biomed Sci Instrum, 20*, 99-100. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6608962

Archambault, J. M., Wiley, J. P., & Bray, R. C. (1995). Exercise loading of tendons and the development of overuse injuries. *Sports Medicine, 20*(2), 77-89. doi:http://dx.doi.org/10.2165/00007256-199520020-00003

Assimacopoulos, D. (1968a). Low intensity negative electric current in the treatment of ulcers of the leg due to chronic venous insufficiency: Preliminary report of three gases. *The American Journal of Surgery, 115*(5), 683-687. doi:http://dx.doi.org/10.1016/0002-9610(68)90101-3

Assimacopoulos, D. (1968b). Wound healing promotion by the use of negative electric current. *The American Surgeon, 34*(6), 423-431. doi:http://dx.doi.org/10.1097%2F00006534-197001000-00053

Bachrach, N. M., Valhmu, W. B., Stazzone, E., Ratcliffe, A., Lai, W. M., & Mow, V. C. (1995). Changes in proteoglycan synthesis of chondrocytes in articular cartilage are associated with the time-dependent changes in their mechanical environment. *Journal of biomechanics, 28*(12), 1561-1569. doi:http://dx.doi.org/10.1016/0021-9290(95)00103-4
Baker, J. R. (1941). A Fiuid for Softening Tissues Embedded in Paraffin Wax. doi:http://dx.doi.org/10.1111/j.1365-2818.1941.tb00890.x
Bax, L., Staes, F., & Verhagen, A. (2005). Does neuromuscular electrical stimulation strengthen the quadriceps femoris? A systematic review of randomised controlled trials. *Sports Medicine, 35*, 191-212. doi:http://dx.doi.org/10.2165/00007256-200535030-00002
Beebe, S. J., White, J., Blackmore, P. F., Deng, Y., Somers, K., & Schoenbach, K. H. (2003). Diverse effects of nanosecond pulsed electric fields on cells and tissues. *DNA and cell biology, 22*(12), 785-796. doi:http://dx.doi.org/10.1089/104454903322624993
Benjamin, M., & Ralphs, J. (1998). Fibrocartilage in tendons and ligaments—an adaptation to compressive load. *The Journal of Anatomy, 193*(4), 481-494. doi:http://dx.doi.org/10.1046/j.1469-7580.1998.19340481.x
Benjamin, M., & Ralphs, J. (2001). Entheses--the bony attachments of tendons and ligaments. *Italian journal of anatomy and embryology= Archivio italiano di anatomia ed embriologia, 106*(2 Suppl 1), 151-157.
Benjamin, M., Toumi, H., Ralphs, J. R., Bydder, G., Best, T., & Milz, S. (2006). Where tendons and ligaments meet bone: attachment sites (‘entheses’) in relation to exercise and/or mechanical load. *Journal of anatomy, 208*(4), 471-490. doi:http://dx.doi.org/10.1111/j.1469-7580.2006.00540.x
Benoliel, A.-M., Kahn-Peries, B., Imbert, J., & Verrando, P. (1997). Insulin stimulates haptotactic migration of human epidermal keratinocytes through activation of NF-kappa B transcription factor. *Journal of Cell Science, 110*(17), 2089-2097. doi:http://dx.doi.org/10.1242/jcs.110.17.2089
Benson, R., McDonnell, S., Knowles, H., Rees, J., Carr, A., & Hulley, P. (2010). Tendinopathy and tears of the rotator cuff are associated with hypoxia and apoptosis. *The Journal of Bone & Joint Surgery British Volume, 92*(3), 448-453. doi:http://dx.doi.org/10.1302/0301-620X.92B3.23074
Berg, R. A., & Kerr, J. S. (1992). Nutritional aspects of collagen metabolism. *Annual review of nutrition, 12*(1), 369-390. doi:http://dx.doi.org/10.1146/annurev.nu.12.070192.002101
Berg, S., Kutra, D., Kroeger, T., Straehle, C. N., Kausler, B. X., Haubold, C., . . . Rudy, M. (2019). Ilastik: interactive machine learning for (bio) image analysis. *Nature methods, 16*(12), 1226-1232. doi:http://dx.doi.org/10.1038/s41592-019-0582-9
Berhanu, P., & Olefsky, J. M. (1981). Effects of insulin and insulin-like agents on the glucose transport system of cultured human fibroblasts. *Diabetes, 30*(6), 523-529. doi:http://dx.doi.org/10.2337/diab.30.6.523
Berridge, M. J. (1995). Calcium signalling and cell proliferation. *Bioessays, 17*(6), 491-500. doi:http://dx.doi.org/10.1002/bies.950170605
Berridge, M. J., Lipp, P., & Bootman, M. D. (2000). The versatility and universality of calcium signalling. *Nature reviews Molecular cell biology, 1*(1), 11-21. doi:http://dx.doi.org/10.1038/35036035
Bi, Y., Ehirchiou, D., Kilts, T. M., Inkson, C. A., Embree, M. C., Sonoyama, W., . . . Zhang, L. (2007). Identification of tendon stem/progenitor cells and the role of

the extracellular matrix in their niche. *Nature medicine, 13*(10), 1219-1227. doi:http://dx.doi.org/10.1038/nm1630

Bigelow, J., Al-Husseini, S., Von Recum, A., & Park, J. (1979). Effect of electrical stimulation on canine skin and percutaneous device: skin interface healing. *Skin interface healing and electrical properties of bone and cartilage. New York: Grune & Stratton, 289*.

Birch, H. L., Peffers, M. J., & Clegg, P. D. (2016). Influence of ageing on tendon homeostasis. *Metabolic influences on risk for tendon disorders*, 247-260. doi:http://dx.doi.org/10.1007/978-3-319-33943-6_24

Blakytny, R., Jude, E. B., Martin Gibson, J., Boulton, A. J., & Ferguson, M. W. (2000). Lack of insulin-like growth factor 1 (IGF1) in the basal keratinocyte layer of diabetic skin and diabetic foot ulcers. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 190*(5), 589-594. doi:http://dx.doi.org/10.1002/(SICI)1096-9896(200004)190:5<589::AID-PATH553>3.0.CO;2-T

Böhme, K., Winterhalter, K. H., & Bruckner, P. (1995). Terminal differentiation of chondrocytes in culture is a spontaneous process and is arrested by transforming growth factor-β2 and basic fibroblast growth factor in synergy. *Experimental cell research, 216*(1), 191-198. doi:http://dx.doi.org/10.1006/excr.1995.1024

Bourguignon, G., & Bourguignon, L. (1987). Electric stimulation of protein and DNA synthesis in human fibroblasts. *The FASEB Journal, 1*(5), 398-402. doi:http://dx.doi.org/10.1096/fasebj.1.5.3678699

Bourguignon, G. J., & Bourguignon, L. Y. (1987). Electric stimulation of protein and DNA synthesis in human fibroblasts. *The FASEB Journal, 1*(5), 398-402. doi:http://dx.doi.org/10.1096/fasebj.1.5.3678699

Bourguignon, G. J., & Bourguignon, L. Y. (1987). Electric stimulation of protein and DNA synthesis in human fibroblasts. *FASEB J, 1*(5), 398-402. doi:http://dx.doi.org/10.1096/fasebj.1.5.3678699

Bourguignon, G. J., Jy, W., & Bourguignon, L. Y. (1989). Electric stimulation of human fibroblasts causes an increase in Ca2+ influx and the exposure of additional insulin receptors. *Journal of cellular physiology, 140*(2), 379-385. doi:http://dx.doi.org/10.1002/jcp.1041400224

Bourguignon, G. J., Jy, W., & Bourguignon, L. Y. (1989). Electric stimulation of human fibroblasts causes an increase in Ca2+ influx and the exposure of additional insulin receptors. *J Cell Physiol, 140*(2), 379-385. doi:http://dx.doi.org/10.1002/jcp.1041400224

Bourguignon, L. Y., Jy, W., Majercik, M. H., & Bourguignon, G. J. (1988). Lymphocyte activation and capping of hormone receptors. *J Cell Biochem, 37*(2), 131-150. doi:http://dx.doi.org/10.1002/jcb.240370202

Brown, M., Gogia, P. P., Sinacore, D. R., & Menton, D. N. (1995). High-voltage galvanic stimulation on wound healing in guinea pigs: longer-term effects. *Archives of physical medicine and rehabilitation, 76*(12), 1134-1137. doi:http://dx.doi.org/10.1016/S0003-9993(95)80122-7

Brown, M., McDonnell, M. K., & Menton, D. N. (1989). Polarity effects on wound healing using electric stimulation in rabbits. *Archives of physical medicine and rehabilitation, 70*(8), 624-627. doi:http://dx.doi.org/10.5555/uri:pii:0003999389900919

Buckwalter, J. A., Mower, D., & Schaeffer, J. (1987). Differences in matrix vesicle concentration among growth plate zones. *Journal of orthopaedic research, 5*(2), 157-163. doi:http://dx.doi.org/10.1002/jor.1100050202
Cadby, J. A., Buehler, E., Godbout, C., van Weeren, P. R., & Snedeker, J. G. (2014). Differences between the cell populations from the peritenon and the tendon core with regard to their potential implication in tendon repair. *PLoS One, 9*(3), e92474. doi:http://dx.doi.org/10.1371/journal.pone.0092474
Cardiff, R. D., Miller, C. H., & Munn, R. J. (2014). Manual hematoxylin and eosin staining of mouse tissue sections. *Cold Spring Harbor protocols, 2014*(6), pdb. prot073411. doi:http://dx.doi.org/10.1101/pdb.prot073411
Catterall, W. A. (2011). Voltage-gated calcium channels. *Cold Spring Harbor perspectives in biology, 3*(8), a003947. doi:http://dx.doi.org/10.1101/cshperspect.a003947
Chainani, P. H., Mena, M. B., Yeritsyan, D., Caro, D., Momenzadeh, K., Galloway, J. L., . . . Nazarian, A. (2024). Successive tendon injury in an in vivo rat overload model induces early damage and acute healing responses. *Frontiers in bioengineering and biotechnology, 12*. doi:http://dx.doi.org/10.3389/fbioe.2024.1327094
Charlier, E., Deroyer, C., Ciregia, F., Malaise, O., Neuville, S., Plener, Z., . . . de Seny, D. (2019). Chondrocyte dedifferentiation and osteoarthritis (OA). *Biochemical pharmacology, 165*, 49-65. doi:http://dx.doi.org/10.1016/j.bcp.2019.02.036
Chatterjee, M., Evans, M. K., Bell, R., Nguyen, P. K., Kamalitdinov, T. B., Korntner, S., . . . Andarawis-Puri, N. (2023). Histological and immunohistochemical guide to tendon tissue. *Journal of Orthopaedic Research®, 41*(10), 2114-2132. doi:http://dx.doi.org/10.1002/jor.25645
Chatterjee, M., Muljadi, P. M., & Andarawis-Puri, N. (2022). The role of the tendon ECM in mechanotransduction: disruption and repair following overuse. *Connective tissue research, 63*(1), 28-42. doi:http://dx.doi.org/10.1080/03008207.2021.1925663
Chen, J., Wang, A., Xu, J., & Zheng, M. (2010). In chronic lateral epicondylitis, apoptosis and autophagic cell death occur in the extensor carpi radialis brevis tendon. *Journal of shoulder and elbow surgery, 19*(3), 355-362. doi:http://dx.doi.org/10.1016/j.jse.2009.07.064
Chen, S.-Y., Jou, I.-M., Ko, P.-Y., Hsu, K.-L., Su, W.-R., Kuo, L.-C., . . . Wu, P.-T. (2022). Amelioration of experimental tendinopathy by lentiviral CD44 gene therapy targeting senescence-associated secretory phenotypes. *Molecular Therapy-Methods & Clinical Development, 26*, 157-168. doi:http://dx.doi.org/10.1016/j.omtm.2022.06.006
Chisari, E., Yaghmour, K., & Khan, W. (2020). The effects of TNF-alpha inhibition on cartilage: a systematic review of preclinical studies. *Osteoarthritis and cartilage, 28*(5), 708-718. doi:http://dx.doi.org/10.1016/j.joca.2019.09.008
Cho, M. R., Marler, J. P., Thatte, H. S., & Golan, D. E. (2002). Control of calcium entry in human fibroblasts by frequency-dependent electrical stimulation. *Front Biosci, 7*(1), 1-8. doi:http://dx.doi.org/10.2741/A733
Cho, M. R., Thatte, H. S., Silvia, M. T., & Golan, D. E. (1999). Transmembrane calcium influx induced by ac electric fields. *The FASEB Journal, 13*(6), 677-683. doi:http://dx.doi.org/10.1096/fasebj.13.6.677

Choi, E.-Y., Lee, J.-H., & JeKal, S.-J. (1995). Effect of High Voltage Pulsed Current Stimulation on Surgical Wound of Rat. *Journal of Korean Physical Therapy Science, 2*(2), 495-508. doi:http://dx.doi.org/10.1589/jpts.27.1485
Chow, C.-W., Rincón, M., & Davis, R. J. (1999). Requirement for transcription factor NFAT in interleukin-2 expression. *Molecular and cellular biology, 19*(3), 2300-2307. doi:http://dx.doi.org/10.1128/MCB.19.3.2300
Cloutier, S., & Newberry, R. C. (2008). Use of a conditioning technique to reduce stress associated with repeated intra-peritoneal injections in laboratory rats. *Applied Animal Behaviour Science, 112*(1-2), 158-173. doi:http://dx.doi.org/10.1016/j.applanim.2007.07.003
Conover, A., Hintz, R., & Rosenfeld, R. (1989). Direct evidence that the insulin receptor mediates a mitogenic response in cultured human fibroblasts. *Hormone and metabolic research, 21*(02), 59-63. doi:http://dx.doi.org/10.1055/s-2007-1009151
Cruz, N. I., Bayrón, F. E., & Suárez, A. J. (1989). Accelerated healing of full-thickness burns by the use of high-voltage pulsed galvanic stimulation in the pig. *Annals of Plastic Surgery, 23*(1), 49-55. doi:http://dx.doi.org/10.1097/00000637-198907000-00009
D'Agostino, M. A., & Olivieri, I. (2006). Enthesitis. *Best Practice & Research Clinical Rheumatology, 20*(3), 473-486. doi:http://dx.doi.org/10.1016/j.berh.2006.03.007
Darrieutort-Laffite, C., Arnolfo, P., Garraud, T., Adrait, A., Couté, Y., Louarn, G., . . . Blanchard, F. (2019). Rotator cuff tenocytes differentiate into hypertrophic chondrocyte-like cells to produce calcium deposits in an alkaline phosphatase-dependent manner. *Journal of Clinical Medicine, 8*(10), 1544. doi:http://dx.doi.org/10.3390/jcm8101544
de Vos, R.-J., Van Der Vlist, A. C., Zwerver, J., Meuffels, D. E., Smithuis, F., Van Ingen, R., . . . Pols, M. (2021). Dutch multidisciplinary guideline on Achilles tendinopathy. *British journal of sports medicine, 55*(20), 1125-1134. doi:http://dx.doi.org/10.1136/bjsports-2020-103867
Dean, B., Franklin, S., & Carr, A. (2012). A systematic review of the histological and molecular changes in rotator cuff disease. *Bone & joint research, 1*(7), 158-166. doi:http://dx.doi.org/10.1302/2046-3758.17.2000115
Demozay, D., Tsunekawa, S., Briaud, I., Shah, R., & Rhodes, C. J. (2011). Specific glucose-induced control of insulin receptor substrate-2 expression is mediated via Ca2+-dependent calcineurin/NFAT signaling in primary pancreatic islet β-cells. *Diabetes, 60*(11), 2892-2902. doi:http://dx.doi.org/10.2337/db11-0341
Denegar, C. R., Saliba, E., & Saliba, S. (2015). *Therapeutic Modalities for Musculoskeletal Injuries, 4E*: Human Kinetics.
Depper, J. M., Leonard, W. J., Drogula, C., Krönke, M., Waldmann, T. A., & Greene, W. C. (1985). Interleukin 2 (IL-2) augments transcription of the IL-2 receptor gene. *Proceedings of the National Academy of Sciences, 82*(12), 4230-4234. doi:http://dx.doi.org/10.1073/pnas.82.12.4230
Dingemanse, R., Randsdorp, M., Koes, B. W., & Huisstede, B. M. (2013). Evidence for the effectiveness of electrophysical modalities for treatment of medial and lateral epicondylitis: a systematic review. *British journal of sports medicine*. doi:http://dx.doi.org/10.1136/bjsports-2012-091513
Dirks, R., & Warden, S. (2011). Models for the study of tendinopathy. *J Musculoskelet Neuronal Interact, 11*(2), 141-149.

Disser, N. P., Piacentini, A. N., De Micheli, A. J., Schonk, M. M., Yao, V. J., Deng, X.-H., . . . Rodeo, S. A. (2022). Achilles Tendons Display Region-Specific Transcriptomic Signatures Associated With Distinct Mechanical Properties. *The American Journal of Sports Medicine, 50*(14), 3866-3874. doi:http://dx.doi.org/10.1177/03635465221128589

Disser, N. P., Sugg, K. B., Talarek, J. R., Sarver, D. C., Rourke, B. J., & Mendias, C. L. (2019). Insulin-like growth factor 1 signaling in tenocytes is required for adult tendon growth. *The FASEB Journal, 33*(11), 12680. doi:http://dx.doi.org/10.1096/fj.201901503R

Docheva, D., Müller, S. A., Majewski, M., & Evans, C. H. (2015). Biologics for tendon repair. *Advanced drug delivery reviews, 84*, 222-239. doi:http://dx.doi.org/10.1016/j.addr.2014.11.015

Dolibog, P. T., Porębska, B., Grzegorczyn, S., Chmielewska, D., Ślęzak, A., & Dolibog, P. (2022). Analgesic and Functional Efficiency of High-Voltage Electrical Stimulation in Patients with Lateral Epicondylitis—A Report with a 180-Day Follow-Up. *Journal of Clinical Medicine, 11*(9), 2571. doi:http://dx.doi.org/10.3390/jcm11092571

Dubé, J., Rochette-Drouin, O., Lévesque, P., Gauvin, R., Roberge, C. J., Auger, F. A., . . . Moulin, V. J. (2012). Human keratinocytes respond to direct current stimulation by increasing intracellular calcium: preferential response of poorly differentiated cells. *Journal of cellular physiology, 227*(6), 2660-2667. doi:http://dx.doi.org/10.1002/jcp.23008

Dunaif, A., Xia, J., Book, C.-B., Schenker, E., & Tang, Z. (1995). Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. *The Journal of clinical investigation, 96*(2), 801-810. doi:http://dx.doi.org/10.1172/JCI118126

Elshazly, F., & Hassan, A. (1998). Influence of High Voltage Pulsed Galvanic Stimulation on Wound Healing in Children with Burn. *Bull. Fac. Ph. Th. Cairo Univ., Vol 3*(1), 61-68.

Falanga, V. (1987). Electrical stimulation increases the expression of fibroblasts receptors for transforming growth factor-beta. *J Invest Dermatol, 88*, A.

Fearon, A., Dahlstrom, J. E., Twin, J., Cook, J., & Scott, A. (2014). The Bonar score revisited: region of evaluation significantly influences the standardized assessment of tendon degeneration. *Journal of science and medicine in sport, 17*(4), 346-350. doi:http://dx.doi.org/10.1016/j.jsams.2013.07.008

Fenwick, S. A., Hazleman, B. L., & Riley, G. P. (2002). The vasculature and its role in the damaged and healing tendon. *Arthritis Research & Therapy, 4*(4), 252. doi:http://dx.doi.org/10.1186/ar416

Fitzgerald, G. K., & Newsome, D. (1993). Treatment of a large infected thoracic spine wound using high voltage pulsed monophasic current. *Physical Therapy, 73*(6), 355-360. doi:http://dx.doi.org/10.1093/ptj/73.6.355

Franek, A., Kostur, R., Polak, A., Taradaj, J., Szlachta, Z., Blaszczak, E., . . . Kucio, C. (2012). Using high-voltage electrical stimulation in the treatment of recalcitrant pressure ulcers: results of a randomized, controlled clinical study. *Ostomy-Wound Management, 58*(3), 30.

Franek, A., Kostur, R., Taradaj, J., Blaszczak, E., Szlachta, Z., Dolibog, P., . . . Polak, A. (2011). Effect of High Voltage Monophasic Stimulation on Pressure Ulcer Healing: Results From a Randomized Controlled Trial. *WOUNDS-A COMPENDIUM OF CLINICAL RESEARCH AND PRACTICE, 23*(1), 15-23.

Fredberg, U., & Stengaard-Pedersen, K. (2008). Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation. *Scandinavian journal of medicine & science in sports, 18*(1), 3-15.

Freeman, T. A., Parvizi, J., Dela Valle, C. J., & Steinbeck, M. J. (2010). Mast cells and hypoxia drive tissue metaplasia and heterotopic ossification in idiopathic arthrofibrosis after total knee arthroplasty. *Fibrogenesis & Tissue Repair, 3*(1), 1-10. doi:http://dx.doi.org/10.1186/1755-1536-3-17

Fujimoto, W. Y., & Williams, R. H. (1974). Insulin action on the cultured human fibroblast: glucose uptake, protein synthesis, RNA synthesis. *Diabetes, 23*(5), 443-448. doi:http://dx.doi.org/10.2337/diab.23.5.443

Gaida, J. E., Bagge, J., Purdam, C., Cook, J., Alfredson, H., & Forsgren, S. (2012). Evidence of the TNF-α system in the human Achilles tendon: expression of TNF-α and TNF receptor at both protein and mRNA levels in the tenocytes. *Cells Tissues Organs, 196*(4), 339-352. doi:http://dx.doi.org/10.1159/000335475

Gains, C. C., Correia, J. C., Baan, G. C., Noort, W., Screen, H. R., & Maas, H. (2020). Force transmission between the gastrocnemius and soleus sub-tendons of the Achilles tendon in rat. *Frontiers in bioengineering and biotechnology, 8*, 700. doi:http://dx.doi.org/10.3389/fbioe.2020.00700

Gassner, R., Buckley, M. J., Georgescu, H., Studer, R., Stefanovich-Racic, M., Piesco, N. P., . . . Agarwal, S. (1999). Cyclic tensile stress exerts antiinflammatory actions on chondrocytes by inhibiting inducible nitric oxide synthase. *The Journal of Immunology, 163*(4), 2187-2192. doi:http://dx.doi.org/10.4049/jimmunol.163.4.2187

Gilcreast, D. M., Stotts, N. A., Froelicher, E. S., Baker, L. L., & Moss, K. M. (1998). Effect of electrical stimulation on foot skin perfusion in persons with or at risk for diabetic foot ulcers. *Wound Repair and Regeneration, 6*(5), 434-441. doi:http://dx.doi.org/10.1046/j.1524-475X.1998.60505.x

Glazebrook, M. A., Wright Jr, J. R., Langman, M., Stanish, W. D., & Lee, J. M. (2008). Histological analysis of achilles tendons in an overuse rat model. *Journal of orthopaedic research, 26*(6), 840-846. doi:http://dx.doi.org/10.1002/jor.20546

Goldman, R., Brewley, B., Zhou, L., & Golden, M. (2003). Electrotherapy reverses inframalleolar ischemia: a retrospective, observational study. 2003b. *Adv Skin Wound Care, 16*(2), 79-89. doi:http://dx.doi.org/10.1097/00129334-200303000-00009

Goldman, R., Rosen, M., Brewley, B., & Golden, M. (2004). Electrotherapy promotes healing and microcirculation of infrapopliteal ischemic wounds: a prospective pilot study. *Adv Skin Wound Care, 17*(6), 284-294. doi:http://dx.doi.org/10.1097/00129334-200407000-00010

Goldman, R. J., Brewley, B. I., Cohen, R., & Rudnick, M. (2003). Use of electrotherapy to reverse expanding cutaneous gangrene in end-stage renal disease. 2003a. *Adv Skin Wound Care, 16*(7), 363-366. doi:http://dx.doi.org/10.1097/00129334-200312000-00013

Goldman, R. J., Brewley, B. I., & Golden, M. A. (2002). Electrotherapy reoxygenates inframalleolar ischemic wounds on diabetic patients: a case series. *Adv Skin Wound Care, 15*(3), 112-120. doi:http://dx.doi.org/10.1097/00129334-200205000-00006

Gomes, R. C., Brandino, H. E., de Sousa, N. T., Santos, M. F., Martinez, R., & Guirro, R. R. (2015). Polarized currents inhibit in vitro growth of bacteria colonizing cutaneous ulcers. *Wound Repair Regen, 23*(3), 403-411. doi:http://dx.doi.org/10.1111/wrr.12296

Gomes, R. C., Guirro, E. C., Gonçalves, A. C., Junior, J. A. F., Junior, L. O. M., & Guirro, R. R. (2018). High-voltage electric stimulation of the donor site of skin grafts accelerates the healing process. A randomized blinded clinical trial. *BURNS, 44*(3), 636-645. doi:http://dx.doi.org/10.1016/j.burns.2017.10.006

Grant, W. T., Wang, G.-J., & Balian, G. (1987). Type X collagen synthesis during endochondral ossification in fracture repair. *Journal of Biological Chemistry, 262*(20), 9844-9849. doi:http://dx.doi.org/10.1016/S0021-9258(18)48010-6

Griffin, J. W., Tooms, R. E., Mendius, R. A., Clifft, J. K., Vander Zwaag, R., & el-Zeky, F. (1991). Efficacy of high voltage pulsed current for healing of pressure ulcers in patients with spinal cord injury. *Physical Therapy 1991 Jun;71(6):433-442*. doi:http://dx.doi.org/10.1093/ptj/71.6.433

Grinstein, M., Dingwall, H. L., O'Connor, L. D., Zou, K., Capellini, T. D., & Galloway, J. L. (2019). A distinct transition from cell growth to physiological homeostasis in the tendon. *Elife, 8*, e48689. doi:http://dx.doi.org/10.7554/eLife.48689

Gui, M. S., Guirro, R. R. d. J., Sakabe, D. I., & Sakabe, F. F. (2013). High voltage pulsed stimulation increases cicatrization of chronic cutaneous ulcers: analysis of six cases. *Fisioterapia e Pesquisa, 20*, 286-292. doi:http://dx.doi.org/10.1590/S1809-29502013000300014

Guilak, F., Meyer, B. C., Ratcliffe, A., & Mow, V. C. (1994). The effects of matrix compression on proteoglycan metabolism in articular cartilage explants. *Osteoarthritis and cartilage, 2*(2), 91-101. doi:http://dx.doi.org/10.1016/S1063-4584(05)80059-7

Handsfield, G. G., Greiner, J., Madl, J., Rog-Zielinska, E. A., Hollville, E., Vanwanseele, B., & Shim, V. (2020). Achilles subtendon structure and behavior as evidenced from tendon imaging and computational modeling. *Frontiers in Sports and Active Living, 2*, 70. doi:http://dx.doi.org/10.3389/fspor.2020.00070

Hardingham, T., Fitton-Jackson, S., & Muir, H. (1972). Replacement of proteoglycans in embryonic chick cartilage in organ culture after treatment with testicular hyaluronidase. *Biochemical Journal, 129*(1), 101-112. doi:http://dx.doi.org/10.1042/bj1290101

Hashimoto, S., Ochs, R. L., Rosen, F., Quach, J., McCabe, G., Solan, J., . . . Lotz, M. (1998). Chondrocyte-derived apoptotic bodies and calcification of articular cartilage. *Proceedings of the National Academy of Sciences, 95*(6), 3094-3099. doi:http://dx.doi.org/10.1073/pnas.95.6.3094

Hawthorne, B. C., Wellington, I. J., Sabitsky, J. T., Murphy, K. V., Karsmarski, O. P., Thomas, R. O., . . . Gulati, S. (2023). Human rotator cuff tears demonstrate an age-dependent increase in markers of cellular senescence and selective removal of senescent cells with dasatinib+ quercetin increases genetic expression of COL1A1 in vitro. *Arthroscopy: The Journal of Arthroscopic & Related Surgery*. doi:http://dx.doi.org/10.1016/j.arthro.2023.05.036

Heath, M. E., & GIBBS, S. B. (1992). High-voltage pulsed galvanic stimulation: effects of frequency of current on blood flow in the human calf muscle. *Clinical Science, 82*(6), 607-613. doi:http://dx.doi.org/10.1042/cs0820607

Hecker, B., Carron, H., & Schwartz, D. P. (1985). Pulsed galvanic stimulation: effects of current frequency and polarity on blood flow in healthy subjects. *Arch Phys*

*Med Rehabil, 66*(6), 369-371. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/4004533

Hehenberger, K., & Hansson, A. (1997). High glucose-induced growth factor resistance in human fibroblasts can be reversed by antioxidants and protein kinase C-inhibitors. *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease, 15*(3), 197-201. doi:http://dx.doi.org/10.1002/(SICI)1099-0844(199709)15:3<197::AID-CBF740>3.0.CO;2-7

Hehenberger, K., Heilborn, J. D., Brismar, K., & Hansson, A. (1998). Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal dermal fibroblasts treated with high glucose is associated with increased formation of L-lactate. *Wound Repair and Regeneration, 6*(2), 135-141. doi:http://dx.doi.org/10.1046/j.1524-475X.1998.60207.x

Hehenberger, K., Kratz, G., Hansson, A., & Brismar, K. (1998). Fibroblasts derived from human chronic diabetic wounds have a decreased proliferation rate, which is recovered by the addition of heparin. *Journal of Dermatological Science, 16*(2), 144-151. doi:http://dx.doi.org/10.1016/S0923-1811(97)00042-X

Heinemeier, K. M., Skovgaard, D., Bayer, M. L., Qvortrup, K., Kjaer, A., Kjaer, M., . . . Kongsgaard, M. (2012). Uphill running improves rat Achilles tendon tissue mechanical properties and alters gene expression without inducing pathological changes. *Journal of applied physiology, 113*(5), 827-836. doi:http://dx.doi.org/10.1152/japplphysiol.00401.2012

Henao-Murillo, L., Pastrama, M.-I., Ito, K., & van Donkelaar, C. C. (2021). The relationship between proteoglycan loss, overloading-induced collagen damage, and cyclic loading in articular cartilage. *Cartilage, 13*(2_suppl), 1501S-1512S. doi:http://dx.doi.org/10.1177/1947603519885005

Herminawaty, D., Defi, I., Probowo, T., & Parwati, T. (2014). Promising treatment for pressure ulcers using high voltage pulsed current stimulation. *Gerontechnology, 13*(2), 202. doi:http://dx.doi.org/10.4017/gt.2014.13.02.266.00

Hidaka, H., Howard, B. V., Kosmakos, F. C., Fields, R. M., Craig, J. W., Bennett, P. H., & Larner, J. (1980). Insulin stimulation of glycogen synthase in cultured human diploid fibroblasts. *Diabetes, 29*(10), 806-810. doi:http://dx.doi.org/10.2337/diacare.20.10.806

Hogan, P. G., Chen, L., Nardone, J., & Rao, A. (2003). Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes & development, 17*(18), 2205-2232. doi:http://dx.doi.org/10.1101/gad.1102703

Holmvall, K., Camper, L., Johansson, S., Kimura, J. H., & Lundgren-Åkerlund, E. (1995). Chondrocyte and chondrosarcoma cell integrins with affinity for collagen type II and their response to mechanical stress. *Experimental cell research, 221*(2), 496-503. doi:http://dx.doi.org/10.1006/excr.1995.1401

Houghton, P. E., Campbell, K. E., Fraser, C. H., Harris, C., Keast, D. H., Potter, P. J., . . . Woodbury, M. G. (2010). Electrical stimulation therapy increases rate of healing of pressure ulcers in community-dwelling people with spinal cord injury. *Archives of Physical Medicine and Rehabilitation 2010 May;91(5):669-678*. doi:http://dx.doi.org/10.1016/j.apmr.2009.12.026

Houghton, P. E., Kincaid, C. B., Lovell, M., Campbell, K. E., Keast, D. H., Woodbury, M. G., & Harris, K. A. (2003). Effect of electrical stimulation on chronic leg

ulcer size and appearance. *Physical Therapy, 83*(1), 17-28. doi:http://dx.doi.org/10.1093/ptj/83.1.17
Howard, B. V., Mott, D. M., Fields, R. M., & Bennett, P. H. (1979). Insulin stimulation of glucose entry in cultured human fibroblasts. *Journal of cellular physiology, 101*(1), 129-138. doi:http://dx.doi.org/10.1002/jcp.1041010115
Hu, S. C.-S., & Lan, C.-C. E. (2016). High-glucose environment disturbs the physiologic functions of keratinocytes: focusing on diabetic wound healing. *Journal of Dermatological Science, 84*(2), 121-127. doi:http://dx.doi.org/10.1016/j.jdermsci.2016.07.008
Huang, J., Ballou, L., & Hasty, K. (2007). Cyclic equibiaxial tensile strain induces both anabolic and catabolic responses in articular chondrocytes. *Gene, 404*(1-2), 101-109.
Iannotti, J., Naidu, S., Noguchi, Y., Hunt, R., & Brighton, C. (1994). Growth plate matrix vesicle biogenesis. The role of intracellular calcium. *Clin Orthop Relat Res*(306), 222-229.
Ichioka, S., Ando, T., Shibata, M., Sekiya, N., & Nakatsuka, T. (2008). Oxygen consumption of keloids and hypertrophic scars. *Annals of Plastic Surgery, 60*(2), 194-197. doi:http://dx.doi.org/10.1097/SAP.0b013e318053ec1d
Iimoto, S., Watanabe, S., Takahashi, T., Shimizu, A., & Yamamoto, H. (2005). The influence of Celecoxib on matrix synthesis by chondrocytes under mechanical stress in vitro. *International Journal of Molecular Medicine, 16*(6), 1083-1088. doi:http://dx.doi.org/10.3892/ijmm.16.6.1083
Imoto, A. M., Peccin, S., Almeida, G. J. M., Saconato, H., & Atallah, Á. N. (2011). Effectiveness of electrical stimulation on rehabilitation after ligament and meniscal injuries: a systematic review. *Sao Paulo Medical Journal, 129*, 414-423. doi:http://dx.doi.org/10.1590/S1516-31802011000600008
Ippolito, E., Pedrini, V. T. A., & Pedrini-Mille, A. (1983). Histochemical properties of cartilage proteoglycans. *Journal of Histochemistry & Cytochemistry, 31*(1), 53-61. doi:http://dx.doi.org/10.1177/31.1.6187804
Irby, A., Gutierrez, J., Chamberlin, C., Thomas, S. J., & Rosen, A. B. (2020). Clinical management of tendinopathy: a systematic review of systematic reviews evaluating the effectiveness of tendinopathy treatments. *Scandinavian journal of medicine & science in sports, 30*(10), 1810-1826. doi:http://dx.doi.org/10.1111/sms.13734
Ireland, D., Harrall, R., Curry, V., Holloway, G., Hackney, R., Hazleman, B., & Riley, G. (2001). Multiple changes in gene expression in chronic human Achilles tendinopathy. *Matrix Biology, 20*(3), 159-169. doi:http://dx.doi.org/10.1016/S0945-053X(01)00128-7
James, R., Kesturu, G., Balian, G., & Chhabra, A. B. (2008). Tendon: biology, biomechanics, repair, growth factors, and evolving treatment options. *The Journal of hand surgery, 33*(1), 102-112. doi:http://dx.doi.org/10.1016/j.jhsa.2007.09.007
Järvinen, M., Jozsa, L., Kannus, P., Järvinen, T., Kvist, M., & Leadbetter, W. (1997). Histopathological findings in chronic tendon disorders. *Scandinavian journal of medicine & science in sports, 7*(2), 86-95. doi:http://dx.doi.org/10.1111/j.1600-0838.1997.tb00124.x
Jelinsky, S. A., Lake, S. P., Archambault, J. M., & Soslowsky, L. J. (2008). Gene expression in rat supraspinatus tendon recovers from overuse with rest. *Clin Orthop Relat Res, 466*(7), 1612-1617. doi:http://dx.doi.org/10.1007/s11999-008-0270-z

Jeon, J.-K., Park, S.-K., & Lee, J.-H. (2015). Effects of high voltage pulsed current stimulation with a visible contraction intensity on expression of TGF-β1 and synthesis of type I collagen in wound-induced white rats. *Journal of physical therapy science, 27*(5), 1485-1490. doi:http://dx.doi.org/10.1589/jpts.27.1485

Jiao, X., Zhang, Y., Li, W., Zhou, X., Chu, W., Li, Y., . . . Gan, Y. (2022). HIF-1α inhibition attenuates severity of Achilles tendinopathy by blocking NF-κB and MAPK pathways. *International Immunopharmacology, 106*, 108543. doi:http://dx.doi.org/10.1016/j.intimp.2022.108543

JONES, I. L., KLÄMFELDT, A., & SANDSTRÖM, T. (1982). The effect of continuous mechanical pressure upon the turnover of articular cartilage proteoglycans in vitro. *Clinical Orthopaedics and Related Research (1976-2007), 165*, 283-289.

Jørgensen, A. E. M., Kjær, M., & Heinemeier, K. M. (2017). The effect of aging and mechanical loading on the metabolism of articular cartilage. *The Journal of rheumatology, 44*(4), 410-417. doi:http://dx.doi.org/10.3899/jrheum.160226

Jozsa, L., Balint, B., & Reffy, A. (1980). Calcifying tendinopathy. *Archives of orthopaedic and traumatic surgery, 97*, 305-307. doi:http://dx.doi.org/10.1007/BF00380713

Jozsa, L., Balint, B., Reffy, A., & Demel, Z. (1982). Hypoxic alterations of tenocytes in degenerative tendinopathy. *Archives of orthopaedic and traumatic surgery, 99*, 243-246. doi:http://dx.doi.org/10.1007/BF00381401

Jozsa, L., Reffy, A., Kannus, P., Demel, S., & Elek, E. (1990). Pathological alterations in human tendons. *Archives of Orthopaedic and Trauma Surgery, 110*, 15-21. doi:http://dx.doi.org/10.1007/BF00431359

Jy, W., Fregien, N., Bourguignon, G., & Bourguignon, L. Y. (1989). Role of Ca2+ in the regulation of hormone receptor exposure during lymphocyte activation. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 983*(2), 153-160. doi:http://dx.doi.org/10.1016/0005-2736(89)90228-9

Kang, E.-J., Roh, J.-S., Lee, J.-S., Yi, C.-H., & Kim, T.-H. (1996). A Comparison of the Inhibitive Effect of High Voltage Pulsed Current Stimulation and Microcurrent Electrical Neuromuscular Stimulation on Bacterial Growth. *Physical Therapy Korea, 3*(1), 12-23.

Kang, R., Tang, D., Lotze, M. T., & Zeh III, H. J. (2013). Autophagy is required for IL-2-mediated fibroblast growth. *Experimental cell research, 319*(4), 556-565. doi:http://dx.doi.org/10.1016/j.yexcr.2012.11.012

Kannus, P., & Jozsa, L. (1991). Histopathological changes preceding spontaneous rupture of a tendon. A controlled study of 891 patients. *JBJS, 73*(10), 1507-1525. doi:http://dx.doi.org/10.2106/00004623-199173100-00009

Karlsson, M., & Björnsson, S. (2001). Quantitation of proteoglycans in biological fluids using Alcian blue. *Proteoglycan Protocols*, 159-173. doi:http://dx.doi.org/10.1385/1-59259-209-0:159

Karsli, P. B., Gurcay, E., Karaahmet, O. Z., & Cakci, A. (2017). High-Voltage electrical stimulation versus ultrasound in the treatment of pressure ulcers. *Advances in skin & wound care, 30*(12), 565-570. doi:http://dx.doi.org/10.1097/01.ASW.0000526606.72489.99

Kato, Y., Iwamoto, M., Koike, T., Suzuki, F., & Takano, Y. (1988). Terminal differentiation and calcification in rabbit chondrocyte cultures grown in centrifuge tubes: regulation by transforming growth factor beta and serum factors. *Proceedings of the National Academy of Sciences, 85*(24), 9552-9556. doi:http://dx.doi.org/10.1073/pnas.85.24.9552

Keller, L. E., Tait Wojno, E. D., Begum, L., & Fortier, L. A. (2023). Interleukin-6 neutralization and regulatory T cells are additive in chondroprotection from IL-1β-induced inflammation. *Journal of Orthopaedic Research®, 41*(5), 942-950. doi:http://dx.doi.org/10.1002/jor.25453
Kendal, A. R., Layton, T., Al-Mossawi, H., Appleton, L., Dakin, S., Brown, R., . . . Carr, A. (2020). Multi-omic single cell analysis resolves novel stromal cell populations in healthy and diseased human tendon. *Scientific Reports, 10*(1), 13939. doi:http://dx.doi.org/10.1038/s41598-020-70786-5
Kiani, C., Chen, L., Wu, Y. J., Yee, A. J., & Yang, B. B. (2002). Structure and function of aggrecan. *Cell research, 12*(1), 19-32. doi:http://dx.doi.org/10.1038/sj.cr.7290106
Kim, T. H., Cho, H.-y., & Lee, S. M. (2014). High-voltage pulsed current stimulation enhances wound healing in diabetic rats by restoring the expression of collagen, α-smooth muscle actin, and TGF-β1. *The Tohoku journal of experimental medicine, 234*(1), 1-6. doi:http://dx.doi.org/10.1620/tjem.234.1
Kincaid, C. B., & Lavoie, K. H. (1989). Inhibition of bacterial growth in vitro following stimulation with high voltage, monophasic, pulsed current. *Phys Ther, 69*(8), 651-655. doi:http://dx.doi.org/10.1093/ptj/69.8.651
Kirsch, T., & Wuthier, R. E. (1994). Stimulation of calcification of growth plate cartilage matrix vesicles by binding to type II and X collagens. *Journal of Biological Chemistry, 269*(15), 11462-11469. doi:http://dx.doi.org/10.1016/S0021-9258(19)78146-0
Kloth, L. C., & Feedar, J. A. (1988). Acceleration of wound healing with high voltage, monophasic, pulsed current. *Phys Ther, 68*(4), 503-508. doi:http://dx.doi.org/10.1093/ptj/68.4.503
Kondratko-Mittnacht, J., Lakes, R., & Vanderby Jr, R. (2015). Shear loads induce cellular damage in tendon fascicles. *Journal of biomechanics, 48*(12), 3299-3305. doi:http://dx.doi.org/10.1016/j.jbiomech.2015.06.006
Konikoff, J. J. (1976). Electrical promotion of soft tissue repairs. *Ann Biomed Eng, 4*(1), 1-5. doi:http://dx.doi.org/10.1007/BF02363553
Koob, T. J., Clark, P. E., Hernandez, D. J., Thurmond, F. A., & Vogel, K. G. (1992). Compression loading in vitro regulates proteoglycan synthesis by tendon fibrocartilage. *Archives of biochemistry and biophysics, 298*(1), 303-312. doi:http://dx.doi.org/10.1016/0003-9861(92)90127-I
Kotturi, M. F., Carlow, D. A., Lee, J. C., Ziltener, H. J., & Jefferies, W. A. (2003). Identification and functional characterization of voltage-dependent calcium channels in T lymphocytes. *Journal of Biological Chemistry, 278*(47), 46949-46960. doi:http://dx.doi.org/10.1074/jbc.M309268200
Kuykendall, C. A., Hutchinson, T. L., & Bloedaum, A. P. (2004). 112 Effects of High Voltage Pulsed Current on Bacterial Viability: An in Vitro Study. *Wound Repair and Regeneration, 12*(2). doi:http://dx.doi.org/10.1111/j.1067-1927.2004.0abstractdf.x
LaCourse, M., Singh, M., & Soo Hoo, J. (2023). Update on Interventional Procedural Considerations for Tendinopathy. *Current Physical Medicine and Rehabilitation Reports*, 1-12. doi:http://dx.doi.org/10.1007/s40141-023-00401-x
Lake, S. P., Ansorge, H. L., & Soslowsky, L. J. (2008). Animal models of tendinopathy. *Disability and rehabilitation, 30*(20-22), 1530-1541. doi:http://dx.doi.org/10.1080/09638280701785460

Lan, C. C., Liu, I. H., Fang, A. H., Wen, C. H., & Wu, C. S. (2008). Hyperglycaemic conditions decrease cultured keratinocyte mobility: implications for impaired wound healing in patients with diabetes. *British Journal of Dermatology, 159*(5), 1103-1115. doi:http://dx.doi.org/10.1111/j.1365-2133.2008.08789.x

Lansdown, A. B. (2002). Calcium: a potential central regulator in wound healing in the skin. *Wound Repair and Regeneration, 10*(5), 271-285. doi:http://dx.doi.org/10.1046/j.1524-475X.2002.10502.x

Lasko, J., Kochik, J., & Serena, T. (2010). Combining acoustic pressure wound therapy with electrical stimulation for treatment of chronic lower-extremity ulcers: A case series. *Advances in skin & wound care, 23*(10), 446-449. doi:http://dx.doi.org/10.1097/01.ASW.0000383223.56900.13

Lee, J.-H., Jekal, S.-J., & Park, R.-J. (2002). Enhance of migration and proliferation of cells from tendon biopsies by high voltage pulsed current stimulation. *Journal of Korean Physical Therapy, 14*(2), 162-171.

Lee, J.-H., Lee, J.-S., & Kil, E.-Y. (2004). Keratinocyte proliferation in aged rat skin by high voltage pulsed current stimulation. *대한의생명과학회지, 10*(4), 361-366.

Lee, J.-H., Park, C.-E., & Park, R.-J. (2010). Electrical Stimulation Induces the Collagen Deposition and TGF-${\beta} $1 mRNA Expression in Skin Wound of Rat. *The Journal of Korean Physical Therapy, 22*(3), 87-92.

Lee, J.-h., Song, I.-y., & Jong-Gyu, K. (2003). Acceleration of wound healing and collagen deposition in rat skin by high voltage pulsed current stimulation. *Journal of Korean Physical Therapy, 15*(4), 1-12.

Lee, W., Leddy, H. A., Chen, Y., Lee, S. H., Zelenski, N. A., McNulty, A. L., . . . Zauscher, S. (2014). Synergy between Piezo1 and Piezo2 channels confers high-strain mechanosensitivity to articular cartilage. *Proceedings of the National Academy of Sciences, 111*(47), E5114-E5122. doi:http://dx.doi.org/10.1073/pnas.1414298111

Legendre, F., Bogdanowicz, P., Boumediene, K., & Pujol, J.-P. (2005). Role of interleukin 6 (IL-6)/IL-6R-induced signal tranducers and activators of transcription and mitogen-activated protein kinase/extracellular. *The Journal of rheumatology, 32*(7), 1307-1316.

Legendre, F., Dudhia, J., Pujol, J.-P., & Bogdanowicz, P. (2003). JAK/STAT but not ERK1/ERK2 pathway mediates interleukin (IL)-6/soluble IL-6R down-regulation of type II collagen, aggrecan core, and link protein transcription in articular chondrocytes: association with a down-regulation of SOX9 expression. *Journal of Biological Chemistry, 278*(5), 2903-2912. doi:http://dx.doi.org/10.1074/jbc.M110773200

Leonard, W. J., Krönke, M., Peffer, N. J., Depper, J. M., & Greene, W. C. (1985). Interleukin 2 receptor gene expression in normal human T lymphocytes. *Proceedings of the National Academy of Sciences, 82*(18), 6281-6285. doi:http://dx.doi.org/10.1073/pnas.82.18.6281

Lerman, O. Z., Galiano, R. D., Armour, M., Levine, J. P., & Gurtner, G. C. (2003). Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. *The American journal of pathology, 162*(1), 303-312. doi:http://dx.doi.org/10.1016/S0002-9440(10)63821-7

Li, B., Li, Y., Tian, S., Wang, H., Wu, H., Zhang, A., & Gao, C. (2015). Anti-inflammatory effects of perioperative dexmedetomidine administered as an adjunct to general anesthesia: a meta-analysis. *Scientific Reports, 5*(1), 12342. doi:http://dx.doi.org/10.1038/srep12342

Li, L., Zhang, J., Zhang, Q., Zhang, D., Xiang, F., Jia, J., . . . Huang, Y. (2019). High glucose suppresses keratinocyte migration through the inhibition of p38 MAPK/autophagy pathway. *Frontiers in Physiology, 10*, 24. doi:http://dx.doi.org/10.3389/fphys.2019.00024

Li, R., Lai, I. K., Pan, J. Z., Zhang, P., & Maze, M. (2020). Dexmedetomidine exerts an anti-inflammatory effect via α2 adrenoceptors to prevent lipopolysaccharide-induced cognitive decline in mice. *Anesthesiology, 133*(2), 393-407. doi:http://dx.doi.org/10.1097/ALN.0000000000003390

Lian, Ø., Scott, A., Engebretsen, L., Bahr, R., Duronio, V., & Khan, K. (2007). Excessive apoptosis in patellar tendinopathy in athletes. *The American Journal of Sports Medicine, 35*(4), 605-611. doi:http://dx.doi.org/10.1177/0363546506295702

Liang, H., Liu, H.-Z., Wang, H.-B., Zhong, J.-Y., Yang, C.-X., & Zhang, B. (2017). Dexmedetomidine protects against cisplatin-induced acute kidney injury in mice through regulating apoptosis and inflammation. *Inflammation Research, 66*, 399-411. doi:http://dx.doi.org/10.1007/s00011-017-1023-9

Liang, L., Stone, R. C., Stojadinovic, O., Ramirez, H., Pastar, I., Maione, A. G., . . . Kirsner, R. S. (2016). Integrative analysis of miRNA and mRNA paired expression profiling of primary fibroblast derived from diabetic foot ulcers reveals multiple impaired cellular functions. *Wound Repair and Regeneration, 24*(6), 943-953. doi:http://dx.doi.org/10.1111/wrr.12470

Liao, Y., Ren, Y., Luo, X., Mirando, A. J., Long, J. T., Leinroth, A., . . . Hilton, M. J. (2022). Interleukin-6 signaling mediates cartilage degradation and pain in posttraumatic osteoarthritis in a sex-specific manner. *Science signaling, 15*(744), eabn7082. doi:http://dx.doi.org/10.1126/scisignal.abn7082

Liu, B., Barbosa-Sampaio, H., Jones, P. M., Persaud, S. J., & Muller, D. S. (2012). The CaMK4/CREB/IRS-2 cascade stimulates proliferation and inhibits apoptosis of β-cells. doi:http://dx.doi.org/10.1371/journal.pone.0045711

Liu, H., Zhang, C., Zhu, S., Lu, P., Zhu, T., Gong, X., . . . Heng, B. C. (2015). Mohawk promotes the tenogenesis of mesenchymal stem cells through activation of the TGFβ signaling pathway. *Stem cells, 33*(2), 443-455. doi:http://dx.doi.org/10.1002/stem.1866

Liu, Y., Petreaca, M., Yao, M., & Martins-Green, M. (2009). Cell and molecular mechanisms of keratinocyte function stimulated by insulin during wound healing. *BMC cell biology, 10*(1), 1-15. doi:http://dx.doi.org/10.1186/1471-2121-10-1

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *methods, 25*(4), 402-408. doi:http://dx.doi.org/10.1006/meth.2001.1262

Long, P., Gassner, R., & Agarwal, S. (2001). Tumor necrosis factor α–dependent proinflammatory gene induction is inhibited by cyclic tensile strain in articular chondrocytes in vitro. *Arthritis & Rheumatism, 44*(10), 2311-2319. doi:http://dx.doi.org/10.1002/1529-0131(200110)44:10<2311::AID-ART393>3.0.CO;2-Q

Loots, M. A., Lamme, E. N., Mekkes, J. R., Bos, J. D., & Middelkoop, E. (1999). Cultured fibroblasts from chronic diabetic wounds on the lower extremity (non-insulin-dependent diabetes mellitus) show disturbed proliferation. *Archives of dermatological research, 291*(2), 93-99. doi:http://dx.doi.org/10.1007/s004030050389

Lories, R. J., & McInnes, I. B. (2012). Primed for inflammation: enthesis-resident T cells. *Nature medicine, 18*(7), 1018-1019. doi:http://dx.doi.org/10.1038/nm.2854
Love, M. R., Palee, S., Chattipakorn, S. C., & Chattipakorn, N. (2018). Effects of electrical stimulation on cell proliferation and apoptosis. *Journal of cellular physiology, 233*(3), 1860-1876. doi:http://dx.doi.org/10.1002/jcp.25975
Luger, A., Schernthaner, G., URBANSKI, A., & Luger, T. (1988). Cytokine production in patients with newly diagnosed insulin-dependent (type I) diabetes mellitus. *European journal of clinical investigation, 18*(3), 233-236. doi:http://dx.doi.org/10.1111/j.1365-2362.1988.tb01251.x
Lui, P., Maffulli, N., Rolf, C., & Smith, R. (2011). What are the validated animal models for tendinopathy? *Scandinavian journal of medicine & science in sports, 21*(1), 3-17. doi:http://dx.doi.org/10.1111/j.1600-0838.2010.01164.x
Lundgreen, K., Lian, Ø., Scott, A., & Engebretsen, L. (2013). Increased levels of apoptosis and p53 in partial-thickness supraspinatus tendon tears. *Knee Surgery, Sports Traumatology, Arthroscopy, 21*, 1636-1641. doi:http://dx.doi.org/10.1007/s00167-012-2226-9
Lundgreen, K., Lian, Ø. B., Engebretsen, L., & Scott, A. (2011). Tenocyte apoptosis in the torn rotator cuff: a primary or secondary pathological event? *British journal of sports medicine, 45*(13), 1035-1039. doi:http://dx.doi.org/10.1136/bjsm.2010.083188
Luo, J., Wang, Z., Tang, C., Yin, Z., Huang, J., Ruan, D., . . . Li, J. (2023). Animal model for tendinopathy. *Journal of Orthopaedic Translation, 42*, 43-56. doi:http://dx.doi.org/10.1016/j.jot.2023.06.005
Maganaris, C. N., Narici, M. V., Almekinders, L. C., & Maffulli, N. (2004). Biomechanics and pathophysiology of overuse tendon injuries. *Sports Medicine, 34*(14), 1005-1017. doi:http://dx.doi.org/10.2165/00007256-200434140-00005
Malghem, J., Omoumi, P., Lecouvet, F., & Vande Berg, B. (2015). Intraosseous migration of tendinous calcifications: cortical erosions, subcortical migration and extensive intramedullary diffusion, a SIMS series. *Skeletal radiology, 44*, 1403-1412. doi:http://dx.doi.org/10.1007/s00256-015-2165-x
Martin, C., & Muir, I. (1990). The role of lymphocytes in wound healing. *British Journal of Plastic Surgery, 43*(6), 655-662. doi:http://dx.doi.org/10.1016/0007-1226(90)90185-3
Mason, G., Wilson, D., Hampton, C., & Würbel, H. (2004). Non-invasively assessing disturbance and stress in laboratory rats by scoring chromodacryorrhoea. *Alternatives to Laboratory Animals, 32*(1_suppl), 153-159. doi:http://dx.doi.org/10.1177/026119290403201s25
Matta, C., Zákány, R., & Mobasheri, A. (2015). Voltage-dependent calcium channels in chondrocytes: roles in health and disease. *Current Rheumatology Reports, 17*, 1-11. doi:http://dx.doi.org/10.1007/s11926-015-0521-4
Meloan, S. N., & Puchtler, H. (1985). Chemical mechanisms of staining methods: von Kossa's technique: what von Kossa really wrote and a modified reaction for selective demonstration of inorganic phosphates. *Journal of Histotechnology, 8*(1), 11-13. doi:http://dx.doi.org/10.1179/his.1985.8.1.11
Mendoza-Naranjo, A., Cormie, P., Serrano, A. E., Wang, C. M., Thrasivoulou, C., Sutcliffe, J. E., . . . Phillips, A. R. (2012). Overexpression of the gap junction protein Cx43 as found in diabetic foot ulcers can retard fibroblast migration.

*Cell biology international, 36*(7), 661-667. doi:http://dx.doi.org/10.1042/CBI20110628
Merriman, H. L., Hegyi, C. A., Albright-Overton, C. R., Carlos, J., Jr., Putnam, R. W., & Mulcare, J. A. (2004). A comparison of four electrical stimulation types on Staphylococcus aureus growth in vitro. *J Rehabil Res Dev, 41*(2), 139-146. doi:http://dx.doi.org/10.1682/JRRD.2004.02.0139
Michlovitz, S. L. (2005). Is there a role for ultrasound and electrical stimulation following injury to tendon and nerve? *Journal of Hand Therapy, 18*(2), 292-296. doi:http://dx.doi.org/10.1197/j.jht.2005.02.013
Mienaltowski, M. J., Adams, S. M., & Birk, D. E. (2013). Regional differences in stem cell/progenitor cell populations from the mouse achilles tendon. *Tissue Engineering Part A, 19*(1-2), 199-210. doi:http://dx.doi.org/10.1089/ten.TEA.2012.0182
Millar, N. L., Reilly, J. H., Kerr, S. C., Campbell, A. L., Little, K. J., Leach, W. J., . . . McInnes, I. B. (2012). Hypoxia: a critical regulator of early human tendinopathy. *Annals of the Rheumatic Diseases, 71*(2), 302-310. doi:http://dx.doi.org/10.1136/ard.2011.154229
Mohr, T., Akers, T. K., & Wessman, H. C. (1987). Effect of high voltage stimulation on blood flow in the rat hind limb. *Phys Ther, 67*(4), 526-533. doi:http://dx.doi.org/10.1093/ptj/67.4.526
Moraska, A., Deak, T., Spencer, R. L., Roth, D., & Fleshner, M. (2000). Treadmill running produces both positive and negative physiological adaptations in Sprague-Dawley rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 279*(4), R1321-R1329. doi:http://dx.doi.org/10.1152/ajpregu.2000.279.4.R1321
Moroni, M., Servin-Vences, M. R., Fleischer, R., Sánchez-Carranza, O., & Lewin, G. R. (2018). Voltage gating of mechanosensitive PIEZO channels. *Nature communications, 9*(1), 1096. doi:http://dx.doi.org/10.1038/s41467-018-03502-7
Morton, D. B., & Griffiths, P. (1985). Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Vet Rec, 116*(16), 431-436. doi:http://dx.doi.org/10.1136/vr.116.16.431
Mosca, M. J., Carr, A. J., Snelling, S. J., Wheway, K., Watkins, B., & Dakin, S. G. (2017). Differential expression of alarmins—S100A9, IL-33, HMGB1 and HIF-1α in supraspinatus tendinopathy before and after treatment. *BMJ Open Sport—Exercise Medicine, 3*(1).
Neckers, L. M., & Cossman, J. (1983). Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin 2. *Proceedings of the National Academy of Sciences, 80*(11), 3494-3498. doi:http://dx.doi.org/10.1073/pnas.80.11.3494
Nickien, M., Heuijerjans, A., Ito, K., & van Donkelaar, C. C. (2018). Comparison between in vitro and in vivo cartilage overloading studies based on a systematic literature review. *Journal of Orthopaedic Research®, 36*(8), 2076-2086. doi:http://dx.doi.org/10.1002/jor.23910
Nirschl, R. P., & Sobel, J. (1981). Conservative treatment of tennis elbow. *The Physician and sportsmedicine, 9*(6), 43-54. doi:http://dx.doi.org/10.1080/00913847.1981.11711096
Nishida, K., Yorimitsu, M., Komiyama, T., Kadota, Y., Tetsunaga, T., Yoshida, A., . . . Ozaki, T. (2008). Interleukin-4 downregulates the cyclic tensile stress-induced

matrix metalloproteinases-13 and cathepsin B expression by rat normal chondrocytes. *Acta Medica Okayama, 62*(2), 119-126. doi:http://dx.doi.org/10.18926/AMO/30956
Omar, M. T., El-Badawy, A. M., Borhan, W., & Nossier, A. (2004). Improvement of edema and hand function in superficial second degree hand burn using electrical stimulation. *Egypt J Plast Reconstr Surg, 28*(2), 141-146.
Onuma, E. K., & Hui, S.-W. (1988). Electric field-directed cell shape changes, displacement, and cytoskeletal reorganization are calcium dependent. *The Journal of cell biology, 106*(6), 2067-2075. doi:http://dx.doi.org/10.1083/jcb.106.6.2067
Ortiz, M. C. S., Villabona, E. H., Lemos, D. M. C., & Castellanos, R. (2014). Effects of low level laser therapy and high voltage stimulation on diabetic wound healing. *Revista de la Universidad Industrial de Santander Salud 2014 May-Aug;46(2):107-117*.
Ozawa, A., Tada, H., Tamai, R., Uehara, A., Watanabe, K., Yamaguchi, T., . . . Sugawara, S. (2003). Expression of IL-2 receptor β and $\gamma$ chains by human gingival fibroblasts and up-regulation of adhesion to neutrophils in response to IL-2. *Journal of leukocyte biology, 74*(3), 352-359. doi:http://dx.doi.org/10.1189/jlb.0103044
Pacifici, M., Golden, E. B., Oshima, O., Shapiro, I. M., Leboy, P. S., & Adams, S. L. (1990). Hypertrophic chondrocytes. The terminal stage of differentiation in the chondrogenic cell lineage? *Annals of the New York Academy of Sciences, 599*, 45-57. doi:http://dx.doi.org/10.1111/j.1749-6632.1990.tb42363.x
Palmoski, M. J., & Brandt, K. D. (1984). Effects of static and cyclic compressive loading on articular cartilage plugs in vitro. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology, 27*(6), 675-681. doi:http://dx.doi.org/10.1002/art.1780270611
Parkkinen, J. J., Lammi, M. J., Helminen, H. J., & Tammi, M. (1992). Local stimulation of proteoglycan synthesis in articular cartilage explants by dynamic compression in vitro. *Journal of orthopaedic research, 10*(5), 610-620. doi:http://dx.doi.org/10.1002/jor.1100100503
Parvizi, J., Parpura, V., Greenleaf, J. F., & Bolander, M. E. (2002). Calcium signaling is required for ultrasound-stimulated aggrecan synthesis by rat chondrocytes. *Journal of orthopaedic research, 20*(1), 51-57. doi:http://dx.doi.org/10.1016/S0736-0266(01)00069-9
Passini, F. S., Jaeger, P. K., Saab, A. S., Hanlon, S., Chittim, N. A., Arlt, M. J., . . . Bollhalder, M. (2021). Shear-stress sensing by PIEZO1 regulates tendon stiffness in rodents and influences jumping performance in humans. *Nature biomedical engineering, 5*(12), 1457-1471. doi:http://dx.doi.org/10.1038/s41551-021-00716-x
Pearce, C. J., Ismail, M., & Calder, J. D. (2009). Is apoptosis the cause of noninsertional achilles tendinopathy? *The American Journal of Sports Medicine, 37*(12), 2440-2444. doi:http://dx.doi.org/10.1177/0363546509340264
Peters, E. J., Lavery, L. A., Armstrong, D. G., & Fleischli, J. G. (2001). Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial. *Archives of physical medicine and rehabilitation, 82*(6), 721-725. doi:http://dx.doi.org/10.1053/apmr.2001.23780
Pierantoni, M., Hammerman, M., Barreto, I. S., Larsson, D., Notermans, T., Bodey, A. J., . . . Isaksson, H. (2023). Spatiotemporal and microstructural

characterization of heterotopic ossification in healing rat Achilles tendons. *The FASEB Journal, 37*(6). doi:http://dx.doi.org/10.1096/fj.202201018RRR

Plaisance, S., Alileche, A., Han, D., Rubinstein, E., Sahraoui, Y., Jasmin, C., & Azzarone, B. (1994). How interleukin-2 can affect human fibroblasts behaviour. *Pathology-Research and Practice, 190*(9-10), 942-949. doi:http://dx.doi.org/10.1016/S0344-0338(11)81000-9

Plaisance, S., Rubinstein, E., Allleche, A., Sahraoui, Y., Krief, P., Augery-Bourget, Y., . . . Azzarone, B. (1992). Expression of the interleukin-2 receptor on human fibroblasts and its biological significance. *International immunology, 4*(7), 739-746. doi:http://dx.doi.org/10.1093/intimm/4.7.739

Polak, A., Kloth, L. C., Blaszczak, E., Taradaj, J., Nawrat-Szoltysik, A., Ickowicz, T., . . . Kucio, C. (2017). The Efficacy of Pressure Ulcer Treatment With Cathodal and Cathodal-Anodal High-Voltage Monophasic Pulsed Current: A Prospective, Randomized, Controlled Clinical Trial. *Phys Ther, 97*(8), 777-789. doi:http://dx.doi.org/10.1093/ptj/pzx052

Polak, A., Kloth, L. C., Blaszczak, E., Taradaj, J., Nawrat-Szoltysik, A., Walczak, A., . . . Kucio, C. (2016). Evaluation of the Healing Progress of Pressure Ulcers Treated with Cathodal High-Voltage Monophasic Pulsed Current: Results of a Prospective, Double-blind, Randomized Clinical Trial. 2016b. *Adv Skin Wound Care, 29*(10), 447-459. doi:http://dx.doi.org/10.1097/01.ASW.0000493164.75337.de

Polak, A., Kucio, C., Kloth, L. C., Paczula, M., Hordynska, E., Ickowicz, T., . . . Franek, A. (2018). A Randomized, Controlled Clinical Study to Assess the Effect of Anodal and Cathodal Electrical Stimulation on Periwound Skin Blood Flow and Pressure Ulcer Size Reduction in Persons with Neurological Injuries. *Ostomy Wound Manage, 64*(2), 10-29. doi:http://dx.doi.org/10.25270/owm.2018.2.1029

Polak, A., Taradaj, J., Nawrat-Szoltysik, A., Stania, M., Dolibog, P., Blaszczak, E., . . . Kucio, C. (2016). Reduction of pressure ulcer size with high-voltage pulsed current and high-frequency ultrasound: a randomised trial. 2016a. *J Wound Care, 25*(12), 742-754. doi:http://dx.doi.org/10.12968/jowc.2016.25.12.742

Puxkandl, R., Zizak, I., Paris, O., Keckes, J., Tesch, W., Bernstorff, S., . . . Fratzl, P. (2002). Viscoelastic properties of collagen: synchrotron radiation investigations and structural model. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 357*(1418), 191-197. doi:http://dx.doi.org/10.1098/rstb.2001.1033

Radpasand, M. (2009). Combination of manipulation, exercise, and physical therapy for the treatment of a 57-year-old woman with lateral epicondylitis. *Journal of manipulative and physiological therapeutics, 32*(2), 166-172. doi:http://dx.doi.org/10.1016/j.jmpt.2008.12.007

Radpasand, M., & Owens, E. (2009). Combined multimodal therapies for chronic tennis elbow: pilot study to test protocols for a randomized clinical trial. *Journal of manipulative and physiological therapeutics, 32*(7), 571-585. doi:http://dx.doi.org/10.1016/j.jmpt.2009.08.010

Ralphs, J., Benjamin, M., Waggett, A., Russell, D., Messner, K., & Gao, J. (1998). Regional differences in cell shape and gap junction expression in rat Achilles tendon: relation to fibrocartilage differentiation. *The Journal of Anatomy, 193*(2), 215-222. doi:http://dx.doi.org/10.1046/j.1469-7580.1998.19320215.x

Rampazo, É. P., Liebano, R. E., Pinfildi, C. E., Folha, R. A., & Ferreira, L. M. (2016). High voltage pulsed current in collagen realignment, synthesis, and

angiogenesis after Achilles tendon partial rupture. *Brazilian Journal of Physical Therapy, 20*, 312-319. doi:http://dx.doi.org/10.1590/bjpt-rbf.2014.0167
Rechler, M. M., & Podskalny, J. M. (1976). Insulin receptors in cultured human fibroblasts. *Diabetes, 25*(4), 250-255. doi:http://dx.doi.org/10.2337/diab.25.4.250
Recio, A. C., Felter, C. E., Schneider, A. C., & McDonald, J. W. (2012). High-voltage electrical stimulation for the management of Stage III and IV pressure ulcers among adults with spinal cord injury: Demonstration of its utility for recalcitrant wounds below the level of injury. *The journal of spinal cord medicine, 35*(1), 58-63. doi:http://dx.doi.org/10.1179/2045772311Y.0000000044
Reem, G. H., & Yeh, N.-H. (1984). Interleukin 2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. *Science, 225*(4660), 429-430. doi:http://dx.doi.org/10.1126/science.6429853
Roach, H., Aigner, T., & Kouri, J. (2004). Chondroptosis: a variant of apoptotic cell death in chondrocytes? *Apoptosis, 9*, 265-277. doi:http://dx.doi.org/10.1023/B:APPT.0000025803.17498.26
Robinson, P. S., Lin, T. W., Reynolds, P. R., Derwin, K. A., Iozzo, R. V., & Soslowsky, L. J. (2004). Strain-rate sensitive mechanical properties of tendon fascicles from mice with genetically engineered alterations in collagen and decorin. *J. Biomech. Eng., 126*(2), 252-257. doi:http://dx.doi.org/10.1115/1.1695570
Rocha-Zavaleta, L., Huitron, C., Cacéres-Cortés, J. R., Alvarado-Moreno, J. A., Valle-Mendiola, A., Soto-Cruz, I., . . . Rangel-Corona, R. (2004). Interleukin-2 (IL-2) receptor-βγ signalling is activated by c-Kit in the absence of IL-2, or by exogenous IL-2 via JAK3/STAT5 in human papillomavirus-associated cervical cancer. *Cellular signalling, 16*(11), 1239-1247. doi:http://dx.doi.org/10.1016/j.cellsig.2004.03.011
Rocio Servin-Vences, M., Moroni, M., Lewin, G. R., & Poole, K. (2017). Direct measurement of TRPV4 and PIEZO1 activity reveals multiple mechanotransduction pathways in chondrocytes. *Elife, 6*, e21074. doi:http://dx.doi.org/10.7554/eLife.21074
Roderick, H. L., & Cook, S. J. (2008). Ca 2+ signalling checkpoints in cancer: remodelling Ca 2+ for cancer cell proliferation and survival. *Nature Reviews Cancer, 8*(5), 361-375. doi:http://dx.doi.org/10.1038/nrc2374
Roffino, S., Camy, C., Foucault-Bertaud, A., Lamy, E., Pithioux, M., & Chopard, A. (2021). Negative impact of disuse and unloading on tendon enthesis structure and function. *Life Sciences in Space Research, 29*, 46-52. doi:http://dx.doi.org/10.1016/j.lssr.2021.03.001
Roncarolo, M., Zoppo, M., Bacchetta, R., Gabiano, C., Sacchetti, C., Cerutti, F., & Tovo, P. A. (1988). Interleukin-2 production and interleukin-2 receptor expression in children with newly diagnosed diabetes. *Clinical immunology and immunopathology, 49*(1), 53-62. doi:http://dx.doi.org/10.1016/0090-1229(88)90094-3
Ross, S. H., & Cantrell, D. A. (2018). Signaling and function of interleukin-2 in T lymphocytes. *Annual review of immunology, 36*, 411-433. doi:http://dx.doi.org/10.1146/annurev-immunol-042617-053352
Rowe, D. W., Starman, B. J., Fujimoto, W. Y., & Williams, R. H. (1977). Abnormalities in proliferation and protein synthesis in skin fibroblast cultures from patients with diabetes mellitus. *Diabetes, 26*(4), 284-290. doi:http://dx.doi.org/10.2337/diab.26.4.284

Rufai, A., Benjamin, M., & Ralphs, J. (1992). Development and ageing of phenotypically distinct fibrocartilages associated with the rat Achilles tendon. *Anatomy and embryology, 186*, 611-618. doi:http://dx.doi.org/10.1007/BF00186984
Rufai, A., Ralphs, J., & Benjamin, M. (1995). Structure and histopathology of the insertional region of the human Achilles tendon. *Journal of orthopaedic research, 13*(4), 585-593. doi:http://dx.doi.org/10.1002/jor.1100130414
Rufai, A., Ralphs, J., & Benjamin, M. (1996). Ultrastructure of fibrocartilages at the insertion of the rat Achilles tendon. *Journal of anatomy, 189*(Pt 1), 185.
Sah, R. L., Doong, J.-Y. H., Grodzinsky, A. J., Plaas, A. H., & Sandy, J. D. (1991). Effects of compression on the loss of newly synthesized proteoglycans and proteins from cartilage explants. *Archives of biochemistry and biophysics, 286*(1), 20-29. doi:http://dx.doi.org/10.1016/0003-9861(91)90004-3
Saito, T., Nakamichi, R., Yoshida, A., Hiranaka, T., Okazaki, Y., Nezu, S., . . . Nishida, K. (2022). The effect of mechanical stress on enthesis homeostasis in a rat Achilles enthesis organ culture model. *Journal of Orthopaedic Research®, 40*(8), 1872-1882. doi:http://dx.doi.org/10.1002/jor.25210
Sakabe, F. F., Sakabe, D. I., Marracini Fernandes, F. R., & Reges, G. (2018). Estimulação pulsada de alta voltagem reduz a área de úlceras de diferentes etiologias: um estudo piloto. *Fisioterapia Brasil, 19*(1). doi:http://dx.doi.org/10.33233/fb.v19i1.2124
Saltiel, A. R., & Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature, 414*(6865), 799-806. doi:http://dx.doi.org/10.1038/414799a
Santana de Souza, A. C., Lomasso Costa, M. M., Vieira Costa, P. H., & Souza da Silva, F. (2017). Efeito da estimulação elétrica de alta voltagem para o tratamento de úlceras por pressão: um estudo experimental de caso único. *Fisioterapia Brasil, 18*(6). doi:http://dx.doi.org/10.33233/fb.v18i6.2046
Schäffer, M., & Barbul, A. (1998). Lymphocyte function in wound healing and following injury. *Journal of British Surgery, 85*(4), 444-460. doi:http://dx.doi.org/10.1046/j.1365-2168.1998.00734.x
Schett, G., Lories, R. J., D'Agostino, M.-A., Elewaut, D., Kirkham, B., Soriano, E. R., & McGonagle, D. (2017). Enthesitis: from pathophysiology to treatment. *Nature Reviews Rheumatology, 13*(12), 731-741. doi:http://dx.doi.org/10.1038/nrrheum.2017.188
Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., . . . Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature methods, 9*(7), 676-682. doi:http://dx.doi.org/10.1038/nmeth.2019
Schneiderman, R., Keret, D., & Maroudas, A. (1986). Effects of mechanical and osmotic pressure on the rate of glycosaminoglycan synthesis in the human adult femoral head cartilage: an in vitro study. *Journal of orthopaedic research, 4*(4), 393-408. doi:http://dx.doi.org/10.1002/jor.1100040402
Schüppel, M., Kürschner, U., Kleuser, U., Schäfer-Korting, M., & Kleuser, B. (2008). Sphingosine 1-phosphate restrains insulin-mediated keratinocyte proliferation via inhibition of Akt through the S1P2 receptor subtype. *Journal of Investigative Dermatology, 128*(7), 1747-1756. doi:http://dx.doi.org/10.1038/sj.jid.5701259
Scott, A., Cook, J. L., Hart, D. A., Walker, D. C., Duronio, V., & Khan, K. M. (2007). Tenocyte responses to mechanical loading in vivo: a role for local insulin-like

growth factor 1 signaling in early tendinosis in rats. *Arthritis & Rheumatism, 56*(3), 871-881. doi:http://dx.doi.org/10.1002/art.22426
Scott, A., Khan, K., Heer, J., Cook, J., Lian, O., & Duronio, V. (2005). High strain mechanical loading rapidly induces tendon apoptosis: an ex vivo rat tibialis anterior model. *British journal of sports medicine, 39*(5), e25-e25. doi:http://dx.doi.org/10.1136/bjsm.2004.015164
Scott, A., Squier, K., Alfredson, H., Bahr, R., Cook, J. L., Coombes, B., . . . Lewis, J. S. (2020). Icon 2019: international scientific tendinopathy symposium consensus: clinical terminology. *British journal of sports medicine, 54*(5), 260-262. doi:http://dx.doi.org/10.1136/bjsports-2019-100885
Selvanetti, A., Cipolla, M., & Puddu, G. (1997). Overuse tendon injuries: basic science and classification. *Operative Techniques in Sports Medicine, 5*(3), 110-117. doi:http://dx.doi.org/10.1016/S1060-1872(97)80031-7
Sharma, P., & Maffulli, N. (2005). Tendon injury and tendinopathy: healing and repair. *JBJS, 87*(1), 187-202. doi:http://dx.doi.org/10.2106/JBJS.D.01850
Sharma, P., & Maffulli, N. (2006). Biology of tendon injury: healing, modeling and remodeling. *Journal of Musculoskeletal and Neuronal Interactions, 6*(2), 181.
Shen, G. (2005). The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage. *Orthodontics & craniofacial research, 8*(1), 11-17. doi:http://dx.doi.org/10.1111/j.1601-6343.2004.00308.x
Shen, S., Alt, A., Wertheimer, E., Gartsbein, M., Kuroki, T., Ohba, M., . . . Tennenbaum, T. (2001). PKCδ Activation: A Divergence Point in the Signaling of Insulin and IGF-1—Induced Proliferation of Skin Keratinocytes. *Diabetes, 50*(2), 255-264. doi:http://dx.doi.org/10.2337/diabetes.50.2.255
Sherlock, J. P., Joyce-Shaikh, B., Turner, S. P., Chao, C.-C., Sathe, M., Grein, J., . . . Yearley, J. H. (2012). IL-23 induces spondyloarthropathy by acting on ROR-γt+ CD3+ CD4− CD8− entheseal resident T cells. *Nature medicine, 18*(7), 1069-1076. doi:http://dx.doi.org/10.1038/nm.2817
Shimizu, A., Watanabe, S., Iimoto, S., & Yamamoto, H. (2004). Interleukin-4 protects matrix synthesis in chondrocytes under excessive mechanical stress in vitro. *Modern Rheumatology, 14*(4), 296-300. doi:http://dx.doi.org/10.3109/s10165-004-0312-7
Silva Ede, F., Martins, C. C., Guirro, E. C., & Guirro, R. R. (2010). High voltage electrical stimulation as an alternative treatment for chronic ulcers of the lower limbs. *An Bras Dermatol, 85*(4), 567-569. doi:http://dx.doi.org/10.1590/s0365-05962010000400025
Soleimanpour, S. A., Crutchlow, M. F., Ferrari, A. M., Raum, J. C., Groff, D. N., Rankin, M. M., . . . Kushner, J. A. (2010). Calcineurin signaling regulates human islet β-cell survival. *Journal of Biological Chemistry, 285*(51), 40050-40059. doi:http://dx.doi.org/10.1074/jbc.M110.154955
Soslowsky, L., Thomopoulos, S., Tun, S., Flanagan, C., Keefer, C., Mastaw, J., & Carpenter, J. n. (2000). Neer Award 1999: Overuse activity injures the supraspinatus tendon in an animal model: A histologic and biomechanical study. *Journal of shoulder and elbow surgery, 9*(2), 79-84. doi:http://dx.doi.org/10.1067/mse.2000.101962
Spravchikov, N., Sizyakov, G., Gartsbein, M., Accili, D., Tennenbaum, T., & Wertheimer, E. (2001). Glucose effects on skin keratinocytes: implications for diabetes skin complications. *Diabetes, 50*(7), 1627-1635. doi:http://dx.doi.org/10.2337/diabetes.50.7.1627

Stauber, T., Blache, U., & Snedeker, J. G. (2020). Tendon tissue microdamage and the limits of intrinsic repair. *Matrix Biology, 85*, 68-79. doi:http://dx.doi.org/10.1016/j.matbio.2019.07.008
Steinmeyer, J., & Knue, S. (1997). The proteoglycan metabolism of mature bovine articular cartilage explants superimposed to continuously applied cyclic mechanical loading. *Biochemical and biophysical research communications, 240*(1), 216-221. doi:http://dx.doi.org/10.1006/bbrc.1997.7641
Stirling, D. R., Swain-Bowden, M. J., Lucas, A. M., Carpenter, A. E., Cimini, B. A., & Goodman, A. (2021). CellProfiler 4: improvements in speed, utility and usability. *BMC bioinformatics, 22*, 1-11. doi:http://dx.doi.org/10.1186/s12859-021-04344-9
Stojadinovic, O., Brem, H., Vouthounis, C., Lee, B., Fallon, J., Stallcup, M., . . . Tomic-Canic, M. (2005). Molecular pathogenesis of chronic wounds: the role of β-catenin and c-myc in the inhibition of epithelialization and wound healing. *The American journal of pathology, 167*(1), 59-69. doi:http://dx.doi.org/10.1016/S0002-9440(10)62953-7
Sudoł-Szopińska, I., Kwiatkowska, B., Prochorec-Sobieszek, M., & Maśliński, W. (2015). Enthesopathies and enthesitis. Part 1. Etiopathogenesis. *Journal of ultrasonography, 15*(60), 72-84. doi:http://dx.doi.org/10.15557/JoU.2015.0006
Sun, P., Enslen, H., Myung, P. S., & Maurer, R. A. (1994). Differential activation of CREB by Ca2+/calmodulin-dependent protein kinases type II and type IV involves phosphorylation of a site that negatively regulates activity. *Genes & development, 8*(21), 2527-2539. doi:http://dx.doi.org/10.1101/gad.8.21.2527
Szuminsky, N. J., Albers, A. C., Unger, P., & Eddy, J. G. (1994). Effect of narrow, pulsed high voltages on bacterial viability. *Phys Ther, 74*(7), 660-667. doi:http://dx.doi.org/10.1093/ptj/74.7.660
Templeton, G. F. (2011). A two-step approach for transforming continuous variables to normal: implications and recommendations for IS research. *Communications of the association for information systems, 28*(1), 4. doi:http://dx.doi.org/10.17705/1CAIS.02804
Thomas, R. S., Clarke, A. R., Duance, V. C., & Blain, E. J. (2011). Effects of Wnt3A and mechanical load on cartilage chondrocyte homeostasis. *Arthritis Research & Therapy, 13*(6), 1-10. doi:http://dx.doi.org/10.1186/ar3536
Thompson, G. L., Roth, C. C., Dalzell, D. R., Kuipers, M. A., & Ibey, B. L. (2014). Calcium influx affects intracellular transport and membrane repair following nanosecond pulsed electric field exposure. *Journal of Biomedical Optics, 19*(5), 055005. doi:http://dx.doi.org/10.1117/1.JBO.19.5.055005
Thouverey, C., Malinowska, A., Balcerzak, M., Strzelecka-Kiliszek, A., Buchet, R., Dadlez, M., & Pikula, S. (2011). Proteomic characterization of biogenesis and functions of matrix vesicles released from mineralizing human osteoblast-like cells. *Journal of proteomics, 74*(7), 1123-1134. doi:http://dx.doi.org/10.1016/j.jprot.2011.04.005
Titushkin, I., & Cho, M. (2009). Regulation of cell cytoskeleton and membrane mechanics by electric field: role of linker proteins. *Biophysical journal, 96*(2), 717-728. doi:http://dx.doi.org/10.1016/j.bpj.2008.09.035
Tomoda, T., Kurashige, T., & Taniguchi, T. (1994). Imbalance of the interleukin 2 system in children with IDDM. *Diabetologia, 37*(5), 476-482. doi:http://dx.doi.org/10.1007/s001250050135
Tuoheti, Y., Itoi, E., Pradhan, R. L., Wakabayashi, I., Takahashi, S., Minagawa, H., . . . Shimada, Y. (2005). Apoptosis in the supraspinatus tendon with stage II

subacromial impingement. *Journal of shoulder and elbow surgery, 14*(5), 535-541. doi:http://dx.doi.org/10.1016/j.jse.2005.01.001
Uhthoff, H. K., & Loehr, J. W. (1997). Calcific tendinopathy of the rotator cuff: pathogenesis, diagnosis, and management. *JAAOS-Journal of the American Academy of Orthopaedic Surgeons, 5*(4), 183-191. doi:http://dx.doi.org/10.5435/00124635-199707000-00001
Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic acids research, 40*(15), e115-e115. doi:http://dx.doi.org/10.1093/nar/gks596
Usui, M. L., Mansbridge, J. N., Carter, W. G., Fujita, M., & Olerud, J. E. (2008). Keratinocyte migration, proliferation, and differentiation in chronic ulcers from patients with diabetes and normal wounds. *Journal of Histochemistry & Cytochemistry, 56*(7), 687-696. doi:http://dx.doi.org/10.1369/jhc.2008.951194
Van der Kraan, P., Davidson, E. B., Blom, A., & Van den Berg, W. (2009). TGF-beta signaling in chondrocyte terminal differentiation and osteoarthritis: modulation and integration of signaling pathways through receptor-Smads. *Osteoarthritis and cartilage, 17*(12), 1539-1545. doi:http://dx.doi.org/10.1016/j.joca.2009.06.008
Van Der Vlist, A. C., Winters, M., Weir, A., Ardern, C. L., Welton, N. J., Caldwell, D. M., . . . De Vos, R.-J. (2020). Which treatment is most effective for patients with Achilles tendinopathy? A living systematic review with network meta-analysis of 29 randomised controlled trials. *British journal of sports medicine*. doi:http://dx.doi.org/10.1136/bjsports-2019-101872
van Haaften, E. E., Ito, K., & van Donkelaar, C. C. (2017). The initial repair response of articular cartilage after mechanically induced damage. *Journal of orthopaedic research, 35*(6), 1265-1273. doi:http://dx.doi.org/10.1002/jor.23382
Verrando, P., & Ortonne, J.-P. (1985). Insulin binding properties of normal and transformed human epidermal cultured keratinocytes. *Journal of Investigative Dermatology, 85*(4), 328-332. doi:http://dx.doi.org/10.1111/1523-1747.ep12276922
Vicenzino, B., de Vos, R.-J., Alfredson, H., Bahr, R., Cook, J. L., Coombes, B. K., . . . Lewis, J. S. (2020). ICON 2019—International Scientific Tendinopathy Symposium Consensus: There are nine core health-related domains for tendinopathy (CORE DOMAINS): Delphi study of healthcare professionals and patients. *British journal of sports medicine, 54*(8), 444-451. doi:http://dx.doi.org/10.1136/bjsports-2019-100894
Waggett, A. D., Ralphs, J. R., Kwan, A. P., Woodnutt, D., & Benjamin, M. (1998). Characterization of collagens and proteoglycans at the insertion of the human Achilles tendon. *Matrix Biology, 16*(8), 457-470. doi:http://dx.doi.org/10.1016/S0945-053X(98)90017-8
Wang, A., Toma, M. A., Ma, J., Li, D., Vij, M., Chu, T., . . . Xu Landén, N. (2020). Circular RNA hsa_circ_0084443 is upregulated in diabetic foot ulcer and modulates keratinocyte migration and proliferation. *Advances in Wound Care, 9*(4), 145-160. doi:http://dx.doi.org/10.1089/wound.2019.0956
Wang, H., Lindborg, C., Lounev, V., Kim, J. H., McCarrick-Walmsley, R., Xu, M., . . . Schipani, E. (2016). Cellular hypoxia promotes heterotopic ossification by amplifying BMP signaling. *Journal of Bone and Mineral Research, 31*(9), 1652-1665. doi:http://dx.doi.org/10.1002/jbmr.2848

Wang, K., Wu, M., Xu, J., Wu, C., Zhang, B., Wang, G., & Ma, D. (2019). Effects of dexmedetomidine on perioperative stress, inflammation, and immune function: systematic review and meta-analysis. *British Journal of Anaesthesia, 123*(6), 777-794. doi:http://dx.doi.org/10.1016/j.bja.2019.07.027

Wang, T., Chen, P., Chen, L., Zhou, Y., Wang, A., Zheng, Q., . . . Zheng, M. H. (2021). Reduction of mechanical loading in tendons induces heterotopic ossification and activation of the β-catenin signaling pathway. *Journal of Orthopaedic Translation, 29*, 42-50. doi:http://dx.doi.org/10.1016/j.jot.2021.03.004

Wang, T., Chen, P., Zheng, M., Wang, A., Lloyd, D., Leys, T., . . . Zheng, M. H. (2018). In vitro loading models for tendon mechanobiology. *Journal of Orthopaedic Research®, 36*(2), 566-575. doi:http://dx.doi.org/10.1002/jor.23752

Wang, W., Xu, J., & Kirsch, T. (2003). Annexin-mediated Ca2+ influx regulates growth plate chondrocyte maturation and apoptosis. *Journal of Biological Chemistry, 278*(6), 3762-3769. doi:http://dx.doi.org/10.1074/jbc.M208868200

Wang, X.-F., Li, M.-L., Fang, Q.-Q., Zhao, W.-Y., Lou, D., Hu, Y.-Y., . . . Tan, W.-Q. (2021). Flexible electrical stimulation device with Chitosan-Vaseline® dressing accelerates wound healing in diabetes. *BIOACTIVE MATERIALS, 6*(1), 230-243. doi:http://dx.doi.org/10.1016/j.bioactmat.2020.08.003

Wang, X., Li, F., Xie, L., Crane, J., Zhen, G., Mishina, Y., . . . Liu, S. (2018). Inhibition of overactive TGF-β attenuates progression of heterotopic ossification in mice. *Nature communications, 9*(1), 551. doi:http://dx.doi.org/10.1038/s41467-018-02988-5

Warden, S. J. (2007). Animal models for the study of tendinopathy. *British journal of sports medicine, 41*(4), 232-240. doi:http://dx.doi.org/10.1136/bjsm.2006.032342

White, J. A., Blackmore, P. F., Schoenbach, K. H., & Beebe, S. J. (2004). Stimulation of capacitative calcium entry in HL-60 cells by nanosecond pulsed electric fields. *Journal of Biological Chemistry, 279*(22), 22964-22972. doi:http://dx.doi.org/10.1074/jbc.M311135200

Wildemann, B., Schmidmaier, G., Ordel, S., Stange, R., Haas, N., & Raschke, M. (2003). Cell proliferation and differentiation during fracture healing are influenced by locally applied IGF-I and TGF-β1: Comparison of two proliferation markers, PCNA and BrdU. *Journal of Biomedical Materials Research Part B: Applied Biomaterials: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 65*(1), 150-156. doi:http://dx.doi.org/10.1002/jbm.b.10512

Wong, Y.-C., & Buck, R. C. (1972). The development of sites of metaplastic change in regenerating tendon. *Zeitschrift fuer Zellforschung und Mikroskopische Anatomie, 134*, 175-181. doi:http://dx.doi.org/10.1007/BF00307151

Wu, B., Chen, J., Rosa, T. D., Yu, Q., Wang, A., Xu, J., & Zheng, M.-H. (2011). Cellular response and extracellular matrix breakdown in rotator cuff tendon rupture. *Archives of Orthopaedic and Trauma Surgery, 131*, 405-411. doi:http://dx.doi.org/10.1007/s00402-010-1157-5

Wu, M., Rubin, A. E., Dai, T., Schloss, R., Usta, O. B., Golberg, A., & Yarmush, M. (2021). High-Voltage, Pulsed Electric Fields Eliminate Pseudomonas aeruginosa Stable Infection in a Mouse Burn Model. *Advances in Wound Care, 10*(9), 477-489. doi:http://dx.doi.org/10.1089/wound.2019.1147

Wuthier, R. E. (1993). Involvement of cellular metabolism of calcium and phosphate in calcification of avian growth plate cartilage. *The Journal of nutrition, 123*, 301-309. doi:http://dx.doi.org/10.1093/jn/123.suppl_2.301
Xiang, H., Hu, B., Li, Z., & Li, J. (2014). Dexmedetomidine controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. *Inflammation, 37*, 1763-1770. doi:http://dx.doi.org/10.1007/s10753-014-9906-1
Xie, F., Wang, J., & Zhang, B. (2023). RefFinder: a web-based tool for comprehensively analyzing and identifying reference genes. *Functional & Integrative Genomics, 23*(2), 1-5. doi:http://dx.doi.org/10.1007/s10142-023-01055-7
Xu, Z., Buckley, M. J., Evans, C. H., & Agarwal, S. (2000). Cyclic tensile strain acts as an antagonist of IL-1β actions in chondrocytes. *The Journal of Immunology, 165*(1), 453-460. doi:http://dx.doi.org/10.4049/jimmunol.165.1.453
Xuan, Y. H., Huang, B. B., Tian, H. S., Chi, L. S., Duan, Y. M., Wang, X., . . . Wei, T. M. (2014). High-glucose inhibits human fibroblast cell migration in wound healing via repression of bFGF-regulating JNK phosphorylation. *PLoS One, 9*(9), e108182. doi:http://dx.doi.org/10.1371/journal.pone.0108182
Yagi, M., Endo, K., Komori, K., & Sekiya, I. (2023). Comparison of the effects of oxidative and inflammatory stresses on rat chondrocyte senescence. *Scientific Reports, 13*(1), 7697. doi:http://dx.doi.org/10.1038/s41598-023-34825-1
Yang, G., Zhu, L., Hou, N., Lan, Y., Wu, X.-M., Zhou, B., . . . Yang, X. (2014). Osteogenic fate of hypertrophic chondrocytes. *Cell research, 24*(10), 1266-1269. doi:http://dx.doi.org/10.1038/cr.2014.111
Yang, L., Tsang, K. Y., Tang, H. C., Chan, D., & Cheah, K. S. (2014). Hypertrophic chondrocytes can become osteoblasts and osteocytes in endochondral bone formation. *Proceedings of the National Academy of Sciences, 111*(33), 12097-12102. doi:http://dx.doi.org/10.1073/pnas.1302703111
Yasumura, S., Lin, W. C., Weidmann, E., Hebda, P., & Whiteside, T. L. (1994). Expression of interleukin 2 receptors on human carcinoma cell lines and tumor growth inhibition by interleukin 2. *International journal of cancer, 59*(2), 225-234. doi:http://dx.doi.org/10.1002/ijc.2910590215
Yuan, J., Murrell, G. A., Wei, A. Q., & Wang, M. X. (2002). Apoptosis in rotator cuff tendonopathy. *Journal of orthopaedic research, 20*(6), 1372-1379. doi:http://dx.doi.org/10.1016/S0736-0266(02)00075-X
Zhang, J., Blackmore, P. F., Hargrave, B. Y., Xiao, S., Beebe, S. J., & Schoenbach, K. H. (2008). Nanosecond pulse electric field (nanopulse): a novel non-ligand agonist for platelet activation. *Archives of biochemistry and biophysics, 471*(2), 240-248. doi:http://dx.doi.org/10.1016/j.abb.2007.12.009
Zhang, J., & Wang, J. H. (2013). The effects of mechanical loading on tendons-an in vivo and in vitro model study. *PLoS One, 8*(8), e71740. doi:http://dx.doi.org/10.1371/journal.pone.0071740
Zhang, T., Wan, L., Xiao, H., Wang, L., Hu, J., & Lu, H. (2023). Single-cell RNA sequencing reveals cellular and molecular heterogeneity in fibrocartilaginous enthesis formation. *Elife, 12*, e85873. doi:http://dx.doi.org/10.7554/eLife.85873
Zheng, R.-s., Yang, Z.-l., Du Yan-xiao, Y. C.-y., Jia, P.-p., & Yuan, X. (2012). Effect of tensile stress on type Ⅱ collagen and aggrecan expression in rat condylar chondrocytes*★. *Chinese Journal of Tissue Engineering Research, 16*(20), 3649. doi:http://dx.doi.org/10.3969/j.issn.1673-8225.2012.20.009
Zhou, K., Krug, K., Stachura, J., Niewczyk, P., Ross, M., Tutuska, J., & Ford, G. (2016). Silver-Collagen Dressing and High-voltage, Pulsed-current Therapy for

the Treatment of Chronic Full-thickness Wounds: A Case Series. 2016b. *Ostomy Wound Manage, 62*(3), 36-44.
Zhou, K., Schenk, R., & Brogan, M. S. (2016). The wound healing trajectory and predictors with combined electric stimulation and conventional care: one outpatient wound care clinic's experience. 2016a. *Eur J Clin Invest, 46*(12), 1017-1023. doi:http://dx.doi.org/10.1111/eci.12685
Zier, K. S., Leo, M. M., Spielman, R. S., & Baker, L. (1984). Decreased synthesis of interleukin-2 (IL-2) in insulin-dependent diabetes mellitus. *Diabetes, 33*(6), 552-555. doi:http://dx.doi.org/10.2337/diab.33.6.552

# Supplementary information

**Overuse-induced tendon injury: an animal model**

**Ground substance**

***Tendon middle third***

Statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and EP groups in the middle-third of tendons; difference between medians= -3.470; Hodges-Lehmann= -2.460; SC= 5.930, n= 47; EP= 2.460, n= 59; U= 830; P= 0.0003. Moreover, a reduction in Alcian-blue density (two-class Otsu) was found between SC and 2R groups: difference between medians= -2.170; Hodges-Lehmann= -1.570; SC= 5.930, n= 47; 2R= 3.760, n= 64; U= 1172; P= 0.0474. Non-statistically significant difference in Alcian-blue density (two-class Otsu) between SC and 4R groups was found; difference between medians= -1.060; Hodges-Lehmann= -1.040; SC= 5.930, n= 47; 4R= 4.870, n= 67; U= 1348; P= 0.1936 (Figure 9).

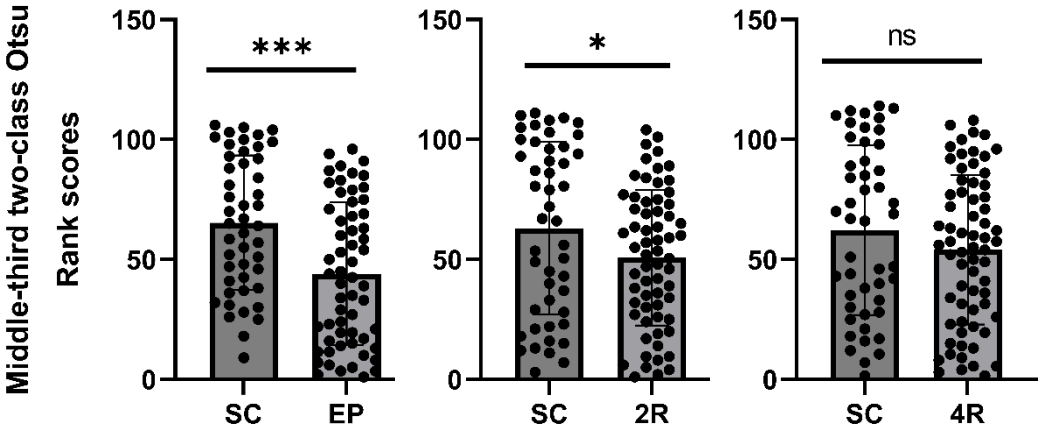



Figure 9. Rank score in middle-third section for the assessment of ground substance content between sedentary control and experimental groups at the three timepoints, measured using two-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

Three-class Otsu Alcian-blue density analysis showed statistically significant differences between SC and EP groups; difference between medians= 1.460; Hodges-Lehmann= 0.9400; SC= 2.190, n= 47; EP= 0.7300, n= 59; U= 901.5; P= 0.0018. Comparing SC with 2R and 4R groups demonstrated non-statistically significant differences; SC vs 2R groups; difference between medians= 0.7300; Hodges-Lehmann= 0.6100; SC= 2.190, n= 47; 2R= 1.460, n= 64; U= 1213; P= 0.0827. SC vs 4R groups results were; difference between medians= 0.6200; Hodges-Lehmann= 0.4500; SC= 2.190, n= 47; 4R= 1.570, n= 67; U= 1332; P= 0.1638 (Figure 10).

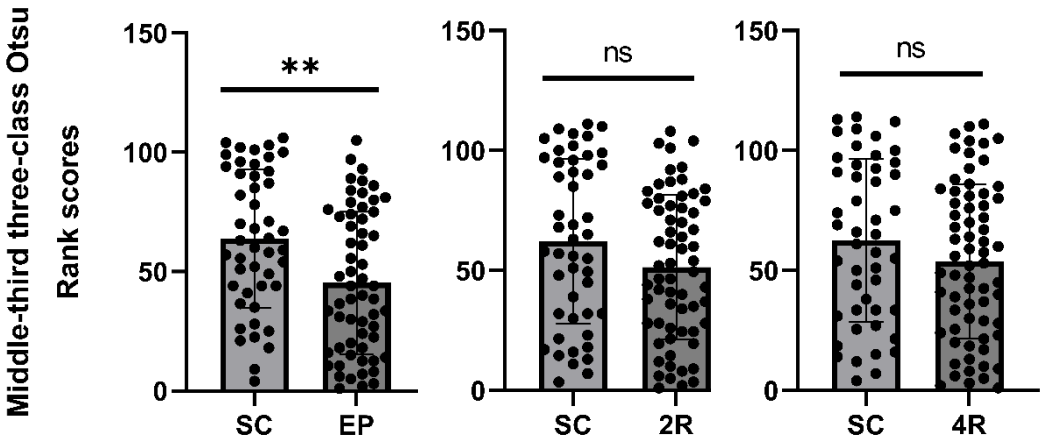



Figure 10. Rank score in middle-third ground substance content between sedentary control and experimental groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.005. Y-axis represents pixel density.

A statistically significant difference was found between EP and 4R groups in tendon middle-third using two-class Otsu but not three-class Otsu; H= 8.479, df= 2, P= 0.0144; mean rank= -28.28; adjusted P= 0.0119. Non-statistically significant differences, however, were identified between EP and 2R or between 2R and 4R groups. Moreover, the differences between EP, 2R, and 4R groups were non-statistically significant in the three-class Otsu analysis (Figure 11).

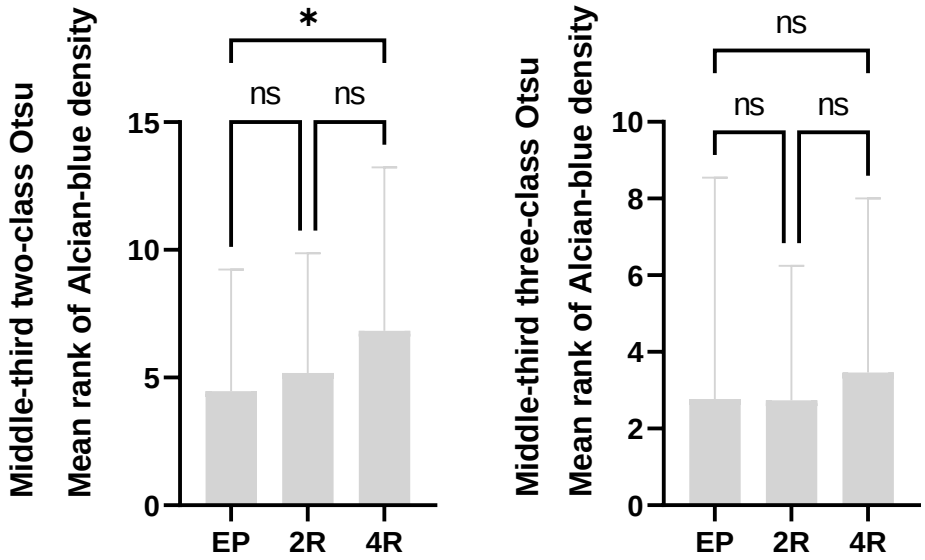



Figure 11. Mean rank score difference in middle-third ground substance content between the three experimental timepoints, measured using two-class and three-class Otsu thresholding methods; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

***Ground substance with mineralized fibrocartilage excluded***

Comparison between SC and EP groups, using the three-class Otsu method and with areas of mineralized fibrocartilage excluded, revealed a statistically significant difference; difference between medians= 1.050; Hodges-Lehmann= 0.6500; SC= 1.740, n= 47; EP= 0.6900, n= 59; U= 938.5; P= 0.0041. Additionally, SC and 2R group difference was statistically significant; difference between medians= 0.7400; Hodges-Lehmann= 0.5300; SC= 1.7400, n= 47; 2R= 1.000, n= 64; U= 1157; P= 0.0381. Non-statistically significant difference, however, was found between SC and 4R groups; difference between medians= 0.6700; Hodges-Lehmann= 0.3700; SC= 1.740, n= 47; 4R= 1.070, n= 67; U= 1304; P= 0.1201 (Figure 13).

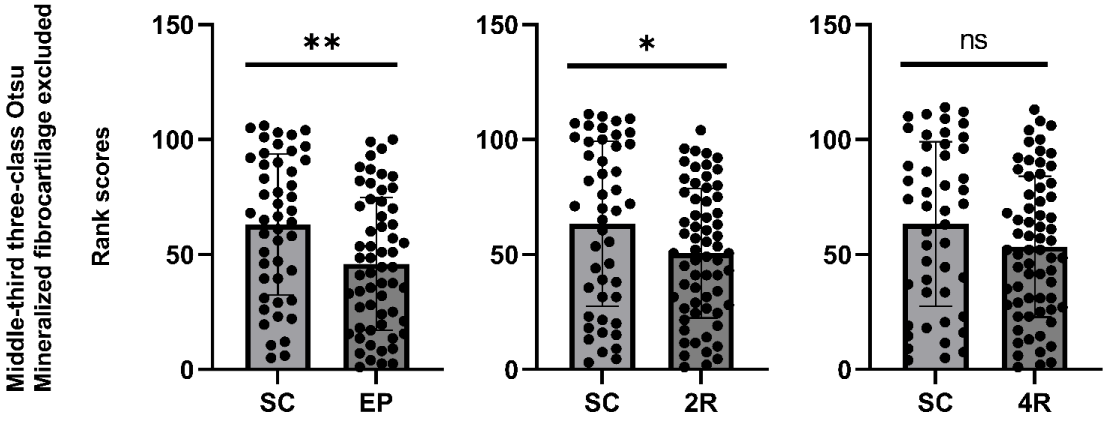



Figure 13. Rank score difference in middle-third ground substance content, with mineralized fibrocartilage excluded, between sedentary control and experimental groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

Within-group comparison, using three-class Otsu method and including tendon middle-third but excluding areas of mineralized fibrocartilage, revealed non-statistically significant difference between EP, 2R and 4R groups; H= 4.168, df= 2, P= 0.1244; EP vs 2R mean rank diff.= -14.75; adjusted P= 0.4117; EP vs 4R mean rank diff.= -19.36, adjusted P= 0.1457; 2R vs 4R mean rank diff.= -4.614, adjusted P= >0.9999 (Figure 14).

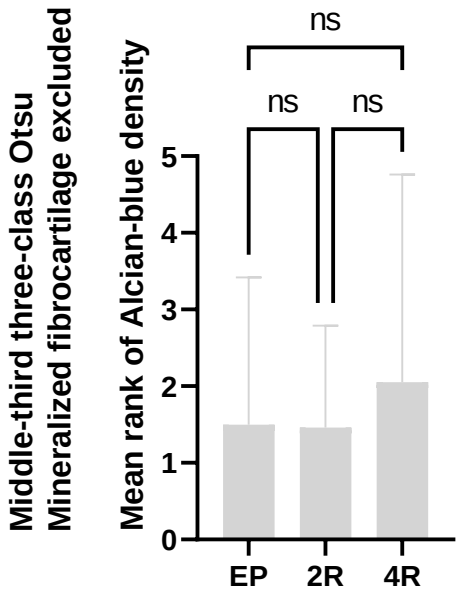



Figure 14. Mean rank score difference in middle-third ground substance content, with mineralized fibrocartilage excluded, between the three experimental timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

***Tendon middle-third ground substance - normalized data***

A statistically significant difference was found between SC and EP groups in the two-class Otsu analysis: difference between means= 5.621 ± 1.488; 95% CI: [2.669, 8.572]; t= 3.777, df= 103; P= 0.0003. The same comparison, moreover, revealed statistically significant differences using the three-class Otsu method: difference between means= 2.882 ± 0.9484; 95% CI: [1.001, 4.763]; t= 3.039, df= 103; P= 0.0030. The comparisons between SC and 2R showed statistically significant differences in two-class Otsu but not three-class Otsu methods. Non-statistically significant differences, however, were found between SC and 4R groups in both two-class and three-class Otsu methods (Figure 12).

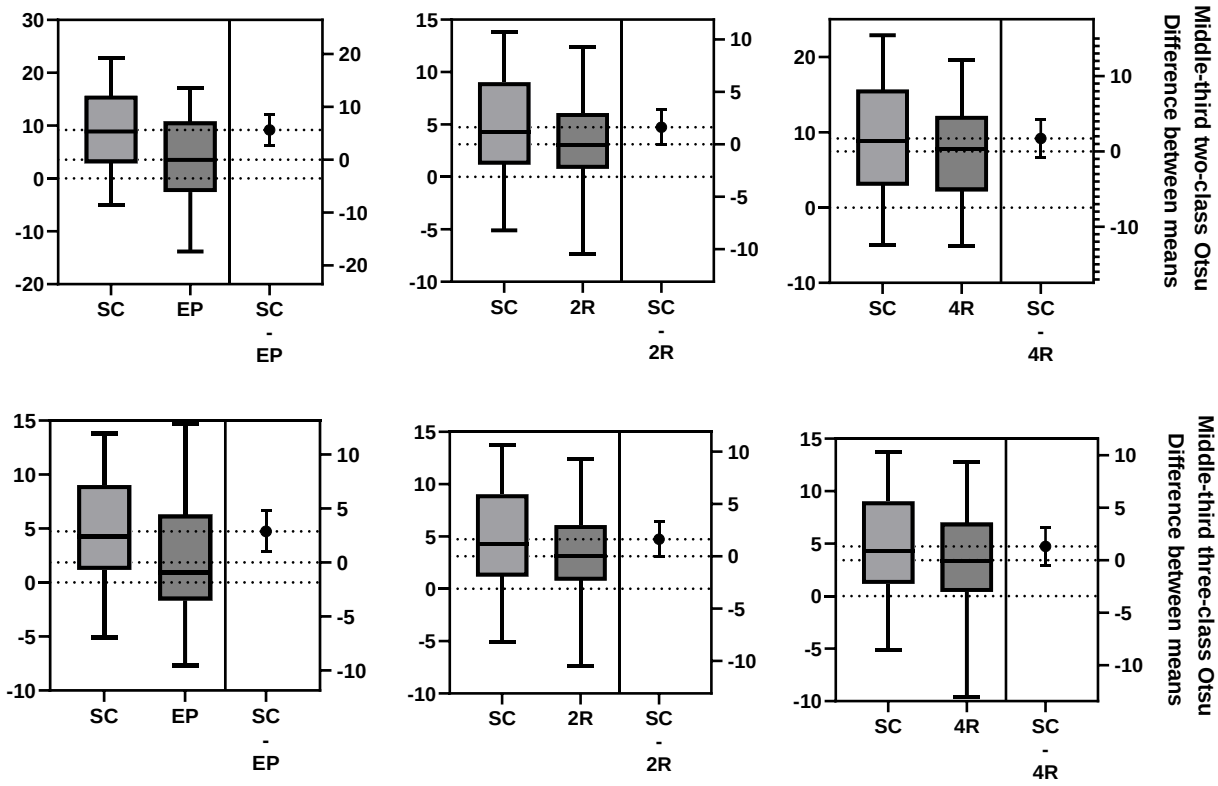



Figure 12. Mean difference in middle-third ground substance content between the sedentary control and experimental groups at three timepoints, measured using two-class and three-class Otsu thresholding methods; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

***Ground substance with mineralized fibrocartilage excluded - normalized data***

A statistically significant difference between SC and EP groups, using three-class Otsu method and analyzing tendon middle-third excluding areas of mineralized fibrocartilage; difference between means= 1.453 ± 0.4911; 95% CI: [0.4795, 2.427]; t= 2.960, df= 103; P= 0.0038. Nonetheless, non-statistically significant difference between SC and 2R groups was found; difference between means= 0.8475 ± 0.4301; 95% CI: [-0.0051, 1.700]; t= 1.970, df= 108; P= 0.0514. Non-statistically significant difference, furthermore, was identified between SC and 4R groups; difference between means= 0.6295 ± 0.4626; 95% CI: [-0.2872, 1.546]; t= 1.361, df= 111; P= 0.1764 (Figure 15).

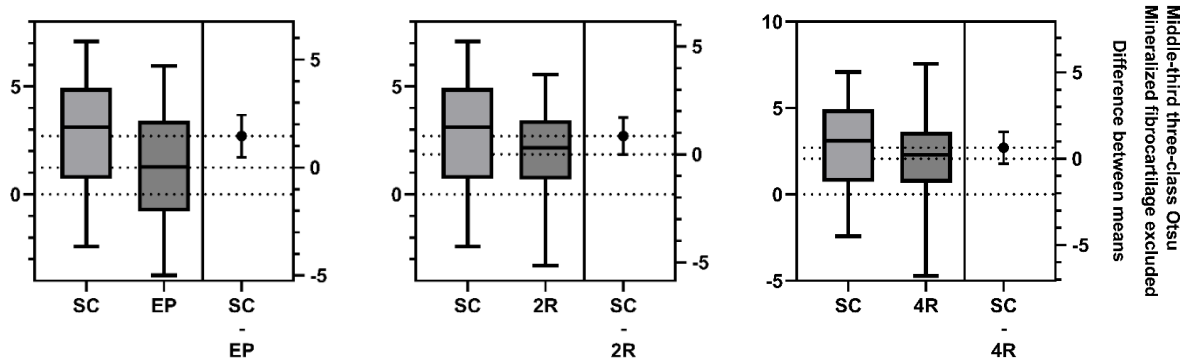



Figure 15. Mean difference in middle-third ground substance content, with mineralized fibrocartilage excluded, between sedentary control and experimental groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

Comparisons between EP, 2R and 4R groups, excluding areas of mineralized fibrocartilage and analyzing middle-third of tendons using three-class Otsu method, indicated non-statistically significant differences between groups; F=2.211; P= 0.1125; factors (EP, 2R, 4R); levels= 3; between-subjects factors; EP vs 2R: mean diff.= -0.6060; 95% CI: [-1.568, 0.3558], P= 0.2987; EP vs 4R: mean diff.= -0.8240; 95% CI: [-1.775, 0.1274], P= 0.1042; 2R vs 4R: mean diff.= -0.2180; 95% CI: [-1.149, 0.7135], P=0.8451 (Figure 16).

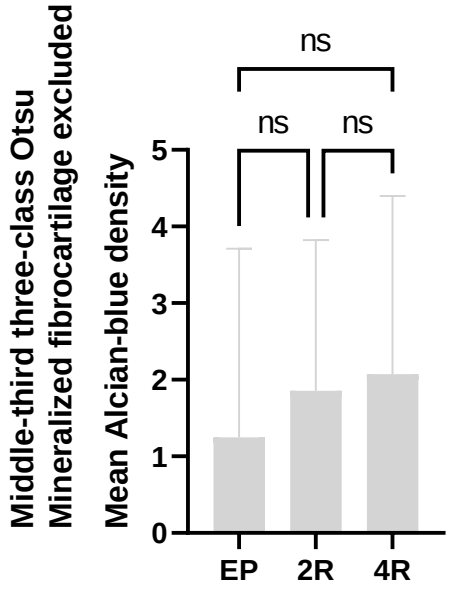



Figure 16. Mean difference in middle-third ground substance content, with mineralized fibrocartilage excluded, between the three experimental timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

***Tendon lower third***

The difference between SC and EP, 2R and 4R groups was non-statistically significant neither in two-class nor three-class Otsu assessments. SC vs EP groups (two-class Otsu) revealed; difference between medians= 3.600; Hodges-Lehmann= 3.510; SC= 13.20, n= 47; EP= 9.600, n= 59; U= 1085; P= 0.0551. SC vs 2R results (two-class Otsu) were; difference between medians= -0.3050; Hodges-Lehmann= -0.1900; SC= 13.20, n= 47; EP= 13.51, n= 64; U= 1476; P= 0.8690. Moreover, SC vs 4R groups (two-class Otsu) showed; difference between medians= 1.020; Hodges-Lehmann= 0.7300; SC= 13.20, n= 47; EP= 12.18, n= 67; U= 1507; P= 0.7009. SC vs EP groups (three-class Otsu) demonstrated; difference between medians= 0.8100; Hodges-Lehmann= 1.170; SC= 3.970, n= 47; EP= 3.160, n= 59; U= 1093; P= 0.0620. SC vs 2R groups results (three-class Otsu) were; difference between medians= -0.5600; Hodges-Lehmann= -0.6700; SC= 3.970, n= 47; EP= 4.530, n= 64; U= 1361; P= 0.3941. SC vs 4R groups (three-class Otsu) showed; difference between medians= -0.4200; Hodges-Lehmann= 0.2200; SC= 3.970, n= 47; EP= 4.390, n= 67; U= 1525; P= 0.7755 (Figure 17).

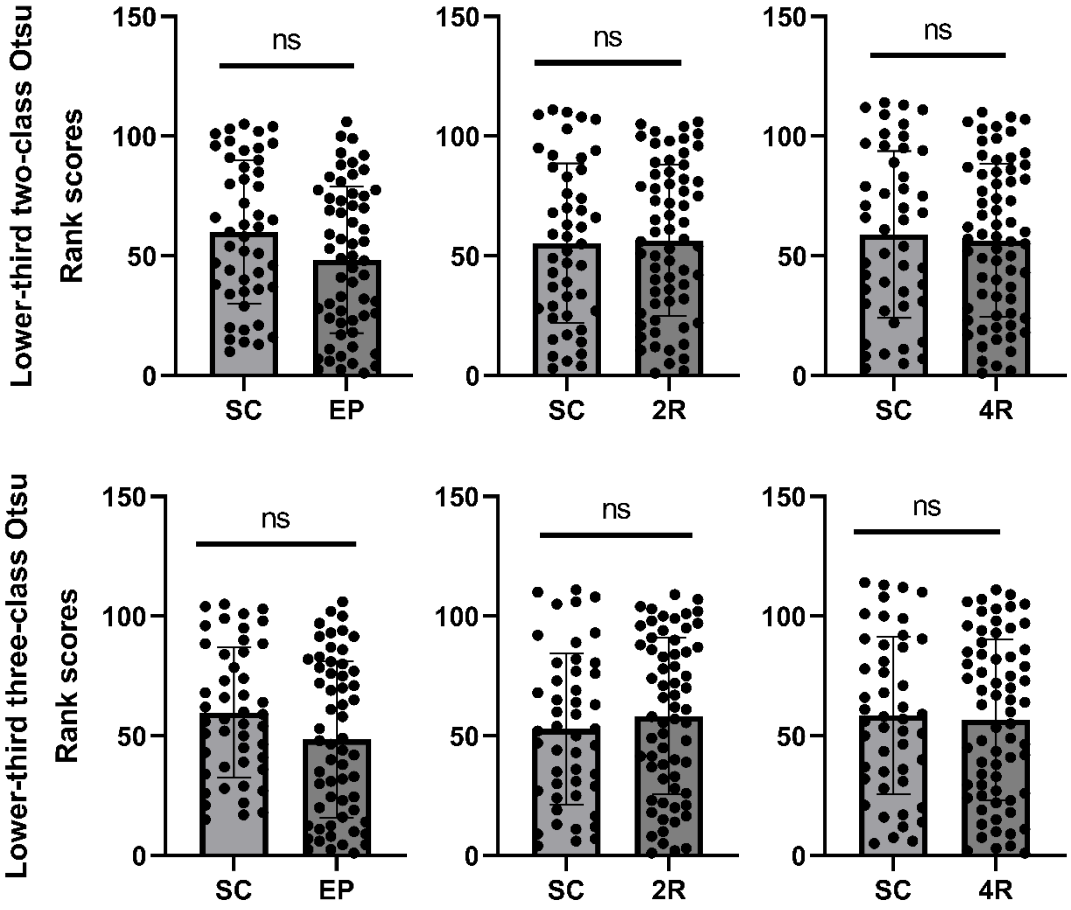



Figure 17. Rank score difference in lower-third ground substance content between sedentary control and experimental groups at the three timepoints, measured using two-class and three-class Otsu thresholding methods; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

Comparison between EP and 2R groups showed a statistically significant difference between groups, with both two-class and three-class Otsu analysis; H= 6.360, df= 2, P= 0.0416; EP vs 2R mean rank diff.= -24.07; adjusted P= 0.0458 (two-class Otsu), and H= 8.650, df= 2, P= 0.0132; EP vs 2R mean rank diff.= -29.14; adjusted P= 0.0100 (three-class Otsu). Non-statistically significant differences were found between EP and 4R, and 2R and 4R groups, using two-class and three-class Otsu methods (Figure 18).

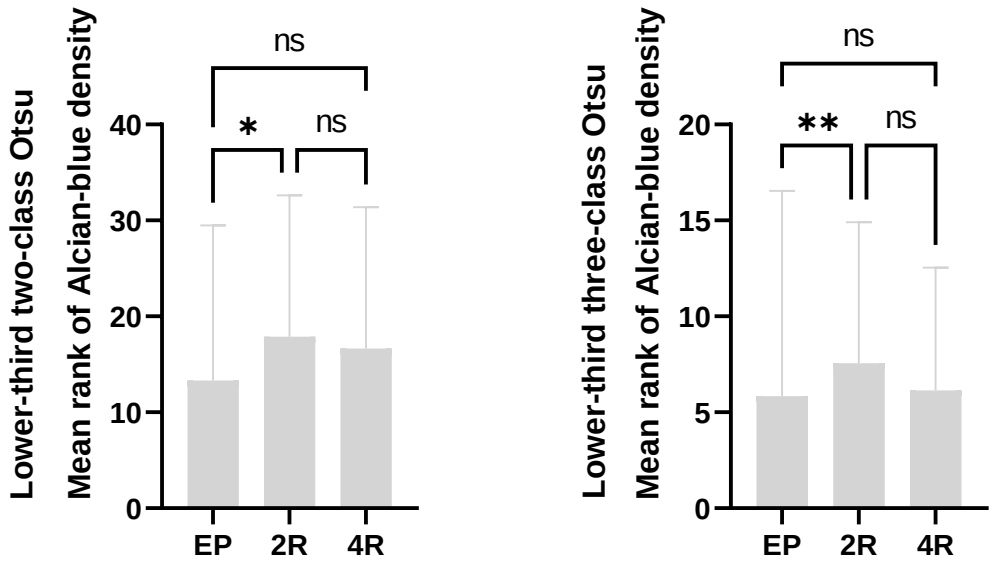



Figure 18. Mean rank score difference in lower-third ground substance content between the three experimental timepoints, measured using two-class and three-class Otsu thresholding methods; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

***Ground substance with mineralized fibrocartilage excluded***

Comparing SC and EP groups revealed a statistically significant difference between the lower-third of tendons, excluding areas of mineralized fibrocartilage and using the three-class Otsu method; difference between medians= 2.210; Hodges-Lehmann= 1.570; SC= 3.640, n= 47; EP= 1.430, n= 59; U= 836; P= 0.0004. Both comparisons, nevertheless, of SC with 2R and 4R groups showed non-statistically significant results; SC vs 2R: difference between medians= 0.8450; Hodges-Lehmann= 0.6100; SC= 3.640, n= 47; 2R= 2.795, n= 64; U= 1292; P= 0.2061; SC vs 4R: difference between medians= 0.4800; Hodges-Lehmann= 0.2700; SC= 3.640, n= 47; = 3.160, n= 67; U= 1486; P= 0.6108 (Figure 20).

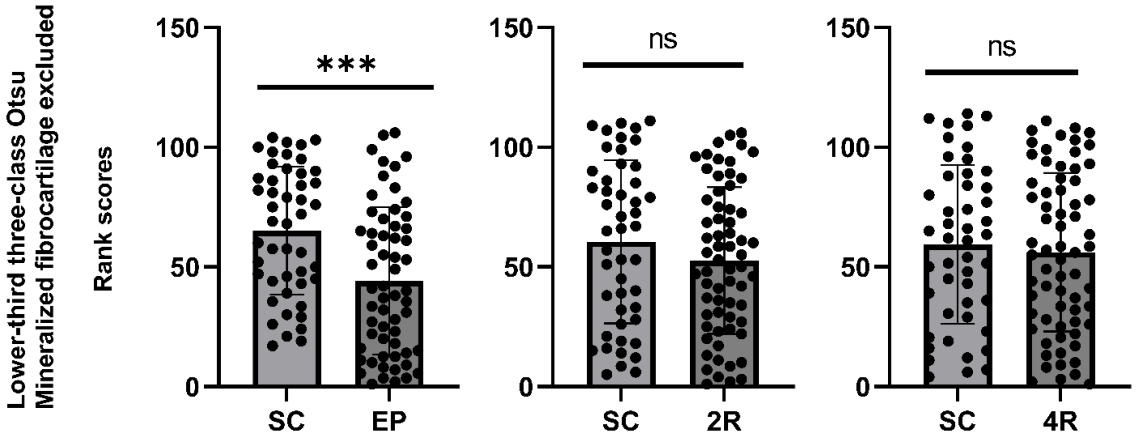



Figure 20. Rank score difference in lower-third ground substance content, with mineralized fibrocartilage excluded, between sedentary control and experimental groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

Statistically significant differences were found both between EP and 2R and, EP and 4R groups, using the three-class Otsu method, excluding areas of mineralized fibrocartilage, and comparing tendon lower-third: H= 12.63, df= 2, P= 0.0018; EP vs 2R mean rank diff.= -26.04; adjusted P= 0.0261, and EP vs 4R: mean rank diff.= -33.57; adjusted P= 0.0019. The comparison of 2R and 4R groups, nevertheless, showed non-statistically significant difference: H= 12.63, df= 2, P= 0.0018; 2R vs 4R mean rank diff.= -7.533; adjusted P= >0.9999 (Figure 21).

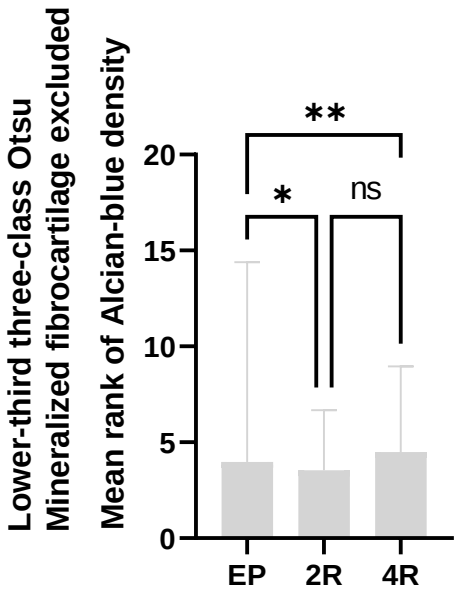



Figure 21. Mean rank score difference in lower-third ground substance content, with mineralized fibrocartilage excluded, between the three experimental timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

***Tendon lower-third ground substance - normalized data***

Statistically significant differences were found between SC and EP, but not between SC and 2R, and SC and 4R groups in tendon lower-third, using two-class and three-class Otsu methods. (Figure 19).

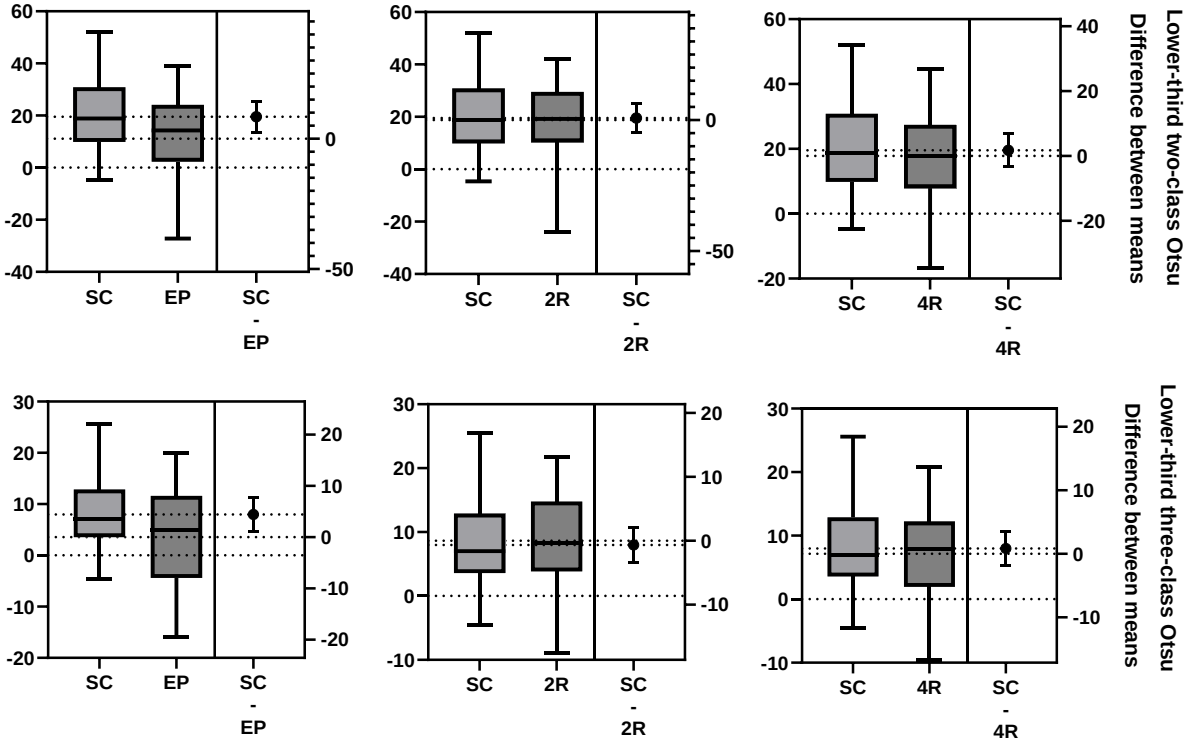



Figure 19. Mean difference in lower-third ground substance content between the sedentary control and experimental groups at three timepoints, measured using two-class and three-class Otsu thresholding methods; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

***Ground substance with mineralized fibrocartilage excluded - normalized data***

Following the normalization of data and excluding mineralized fibrocartilage areas, the analysis of tendon lower-third using three-class Otsu thresholding method revealed a statistically significant difference between SC and EP groups: difference between means= 0.2762 ± 0.06625; 95% CI: [0.1448, 0.4076]; t= 4.169, df= 103; P= <0.0001. However, comparing SC with 2R and 4R groups did not show statistically significant differences (Figure 22).

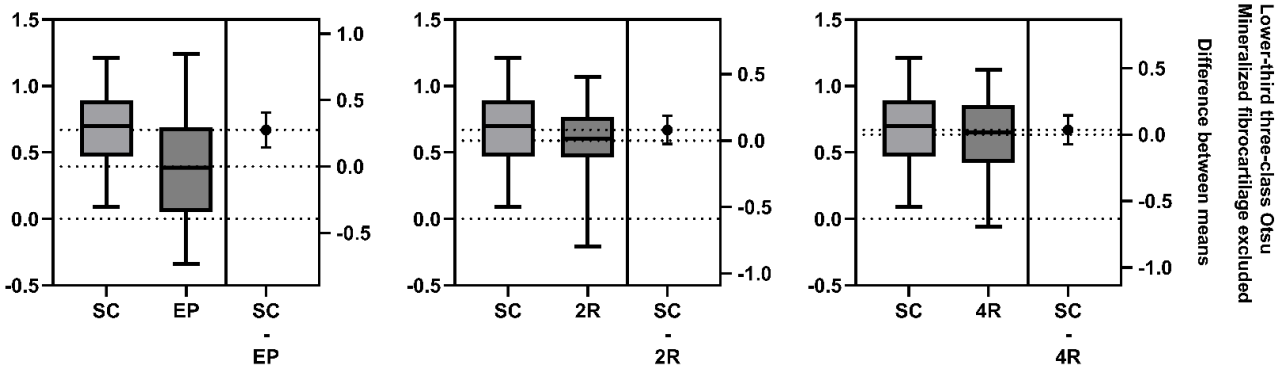



Figure 22. Mean difference in lower-third ground substance content, with mineralized fibrocartilage excluded, between sedentary control and experimental groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

Statistically significant differences were identified between EP and 2R, and EP and 4R groups, following the exclusion of mineralized fibrocartilage areas and analyzing tendon lower-third, using three-class Otsu method: F=10.16; P= <0.0001; factors (EP, 2R, 4R); levels= 3; between-subjects factors; EP vs 2R: mean diff.= -0.1946; 95% CI: [-0.3281, -0.6122], P= 0.0020; EP vs 4R: EP vs 4R: mean diff.= -0.2396; 95% CI: [-0.3716, -0.1076], P= <0.0001; 2R vs 4R: mean diff.= -0.04500; 95% CI: [-0.1736, 0.08364], P= 0.6871 (Figure 23).

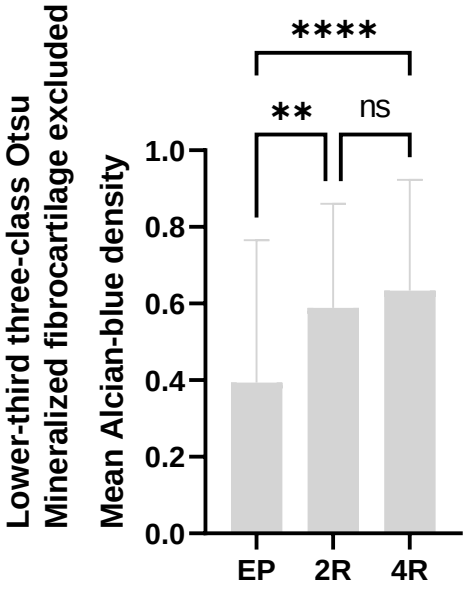



Figure 23. Mean difference in lower-third ground substance content, with mineralized fibrocartilage excluded, between the three experimental timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y-axis represents pixel density.

**Cellularity**

Non-statistically significant differences were found in both the tendon middle-third and lower-third analyses of cellularity between EP, 2R and 4R groups (Figure 24).

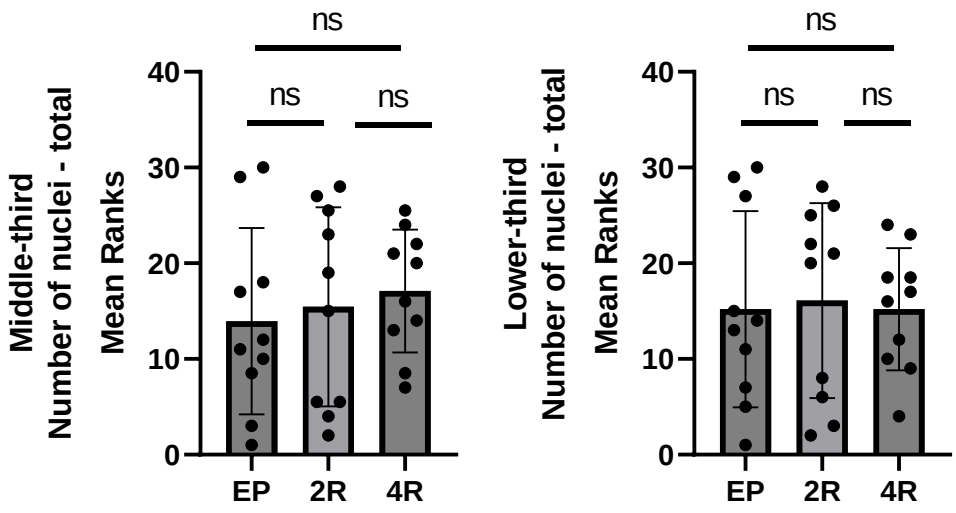



Figure 24. Mean rank difference in nuclei number between the three timepoints of the experimental groups in middle and lower-thirds analyses; error bars represent 95% confidence interval; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05.

Non-statistically significant difference in cellularity in tendon middle-third was found when SC group was compared with EP, 2R and 4R groups; SC vs EP: difference between means= -81.55 ± 64.12; 95% CI: [-217.5, 54.38]; t= 1.272, df= 16; P= 0.2216; SC vs 2R: difference between means= -75.35 ± 56.29; 95% CI: [-194.7, 43.97]; t= 1.339, df= 16; P= 0.1994; SC vs 4R: difference between means= -75.75 ± 47.42; 95% CI: [-176.3, 24.78]; t= 1.597, df= 16; P= 0.1297.

Data of the lower-third, nevertheless, indicated statistically significant differences between SC and 4R groups, but not EP and 2R groups; SC vs EP: difference between means= -123.9 ± 78.75; 95% CI: [-290.8, 43.06]; t= 1.573, df= 16; P= 0.1353; SC vs 2R: difference between means= -115.8 ± 72.10; 95% CI: [-268.6, 37.06]; t=1.606, df= 16; P= 0.1279; and SC vs 4R: difference between means= -138.9 ± 64.02; 95% CI: [-274.6, -3.157]; t=2.169, df=16; P= 0.0455 (Figure 25).

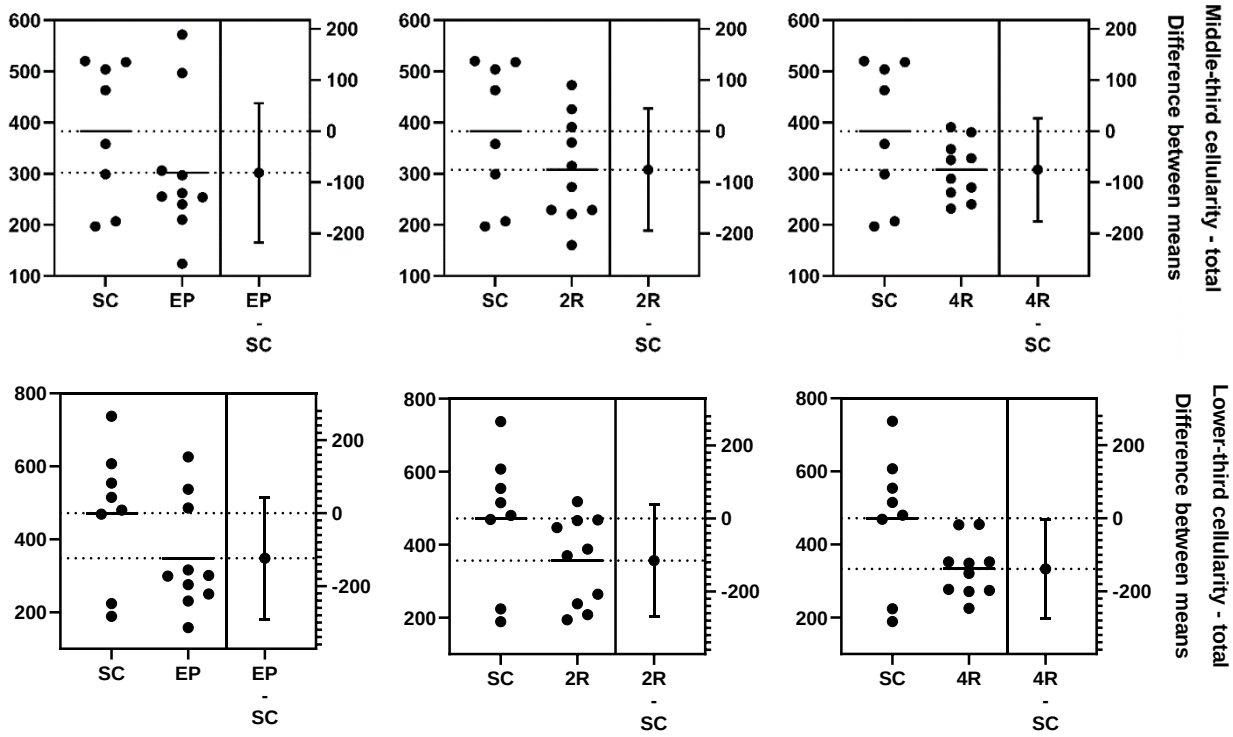



Figure 25. Mean difference in nuclei number between the sedentary control and experimental groups at the three timepoints in middle and lower-thirds analyses; error bars represent 95% confidence interval; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05. Y axis represents number of nuclei.

**Circularity**

Non-statistically significant differences were found between SC and EP, 2R and 4R groups in tendon middle-third nor lower-third in circularity (Figure 26).

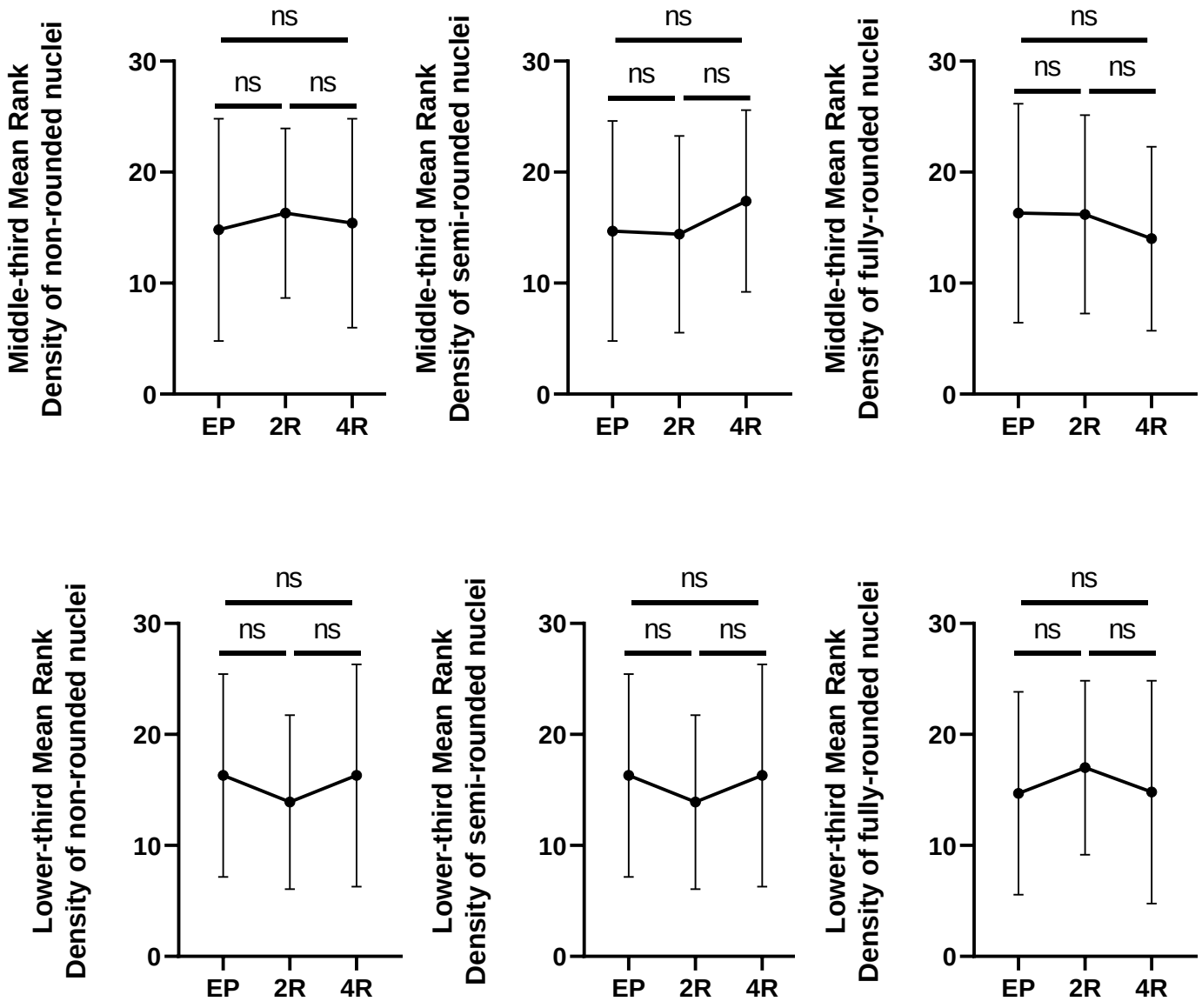



Figure 26. Mean rank difference in nuclei roundedness between the three timepoints of the experimental groups in middle and lower-thirds analyses; scale error bars represent 95% confidence interval; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05.

## Fiber alignment

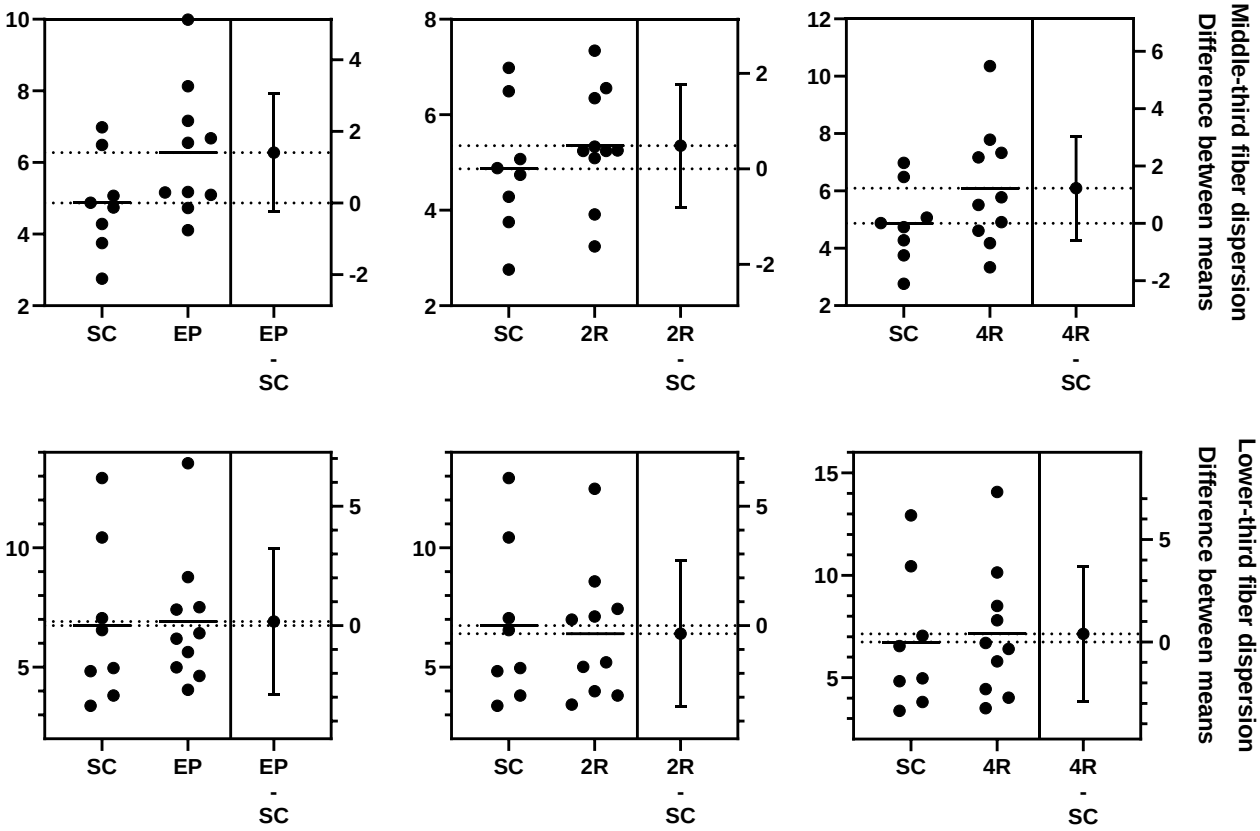



Figure 27. Mean difference in fiber dispersion between the sedentary control and experimental groups at the three timepoints in middle and lower-thirds analyses; error bars represent 95% confidence interval; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05.

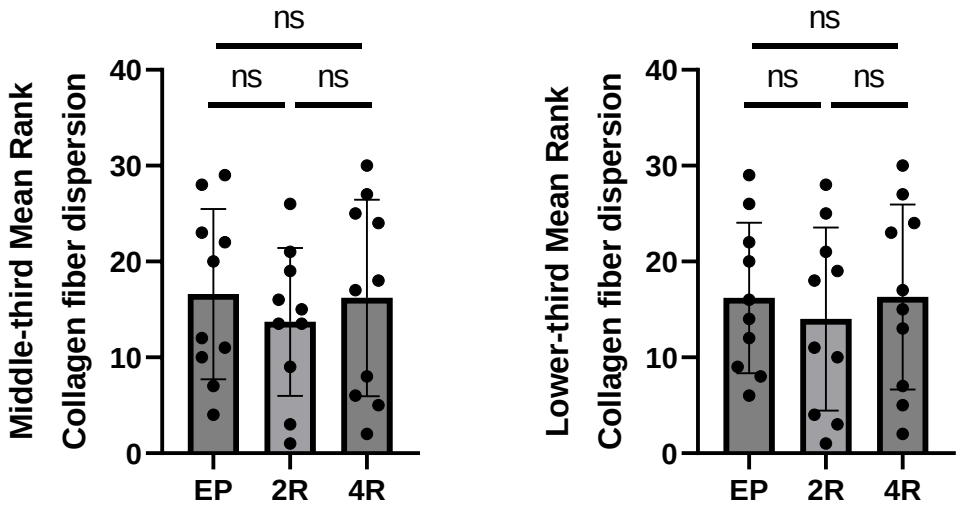
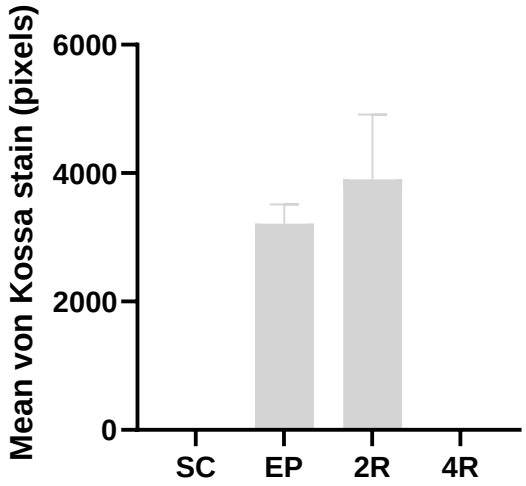



Figure 28. Mean rank difference in fiber dispersion between the three timepoints of the experimental group in middle and lower-thirds analyses; error bars represent 95% confidence interval; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest. P<0.05.

**Calcification**



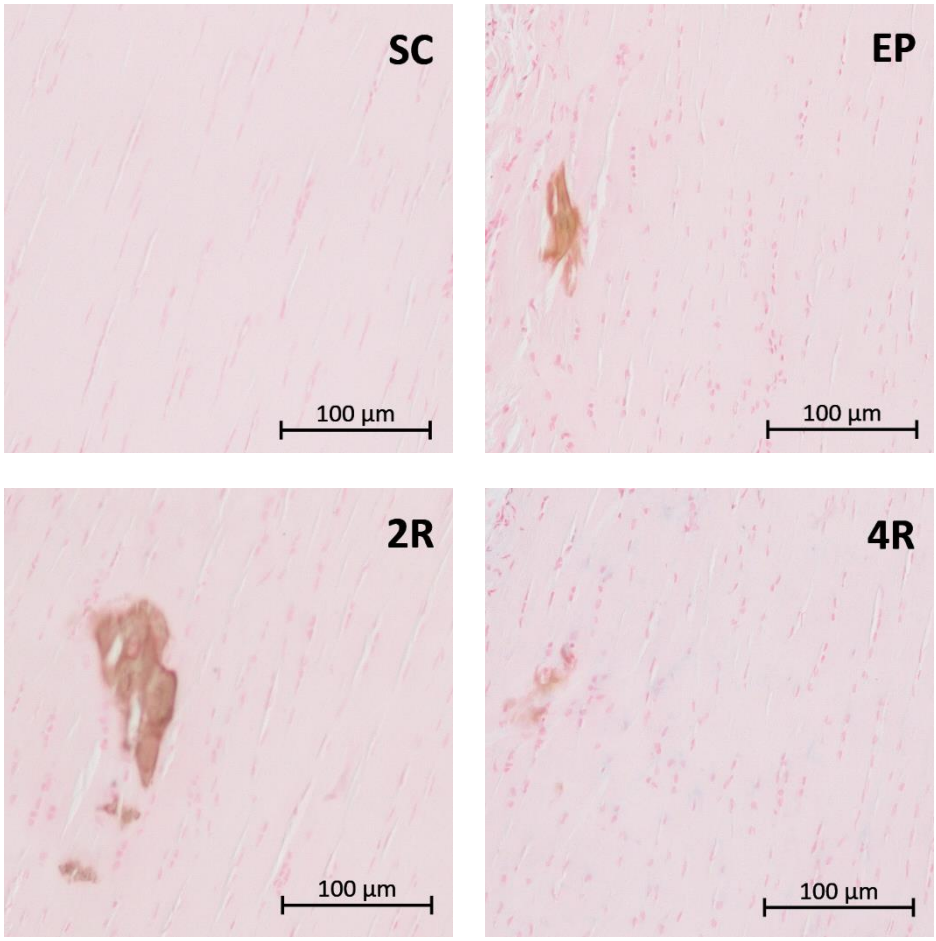




Figure 29. Mean pixels and representative images of von-Kossa-stained samples from sedentary control and experimental groups; SC, sedentary control; EP, end of protocol; 2R, two weeks of rest; 4R, four weeks of rest; dimensions 700×700 pixels; magnification = 10x; scale bar= 100 µm

**Real-time polymerase chain reaction analysis**

Most of the transcript expressions of the *p16* and *p21* target genes were "undetermined", and consequently, these results were unobtainable. Nonetheless, the results of the few samples of these genes that were "determined" are available in the supplementary information. One of the samples, in the 2R group, was found to consistently have much higher values than all other samples, and across almost all genes. Therefore, this sample was considered an outlier, and was excluded in a secondary analysis. The differences between experimental groups were non-statistically significant for all target genes, with exception to *MKX* gene (Figure 30). The difference between 2R and 4R groups in *MKX* gene expression was found to be statistically significant; H= 6.785, df= 2, P= 0.0179; 2R vs. 4R: mean rank diff= 7.000; adjusted P= 0.0331 (Figure 31).

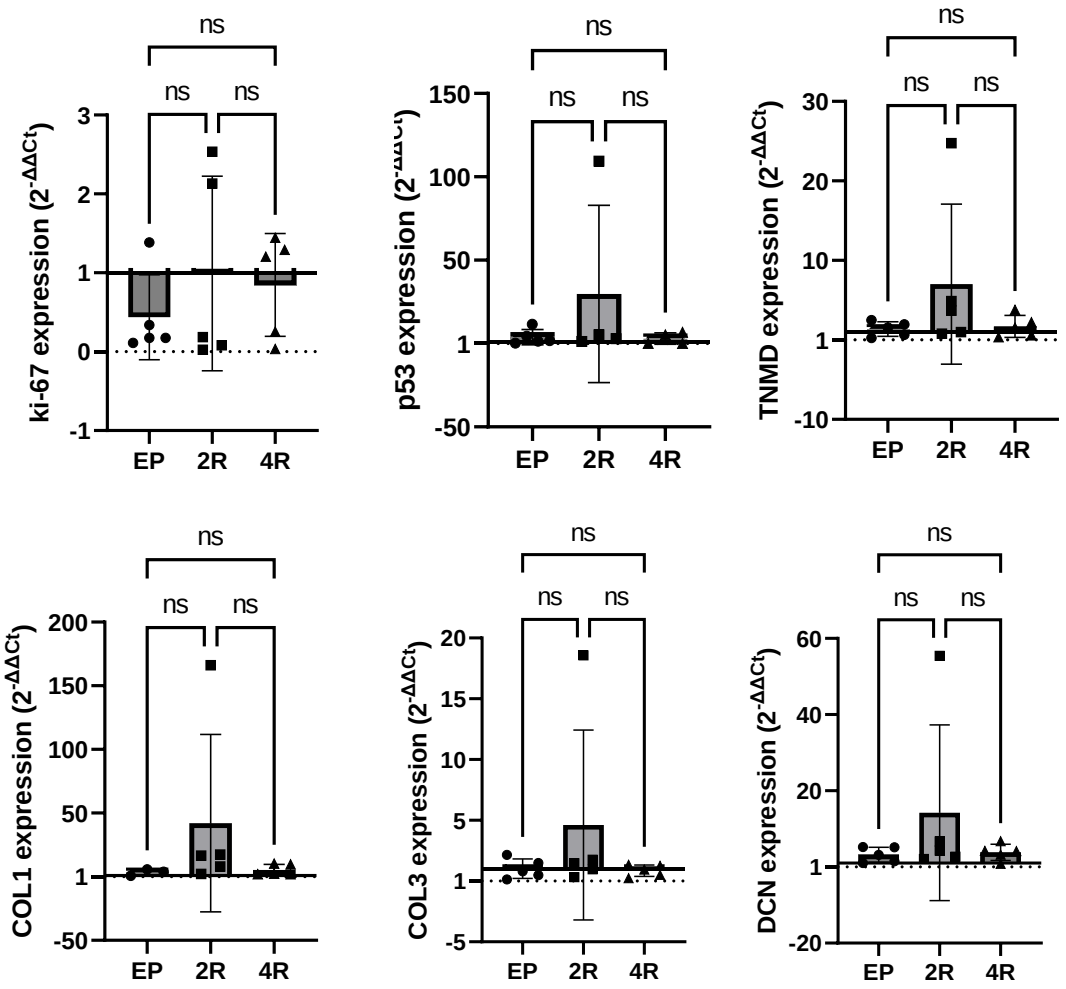



Figure 30. Relative gene expression results calculated using the $2^{-\Delta\Delta C}{}_{T}$ method; results are with the outlier included. Error bars represent mean ± standard error of the mean (SE) of three independent experiments.

At 4R, about 15% reduction in *Ki-67*, almost 3-fold increase in *p53*, about 70% increase in *TNMD*, about 5-fold increase in *Col1*, about 15% reduction in *Col3*, almost 4-fold increase in *DCN*, almost 1-fold reduction in *MKX*.

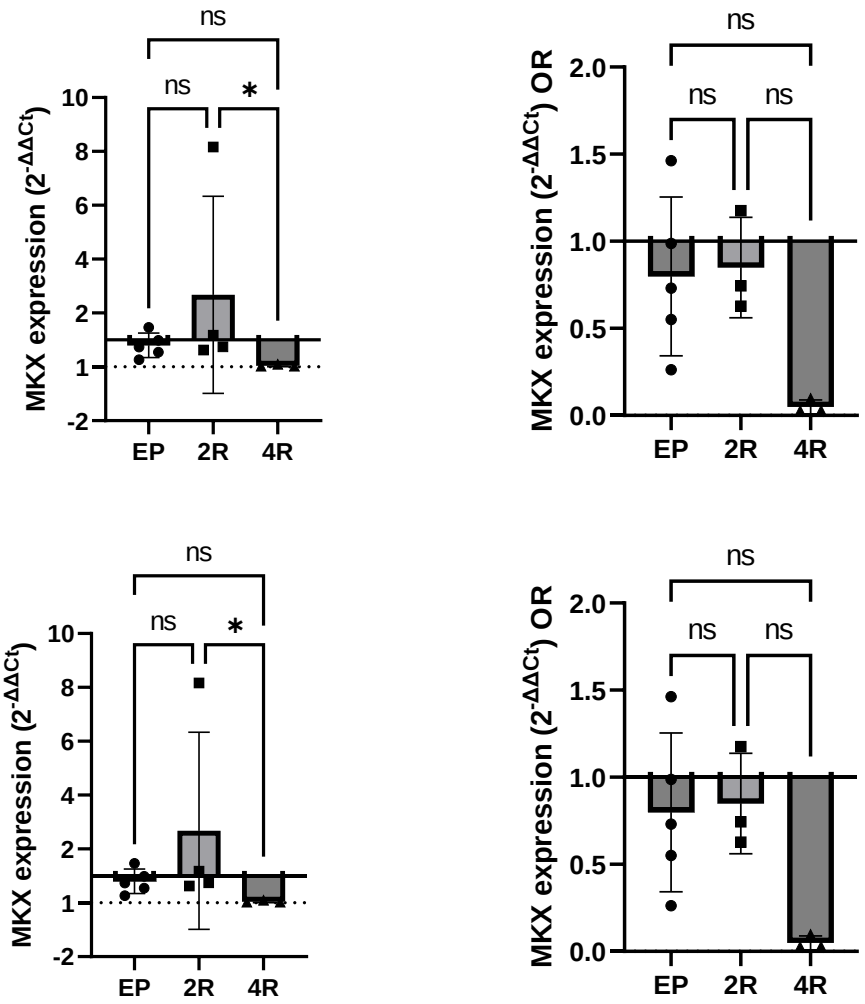



Figure 31. Relative gene expression results as calculated using the $2^{-\Delta\Delta C}{}_{T}$ method; results are with outlier included (left) and excluded (right). Error bars represent mean ± standard error of the mean (SE) of three independent experiments.

**Gene expression results – outlier removed**

At 4R, about 15% reduction in *Ki-67*, about 3-fold increase in *p53*, almost 70% increase in *TNMD*, about 5-fold increase in *Col1*, about 15% reduction in *Col3*, almost 4-fold increase in *DCN*, almost 1-fold reduction in *MKX*. Non-statistically significant differences were found between all groups, for all genes (Figures 31 and 32).

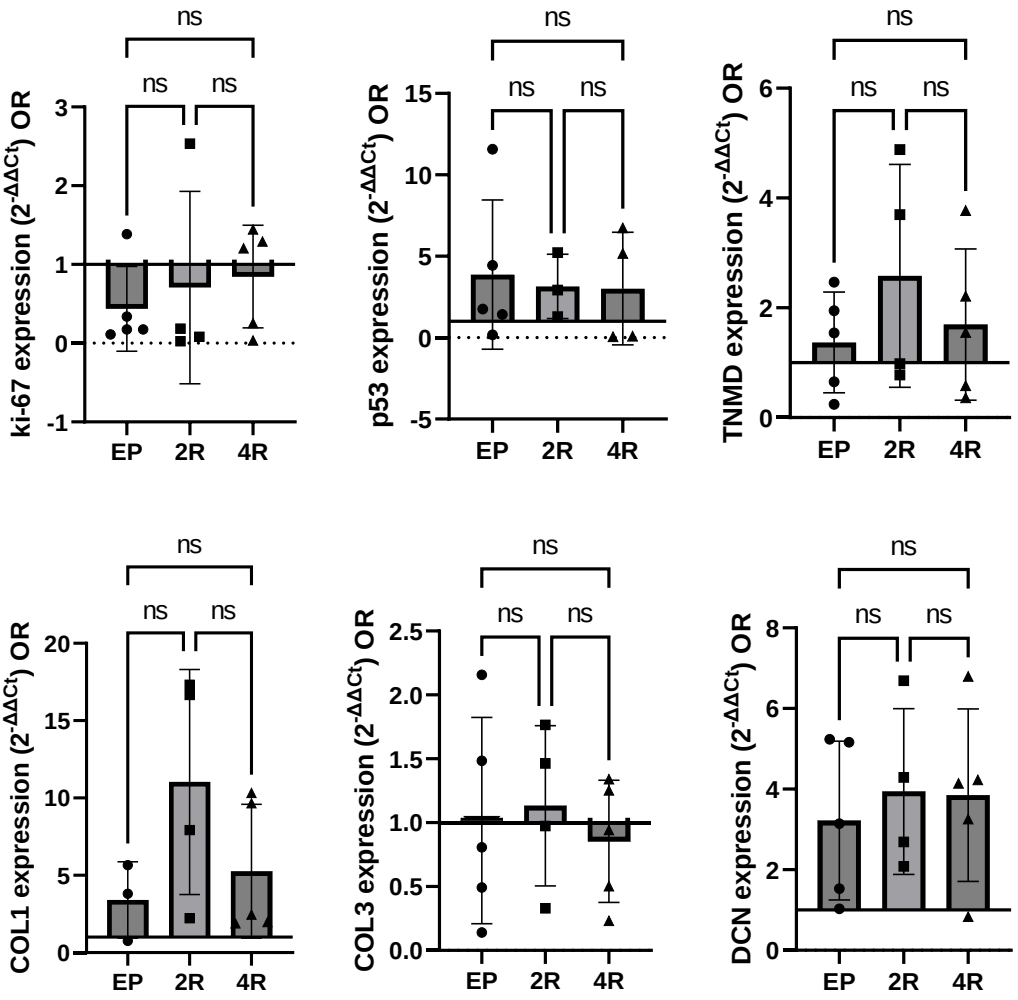



Figure 32. Relative gene expression results as calculated using the $2^{-\Delta\Delta C}{}_{T}$ method; results are with outlier removed. Error bars represent mean ± standard error of the mean (SE) of three independent experiments.

**The effect of HVMPC on Achilles tendon overuse injury in rats**

None of the humane endpoints were reached. Aggressive behavior to inmates was observed in 3 rats following the first application of depilatory cream. All rats received the designated interventions, with exception to one rat in the sham group, that died one week before its due euthanasia date. Autopsy of that animal did not reveal any signs of colitis or peritonitis.

Slight transient chromodacryorrhoea, scoring 1-2 on the scoring system of quantifying chromodacryorrhoea (Mason et al., 2004), was observed only six times (in three rats) throughout the running protocol (during the last two weeks), which may indicate that the protocol was well tolerated. No chromodacryorrhoea was observed during the application of HVMPC intervention or in response to injections. Acute dermal irritation due to depilator use was assessed using the Draize dermal irritation scoring system (Administration et al., 1959), and the grading was found to range between 1-2 indicating very slight to well-defined erythema. Examples of dermal irritation are provided in the supplementary data.

Five rats (three experimental, and two control) did not achieve adequate sedation during the study; compensatory sessions were administered to two rats (one experimental, and one control), additional sedative doses were administered to two rats (experimental group) in order to complete the stimulation session, and one rat in the experimental group was not administered additional doses (missed about 20 minutes of intervention).

Non-statistically significant differences were found between groups with regard to mean body weight and chow consumption at baseline. Baseline body weight and chow consumption were recorded in the sedentary control (SC) group and sham electrical stimulation (Sham ES) for 4 weeks (Sham ES 4W), for 6 weeks (Sham ES 6W), and for 8 weeks (Sham ES 8W), and electrical stimulation (ES) for 4 weeks (ES 4W), for 6 weeks (ES 6W), and for 8 weeks (ES 8W). Mean differences in baseline body weight were -32.25; 95% CI: [-145.5, 80.99]; P= 0.9930, -6.000; 95% CI: [-113.4, 101.4]; P= >0.9999, and 30.80; 95% CI: [-76.63, 138.2]; P= 0.9926 between SC and Sham ES 4W, Sham ES 6W and Sham ES 8W, respectively. Moreover, differences between Sham ES 4W and ES 4W, Sham ES 6W and ES 6W, and Sham ES 8W and ES 8W

were -3.250; 95% CI: [-110.0, 116.5]; P= >0.9999, 29.50; 95% CI: [-77.93, 136.9]; P= 0.9946; and 38.20; 95% CI: [-63.09, 139.5]; P= 0.9546, respectively (Figures 33 and 34).

Mean rank differences in baseline chow consumption were non-statistically significant between groups; H= 14.10, P= 0.0285; the SC group vs Sham ES 4W, Sham ES 6W and Sham ES 8W results were -8.500; Z= 1.284; P= >0.9999, 5.700; Z= 0.9077; P= >0.9999, and 10.90; Z= 1.736, P= >0.9999. Differences between Sham ES 4W and ES 4W, Sham ES 6W and ES 6W, and Sham ES 8W and ES 8W were -1.500; Z= 0.2266; P= >0.9999; -5.800; Z= 0.9797; P= >0.9999; and 8.600; Z= 1.453; P= >0.9999, respectively (Figures 33 and 35).

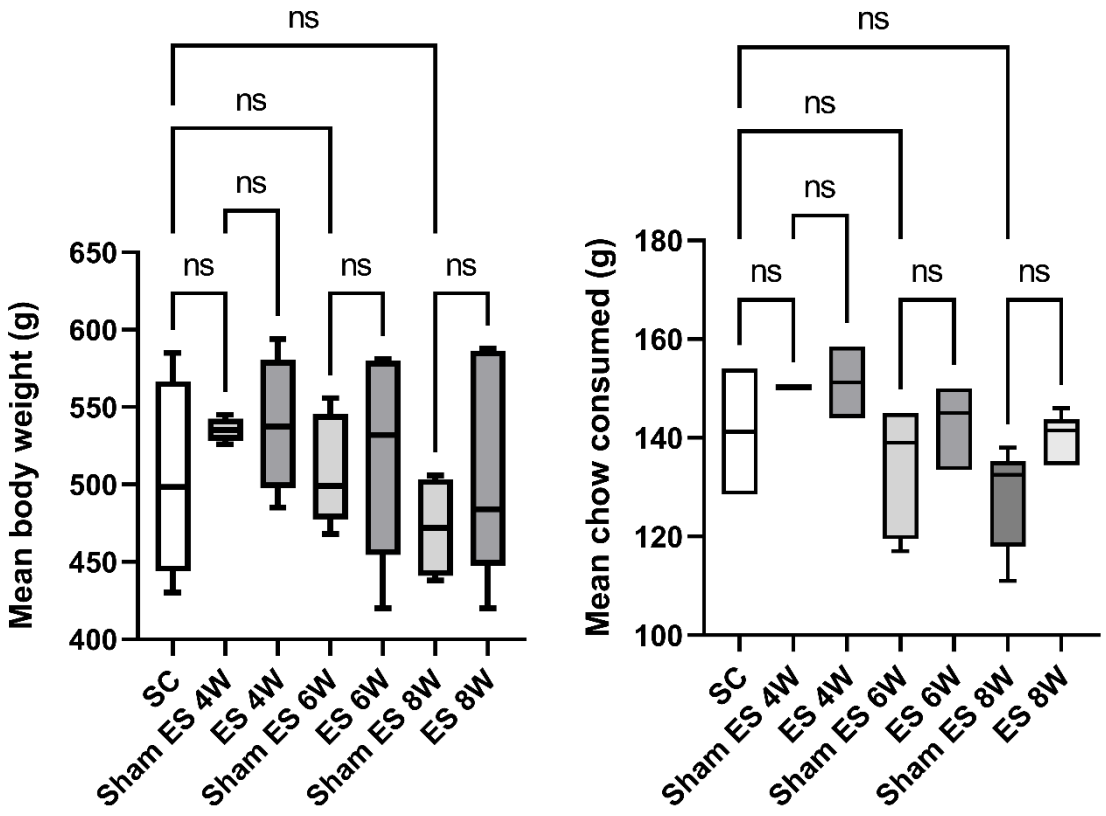



Figure 33. Mean body weight and chow consumption at baseline; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively; ns, non-statistically significant difference; g, grams; scale bars represent 95% confidence interval.

## Body weight

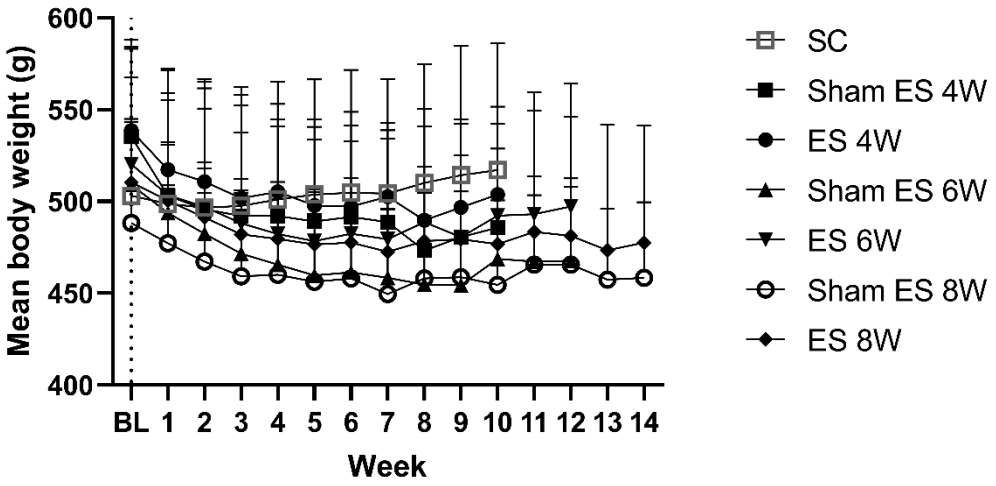



Figure 34. Change in mean body weight over the study span; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively; g, grams; note the minimal gain in body weight following the end of running protocol at 6 weeks.

## Chow consumption

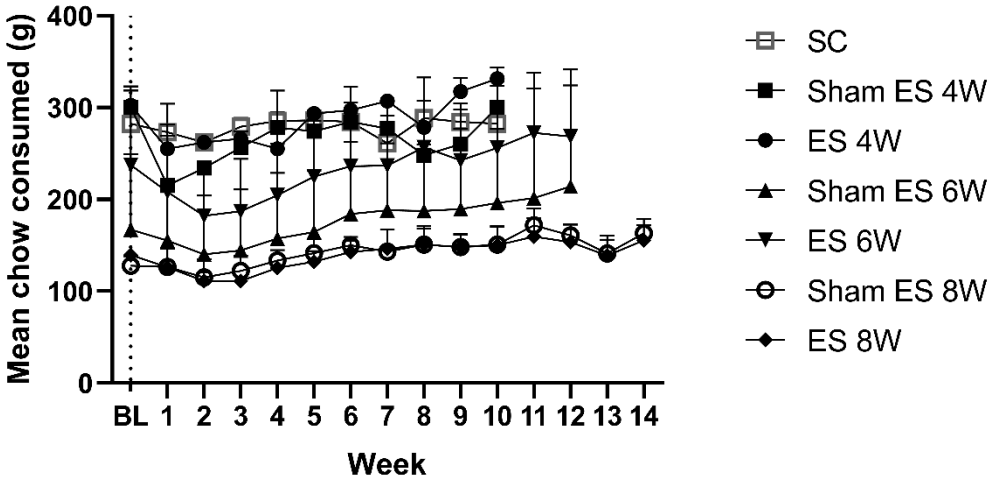



Figure 35. Change in mean chow consumption over the study span; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively; g, grams; note the minimal increase in chow consumption following the end of running protocol at 6 weeks.

**Ground substance**

***Tendon middle third***

Statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and Sham ES 4W groups in the middle-third of tendons; difference between medians= -4.280; Hodges-Lehmann= -3.020; SC= 5.930, n= 47; Sham ES 4W= 1.650, n= 43; U= 470; P= <0.0001. Moreover, a statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and Sham ES 6W groups: difference between medians= -2.450; Hodges-Lehmann= -1.640; SC= 5.930, n= 47; Sham ES 6W= 3.480, n= 55; U= 974.5; P= 0.0325. Furthermore, a statistically significant difference in Alcian-blue density (two-class Otsu) between SC and Sham ES 8W groups was found; difference between medians= -2.320; Hodges-Lehmann= -2.380; SC= 5.930, n= 47; Sham ES 8W= 3.610, n= 59; U= 927.5; P= 0.0033 (Figure 36).

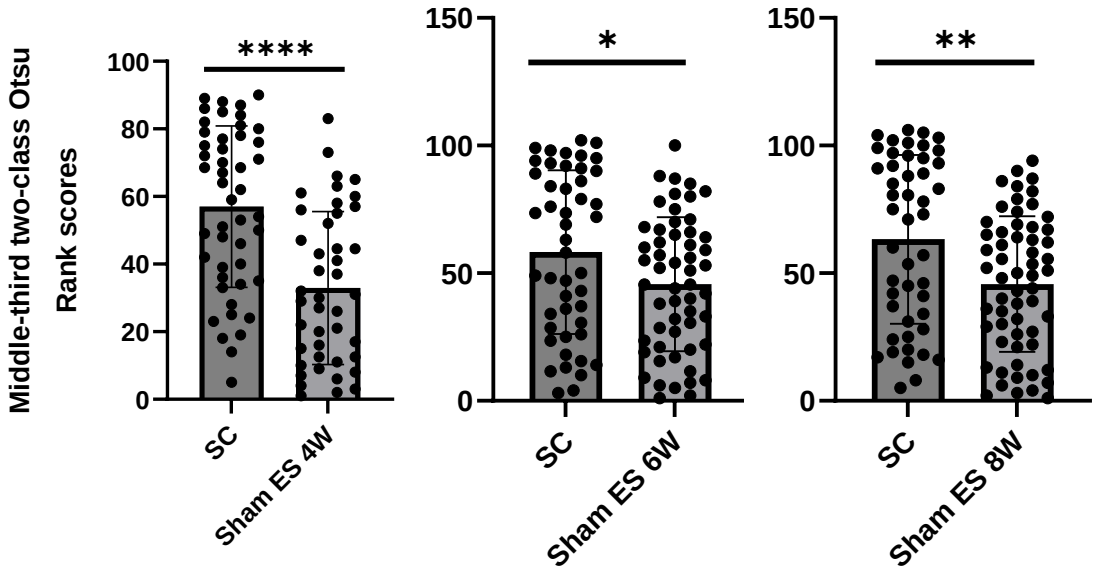



Figure 36. Rank score difference in middle-third ground substance content between sedentary control and sham groups at the three timepoints, measured using two-class Otsu thresholding method; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

Three-class Otsu Alcian-blue density analysis of middle-third of tendons showed statistically significant difference between SC and Sham ES 4W groups; difference between medians= -1.420; Hodges-Lehmann= -1.330; SC= 2.190, n= 47; Sham ES 4W= 0.7700, n= 43; U= 515; P= <0.0001. Non-statistically significant difference in Alcian-blue density (three-class Otsu) was found between SC and Sham ES 6W groups: difference between medians= -0.3900; Hodges-Lehmann= -0.6200; SC= 2.190, n= 47; Sham ES 6W= 1.800, n= 55; U= 1045; P= 0.0964. However, a statistically significant difference in Alcian-blue density (three-class Otsu) between SC and Sham ES 8W groups was found; difference between medians= -1.100; Hodges-Lehmann= -1.000; SC= 2.190, n= 47; Sham ES 8W= 1.090, n= 59; U= 930.5; P= 0.0035 (Figure 38).

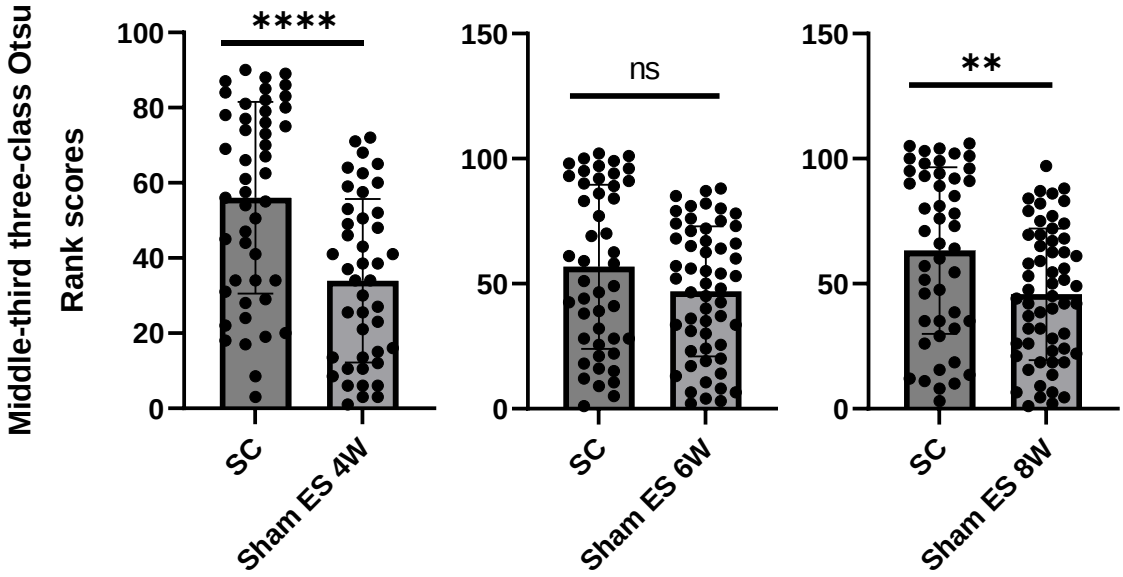



Figure 38. Rank score difference in middle-third ground substance content between sedentary control and sham groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

The differences between Sham ES 4W and ES 4W, Sham ES 6W and ES 6W, and Sham ES 8W and ES 8W were all non-statistically significant (Figure 40).

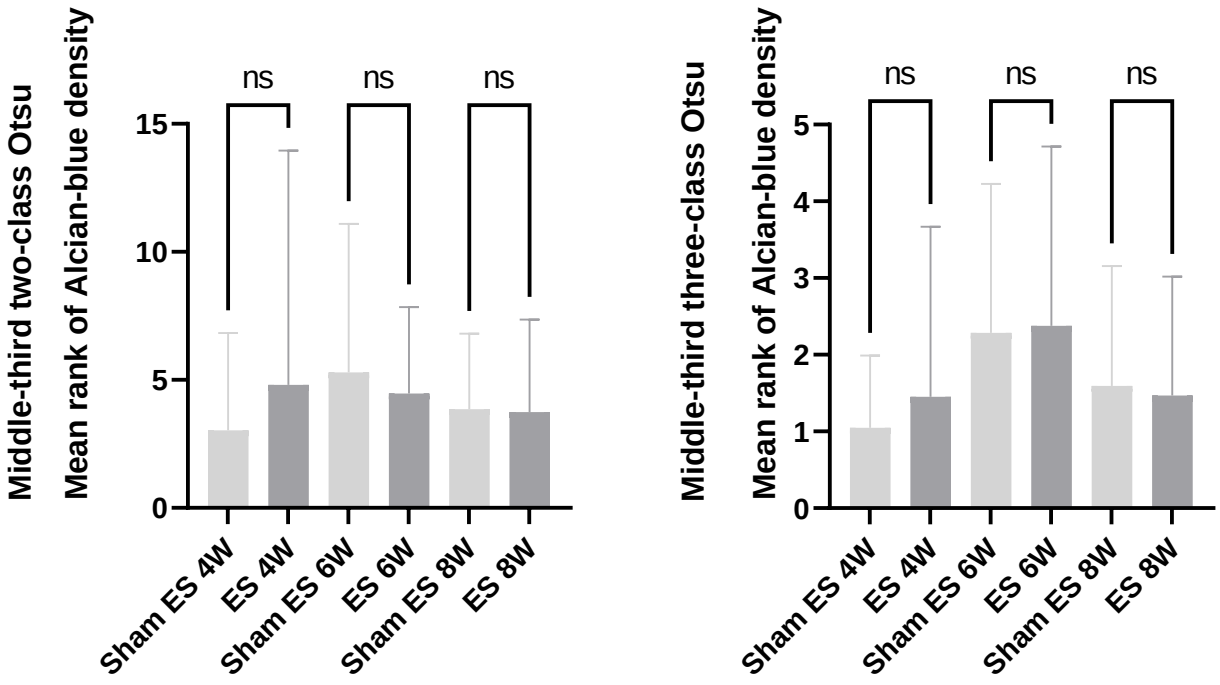



Figure 40. Mean rank score difference in middle-third ground substance content within sham, and within stimulation groups, at the three timepoints, measured using two-class and three-class Otsu thresholding methods; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. $P<0.05$. Y-axis represents pixel density.

***Ground substance with mineralized fibrocartilage excluded***

Comparison between SC and Sham ES 4W groups, using the three-class Otsu method and with areas of mineralized fibrocartilage excluded, revealed a statistically significant difference; difference between medians= -1.180; Hodges-Lehmann= -1.140; SC= 1.740, n= 47; Sham ES 4W= 0.5600, n= 43; U= 446; P= <0.0001. Additionally, SC and Sham ES 6W group difference was statistically significant; difference between medians= -0.8600; Hodges-Lehmann= -0.7800; SC= 1.740, n= 47; Sham ES 6W= 0.8800, n= 55; U= 857; P= 0.0032. A statistically significant difference, furthermore, was found between SC and Sham ES 8W groups; difference between medians= -1.100; Hodges-Lehmann= -0.8700; SC= 1.740, n= 47; Sham ES 8W= 0.6400, n= 59; U= 857; P= 0.0007 (Figure 43).

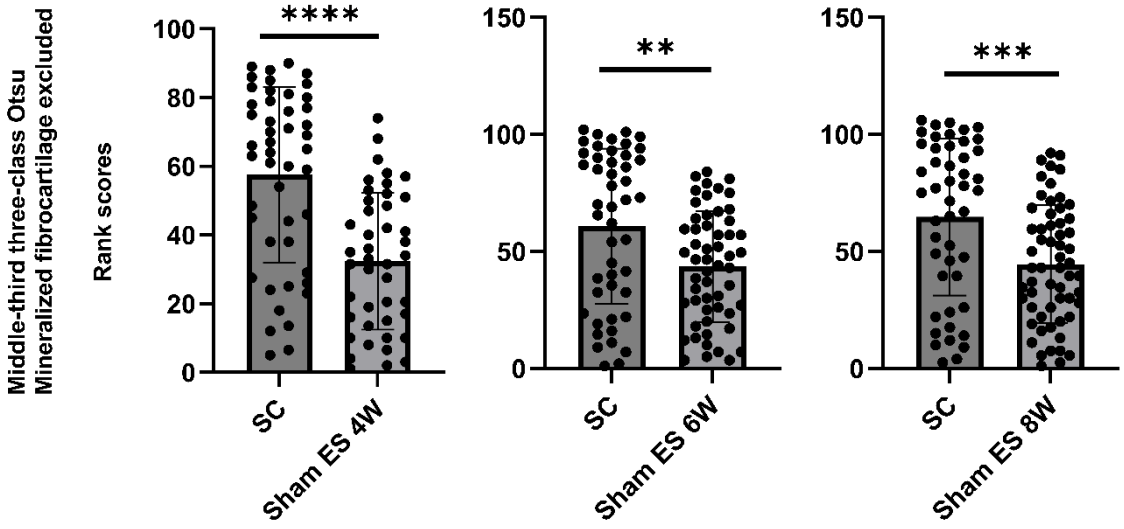



Figure 43. Rank score difference in middle-third ground substance content, with mineralized fibrocartilage excluded, between sedentary control and sham groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

Using three-class Otsu method and including tendon middle-third but excluding areas of mineralized fibrocartilage, between-group comparison revealed non-statistically significant difference between any of the groups (Figure 44).

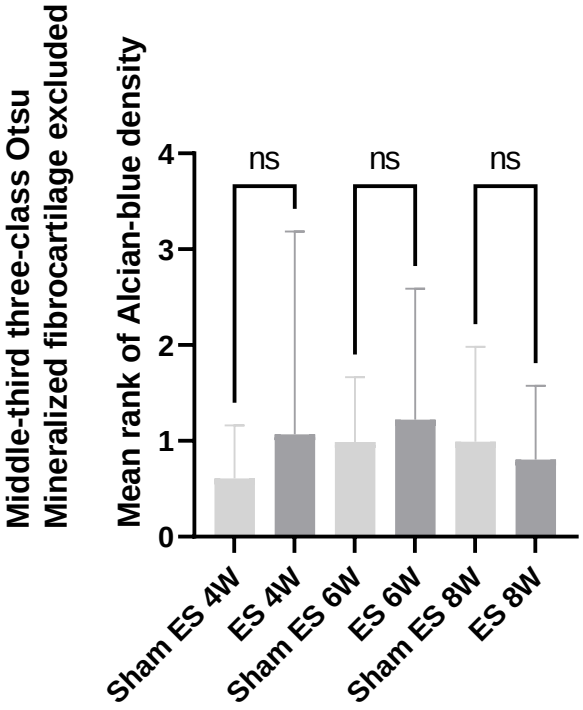



Figure 44. Mean rank score difference in middle-third ground substance content, with mineralized fibrocartilage excluded, within sham groups, and within stimulation groups, at the three experimental timepoints, measured using three-class Otsu thresholding method; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. $P<0.05$. Y-axis represents pixel density.

***Tendon middle-third ground substance - normalized data***

A statistically significant difference was found between SC and Sham ES 4W groups in the two-class Otsu analysis: difference between means= -6.946 ± 1.417; 95% CI: [-9.762, -4.130]; t=4.903, df=87; P= <0.0001. The same comparison, moreover, using the three-class Otsu method revealed statistically significant differences: difference between means= -4.220 ± 0.8880; 95% CI: [-5.985, -2.455]; t= 4.752, df= 87; P= <0.0001. The comparison between SC and Sham ES 6W groups was statistically significant using two-class but not three-class Otsu method. The difference between SC and Sham ES 8W groups was statistically significant using two-class Otsu method; difference between means= -4.651 ± 1.248; 95% CI: [-7.126, -2.177]; t= 3.728, df= 103; P= 0.0003, and statistically significant using three-class Otsu; difference between means= -2.724 ± 0.7930; 95% CI: [-4.296, -1.151]; t= 3.435, df= 103; P= 0.0009. (Figure 41).

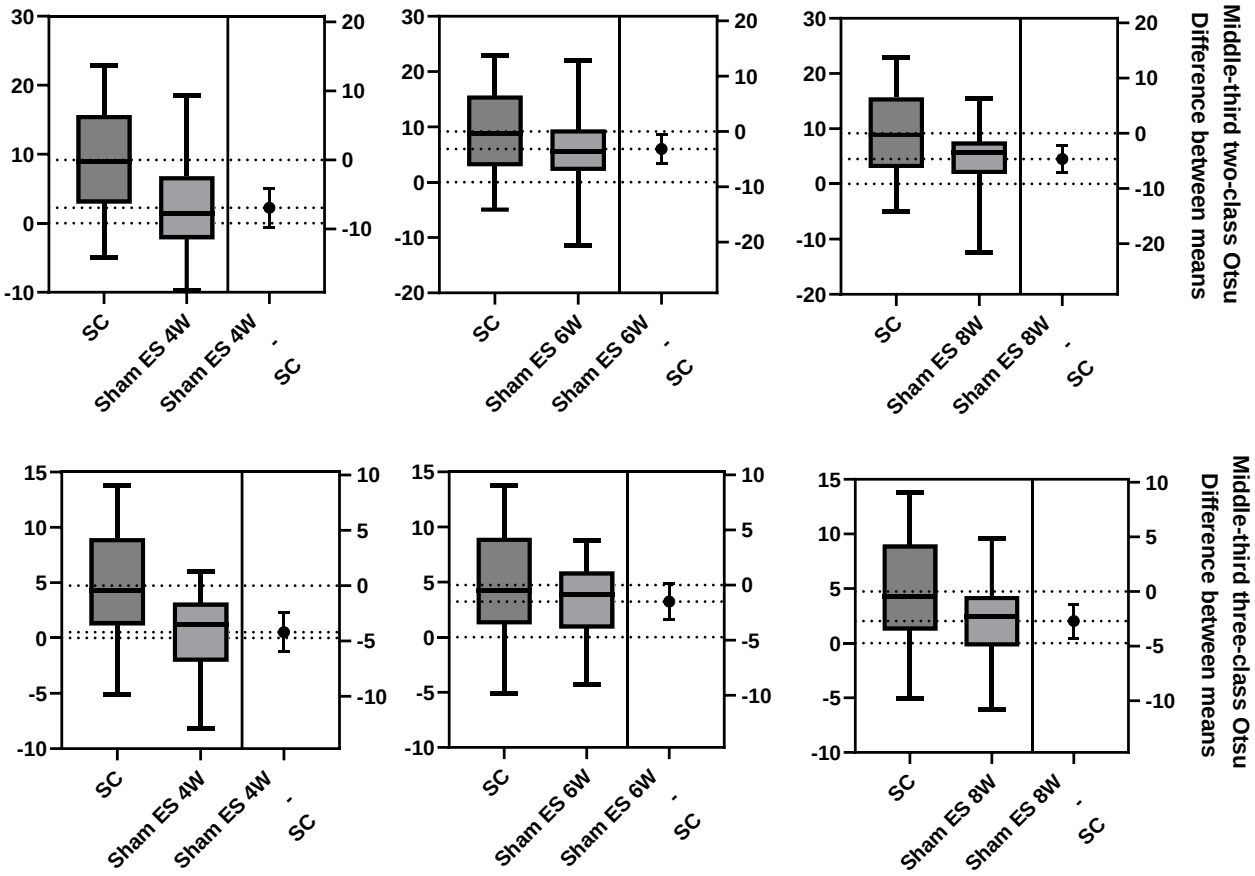



Figure 41. Mean difference in middle-third ground substance content between the sedentary control and sham groups at the three timepoints, measured using two-class and three-class Otsu thresholding methods; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

***Ground substance with mineralized fibrocartilage excluded - normalized data***

A statistically significant difference between SC and Sham ES 4W groups, using three-class Otsu method and analyzing tendon middle-third excluding areas of mineralized fibrocartilage; difference between means= -2.479 ± 0.4740; 95% CI: [-3.421, -1.537]; t= 5.231, df= 87; P= <0.0001. A statistically significant difference between SC and Sham ES 6W groups was found; difference between means= -1.257 ± 0.4165; 95% CI: [-2.083, -0.4301]; t= 3.017, df= 99; P= 0.0032. Furthermore, a statistically significant difference, was identified between SC and Sham ES 8W groups; difference between means= -1.532 ± 0.4181; 95% CI: [-2.361, -0.7025]; t= 3.664, df= 103; P= 0.0004. (Figure 45).

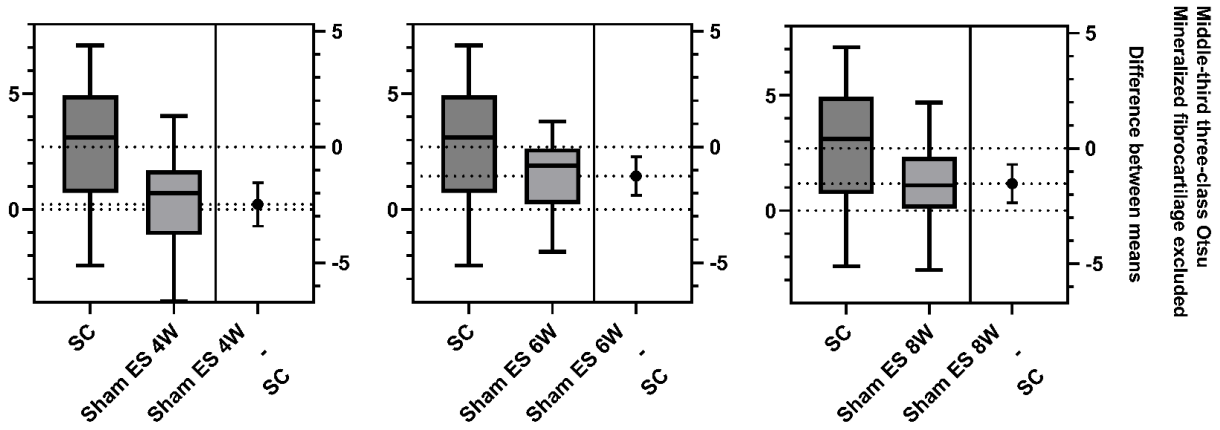



Figure 45. Mean difference in middle-third ground substance content, with mineralized fibrocartilage excluded, between sedentary control and sham groups, and between sham and stimulation groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

Comparisons between Sham ES 4W and ES 4W groups, Sham ES 6W and ES 6W, and Sham ES 8W groups and ES 8W groups, excluding areas of mineralized fibrocartilage and analyzing middle-third of tendons using three-class Otsu method, indicated non-statistically significant differences between groups.

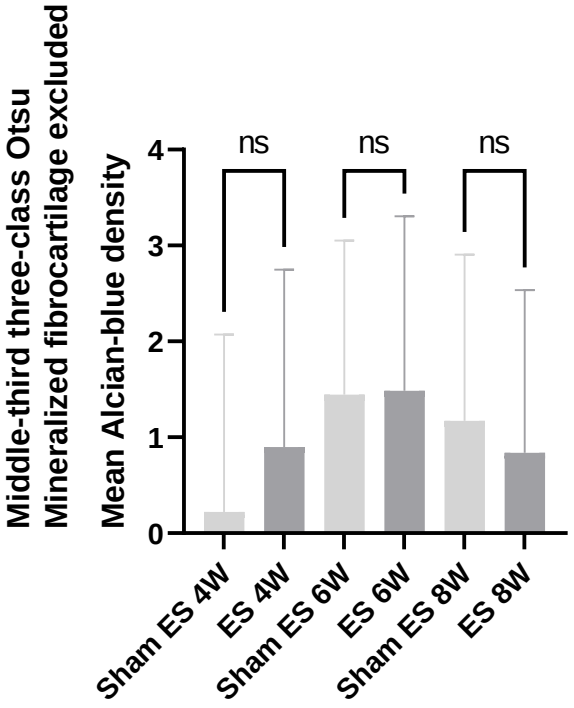



Figure 46. Mean difference in middle-third ground substance content, with mineralized fibrocartilage excluded, within sham groups, and within stimulation groups, at the three experimental timepoints, measured using three-class Otsu thresholding method; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. $P<0.05$. Y-axis represents pixel density.

***Tendon lower third***

Statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and Sham ES 4W groups in the lower-third of tendons; difference between medians= -9.390; Hodges-Lehmann= -8.440; SC= 13.20, n= 47; Sham ES 4W= 3.810, n= 43; U= 298; P= <0.0001. Moreover, a statistically significant reduction in Alcian-blue density (two-class Otsu) was found between SC and Sham ES 6W groups: difference between medians= -4.790; Hodges-Lehmann= -4.240; SC= 13.20, n= 47; Sham ES 6W= 8.410, n= 55; U= 891.5; P= 0.0068. Furthermore, a statistically significant difference in Alcian-blue density (two-class Otsu) between SC and Sham ES 8W groups was found; difference between medians= -7.280; Hodges-Lehmann= -5.980; SC= 13.20, n= 47; Sham ES 8W= -5.980, n= 59; U= 753; P= <0.0001 (Figure 47).

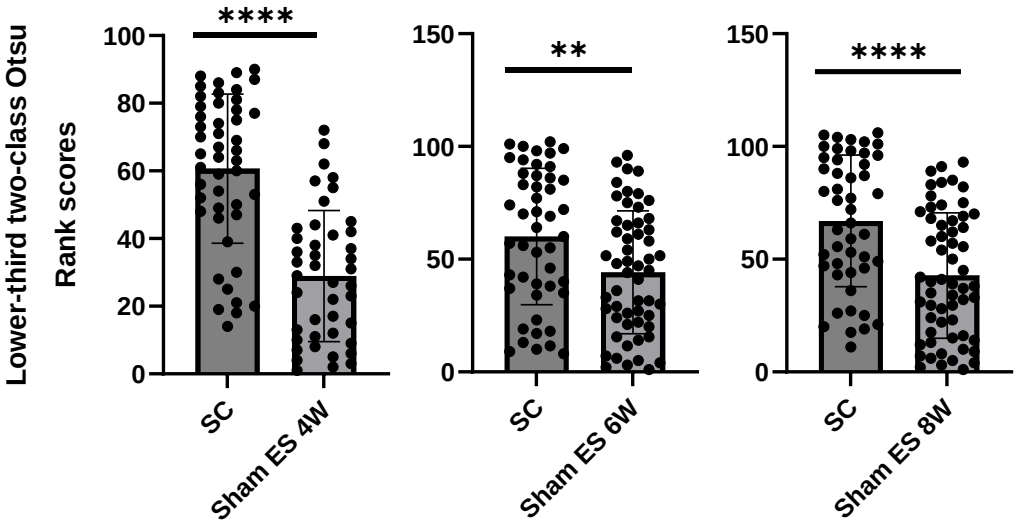



Figure 47. Rank score difference in lower-third ground substance content between sedentary control and sham groups at the three timepoints, measured using two-class Otsu thresholding method; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

Three-class Otsu Alcian-blue density analysis of lower-third of tendons showed statistically significant difference between SC and Sham ES 4W groups; difference between medians= -2.830; Hodges-Lehmann= -2.930; SC= 3.970, n= 47; Sham ES 4W= 1.140, n= 43; U= 299; P= <0.0001. Statistically significant difference in Alcian-blue density (three-class Otsu) was found between SC and Sham ES 6W groups: difference between medians= -0.9200; Hodges-Lehmann= -1.290; SC= 3.970, n= 47; Sham ES 6W= 3.050, n= 55; U= 973; P= 0.0317. Moreover, a statistically significant difference in Alcian-blue density (three-class Otsu) between SC and Sham ES 8W groups was found; difference between medians= -2.140; Hodges-Lehmann= -2.130; SC= 3.970, n= 47; Sham ES 8W= 1.830, n= 59; U= 767; P= <0.0001 (Figure 49).

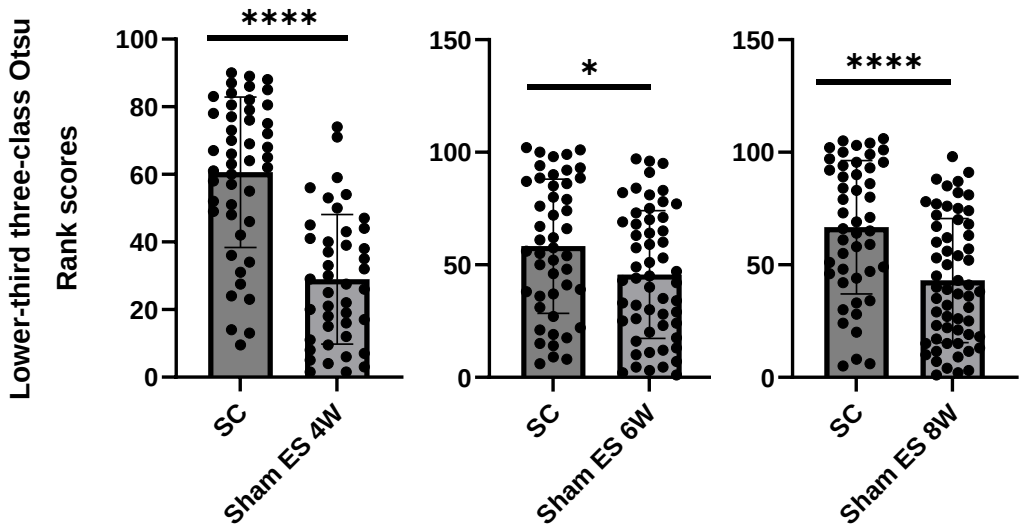



Figure 49. Rank score difference in lower-third ground substance content between sedentary control and sham groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

The differences between Sham ES 4W and ES 4W, Sham ES 6W and ES 6W, and Sham ES 8W and Sham ES 8W were all non-statistically significant (Figure 51).

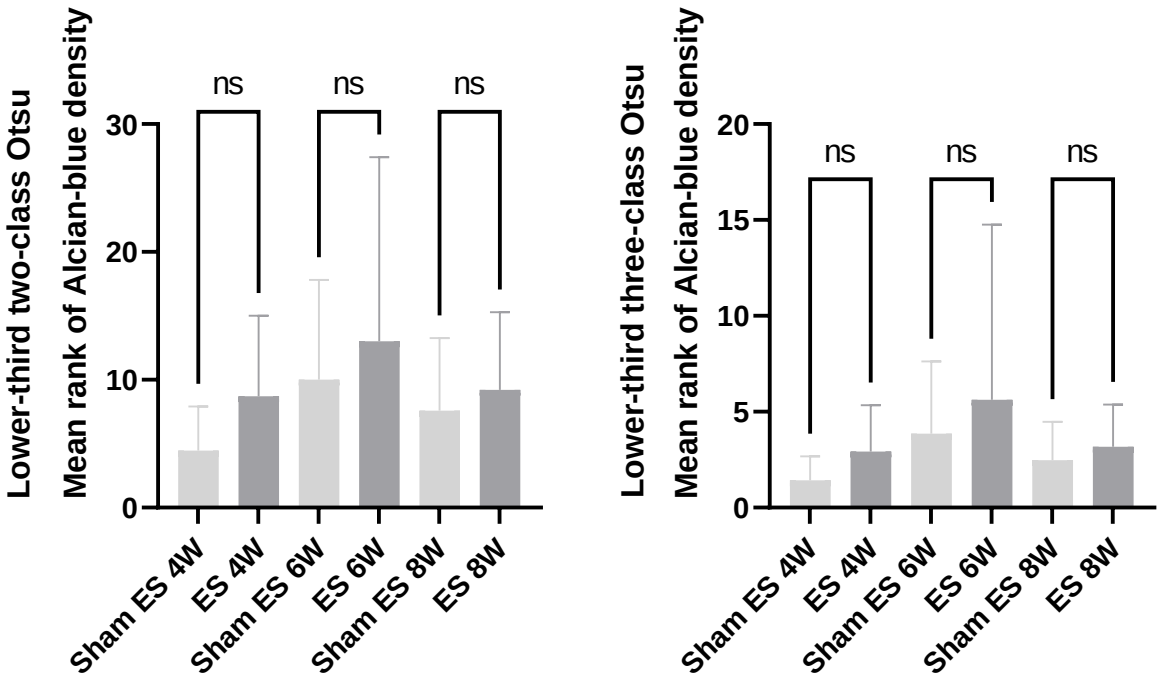



Figure 51. Mean rank score difference in lower-third ground substance content within sham, and within stimulation groups, at the three timepoints, measured using two-class and three-class Otsu thresholding methods; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. $P<0.05$. Y-axis represents pixel density.

***Ground substance with mineralized fibrocartilage excluded***

Comparison between SC and Sham ES 4W groups, using the three-class Otsu method and with areas of mineralized fibrocartilage excluded, showed a statistically significant difference; difference between medians= -2.880; Hodges-Lehmann= -2.520; SC= 3.640, n= 47; Sham ES 4W= 0.7600, n= 43; U= 262.5; P= <0.0001. Additionally, SC and Sham ES 6W group difference was statistically significant; difference between medians= -2.120; Hodges-Lehmann= -1.690; SC= 3.640, n= 47; Sham ES 6W= 1.520, n= 55; U= 691; P= <0.0001. A statistically significant difference, furthermore, was found between SC and Sham ES 8W groups; difference between medians= -2.010; Hodges-Lehmann= -1.730; SC= 3.640, n= 47; Sham ES 8W= 1.630, n= 59; U= 727.5; P= <0.0001 (Figure 54).

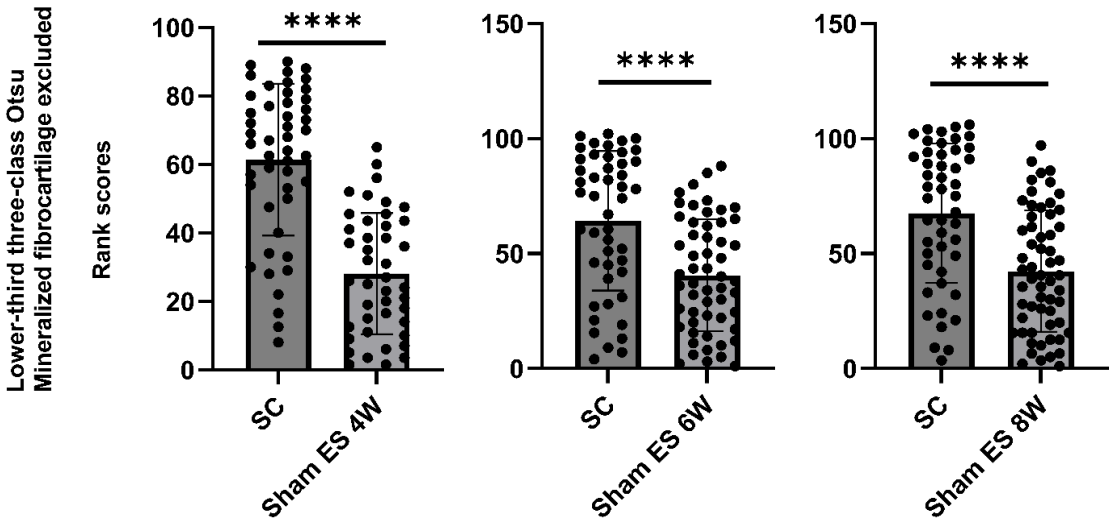



Figure 54. Rank score difference in lower-third ground substance content, with mineralized fibrocartilage excluded, between sedentary control and sham groups at the three timepoints, measured using three-class Otsu thresholding method; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

Using three-class Otsu method and including tendon lower-third but excluding areas of mineralized fibrocartilage, between-group comparison revealed non-statistically significant difference between any of the groups, except for between Sham ES 4W vs ES 4W; mean rank diff.= 75.55; adjusted P= 0.0018; H= 36.40, df= 5, P= <0.0001; (Figure 56).

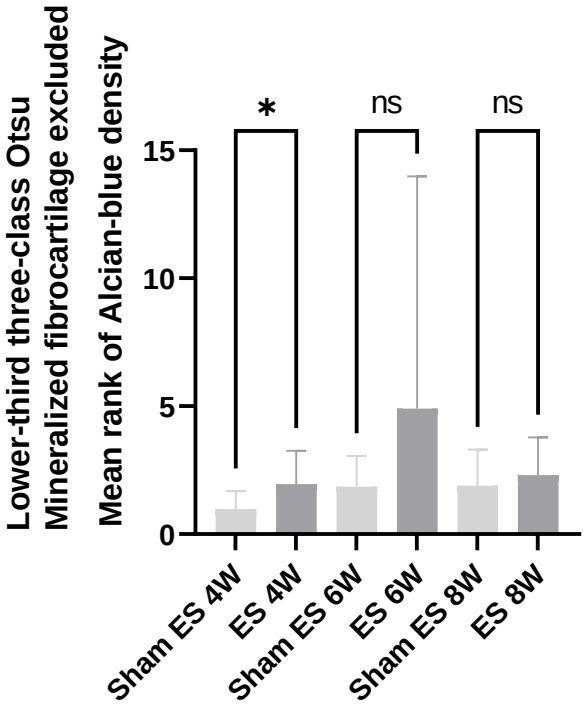



Figure 56. Mean rank score difference in lower-third ground substance content, with mineralized fibrocartilage excluded, within sham groups, and within stimulation groups, at the three experimental timepoints, measured using three-class Otsu thresholding method; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

***Tendon lower-third ground substance - normalized data***

A statistically significant difference was found between SC and Sham ES 4W groups in the two-class Otsu analysis: difference between means= -18.26 ± 2.624; 95% CI: [-23.48, -13.05]; t= 6.960, df= 88; P= <0.0001. The same comparison, moreover, using the three-class Otsu method revealed statistically significant differences: difference between means= -8.711 ± 1.281; 95% CI: [-11.26, -6.165]; t= 6.801, df= 88; P= <0.0001. The comparison between SC and Sham ES 6W groups showed statistically significant differences using both two-class and three-class Otsu methods. The difference between SC and Sham ES 8W groups was statistically significant using two-class Otsu method; difference between means= -12.15 ± 2.541; 95% CI: [-17.19, -7.109]; t= 4.780, df= 104; P= <0.0001, and three-class Otsu; difference between means= -5.517 ± 1.185; 95% CI: [-7.866, -3.168]; t= 4.657, df= 104; P= <0.0001. (Figure 52).

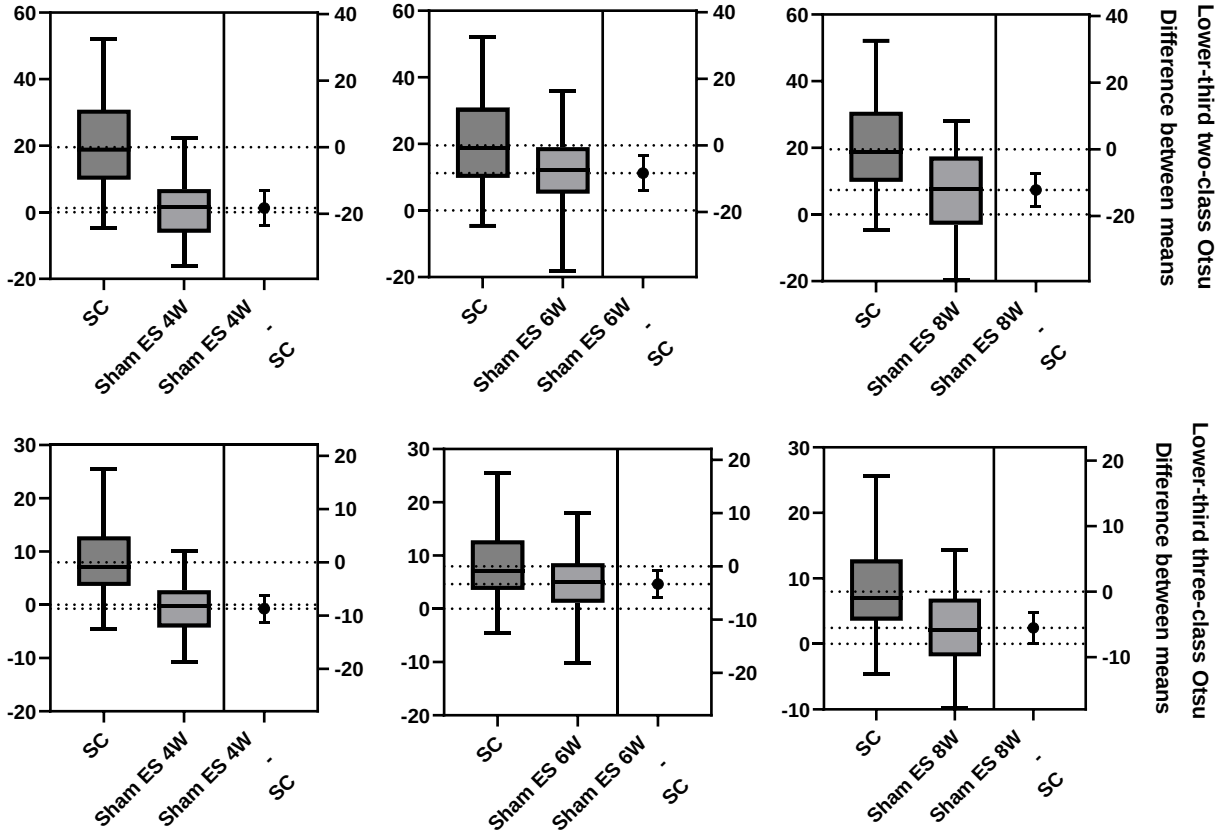



Figure 52. Mean difference in lower-third ground substance content between the sedentary control and sham groups at the three timepoints, measured using two-class and three-class Otsu thresholding methods; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

***Ground substance with mineralized fibrocartilage excluded - normalized data***

A statistically significant difference was found between SC and Sham ES 4W groups in the two-class Otsu analysis: difference between means= -0.4016 ± 0.05145; 95% CI: [-0.5039, -0.2994]; t= 7.806, df= 88; P= <0.0001. The comparison between SC and Sham ES 6W groups using three-class Otsu method was statistically significant; difference between means= -0.2329 ± 0.04909; 95% CI: [-0.3303, -0.1355]; t= 4.745, df= 100; P= <0.0001. The difference between SC and Sham ES 8W groups was statistically significant using three-class Otsu method; difference between means= -0.2371 ± 0.04968; 95% CI: [-0.3356, -0.1386]; t= 4.773, df= 104; P= <0.0001 (Figure 57).

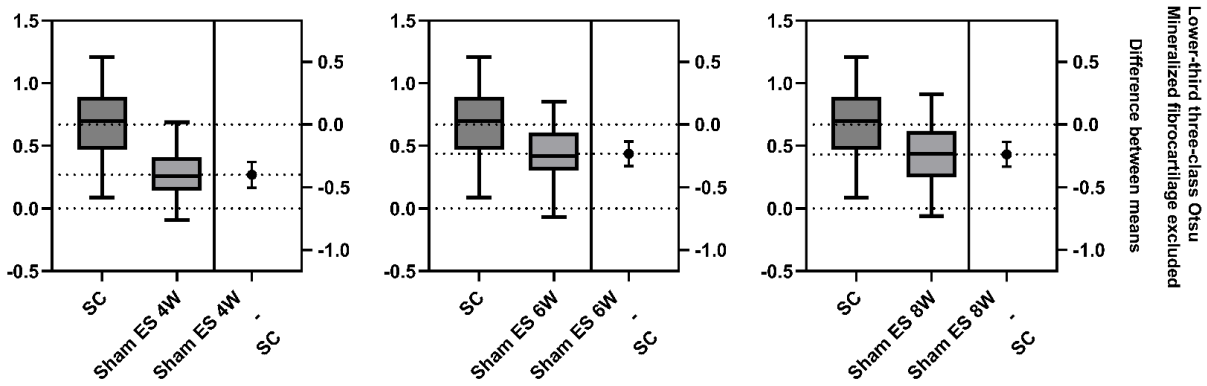



Figure 57. Mean difference in lower-third ground substance content, with mineralized fibrocartilage excluded, between sedentary control and sham groups measured using three-class Otsu thresholding method; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

Comparisons between Sham ES 4W, Sham ES 6W and Sham ES 8W groups, and ES 4W, ES 6W and ES 8W groups, excluding areas of mineralized fibrocartilage and analyzing lower-third of tendons using three-class Otsu method, indicated statistically significant differences between groups; Sham ES 4W vs ES 4W: mean diff.= 0.1894; 95% CI: [0.04732, 0.3314], P= 0.0022.; F=8.429; P= <0.0001; factors (Sham ES 4W, Sham ES 6W, Sham ES 8W, ES 4W, ES 6W, and ES 8W); levels= 6; between-subjects factors; All the other groups did not show statistically significant differences (Figure 59).

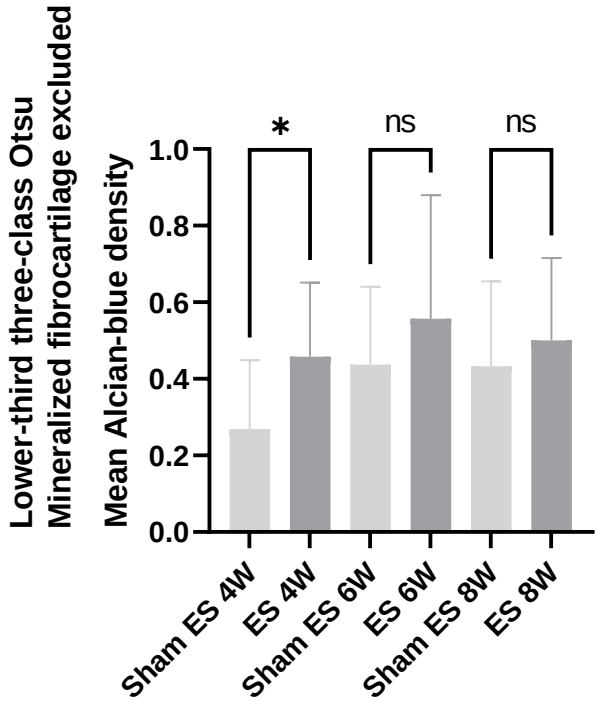



Figure 59. Mean difference in lower-third ground substance content, with mineralized fibrocartilage excluded, within sham groups, and within stimulation groups, at the three experimental timepoints, measured using three-class Otsu thresholding method; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P<0.05. Y-axis represents pixel density.

## Cellularity

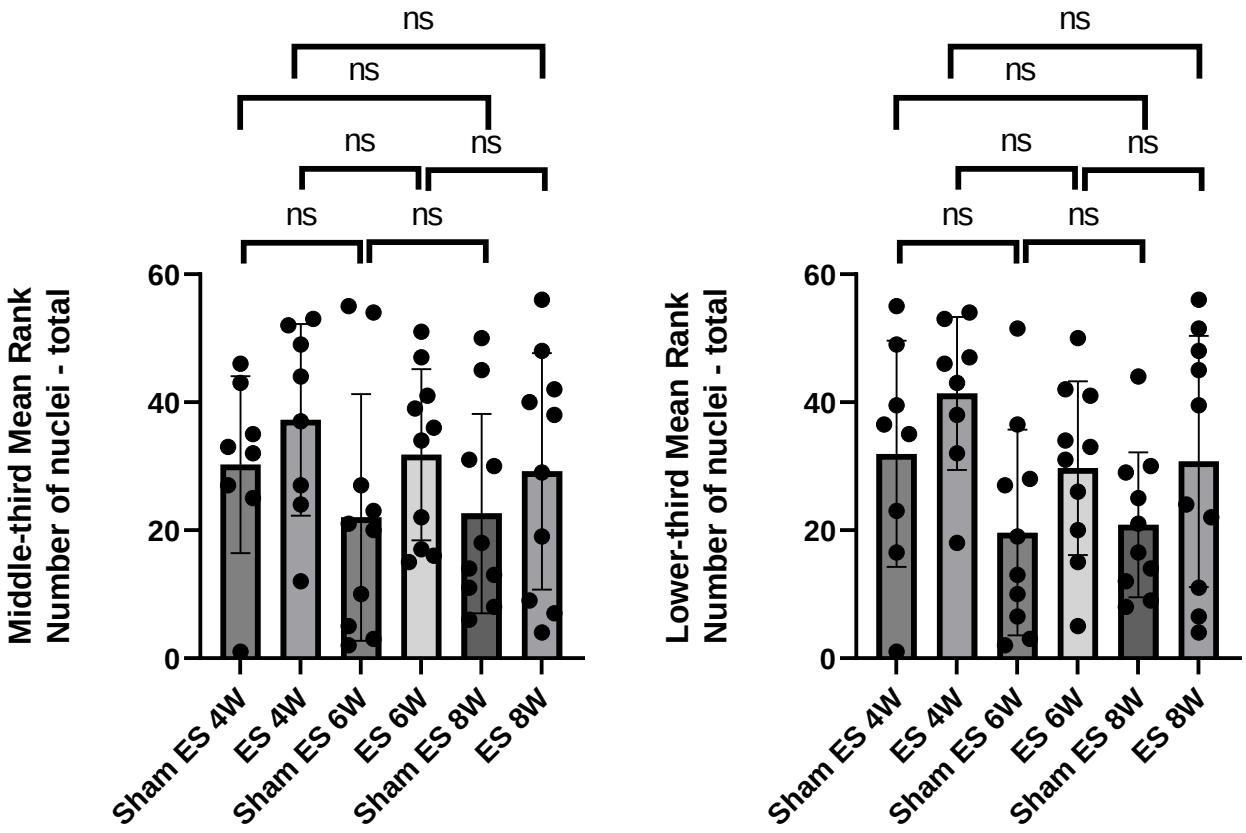



Figure 60. Mean rank difference in nuclei number between the three timepoints within the sham and stimulation groups in middle and lower-thirds analyses; scale bars represent 95% confidence interval; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. $P<0.05$

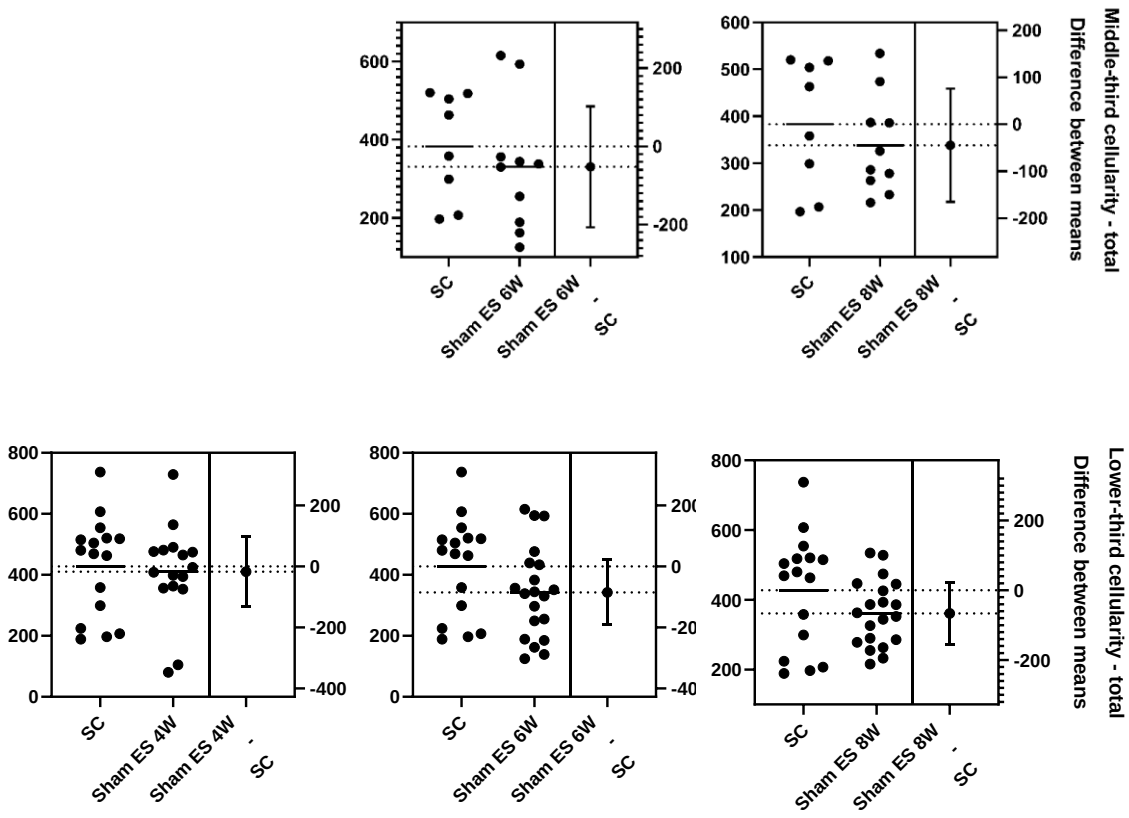



Figure 61. Mean difference in nuclei number between the sedentary control and sham groups at the three timepoints in middle and lower-thirds analyses; scale bars represent 95% confidence interval; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P<0.05.

## Circularity

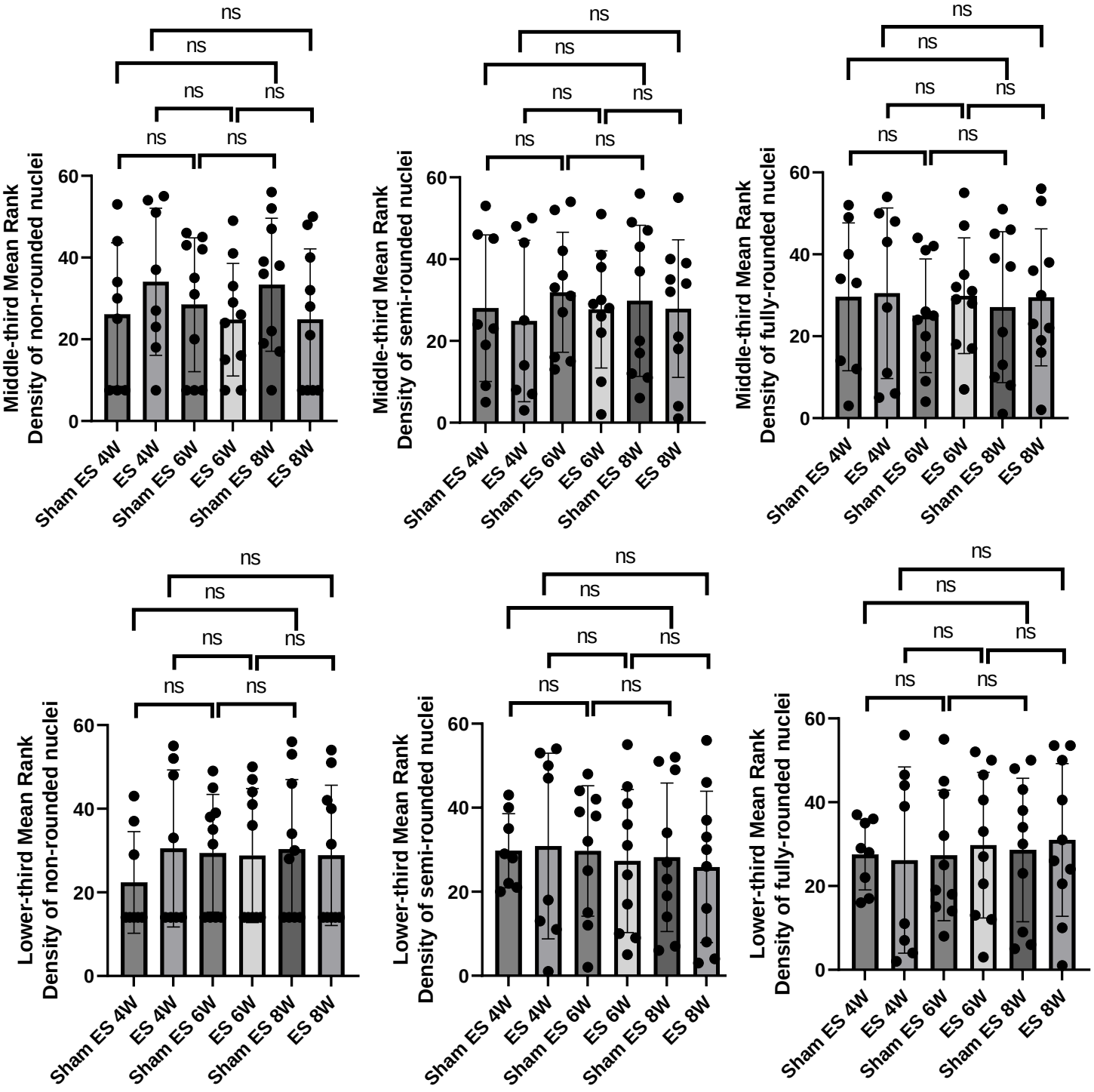



Figure 62. Mean rank difference in nuclei roundedness between the three timepoints of the sham and stimulation groups in middle and lower-thirds analyses; scale bars represent 95% confidence interval; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P<0.05

## Fiber alignment

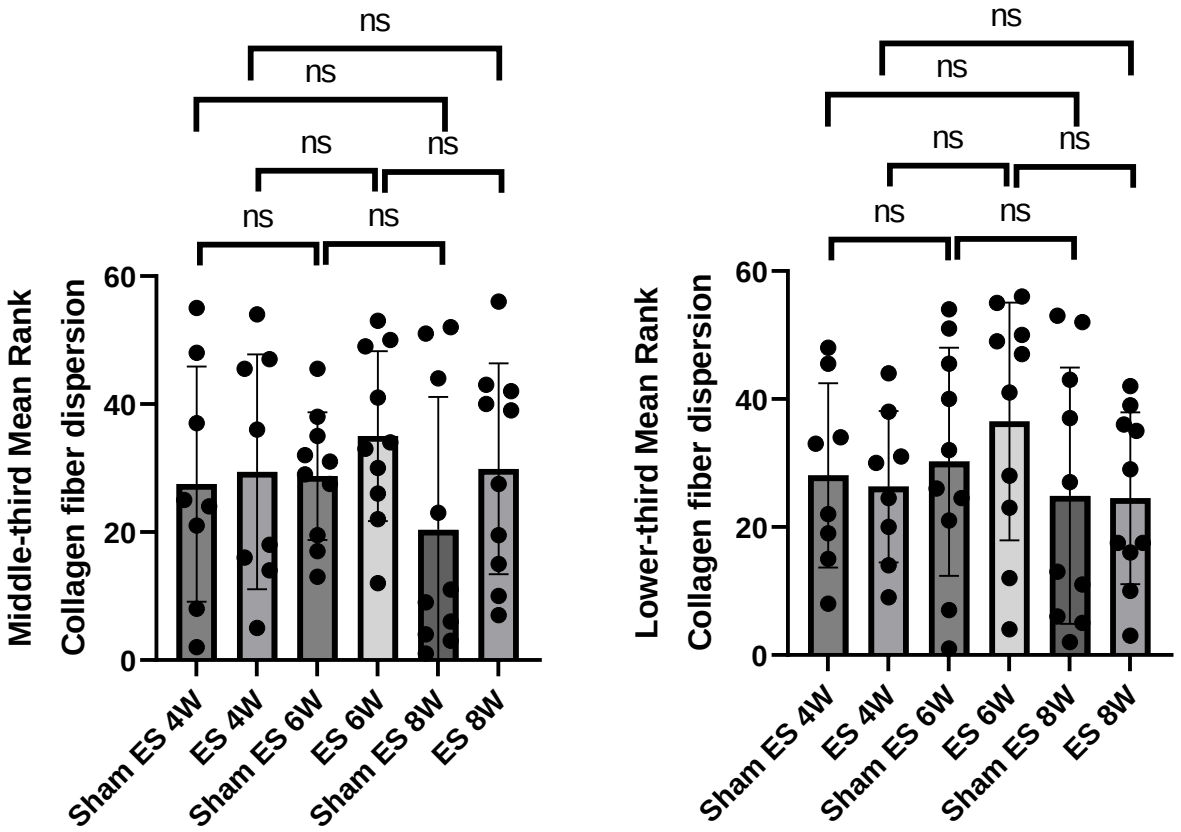



Figure 63. Mean rank difference in fiber dispersion within the sham group, and within the stimulation group at the three timepoints in middle and lower-thirds analyses; scale bars represent 95% confidence interval; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. $P<0.05$

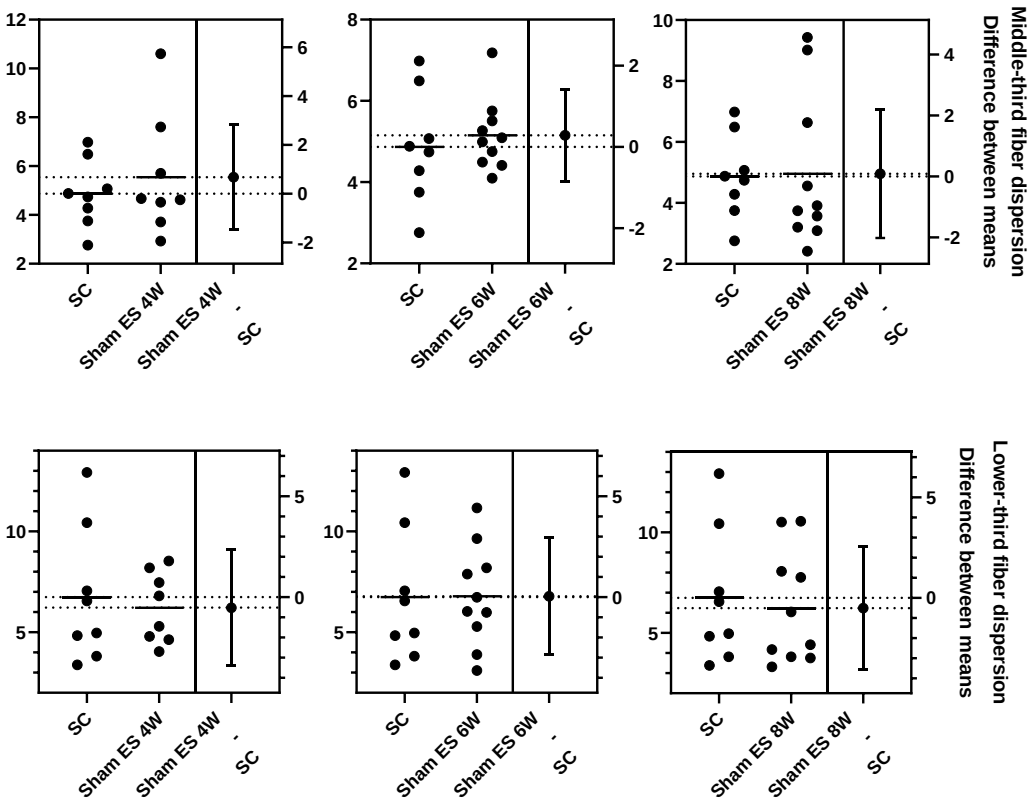
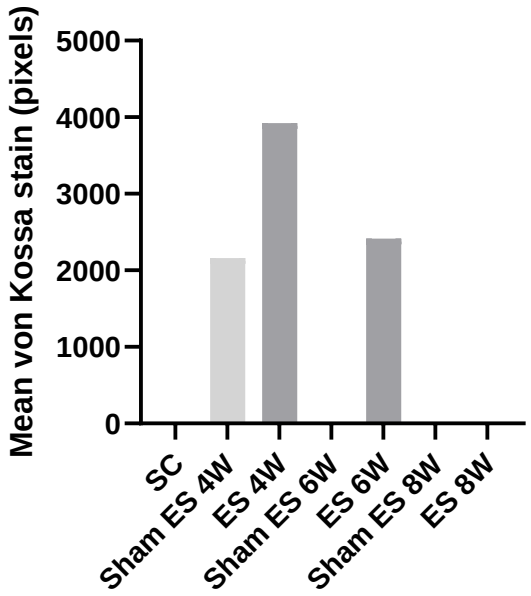



Figure 64. Mean difference in fiber dispersion between the sedentary control and sham groups at the three timepoints in middle and lower-thirds analyses; scale bars represent 95% confidence interval; SC, sedentary control; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively. P<0.05

## Calcification



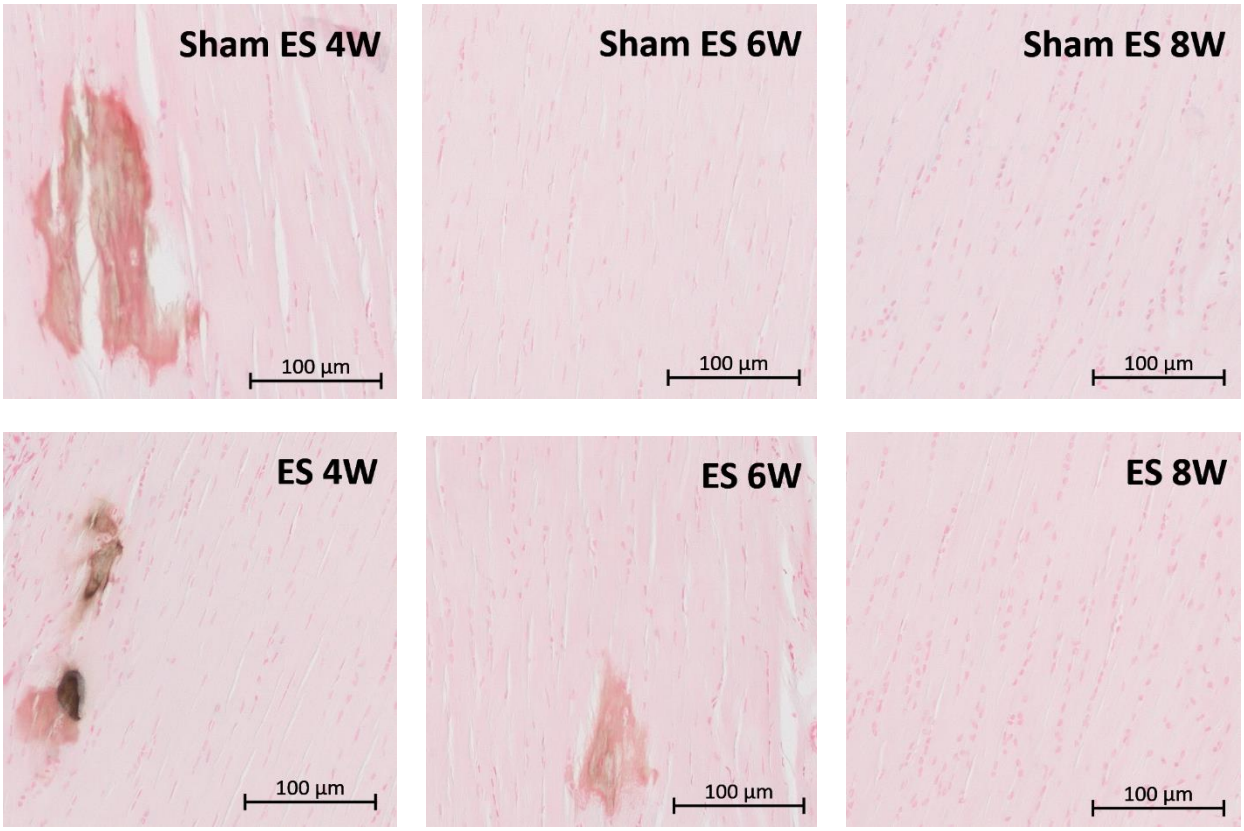




Figure 65. Mean pixels of von-Kossa-stained samples from sham and stimulation groups; Sham ES 4W, 6W and 8W, sham stimulation for 4, 6 and 8 weeks, respectively; ES 4W, ES 6W and ES 8W, electrical stimulation for 4, 6 and 8 weeks, respectively; dimensions 700×700 pixels; magnification = 10x; scale bar= 100 µm

**Histological staining protocols**

Modified regressive Hematoxylin and Eosin staining protocol

1. Xylene: 5 min X 3
2. Ethanol 100%: 5 min
3. Ethanol 95%: 5 min
4. Ethanol 75%: 5 min
5. Distilled water: 5 min
6. Eosin stain: 30 sec
7. Overnight differentiation in distilled water
8. Acetic acid 1%: 15 min
9. Hematoxylin stain: 8 min
10. Distilled water: rinse X 3
11. Distilled water: 5 min
12. Running water: 30 min
13. Distlled water rinse
14. Acid-alcohol: 30 sec
15. Running water: 5 min
16. Distilled water rinse
17. Microscope assessment
18. Bluing reagent: 0.1% sodium bicarbonate solution: 30 sec
19. Running water: 5 min
20. Distilled water rinse
21. Ethanol 75%: 5 min
22. Ethanol 95%: 5 min
23. Ethanol 100%: 5 min X 2
24. Xylene: 30 min X 3
25. Mounting: ROTI® Histokitt (Carl Roth)

Von Kossa and Alcian blue staining protocols

1. Xylene: 5 min X 3
2. Ethanol 100%: 5 min
3. Ethanol 95%: 5 min
4. Ethanol 75%: 5 min
5. Distilled water: 5 min
6. Von Kossa reagent 1 (silver nitrate solution): 5 min under exposure to light (25 Watt incandescent lamp placed about 35 cm away from the samples)
7. Running water: 3 min
8. Von Kossa reagent 2 (sodium thiosulfate solution): 3 min
9. Running water: 1 min
10. Distilled water rinse
11. Alcian blue stain: 30 min
12. Running water: 5 min
13. Distilled water rinse
14. Nuclear fast red stain (Nuclear fast red-aluminium sulfate solution 0.1%): 7 min
15. Distilled water rinse
16. Ethanol 75%: 5 min
17. Ethanol 95%: 5 min
18. Ethanol 100%: 5 min X 2
19. Xylene: 30 min X 3
20. Mounting: ROTI® Histokitt (Carl Roth)

**Polymerase chain reaction supplementary data**

**RNA quantification results and relative expression level of target genes**

| 260/280 | Sample ID | ng/µL | RNA µg/µL | uL RNA (=150ng RNA) | $H_2O$ |
|---|---|---|---|---|---|
| 1.91 | A1 | 59.90 | 0.060 | 2.50 | 12.50 |
| 1.91 | A3 | 59.01 | 0.059 | 2.54 | 12.46 |
| 1.92 | A5 | 22.12 | 0.022 | 6.78 | 8.22 |
| 1.95 | A7 | 34.09 | 0.034 | 4.40 | 10.60 |
| 2.00 | A9 | 75.17 | 0.075 | 2.00 | 13.00 |
| 1.95 | B1 | 37.29 | 0.037 | 4.02 | 10.98 |
| 1.89 | B3 | 35.25 | 0.035 | 4.26 | 10.74 |
| 1.91 | B5 | 34.35 | 0.034 | 4.37 | 10.63 |
| 1.90 | B7 | 49.76 | 0.050 | 3.01 | 11.99 |
| 2.02 | B9 | 52.99 | 0.053 | 2.83 | 12.17 |
| 1.85 | C1 | 33.94 | 0.034 | 4.42 | 10.58 |
| 1.94 | C3 | 43.89 | 0.044 | 3.42 | 11.58 |
| 1.86 | C5 | 44.19 | 0.044 | 3.39 | 11.61 |
| 1.97 | C7 | 34.73 | 0.035 | 4.32 | 10.68 |
| 1.93 | C9 | 87.37 | 0.087 | 1.72 | 13.28 |
| 2.01 | D2 | 20.88 | 0.021 | 7.18 | 7.82 |
| 2.03 | D4 | 58.98 | 0.059 | 2.54 | 12.46 |
| 2.04 | D6 | 46.61 | 0.047 | 3.22 | 11.78 |
| 2.01 | D8 | 82.83 | 0.083 | 1.81 | 13.19 |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | ki-67 | 28.79271 | | 3.34 | | | -0.47 | 1.386 | 0.437 | 0.537 |
| | | A3 | GAPDH | 27.03984 | | A3 | ki-67 | 33.37111 | | 6.33 | | | 2.52 | 0.175 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | ki-67 | 34.69514 | | 5.38 | | | 1.57 | 0.337 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | ki-67 | 35.18807 | | 6.33 | | | 2.51 | 0.175 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | ki-67 | 34.99658 | | 6.97 | | | 3.16 | 0.112 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | ki-67 | 34.43713 | | 7.43 | | | 3.61 | 0.082 | 0.992 | 1.234 |
| | | B3 | GAPDH | 28.48671 | | B3 | ki-67 | 37.54718 | | 9.06 | | | 5.24 | 0.026 | | |
| | | B5 | GAPDH | 31.86955 | | B5 | ki-67 | 34.59336 | | 2.72 | | | -1.09 | 2.132 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | ki-67 | 31.1037 | | 2.47 | | | -1.34 | 2.535 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | ki-67 | 33.57014 | | 6.26 | | | 2.44 | 0.184 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | ki-67 | 32.0667 | | 3.45 | | | -0.37 | 1.292 | 0.846 | 0.651 |
| | | C3 | GAPDH | 28.66757 | | C3 | ki-67 | 34.46207 | | 5.79 | | | 1.98 | 0.254 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | ki-67 | 30.69383 | | 3.28 | | | -0.53 | 1.446 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | ki-67 | 29.11649 | | 3.55 | | | -0.27 | 1.204 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | ki-67 | 36.48237 | | 8.67 | | | 4.85 | 0.035 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D4 | GAPDH | 28.78433 | | D4 | ki-67 | 30.98717 | | | 2.20 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | ki-67 | 30.90082 | | | 2.31 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | ki-67 | 36.14339 | | | 6.93 | 3.82 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | | | A1 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | A3 | GAPDH | | | A3 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | A5 | GAPDH | | | A5 | p16 | | | 0.00 | | | -6.61 | 97.449 | 78.235 | 42.964 |
| | | A7 | GAPDH | 28.85837 | | A7 | p16 | 35.0023 | | 6.14 | | | -0.46 | 1.378 | | |
| | | A9 | GAPDH | | | A9 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | Group 2R | B1 | GAPDH | | | B1 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | B3 | GAPDH | | | B3 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | B5 | GAPDH | 31.86955 | | B5 | p16 | 41.41182 | | 9.54 | | | 2.94 | 0.131 | 77.986 | 43.522 |
| | | B7 | GAPDH | | | B7 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | B9 | GAPDH | | | B9 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | Group 4R | C1 | GAPDH | | | C1 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | C3 | GAPDH | 28.66757 | | C3 | p16 | 33.15614 | | 4.49 | | | -2.12 | 4.341 | | |
| | | C5 | GAPDH | | | C5 | p16 | | | 0.00 | | | -6.61 | 97.449 | 59.339 | 52.207 |
| | | C7 | GAPDH | | | C7 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | p16 | 41.46399 | | 13.65 | | | 7.04 | 0.008 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | 27.87852 | | D2 | p16 | 39.97389 | | | 12.10 | | | | | |
| | | D4 | GAPDH | | | D4 | p16 | | | | 0.00 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | p16 | 42.91961 | | | 14.33 | | | | | |
| | | D8 | GAPDH | | | D8 | p16 | | | | 0.00 | 6.61 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | p21 | 36.56406 | | 11.12 | | | 9.20 | 0.002 | | |
| | | A3 | GAPDH | 27.03984 | | A3 | p21 | 36.67815 | | 9.64 | | | 7.72 | 0.005 | | |
| | | A5 | GAPDH | | | A5 | p21 | | | 0.00 | | | -1.92 | 3.773 | 1.511 | 2.066 |
| | | A7 | GAPDH | 28.85837 | | A7 | p21 | 42.93256 | | 14.07 | | | 12.16 | 0.000 | | |
| | | A9 | GAPDH | | | A9 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | p21 | 37.82612 | | 10.82 | | | 8.90 | 0.002 | | |
| | | B3 | GAPDH | | | B3 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | | B5 | GAPDH | | | B5 | p21 | | | 0.00 | | | -1.92 | 3.773 | 2.266 | 2.064 |
| | | B7 | GAPDH | 28.62938 | | B7 | p21 | 37.71732 | | 9.09 | | | 7.17 | 0.007 | | |
| | | B9 | GAPDH | | | B9 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | Group 4R | C1 | GAPDH | | | C1 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | | C3 | GAPDH | | | C3 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | | C5 | GAPDH | | | C5 | p21 | | | 0.00 | | | -1.92 | 3.773 | 3.021 | 1.683 |
| | | C7 | GAPDH | | | C7 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | p21 | 36.4269 | | 8.61 | | | 6.70 | 0.010 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | | | D2 | p21 | | | | 0.00 | | | | | |
| | | D4 | GAPDH | | | D4 | p21 | | | | 0.00 | | | | | |
| | | D6 | GAPDH | | | D6 | p21 | | | | 0.00 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | p21 | 36.8735 | | | 7.66 | 1.92 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | p53 | 35.59641 | | 10.15 | | | -0.50 | 1.418 | 3.874 | 4.580 |
| | | A3 | GAPDH | 27.03984 | | A3 | p53 | 35.54187 | | 8.50 | | | -2.15 | 4.439 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | p53 | 39.15534 | | 9.84 | | | -0.81 | 1.752 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | p53 | 41.99088 | | 13.13 | | | 2.48 | 0.179 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | p53 | 35.14136 | | 7.12 | | | -3.53 | 11.580 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | p53 | 36.11284 | | 9.10 | | | -1.55 | 2.928 | 29.733 | 53.199 |
| | | B3 | GAPDH | 28.48671 | | B3 | p53 | 38.77776 | | 10.29 | | | -0.36 | 1.285 | | |
| | | B5 | GAPDH | 31.86955 | | B5 | p53 | 35.74723 | | 3.88 | | | -6.77 | 109.495 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | p53 | 36.89615 | | 8.27 | | | -2.39 | 5.226 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | p53 | 36.9022 | | 8.28 | | | -2.37 | 5.172 | 3.012 | 3.456 |
| | | C3 | GAPDH | 28.66757 | | C3 | p53 | 36.56816 | | 7.90 | | | -2.75 | 6.736 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | p53 | 40.37586 | | 14.81 | | | 4.15 | 0.056 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | p53 | 42.00975 | | 14.20 | | | 3.54 | 0.086 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D6 | GAPDH | 28.58866 | | D6 | p53 | 42.94649 | | | 14.36 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | p53 | 36.15712 | | | 6.95 | 10.65 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | TNMD | 33.53322 | | 8.09 | | | 0.62 | 0.651 | | |
| | | A3 | GAPDH | 27.03984 | | A3 | TNMD | 33.20132 | | 6.16 | | | -1.30 | 2.469 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | TNMD | 36.15384 | | 6.84 | | | -0.62 | 1.540 | 1.369 | 0.917 |
| | | A7 | GAPDH | 28.85837 | | A7 | TNMD | 35.36386 | | 6.51 | | | -0.96 | 1.945 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | TNMD | 37.54929 | | 9.53 | | | 2.06 | 0.240 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | TNMD | 34.50532 | | 7.49 | | | 0.03 | 0.980 | | |
| | | B3 | GAPDH | 28.48671 | | B3 | TNMD | 34.06559 | | 5.58 | | | -1.89 | 3.697 | | |
| | | B5 | GAPDH | 31.86955 | | B5 | TNMD | 34.70494 | | 2.84 | | | -4.63 | 24.760 | 7.020 | 10.072 |
| | | B7 | GAPDH | 28.62938 | | B7 | TNMD | 33.80657 | | 5.18 | | | -2.29 | 4.884 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | TNMD | 35.14567 | | 7.83 | | | 0.37 | 0.776 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | TNMD | 34.17124 | | 5.55 | | | -1.91 | 3.770 | | |
| | | C3 | GAPDH | 28.66757 | | C3 | TNMD | 34.98902 | | 6.32 | | | -1.14 | 2.210 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | TNMD | 35.64849 | | 8.24 | | | 0.77 | 0.585 | 1.696 | 1.378 |
| | | C7 | GAPDH | 25.56867 | | C7 | TNMD | 34.49539 | | 8.93 | | | 1.46 | 0.363 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | TNMD | 34.64517 | | 6.83 | | | -0.63 | 1.552 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D4 | GAPDH | 28.78433 | | D4 | TNMD | 36.17414 | | | 7.39 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | TNMD | 36.12954 | | | 7.54 | 7.47 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | COL1 | 29.45222 | | 4.00 | | | 0.39 | 0.766 | 3.410 | 2.470 |
| | | A5 | GAPDH | 29.31165 | | A5 | COL1 | 31.00254 | | 1.69 | | | -1.93 | 3.806 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | COL1 | 29.9769 | | 1.12 | | | -2.50 | 5.659 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | COL1 | 29.47959 | | 2.47 | | | -1.15 | 2.219 | 42.062 | 69.664 |
| | | B3 | GAPDH | 28.48671 | | B3 | COL1 | 28.04687 | | -0.44 | | | -4.06 | 16.666 | | |
| | | B5 | GAPDH | 31.86955 | | B5 | COL1 | 28.11206 | | -3.76 | | | -7.38 | 166.170 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | COL1 | 28.13363 | | -0.50 | | | -4.11 | 17.325 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | COL1 | 27.94723 | | 0.63 | | | -2.99 | 7.928 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | COL1 | 28.9666 | | 0.35 | | | -3.27 | 9.665 | 5.264 | 4.330 |
| | | C3 | GAPDH | 28.66757 | | C3 | COL1 | 28.91865 | | 0.25 | | | -3.37 | 10.324 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | COL1 | 30.11133 | | 2.70 | | | -0.92 | 1.889 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | COL1 | 28.1999 | | 2.63 | | | -0.99 | 1.983 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | COL1 | 30.13552 | | 2.32 | | | -1.30 | 2.458 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | 27.87852 | | D2 | COL1 | 34.96771 | | | 7.09 | | | | | |
| | | D4 | GAPDH | 28.78433 | | D4 | COL1 | 32.6948 | | | 3.91 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | COL1 | 30.45726 | | | 1.87 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | COL1 | 30.81798 | | | 1.61 | 3.62 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | COL3 | 30.76696 | | 5.32 | | | 2.83 | 0.140 | | |
| | | A3 | GAPDH | 27.03984 | | A3 | COL3 | 30.5454 | | 3.51 | | | 1.02 | 0.493 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | COL3 | 30.68809 | | 1.38 | | | -1.11 | 2.157 | 1.016 | 0.807 |
| | | A7 | GAPDH | 28.85837 | | A7 | COL3 | 30.77384 | | 1.92 | | | -0.57 | 1.484 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | COL3 | 30.81748 | | 2.79 | | | 0.31 | 0.807 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | COL3 | 31.09509 | | 4.08 | | | 1.60 | 0.330 | | |
| | | B3 | GAPDH | 28.48671 | | B3 | COL3 | 30.15251 | | 1.67 | | | -0.82 | 1.765 | | |
| | | B5 | GAPDH | 31.86955 | | B5 | COL3 | 30.14004 | | -1.73 | | | -4.21 | 18.570 | 4.620 | 7.817 |
| | | B7 | GAPDH | 28.62938 | | B7 | COL3 | 30.56591 | | 1.94 | | | -0.55 | 1.463 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | COL3 | 29.84025 | | 2.53 | | | 0.04 | 0.973 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | COL3 | 30.68336 | | 2.06 | | | -0.42 | 1.340 | | |
| | | C3 | GAPDH | 28.66757 | | C3 | COL3 | 30.82769 | | 2.16 | | | -0.33 | 1.253 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | COL3 | 30.88609 | | 3.48 | | | 0.99 | 0.503 | 0.854 | 0.478 |
| | | C7 | GAPDH | 25.56867 | | C7 | COL3 | 30.16292 | | 4.59 | | | 2.11 | 0.232 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | COL3 | 30.38554 | | 2.57 | | | 0.09 | 0.942 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | 27.87852 | | D2 | COL3 | 31.68917 | | | 3.81 | | | | | |
| | | D4 | GAPDH | 28.78433 | | D4 | COL3 | 31.84044 | | | 3.06 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | COL3 | 30.09009 | | | 1.50 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | COL3 | 30.78342 | | | 1.57 | 2.49 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | DCN | 32.47248 | | 7.02 | | | -0.04 | 1.027 | | |
| | | A3 | GAPDH | 27.03984 | | A3 | DCN | 31.73548 | | 4.70 | | | -2.37 | 5.160 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | DCN | 33.98621 | | 4.67 | | | -2.39 | 5.236 | 3.219 | 1.969 |
| | | A7 | GAPDH | 28.85837 | | A7 | DCN | 34.26943 | | 5.41 | | | -1.65 | 3.143 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | DCN | 34.4736 | | 6.45 | | | -0.61 | 1.528 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | DCN | 32.64434 | | 5.63 | | | -1.43 | 2.693 | | |
| | | B3 | GAPDH | 28.48671 | | B3 | DCN | 32.80717 | | 4.32 | | | -2.74 | 6.693 | | |
| | | B5 | GAPDH | 31.86955 | | B5 | DCN | 33.14062 | | 1.27 | | | -5.79 | 55.409 | 14.234 | 23.087 |
| | | B7 | GAPDH | 28.62938 | | B7 | DCN | 33.59093 | | 4.96 | | | -2.10 | 4.292 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | DCN | 33.319 | | 6.00 | | | -1.06 | 2.084 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | DCN | 33.60317 | | 4.98 | | | -2.08 | 4.229 | | |
| | | C3 | GAPDH | 28.66757 | | C3 | DCN | 32.96638 | | 4.30 | | | -2.76 | 6.794 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | DCN | 32.76944 | | 5.36 | | | -1.70 | 3.258 | 3.851 | 2.141 |
| | | C7 | GAPDH | 25.56867 | | C7 | DCN | 32.89359 | | 7.32 | | | 0.26 | 0.834 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | DCN | 32.82735 | | 5.01 | | | -2.05 | 4.141 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D4 | GAPDH | 28.78433 | | D4 | DCN | 36.51727 | | | 7.73 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | DCN | 34.01641 | | | 5.43 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | DCN | 37.23882 | | | 8.03 | 7.06 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibra | CT calibra | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | SCX | 36.72131 | | 11.27 | | | 18.47 | 0.000 | | |
| | | A3 | GAPDH | 27.03984 | | A3 | SCX | 38.11598 | | 11.08 | | | 18.27 | 0.000 | | |
| | | A5 | GAPDH | | | A5 | SCX | | | 0.00 | | | 7.20 | 0.007 | 0.001 | 0.003 |
| | | A7 | GAPDH | 28.85837 | | A7 | SCX | 36.30634 | | 7.45 | | | 14.64 | 0.000 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | SCX | 37.39471 | | 9.37 | | | 16.57 | 0.000 | | |
| | Group 2R | B1 | GAPDH | | | B1 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | | B3 | GAPDH | | | B3 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | | B5 | GAPDH | | | B5 | SCX | | | 0.00 | | | 7.20 | 0.007 | 0.007 | 0.000 |
| | | B7 | GAPDH | | | B7 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | | B9 | GAPDH | | | B9 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | SCX | 37.53539 | | 8.91 | | | 16.11 | 0.000 | | |
| | | C3 | GAPDH | | | C3 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | | C5 | GAPDH | | | C5 | SCX | | | 0.00 | | | 7.20 | 0.007 | 0.004 | 0.004 |
| | | C7 | GAPDH | 25.56867 | | C7 | SCX | 36.6939 | | 11.13 | | | 18.32 | 0.000 | | |
| | | C9 | GAPDH | | | C9 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | | | D2 | 43 | | | | 0.00 | | | | | |
| | | D4 | GAPDH | 28.78433 | | D4 | | | | | -28.78 | | | | | |
| | | D6 | GAPDH | | | D6 | | | | | 0.00 | | | | | |
| | | D8 | GAPDH | | | D8 | | | | | 0.00 | -7.20 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibr | CT calibr | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | MKX | 34.02941 | | 8.58 | | | 1.93 | 0.262 | 0.798 | 0.455 |
| | | A3 | GAPDH | 27.03984 | | A3 | MKX | 33.14282 | | 6.10 | | | -0.55 | 1.462 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | MKX | 36.41442 | | 7.10 | | | 0.45 | 0.731 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | MKX | 35.52704 | | 6.67 | | | 0.02 | 0.987 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | MKX | 35.53556 | | 7.51 | | | 0.86 | 0.550 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | MKX | 34.33388 | | 7.32 | | | 0.67 | 0.627 | 2.677 | 3.663 |
| | | B3 | GAPDH | 28.48671 | | B3 | MKX | 34.90285 | | 6.42 | | | -0.23 | 1.176 | | |
| | | B5 | GAPDH | 31.86955 | | B5 | MKX | 35.49146 | | 3.62 | | | -3.03 | 8.160 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | MKX | 35.70391 | | 7.07 | | | 0.42 | 0.745 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | MKX | 40.4739 | | 11.85 | | | 5.20 | 0.027 | 0.050 | 0.040 |
| | | C5 | GAPDH | 27.41017 | | C5 | MKX | 39.30689 | | 11.90 | | | 5.25 | 0.026 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | MKX | 35.60669 | | 10.04 | | | 3.39 | 0.096 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D4 | GAPDH | 28.78433 | | D4 | MKX | 35.50887 | | | 6.72 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | MKX | 35.78663 | | | 6.58 | 6.65 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | ki-67 | 28.79271 | | 3.34 | | | -0.47 | 1.386 | 0.437 | 0.537 |
| | | A3 | GAPDH | 27.03984 | | A3 | ki-67 | 33.37111 | | 6.33 | | | 2.52 | 0.175 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | ki-67 | 34.69514 | | 5.38 | | | 1.57 | 0.337 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | ki-67 | 35.18807 | | 6.33 | | | 2.51 | 0.175 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | ki-67 | 34.99658 | | 6.97 | | | 3.16 | 0.112 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | ki-67 | 34.43713 | | 7.43 | | | 3.61 | 0.082 | 0.707 | 1.220 |
| | | B3 | GAPDH | 28.48671 | | B3 | ki-67 | 37.54718 | | 9.06 | | | 5.24 | 0.026 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | ki-67 | 31.1037 | | 2.47 | | | -1.34 | 2.535 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | ki-67 | 33.57014 | | 6.26 | | | 2.44 | 0.184 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | ki-67 | 32.0667 | | 3.45 | | | -0.37 | 1.292 | 0.846 | 0.651 |
| | | C3 | GAPDH | 28.66757 | | C3 | ki-67 | 34.46207 | | 5.79 | | | 1.98 | 0.254 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | ki-67 | 30.69383 | | 3.28 | | | -0.53 | 1.446 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | ki-67 | 29.11649 | | 3.55 | | | -0.27 | 1.204 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | ki-67 | 36.48237 | | 8.67 | | | 4.85 | 0.035 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D4 | GAPDH | 28.78433 | | D4 | ki-67 | 30.98717 | | | 2.20 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | ki-67 | 30.90082 | | | 2.31 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | ki-67 | 36.14339 | | | 6.93 | 3.82 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | | | A1 | p16 | | | 0.00 | | | -6.61 | 97.449 | 78.235 | 42.964 |
| | | A3 | GAPDH | | | A3 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | A5 | GAPDH | | | A5 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | p16 | 35.0023 | | 6.14 | | | -0.46 | 1.378 | | |
| | | A9 | GAPDH | | | A9 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | Group 2R | B1 | GAPDH | | | B1 | p16 | | | 0.00 | | | -6.61 | 97.449 | 77.986 | 43.522 |
| | | B3 | GAPDH | | | B3 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | B5 | GAPDH | 31.86955 | | B5 | p16 | 41.41182 | | 9.54 | | | 2.94 | 0.131 | | |
| | | B7 | GAPDH | | | B7 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | B9 | GAPDH | | | B9 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | Group 4R | C1 | GAPDH | | | C1 | p16 | | | 0.00 | | | -6.61 | 97.449 | 59.339 | 52.207 |
| | | C3 | GAPDH | 28.66757 | | C3 | p16 | 33.15614 | | 4.49 | | | -2.12 | 4.341 | | |
| | | C5 | GAPDH | | | C5 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | C7 | GAPDH | | | C7 | p16 | | | 0.00 | | | -6.61 | 97.449 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | p16 | 41.46399 | | 13.65 | | | 7.04 | 0.008 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | 27.87852 | | D2 | p16 | 39.97389 | | | 12.10 | | | | | |
| | | D4 | GAPDH | | | D4 | p16 | | | | 0.00 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | p16 | 42.91961 | | | 14.33 | | | | | |
| | | D8 | GAPDH | | | D8 | p16 | | | | 0.00 | 6.61 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | p21 | 36.56406 | | 11.12 | | | 9.20 | 0.002 | | |
| | | A3 | GAPDH | 27.03984 | | A3 | p21 | 36.67815 | | 9.64 | | | 7.72 | 0.005 | | |
| | | A5 | GAPDH | | | A5 | p21 | | | 0.00 | | | -1.92 | 3.773 | 1.511 | 2.066 |
| | | A7 | GAPDH | 28.85837 | | A7 | p21 | 42.93256 | | 14.07 | | | 12.16 | 0.000 | | |
| | | A9 | GAPDH | | | A9 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | p21 | 37.82612 | | 10.82 | | | 8.90 | 0.002 | | |
| | | B3 | GAPDH | | | B3 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | | B5 | GAPDH | | | B5 | p21 | | | 0.00 | | | -1.92 | 3.773 | 2.266 | 2.064 |
| | | B7 | GAPDH | 28.62938 | | B7 | p21 | 37.71732 | | 9.09 | | | 7.17 | 0.007 | | |
| | | B9 | GAPDH | | | B9 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | Group 4R | C1 | GAPDH | | | C1 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | | C3 | GAPDH | | | C3 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | | C5 | GAPDH | | | C5 | p21 | | | 0.00 | | | -1.92 | 3.773 | 3.021 | 1.683 |
| | | C7 | GAPDH | | | C7 | p21 | | | 0.00 | | | -1.92 | 3.773 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | p21 | 36.4269 | | 8.61 | | | 6.70 | 0.010 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | | | D2 | p21 | | | | 0.00 | | | | | |
| | | D4 | GAPDH | | | D4 | p21 | | | | 0.00 | | | | | |
| | | D6 | GAPDH | | | D6 | p21 | | | | 0.00 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | p21 | 36.8735 | | | 7.66 | 1.92 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | p53 | 35.59641 | | 10.15 | | | -0.50 | 1.418 | 3.874 | 4.580 |
| | | A3 | GAPDH | 27.03984 | | A3 | p53 | 35.54187 | | 8.50 | | | -2.15 | 4.439 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | p53 | 39.15534 | | 9.84 | | | -0.81 | 1.752 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | p53 | 41.99088 | | 13.13 | | | 2.48 | 0.179 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | p53 | 35.14136 | | 7.12 | | | -3.53 | 11.580 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | p53 | 36.11284 | | 9.10 | | | -1.55 | 2.928 | 3.146 | 1.980 |
| | | B3 | GAPDH | 28.48671 | | B3 | p53 | 38.77776 | | 10.29 | | | -0.36 | 1.285 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | p53 | 36.89615 | | 8.27 | | | -2.39 | 5.226 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | p53 | 36.9022 | | 8.28 | | | -2.37 | 5.172 | 3.012 | 3.456 |
| | | C3 | GAPDH | 28.66757 | | C3 | p53 | 36.56816 | | 7.90 | | | -2.75 | 6.736 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | p53 | 40.37586 | | 14.81 | | | 4.15 | 0.056 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | p53 | 42.00975 | | 14.20 | | | 3.54 | 0.086 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D6 | GAPDH | 28.58866 | | D6 | p53 | 42.94649 | | | 14.36 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | p53 | 36.15712 | | | 6.95 | 10.65 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | TNMD | 33.53322 | | 8.09 | | | 0.62 | 0.651 | 1.369 | 0.917 |
| | | A3 | GAPDH | 27.03984 | | A3 | TNMD | 33.20132 | | 6.16 | | | -1.30 | 2.469 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | TNMD | 36.15384 | | 6.84 | | | -0.62 | 1.540 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | TNMD | 35.36386 | | 6.51 | | | -0.96 | 1.945 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | TNMD | 37.54929 | | 9.53 | | | 2.06 | 0.240 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | TNMD | 34.50532 | | 7.49 | | | 0.03 | 0.980 | 2.584 | 2.031 |
| | | B3 | GAPDH | 28.48671 | | B3 | TNMD | 34.06559 | | 5.58 | | | -1.89 | 3.697 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | TNMD | 33.80657 | | 5.18 | | | -2.29 | 4.884 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | TNMD | 35.14567 | | 7.83 | | | 0.37 | 0.776 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | TNMD | 34.17124 | | 5.55 | | | -1.91 | 3.770 | 1.696 | 1.378 |
| | | C3 | GAPDH | 28.66757 | | C3 | TNMD | 34.98902 | | 6.32 | | | -1.14 | 2.210 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | TNMD | 35.64849 | | 8.24 | | | 0.77 | 0.585 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | TNMD | 34.49539 | | 8.93 | | | 1.46 | 0.363 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | TNMD | 34.64517 | | 6.83 | | | -0.63 | 1.552 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D4 | GAPDH | 28.78433 | | D4 | TNMD | 36.17414 | | | 7.39 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | TNMD | 36.12954 | | | 7.54 | 7.47 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | COL1 | 29.45222 | | 4.00 | | | 0.39 | 0.766 | 3.410 | 2.470 |
| | | A5 | GAPDH | 29.31165 | | A5 | COL1 | 31.00254 | | 1.69 | | | -1.93 | 3.806 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | COL1 | 29.9769 | | 1.12 | | | -2.50 | 5.659 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | COL1 | 29.47959 | | 2.47 | | | -1.15 | 2.219 | 11.034 | 7.272 |
| | | B3 | GAPDH | 28.48671 | | B3 | COL1 | 28.04687 | | -0.44 | | | -4.06 | 16.666 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | COL1 | 28.13363 | | -0.50 | | | -4.11 | 17.325 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | COL1 | 27.94723 | | 0.63 | | | -2.99 | 7.928 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | COL1 | 28.9666 | | 0.35 | | | -3.27 | 9.665 | 5.264 | 4.330 |
| | | C3 | GAPDH | 28.66757 | | C3 | COL1 | 28.91865 | | 0.25 | | | -3.37 | 10.324 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | COL1 | 30.11133 | | 2.70 | | | -0.92 | 1.889 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | COL1 | 28.1999 | | 2.63 | | | -0.99 | 1.983 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | COL1 | 30.13552 | | 2.32 | | | -1.30 | 2.458 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | 27.87852 | | D2 | COL1 | 34.96771 | | | 7.09 | | | | | |
| | | D4 | GAPDH | 28.78433 | | D4 | COL1 | 32.6948 | | | 3.91 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | COL1 | 30.45726 | | | 1.87 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | COL1 | 30.81798 | | | 1.61 | 3.62 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | COL3 | 30.76696 | | 5.32 | | | 2.83 | 0.140 | 1.016 | 0.807 |
| | | A3 | GAPDH | 27.03984 | | A3 | COL3 | 30.5454 | | 3.51 | | | 1.02 | 0.493 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | COL3 | 30.68809 | | 1.38 | | | -1.11 | 2.157 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | COL3 | 30.77384 | | 1.92 | | | -0.57 | 1.484 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | COL3 | 30.81748 | | 2.79 | | | 0.31 | 0.807 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | COL3 | 31.09509 | | 4.08 | | | 1.60 | 0.330 | 1.133 | 0.627 |
| | | B3 | GAPDH | 28.48671 | | B3 | COL3 | 30.15251 | | 1.67 | | | -0.82 | 1.765 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | COL3 | 30.56591 | | 1.94 | | | -0.55 | 1.463 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | COL3 | 29.84025 | | 2.53 | | | 0.04 | 0.973 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | COL3 | 30.68336 | | 2.06 | | | -0.42 | 1.340 | 0.854 | 0.478 |
| | | C3 | GAPDH | 28.66757 | | C3 | COL3 | 30.82769 | | 2.16 | | | -0.33 | 1.253 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | COL3 | 30.88609 | | 3.48 | | | 0.99 | 0.503 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | COL3 | 30.16292 | | 4.59 | | | 2.11 | 0.232 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | COL3 | 30.38554 | | 2.57 | | | 0.09 | 0.942 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | 27.87852 | | D2 | COL3 | 31.68917 | | | 3.81 | | | | | |
| | | D4 | GAPDH | 28.78433 | | D4 | COL3 | 31.84044 | | | 3.06 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | COL3 | 30.09009 | | | 1.50 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | COL3 | 30.78342 | | | 1.57 | 2.49 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibrat | CT calibrat | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | DCN | 32.47248 | | 7.02 | | | -0.04 | 1.027 | 3.219 | 1.969 |
| | | A3 | GAPDH | 27.03984 | | A3 | DCN | 31.73548 | | 4.70 | | | -2.37 | 5.160 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | DCN | 33.98621 | | 4.67 | | | -2.39 | 5.236 | | |
| | | A7 | GAPDH | 28.85837 | | A7 | DCN | 34.26943 | | 5.41 | | | -1.65 | 3.143 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | DCN | 34.4736 | | 6.45 | | | -0.61 | 1.528 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | DCN | 32.64434 | | 5.63 | | | -1.43 | 2.693 | 3.940 | 2.058 |
| | | B3 | GAPDH | 28.48671 | | B3 | DCN | 32.80717 | | 4.32 | | | -2.74 | 6.693 | | |
| | | B7 | GAPDH | 28.62938 | | B7 | DCN | 33.59093 | | 4.96 | | | -2.10 | 4.292 | | |
| | | B9 | GAPDH | 27.31509 | | B9 | DCN | 33.319 | | 6.00 | | | -1.06 | 2.084 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | DCN | 33.60317 | | 4.98 | | | -2.08 | 4.229 | 3.851 | 2.141 |
| | | C3 | GAPDH | 28.66757 | | C3 | DCN | 32.96638 | | 4.30 | | | -2.76 | 6.794 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | DCN | 32.76944 | | 5.36 | | | -1.70 | 3.258 | | |
| | | C7 | GAPDH | 25.56867 | | C7 | DCN | 32.89359 | | 7.32 | | | 0.26 | 0.834 | | |
| | | C9 | GAPDH | 27.81408 | | C9 | DCN | 32.82735 | | 5.01 | | | -2.05 | 4.141 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D4 | GAPDH | 28.78433 | | D4 | DCN | 36.51727 | | | 7.73 | | | | | |
| | | D6 | GAPDH | 28.58866 | | D6 | DCN | 34.01641 | | | 5.43 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | DCN | 37.23882 | | | 8.03 | 7.06 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | SCX | Ct SYBR | | CT | CT calibr | CT calibr | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A1 | GAPDH | 25.4478 | | A1 | SCX | 36.72131 | | 11.27 | | | 18.47 | 0.000 | | |
| | | A3 | GAPDH | 27.03984 | | A3 | SCX | 38.11598 | | 11.08 | | | 18.27 | 0.000 | | |
| | Group EP | A5 | GAPDH | | | A5 | SCX | | | 0.00 | | | 7.20 | 0.007 | 0.001 | 0.003 |
| | | A7 | GAPDH | 28.85837 | | A7 | SCX | 36.30634 | | 7.45 | | | 14.64 | 0.000 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | SCX | 37.39471 | | 9.37 | | | 16.57 | 0.000 | | |
| | | B1 | GAPDH | | | B1 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | | B3 | GAPDH | | | B3 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | Group 2R | B5 | GAPDH | | | B5 | SCX | | | 0.00 | | | 7.20 | 0.007 | 0.007 | 0.000 |
| | | B7 | GAPDH | | | B7 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | | B9 | GAPDH | | | B9 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | | C1 | GAPDH | 28.62041 | | C1 | SCX | 37.53539 | | 8.91 | | | 16.11 | 0.000 | | |
| | | C3 | GAPDH | | | C3 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | Group 4R | C5 | GAPDH | | | C5 | SCX | | | 0.00 | | | 7.20 | 0.007 | 0.004 | 0.004 |
| | | C7 | GAPDH | 25.56867 | | C7 | SCX | 36.6939 | | 11.13 | | | 18.32 | 0.000 | | |
| | | C9 | GAPDH | | | C9 | SCX | | | 0.00 | | | 7.20 | 0.007 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D2 | GAPDH | | | D2 | 43 | | | | 0.00 | | | | | |
| | | D4 | GAPDH | 28.78433 | | D4 | | | | | -28.78 | | | | | |
| | | D6 | GAPDH | | | D6 | | | | | 0.00 | | | | | |
| | | D8 | GAPDH | | | D8 | | | | | 0.00 | -7.20 | | | | |

| | Sample ID | Pos | Name | Ct SYBR | | Pos | Name | Ct SYBR | | CT | CT calibr | CT calibr | CT | 2^- CT | Average | STDEV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group EP | A1 | GAPDH | 25.4478 | | A1 | MKX | 34.02941 | | 8.58 | | | 1.93 | 0.262 | | |
| | | A3 | GAPDH | 27.03984 | | A3 | MKX | 33.14282 | | 6.10 | | | -0.55 | 1.462 | | |
| | | A5 | GAPDH | 29.31165 | | A5 | MKX | 36.41442 | | 7.10 | | | 0.45 | 0.731 | 0.798 | 0.455 |
| | | A7 | GAPDH | 28.85837 | | A7 | MKX | 35.52704 | | 6.67 | | | 0.02 | 0.987 | | |
| | | A9 | GAPDH | 28.02253 | | A9 | MKX | 35.53556 | | 7.51 | | | 0.86 | 0.550 | | |
| | Group 2R | B1 | GAPDH | 27.01035 | | B1 | MKX | 34.33388 | | 7.32 | | | 0.67 | 0.627 | | |
| | | B3 | GAPDH | 28.48671 | | B3 | MKX | 34.90285 | | 6.42 | | | -0.23 | 1.176 | 0.850 | 0.289 |
| | | B7 | GAPDH | 28.62938 | | B7 | MKX | 35.70391 | | 7.07 | | | 0.42 | 0.745 | | |
| | Group 4R | C1 | GAPDH | 28.62041 | | C1 | MKX | 40.4739 | | 11.85 | | | 5.20 | 0.027 | | |
| | | C5 | GAPDH | 27.41017 | | C5 | MKX | 39.30689 | | 11.90 | | | 5.25 | 0.026 | 0.050 | 0.040 |
| | | C7 | GAPDH | 25.56867 | | C7 | MKX | 35.60669 | | 10.04 | | | 3.39 | 0.096 | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| | Control | D4 | GAPDH | 28.78433 | | D4 | MKX | 35.50887 | | | 6.72 | | | | | |
| | | D8 | GAPDH | 29.21015 | | D8 | MKX | 35.78663 | | | 6.58 | 6.65 | | | | |

**PCR Primer sequences**

rRpl13a_Fw

Sequence (5’ to 3’): AACCGACTTGGCTCCATTGT

rRpl13a_Rv

Sequence (5’ to 3’): TGGGTTCACACCAAGAGTCC

[redacted]

# Invitrogen Custom Primers

## *Certificate of Analysis*

**Primer 1:**

Primer Name: rCol1a2_Fw
Researcher: MR
Sequence (5' to 3'): ACT CAG CCA CCC AGA GTG GAA
Molecular Weight (µg/µmole): 6410.2
Micromolar Extinction Coeff. (OD/µmol) 236.5

Primer Number: N3758B11 ( B11 )
Primer Length: 21
Scale of Synthesis: 25n mol
µg per OD: 27.1
nmoles per OD: 4.2

| **Purity** | **Desalted** |
|---|---|
| **Tm (1 M Na+)** | **73** ] 62 |
| **Tm (50 mM Na+)** | **51** ] |
| **% GC** | **57** |

| | |
|---|---|
| **OD's** | **6.50** |
| **µg's*** | **176.18** |
| **nmoles** | **27.5** 275 µL |
| **Coupling Eff.** | **97%** |

**Notes:** Primer 3 blast: Tm = 63.14

**Primer 2:**

Primer Name: rCol1a2_Rv
Researcher: MR
Sequence (5' to 3'): TTG ACA GGT TGG GCC TGG A
Molecular Weight (µg/µmole): 5900.8
Micromolar Extinction Coeff. (OD/µmol) 209.0

Primer Number: N3758B12 ( B12 )
Primer Length: 19
Scale of Synthesis: 25n mol
µg per OD: 28.2
nmoles per OD: 4.8

| **Purity** | **Desalted** |
|---|---|
| **Tm (1 M Na+)** | **70** ] 59 |
| **Tm (50 mM Na+)** | **48** ] |
| **% GC** | **58** |

| | |
|---|---|
| **OD's** | **6.00** |
| **µg's*** | **169.40** |
| **nmoles** | **28.7** 287 µL |
| **Coupling Eff.** | **97%** |

**Notes:** Primer 3 blast: Tm = 61.08

**Primer 3:**

Primer Name: rScx_Fw
Researcher: MR
Sequence (5' to 3'): AGC CCA AAC AGA TCT GCA CCT T
Molecular Weight (µg/µmole): 6649.4
Micromolar Extinction Coeff. (OD/µmol) 238.9

Primer Number: N3758C01 ( C01 )
Primer Length: 22
Scale of Synthesis: 25n mol
µg per OD: 27.8
nmoles per OD: 4.2

| **Purity** | **Desalted** |
|---|---|
| **Tm (1 M Na+)** | **71** ] 60,5 |
| **Tm (50 mM Na+)** | **50** ] |
| **% GC** | **50** |

| | |
|---|---|
| **OD's** | **6.20** |
| **µg's*** | **172.57** |
| **nmoles** | **26.0** 260 µL |
| **Coupling Eff.** | **98%** |

**Notes:** Primer 3 blast: Tm = 62.49

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers
## *Certificate of Analysis*

**Primer 4:**

Primer Name: rScx_Rv
Researcher: MR
Sequence (5' to 3'): CTT CCA CCT TCA CTA GTG GCA TCA
Molecular Weight (µg/µmole): 7224.8
Micromolar Extinction Coeff (OD/µmol) 243.6

| Purity | Desalted |
|---|---|
| Tm (1 M Na+) | 74 ] 63 |
| Tm (50 mM Na+) | 52 ] |
| % GC | 50 |

Notes: Primer 3 blast : Tm = 62.38

Primer Number: N3758C02 ( C02 )
Primer Length: 24
Scale of Synthesis: 25n mol

µg per OD: 29.7
nmoles per OD: 4.1

| | |
|---|---|
| OD's | 6.60 |
| µg's* | 195.75 |
| nmoles | 27.1 |
| Coupling Eff. | 97% |

271 µL

**Primer 5:**

Primer Name: rTnmd_Fw
Researcher: MR
Sequence (5' to 3'): ATG GGT GGT CCC ACA AGT GAA
Molecular Weight (µg/µmole): 6496.2
Micromolar Extinction Coeff (OD/µmol) 241.2

| Purity | Desalted |
|---|---|
| Tm (1 M Na+) | 71 ] 60 |
| Tm (50 mM Na+) | 49 ] |
| % GC | 52 |

Notes: Primer 3 blast: Tm = 61.61

Primer Number: N3758C03 ( C03 )
Primer Length: 21
Scale of Synthesis: 25n mol

µg per OD: 26.9
nmoles per OD: 4.2

| | |
|---|---|
| OD's | 6.70 |
| µg's* | 180.45 |
| nmoles | 27.8 |
| Coupling Eff. | 97% |

278 µL

**Primer 6:**

Primer Name: rTnmd_Rv
Researcher: MR
Sequence (5' to 3'): CTC TCA TCC AGC ATG GGA TCA A
Molecular Weight (µg/µmole): 6680.4
Micromolar Extinction Coeff (OD/µmol) 237.3

| Purity | Desalted |
|---|---|
| Tm (1 M Na+) | 71 ] 60,5 |
| Tm (50 mM Na+) | 50 ] |
| % GC | 50 |

Notes: Primer 3 blast: Tm = 59.89

Primer Number: N3758C04 ( C04 )
Primer Length: 22
Scale of Synthesis: 25n mol

µg per OD: 28.2
nmoles per OD: 4.2

| | |
|---|---|
| OD's | 5.70 |
| µg's* | 160.46 |
| nmoles | 24.0 |
| Coupling Eff. | 99% |

240 µL

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers

## *Certificate of Analysis*

**Primer 7:**

| | | | | |
|---|---|---|---|---|
| Primer Name: | rDcn_Fw | Primer Number: | N3758C05 | ( C05 ) |
| Researcher: | MR | Primer Length: | 23 | |
| Sequence (5' to 3'): | GAC TCC ACG ACA ATG AGA TCA CC | Scale of Synthesis: | 25n mol | |
| Molecular Weight (µg/µmole): | 6987.6 | µg per OD: | 27.2 | |
| Micromolar Extinction Coeff (OD/µmol) | 256.7 | nmoles per OD: | 3.9 | |
| **Purity** | **Desalted** | **OD's** | **5.90** | |
| **Tm (1 M Na+)** | **74** | **µg's*** | **160.60** | |
| **Tm (50 mM Na+)** | **52** | **nmoles** | **23.0** | 230 uL |
| **% GC** | **52** | **Coupling Eff.** | **98%** | |

**Notes:** Primer 3 blast: 60.98

**Primer 8:**

| | | | | |
|---|---|---|---|---|
| Primer Name: | rDcn_Rv | Primer Number: | N3758C06 | ( C06 ) |
| Researcher: | MR | Primer Length: | 21 | |
| Sequence (5' to 3'): | GTT GCC ATC CAG ATG CAG TTC | Scale of Synthesis: | 25n mol | |
| Molecular Weight (µg/µmole): | 6398.2 | µg per OD: | 29.0 | |
| Micromolar Extinction Coeff (OD/µmol) | 220.4 | nmoles per OD: | 4.5 | |
| **Purity** | **Desalted** | **OD's** | **5.80** | |
| **Tm (1 M Na+)** | **71** | **µg's*** | **168.37** | |
| **Tm (50 mM Na+)** | **49** | **nmoles** | **26.3** | 263 uL |
| **% GC** | **52** | **Coupling Eff.** | **99%** | |

**Notes:** Primer 3 blast: 59.87

**Primer 9:**

| | | | | |
|---|---|---|---|---|
| Primer Name: | rCol3a1_Fw | Primer Number: | N3758C07 | ( C07 ) |
| Researcher: | MR | Primer Length: | 20 | |
| Sequence (5' to 3'): | GAG GAA TGG GTG GCT ATC CT | Scale of Synthesis: | 25n mol | |
| Molecular Weight (µg/µmole): | 6214.0 | µg per OD: | 27.7 | |
| Micromolar Extinction Coeff (OD/µmol) | 224.3 | nmoles per OD: | 4.5 | |
| **Purity** | **Desalted** | **OD's** | **8.10** | |
| **Tm (1 M Na+)** | **70** | **µg's*** | **224.40** | |
| **Tm (50 mM Na+)** | **49** | **nmoles** | **36.1** | 361 uL |
| **% GC** | **55** | **Coupling Eff.** | **98%** | |

**Notes:** Primer 3 blast: 58.56

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers

## *Certificate of Analysis*

**Primer 10:**

| | | | |
|---|---|---|---|
| Primer Name: | rCol3a1_Rv | Primer Number: | N3758C08 ( C08 ) |
| Researcher: | MR | Primer Length: | 21 |
| Sequence (5' to 3'): | GGT ATC CAG GAG AAC CAG GAG | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6530.2 | µg per OD: | 26.2 |
| Micromolar Extinction Coeff (OD/µmol) | 249.7 | nmoles per OD: | 4.0 |
| **Purity** | **Desalted** | **OD's** | **9.40** |
| **Tm (1 M Na+)** | **73** | **µg's*** | **245.83** |
| **Tm (50 mM Na+)** | **51** | **nmoles** | **37.6** 376 uL |
| **% GC** | **57** | **Coupling Eff.** | **98%** |

**Notes:** Primer 3 blast: Tm = 58.96

**Primer 11:**

| | | | |
|---|---|---|---|
| Primer Name: | rMkx_Fw | Primer Number: | N3758C09 ( C09 ) |
| Researcher: | MR | Primer Length: | 22 |
| Sequence (5' to 3'): | TTT ACA AGC ACC GTG ACA ACC C | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6649.4 | µg per OD: | 27.8 |
| Micromolar Extinction Coeff (OD/µmol) | 238.9 | nmoles per OD: | 4.2 |
| **Purity** | **Desalted** | **OD's** | **7.00** |
| **Tm (1 M Na+)** | **71** | **µg's*** | **194.83** |
| **Tm (50 mM Na+)** | **50** | **nmoles** | **29.3** 293 uL |
| **% GC** | **50** | **Coupling Eff.** | **98%** |

**Notes:** Primer 3 blast: Tm = 61.58

**Primer 12:**

| | | | |
|---|---|---|---|
| Primer Name: | rMkx_Rv | Primer Number: | N3758C10 ( C10 ) |
| Researcher: | MR | Primer Length: | 22 |
| Sequence (5' to 3'): | ACA GTG TTC TTC AGC CGT CGT C | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6678.4 | µg per OD: | 30.1 |
| Micromolar Extinction Coeff (OD/µmol) | 221.8 | nmoles per OD: | 4.5 |
| **Purity** | **Desalted** | **OD's** | **5.70** |
| **Tm (1 M Na+)** | **73** | **µg's*** | **171.63** |
| **Tm (50 mM Na+)** | **52** | **nmoles** | **25.7** 257 uL |
| **% GC** | **55** | **Coupling Eff.** | **98%** |

**Notes:** Primer 3 blast: Tm = 63.02

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

# Invitrogen Custom Primers
## *Certificate of Analysis*

[redacted]

**Primer 19:**

| | | | |
|---|---|---|---|
| Primer Name: | rGapdh_Fw | Primer Number: | N3758D05 ( D05 ) |
| Researcher: | MR | Primer Length: | 20 |
| Sequence (5' to 3'): | GGC AAG TTC AAT GGC ACA GT | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6167.0 | µg per OD: | 26.9 |
| Micromolar Extinction Coeff (OD/µmol) | 229.4 | nmoles per OD: | 4.4 |
| **Purity** | **Desalted** | **OD's** | **7.10** |
| **Tm (1 M Na+)** | **68** | **µg's*** | **190.87** |
| **Tm (50 mM Na+)** | **47** | **nmoles** | **31.0** |
| **% GC** | **50** | **Coupling Eff.** | **99%** |

**Notes:**

**Primer 20:**

| | | | |
|---|---|---|---|
| Primer Name: | rGapdh_Rv | Primer Number: | N3758D06 ( D06 ) |
| Researcher: | MR | Primer Length: | 22 |
| Sequence (5' to 3'): | TGG TGA AGA CGC CAG TAG ACT C | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6785.4 | µg per OD: | 27.3 |
| Micromolar Extinction Coeff (OD/µmol) | 248.6 | nmoles per OD: | 4.0 |
| **Purity** | **Desalted** | **OD's** | **6.80** |
| **Tm (1 M Na+)** | **73** | **µg's*** | **185.60** |
| **Tm (50 mM Na+)** | **52** | **nmoles** | **27.3** |
| **% GC** | **55** | **Coupling Eff.** | **98%** |

**Notes:**

**Primer 21:**

| | | | |
|---|---|---|---|
| Primer Name: | rGAPDH_Fw nmol 28.8 → 288 µL | Primer Number: | N3758D07 ( D07 ) ← |
| Researcher: | MR | Primer Length: | 20 |
| Sequence (5' to 3'): | GGC AAG TTC AAT GGC ACA GT | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6167.0 | µg per OD: | 26.9 |
| Micromolar Extinction Coeff (OD/µmol) | 229.4 | nmoles per OD: | 4.4 |
| **Purity** | **Desalted** | **OD's** | **6.60** |
| **Tm (1 M Na+)** | **68** | **µg's*** | **177.43** |
| **Tm (50 mM Na+)** | **47** | **nmoles** | **28.8** |
| **% GC** | **50** | **Coupling Eff.** | **99%** |

**Notes:** Primer 3 blast : Tm = 59.32

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers

## *Certificate of Analysis*

**Primer 22:**

Primer Name: rGAPDH_Rv nmol 25.7 → 257 µL

Researcher: MR

Sequence (5' to 3'): TGG TGA AGA CGC CAG TAG ACT C

| | |
|---|---|
| Molecular Weight (µg/µmole): | 6785.4 |
| Micromolar Extinction Coeff (OD/µmol) | 248.6 |
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **73** |
| **Tm (50 mM Na+)** | **52** |
| **% GC** | **55** |

Primer Number: N3758D08 ( D08 ) ←

Primer Length: 22

Scale of Synthesis: 25n mol

| | |
|---|---|
| µg per OD: | 27.3 |
| nmoles per OD: | 4.0 |
| **OD's** | **6.40** |
| **µg's*** | **174.68** |
| **nmoles** | **25.7** |
| **Coupling Eff.** | **98%** |

**Notes:** Primer 3 blast : Tm = 61.71

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers
## *Certificate of Analysis*

**Primer 1:**

Primer Name: r p21waf1_F nmol 32.6 → 100 µM stock → 326 µL RNAse free H2O
Researcher: MR
Sequence (5' to 3'): TCT TGC ACT CTG GTG TCT CA

| | |
|---|---|
| Molecular Weight (µg/µmole): | 6051.0 |
| Micromolar Extinction Coeff (OD/µmol) | 196.6 |

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **68** |
| **Tm (50 mM Na+)** | **47** |
| **% GC** | **50** |

Primer Number: N3020G12 ( G12 )
Primer Length: 20
Scale of Synthesis: 25n mol

| | |
|---|---|
| µg per OD: | 30.8 |
| nmoles per OD: | 5.1 |
| **OD's** | **6.40** |
| **µg's*** | **196.98** |
| **nmoles** | **32.6** |
| **Coupling Eff.** | **99%** |

**Notes:** Primer 3 blast: Tm = 58.59

**Primer 2:**

Primer Name: r p21waf1_R nmol 40.6 → 406 µL RNAse free H2O
Researcher: MR
Sequence (5' to 3'): GGG CTT TCT CTT GCA GAA G

| | |
|---|---|
| Molecular Weight (µg/µmole): | 5835.8 |
| Micromolar Extinction Coeff (OD/µmol) | 202.1 |

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **68** |
| **Tm (50 mM Na+)** | **46** |
| **% GC** | **53** |

Primer Number: N3020H01 ( H01 )
Primer Length: 19
Scale of Synthesis: 25n mol

| | |
|---|---|
| µg per OD: | 28.9 |
| nmoles per OD: | 5.0 |
| **OD's** | **8.20** |
| **µg's*** | **236.78** |
| **nmoles** | **40.6** |
| **Coupling Eff.** | **99%** |

**Notes:** Primer 3 blast: Tm = 56.21

**Primer 3:**

Primer Name: r Ki67_F nmol 24.6 → 246 µL "
Researcher: MR
Sequence (5' to 3'): CTG CAG AGA AGG TTG GGA TAA A

| | |
|---|---|
| Molecular Weight (µg/µmole): | 6873.4 |
| Micromolar Extinction Coeff (OD/µmol) | 268.8 |

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **69** |
| **Tm (50 mM Na+)** | **48** |
| **% GC** | **45** |

Primer Number: N3020H02 ( H02 )
Primer Length: 22
Scale of Synthesis: 25n mol

| | |
|---|---|
| µg per OD: | 25.6 |
| nmoles per OD: | 3.7 |
| **OD's** | **6.60** |
| **µg's*** | **168.77** |
| **nmoles** | **24.6** |
| **Coupling Eff.** | **99%** |

**Notes:** Primer 3 Blast: Tm = 58.04

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers

## *Certificate of Analysis*

**Primer 4:**

Primer Name: r Ki67_R nmol 21.8 → 218 µL RNAse free water
Researcher: MR
Sequence (5' to 3'): CTG ACT TTG CCC AGA GAT GAA
Molecular Weight (µg/µmole): 6431.2
Micromolar Extinction Coeff (OD/µmol) 234.3

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **69** |
| **Tm (50 mM Na+)** | **47** |
| **% GC** | **48** |

**Notes:** Primer 3 blast : Tm = 57.94

Primer Number: N3020H03 ( H03 )
Primer Length: 21
Scale of Synthesis: 25n mol

µg per OD: 27.5
nmoles per OD: 4.3

| | |
|---|---|
| **OD's** | **5.10** |
| **µg's*** | **139.99** |
| **nmoles** | **21.8** |
| **Coupling Eff.** | **98%** |

**Primer 5:**

Primer Name: r p16-INK4a_F nmol 22.1 → 221 µL "
Researcher: MR
Sequence (5' to 3'): ACC AAA CGC CCC GAA CA
Molecular Weight (µg/µmole): 5103.4
Micromolar Extinction Coeff (OD/µmol) 189.9

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **66** |
| **Tm (50 mM Na+)** | **44** |
| **% GC** | **59** |

**Notes:** Primer 3 blast : Tm = 59.42

Primer Number: N3020H04 ( H04 )
Primer Length: 17
Scale of Synthesis: 25n mol

µg per OD: 26.9
nmoles per OD: 5.3

| | |
|---|---|
| **OD's** | **4.20** |
| **µg's*** | **112.87** |
| **nmoles** | **22.1** |
| **Coupling Eff.** | **92%** |

**Primer 6:**

Primer Name: r p16-INK4a_R nmol 28.3 → 283 µL "
Researcher: MR
Sequence (5' to 3'): GAG AGC TGC CAC TTT GAC GT
Molecular Weight (µg/µmole): 6134.0
Micromolar Extinction Coeff (OD/µmol) 215.5

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **70** |
| **Tm (50 mM Na+)** | **49** |
| **% GC** | **55** |

**Notes:** Primer 3 blast : Tm = 60.60

Primer Number: N3020H05 ( H05 )
Primer Length: 20
Scale of Synthesis: 25n mol

µg per OD: 28.5
nmoles per OD: 4.6

| | |
|---|---|
| **OD's** | **6.10** |
| **µg's*** | **173.63** |
| **nmoles** | **28.3** |
| **Coupling Eff.** | **99%** |

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers
## *Certificate of Analysis*

**Primer 7:**

Primer Name: r p53_F nmol 21.2 → 212 µL RNAse free water
Researcher: MR
Sequence (5' to 3'): CTG GAC GAC AGG CAG ACT TT
Molecular Weight (µg/µmole): 6143.0
Micromolar Extinction Coeff (OD/µmol) 221.5

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **70** |
| **Tm (50 mM Na+)** | **49** |
| **% GC** | **55** |

Primer Number: N3020H06 ( H06 )
Primer Length: 20
Scale of Synthesis: 25n mol
µg per OD: 27.7
nmoles per OD: 4.5

| | |
|---|---|
| **OD's** | **4.70** |
| **µg's*** | **130.35** |
| **nmoles** | **21.2** |
| **Coupling Eff.** | **99%** |

**Notes:** Primer 3 blast: Tm = 59.97

**Primer 8:**

Primer Name: r p53_R nmol 24 → 240 µL
Researcher: MR
Sequence (5' to 3'): GCA CAA ACA CGA ACC TCA AA
Molecular Weight (µg/µmole): 6058.0
Micromolar Extinction Coeff (OD/µmol) 237.7

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **66** |
| **Tm (50 mM Na+)** | **45** |
| **% GC** | **45** |

Primer Number: N3020H07 ( H07 )
Primer Length: 20
Scale of Synthesis: 25n mol
µg per OD: 25.5
nmoles per OD: 4.2

| | |
|---|---|
| **OD's** | **5.70** |
| **µg's*** | **145.27** |
| **nmoles** | **24.0** |
| **Coupling Eff.** | **97%** |

**Notes:** Primer 3 blast: Tm = 57.45

**Primer 9:**

Primer Name: r Caspase-3_F
Researcher: MR
Sequence (5' to 3'): GGT ATT GAG ACA GAC AGT GG
Molecular Weight (µg/µmole): 6247.0
Micromolar Extinction Coeff (OD/µmol) 238.2

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **68** |
| **Tm (50 mM Na+)** | **47** |
| **% GC** | **50** |

Primer Number: N3020H08 ( H08 )
Primer Length: 20
Scale of Synthesis: 25n mol
µg per OD: 26.2
nmoles per OD: 4.2

| | |
|---|---|
| **OD's** | **8.80** |
| **µg's*** | **230.79** |
| **nmoles** | **37.0** → 370 µL |
| **Coupling Eff.** | **99%** |

**Notes:**

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers

## *Certificate of Analysis*

**Primer 10:**

Primer Name: r Caspase-3_R
Researcher: MR
Sequence (5' to 3'): CAT GGG ATC TGT TTC TTT GC
Molecular Weight (µg/µmole): 6106.0
Micromolar Extinction Coeff (OD/µmol) 202.9

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **66** |
| **Tm (50 mM Na+)** | **45** |
| **% GC** | **45** |

**Notes:**

Primer Number: N3020H09 ( H09 )
Primer Length: 20
Scale of Synthesis: 25n mol

µg per OD: 30.1
nmoles per OD: 4.9

| | |
|---|---|
| **OD's** | **5.90** |
| **µg's*** | **177.55** |
| **nmoles** | **29.1** → 291 µL |
| **Coupling Eff.** | **99%** |

**Primer 11:**

Primer Name: r Caspase-9_F
Researcher: MR
Sequence (5' to 3'): ACA AGG CCT TCG ACA GTG
Molecular Weight (µg/µmole): 5509.6
Micromolar Extinction Coeff (OD/µmol) 200.4

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **67** |
| **Tm (50 mM Na+)** | **45** |
| **% GC** | **56** |

**Notes:**

Primer Number: N3020H10 ( H10 )
Primer Length: 18
Scale of Synthesis: 25n mol

µg per OD: 27.5
nmoles per OD: 5.0

| | |
|---|---|
| **OD's** | **7.90** |
| **µg's*** | **217.19** |
| **nmoles** | **39.4** → 394 µL |
| **Coupling Eff.** | **96%** |

**Primer 12:**

Primer Name: r Caspase-9_R
Researcher: MR
Sequence (5' to 3'): GTA CCA GGA ACC GCT CTT
Molecular Weight (µg/µmole): 5460.6
Micromolar Extinction Coeff (OD/µmol) 190.0

| | |
|---|---|
| **Purity** | **Desalted** |
| **Tm (1 M Na+)** | **67** |
| **Tm (50 mM Na+)** | **45** |
| **% GC** | **56** |

**Notes:**

Primer Number: N3020H11 ( H11 )
Primer Length: 18
Scale of Synthesis: 25n mol

µg per OD: 28.7
nmoles per OD: 5.3

| | |
|---|---|
| **OD's** | **5.20** |
| **µg's*** | **149.45** |
| **nmoles** | **27.4** → 274 µL |
| **Coupling Eff.** | **93%** |

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers

## *Certificate of Analysis*

**Primer 13:**

| | | | |
|---|---|---|---|
| Primer Name: | rCaspase7_Fw | Primer Number: | N3758C11 ( C11 ) |
| Researcher: | MR | Primer Length: | 22 |
| Sequence (5' to 3'): | TAT CAA CGA CAC CGA CGC TAA T | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6673.4 | µg per OD: | 27.0 |
| Micromolar Extinction Coeff(OD/µmol) | 246.8 | nmoles per OD: | 4.1 |
| **Purity** | **Desalted** | **OD's** | **8.20** |
| **Tm (1 M Na+)** | **69** | **µg's*** | **221.73** |
| **Tm (50 mM Na+)** | **48** | **nmoles** | **33.2** |
| **% GC** | **45** | **Coupling Eff.** | **98%** |

**Notes:**

**Primer 14:**

| | | | |
|---|---|---|---|
| Primer Name: | rCaspase7_Rv | Primer Number: | N3758C12 ( C12 ) |
| Researcher: | MR | Primer Length: | 20 |
| Sequence (5' to 3'): | GCC TGG AAC CGT GGA GTA AG | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6208.0 | µg per OD: | 27.2 |
| Micromolar Extinction Coeff(OD/µmol) | 228.4 | nmoles per OD: | 4.4 |
| **Purity** | **Desalted** | **OD's** | **9.50** |
| **Tm (1 M Na+)** | **72** | **µg's*** | **258.21** |
| **Tm (50 mM Na+)** | **51** | **nmoles** | **41.6** |
| **% GC** | **60** | **Coupling Eff.** | **98%** |

**Notes:**

**Primer 15:**

| | | | |
|---|---|---|---|
| Primer Name: | Piezo1-710_Fw | Primer Number: | N3758D01 ( D01 ) |
| Researcher: | MR | Primer Length: | 19 |
| Sequence (5' to 3'): | ACG CCT CAC AAG GAA AGC C | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 5776.8 | µg per OD: | 26.8 |
| Micromolar Extinction Coeff(OD/µmol) | 215.4 | nmoles per OD: | 4.6 |
| **Purity** | **Desalted** | **OD's** | **5.20** |
| **Tm (1 M Na+)** | **70** | **µg's*** | **139.46** |
| **Tm (50 mM Na+)** | **48** | **nmoles** | **24.1** |
| **% GC** | **58** | **Coupling Eff.** | **98%** |

**Notes:**

**FOR LABORATORY RESEARCH USE ONLY.**
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

[redacted]

# Invitrogen Custom Primers

## *Certificate of Analysis*

**Primer 16:**

| | | | | |
|---|---|---|---|---|
| Primer Name: | Piezo1-710_Rv | | Primer Number: | N3758D02 ( D02 ) |
| Researcher: | MR | | Primer Length: | 17 |
| Sequence (5' to 3'): | CCG TGA TCC GGA AGC GA | | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 5221.4 | | µg per OD: | 27.8 |
| Micromolar Extinction Coeff (OD/µmol) | 187.6 | | nmoles per OD: | 5.3 |
| **Purity** | **Desalted** | | **OD's** | **5.20** |
| **Tm (1 M Na+)** | **68** | | **µg's*** | **144.73** |
| **Tm (50 mM Na+)** | **47** | | **nmoles** | **27.7** |
| **% GC** | **65** | | **Coupling Eff.** | **98%** |

**Notes:**

**Primer 17:**

| | | | | |
|---|---|---|---|---|
| Primer Name: | rHIF-1a_Fw | | Primer Number: | N3758D03 ( D03 ) |
| Researcher: | MR | | Primer Length: | 20 |
| Sequence (5' to 3'): | AAC AGG ATG GAA TGG AGC AG | | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6265.0 | | µg per OD: | 25.0 |
| Micromolar Extinction Coeff (OD/µmol) | 250.2 | | nmoles per OD: | 4.0 |
| **Purity** | **Desalted** | | **OD's** | **8.30** |
| **Tm (1 M Na+)** | **68** | | **µg's*** | **207.83** |
| **Tm (50 mM Na+)** | **47** | | **nmoles** | **33.2** |
| **% GC** | **50** | | **Coupling Eff.** | **98%** |

**Notes:**

**Primer 18:**

| | | | | |
|---|---|---|---|---|
| Primer Name: | rHIF-1a_Rv | | Primer Number: | N3758D04 ( D04 ) |
| Researcher: | MR | | Primer Length: | 20 |
| Sequence (5' to 3'): | CTG CCT TGT ATG GGA GCA TT | | Scale of Synthesis: | 25n mol |
| Molecular Weight (µg/µmole): | 6140.0 | | µg per OD: | 29.0 |
| Micromolar Extinction Coeff (OD/µmol) | 211.4 | | nmoles per OD: | 4.7 |
| **Purity** | **Desalted** | | **OD's** | **6.80** |
| **Tm (1 M Na+)** | **68** | | **µg's*** | **197.50** |
| **Tm (50 mM Na+)** | **47** | | **nmoles** | **32.2** |
| **% GC** | **50** | | **Coupling Eff.** | **99%** |

**Notes:**

FOR LABORATORY RESEARCH USE ONLY.
**CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.**

invitrogen
by Thermo Fisher Scientific

## Ethical approval declaration

U.PORTO
FACULDADE DE DESPORTO
UNIVERSIDADE DO PORTO

Comissão de Ética

# Declaração

Para os devidos efeitos declara-se que o projeto CEFADE 22 2020 intitulado "High Voltage Monophasic Pulsed Current for Treating Chronic Tendinopathy", submetido à Comissão de Ética da Faculdade de Desporto da Universidade do Porto, por Beshoy Adel Abdalla Girgis, foi aprovado por ter em conta os requisitos éticos recomendados.

Porto, 11 de novembro de 2020.

A Presidente da Comissão de Ética



Zélia Maria Matos de Almeida Roque Pinto

Rua Dr. Plácido Costa, 91
4200 Porto, Portugal
Telefone 351220425200
Telefax 351225500689

# Chapter IV

# *General discussion*

*General discussion*

Of the cells present in connective tissue proper, which are mainly formed of collagen, a single cell type is known to synthesize and maintain their extracellular matrix; the fibroblast (McKee et al., 2019). Consequently, senescence and/or apoptosis of these cells, due to excessive mechanical stress and/or sustained inflammatory state, might contribute to tissue damage. Conversely, restoration of fibroblast function and/or number may aid tissue healing.

Some of the existing physical therapy interventions for tendinopathy, if considered a single disease entity, may be supported by moderate-certainty evidence, with effects being mainly short term, statistical, or qualitative. Existing evidence, moreover, may be insufficient to either support or refute the efficacy of many physical therapy interventions for chronic lateral elbow tendinopathy. Eccentric exercise is currently the preferred method of treatment, and may be more effective than other exercise forms, albeit with low to very low certainty (Merry et al., 2024; Patricio Cordeiro et al., 2024; Prudêncio et al., 2023). However, eccentric exercise was not proven superior to other forms of exercise or other modalities (Rees et al., 2009; Rompe et al., 2008; Rompe et al., 2007). This shows that none of these modalities is effective enough, as if one treatment was potent for treating tendinopathy, it would have predominated (Weber et al., 2015). The lack of head-to-head studies between different modalities may be a contributing factor to this dilemma. A need, therefore, remains to search for an effective treatment of this condition.

The latest conjoint update of the European Pressure Ulcer Advisory Panel (EPUAP), the National Pressure Injury Advisory Panel (NPIAP), and the Pan Pacific Pressure Injury Alliance (PPPIA) on the management of pressure ulcers stated that *pulsed current* electrical stimulation is supported by evidence level “A” for the facilitation of wound healing in recalcitrant stage II, and stage III or IV pressure injuries (Emily, 2019; Kottner et al., 2019). The role of electrical stimulation (ES) in improving wound healing has been described in the literature (Houghton, 2017; Kloth et al., 2020; Zulbaran-Rojas et al., 2021). Several systematic reviews, moreover, have reported evidence that support the application of electrical stimulation (Khouri et al., 2017; Koel & Houghton, 2014; Lala et al., 2016), and *monophasic pulsed current* electrical stimulation in

particular for the treatment of pressure ulcers (Kawasaki et al., 2014; Khouri et al., 2017).

High-voltage monophasic pulsed current (HVMPC) may reduce wound surface area and the probability of worsened healing of pressure ulcers. HVMPC may additionally increase complete healing probability of pressure ulcers. The observation of these beneficial effects in wounds and ulcers with the pathophysiologic factors commonly present in diabetic ulcers might additionally suggest its potential benefit in these cases. The current results, likewise, may indicate that tenocyte function might improve in response to HVMPC intervention.

The pathophysiology of pressure ulcers might include senescence and/or apoptosis of dermal fibroblasts. An overlap between diabetic and pressure ulcers' definitions and pathology may exist, and it is becoming increasingly evident that cellular senescence may have a role in diabetic ulcers (Tomic-Canic & DiPietro, 2019; Wilkinson et al., 2019; Yu et al., 2024). Indeed, the present results, similarly, may suggest that overuse-induced tendon injury might involve senescence and/or apoptosis of tenocytes. Moreover, the results may indicate that tenocyte senescence and/or apoptosis might be followed by rebound increase in cellular function which might be eventually followed by rebound proliferation (Chainani et al., 2024).

Tendinopathy may occur due to overuse, which, if it is indeed similar to overuse-induced tendon injury in rats, might be possibly preceded by tenocyte senescence and/or apoptosis. The results of the present studies, therefore, might suggest that the classic features of tenocyte proliferation, fibrocartilaginous metaplasia, proteoglycan increase, and angiogenesis might in fact represent a healing response to injury rather than injury itself. Consequently, these classic features of tendinopathy might be a rebound attempt to achieve healing, and the pathophysiology of tendinopathy might include both injury and healing phases.

# Chapter V

## *Main conclusions*

*Main conclusions*

I. Similarities, with respect to excessive mechanical stress with insufficient recovery, aging, and sustained inflammation might be responsible for the development of both pressure ulcers and tendinopathies. Moreover, the pathophysiology of both conditions might involve ischemia and hypoxia, cellular senescence and apoptosis, and extracellular matrix degradation.

II. Moderate-certainty evidence may support short-term statistically and potentially clinical efficacy of low-level laser therapy, statisitical efficacy of extracorporeal shockwave therapy, and qualitative efficacy of eccentric exercise therapy. Caution is however, advised when interpreting the results due to possible differences in tendinopathy pathology at each region.

III. Insufficient evidence to support or refute the effectiveness of many of the current physical therapy interventions for chronic lateral elbow tendinopathy were found. Nevertheless, the results might suggest immediate efficacy of mobilization with movement of the elbow joint and short-term efficacy of myofascial release technique. A pilot feasibility study, therefore, might aid the exploration of HVMPC efficacy and safety, which might facilitate clinical translation.

IV. Level I, II and IV evidence studies have consistently indicated that high-voltage monophasic pulsed current plus standard wound care were more effective than standard wound care alone or standard wound care plus sham high-voltage monophasic pulsed current in treating stage II-IV pressure ulcers. Level I evidence studies showed that high-voltage monophasic pulsed current intervention improved the healing of pressure ulcers (reduced wound surface area), and combined with standard wound care, increased the probability of complete healing and almost eliminated the probability of worsening of healing. high-voltage monophasic pulsed current intervention was shown to be relatively safe, with rare adverse reactions.

V. Although conclusive evidence regarding the effect of high-voltage monophasic pulsed current on diabetic ulcers was not found, collateral evidence might suggest a potential benefit. Direct evidence, with moderate certainty, may support its efficacy in treating pressure ulcers, albeit few adverse reactions were reported. Other observations, moreover, might indicate that this efficacy may not be limited to pressure ulcers. Nonetheless, several aspects remain to be clarified for safe and effective application of electrical stimulation for wound healing.

VI. An animal model of overuse tendon injury based on a treadmill running protocol was developed in rats. Reduction in cellular function and/or number, due to senescence and/or apoptosis, might explain reduced ground substance synthesis, particularly of carboxylated and sulfated glycosaminoglycans. These changes might represent precursory alterations that may precede tendinopathy and might correlate with the “lag phase” of tensile strength recovery. Moreover, the “rebound phenomenon”, which might correlate with reactive hyperemia, was not observed throughout the entire study period; this might suggest that complete recovery did not occur. Calcification was reproducibly induced by overuse tendon injury, and its resorption may occur spontaneously with rest. Therefore, the results may indicate that a reproducible cellular injury of Achilles tendons was sustained for at least 15 days following the end of protocol and up to 60 days with co-administration of dexmedetomidine injections. High-voltage monophasic pulsed current might improve cell activity and function, and might enhance calcification under certain conditions. These responses, moreover, occurred despite slower healing, possibly due to restraint and/or injections, which might correlate with lower body weight and dietary intake.

*Future perspectives*

Other connective tissue conditions with failed healing response might benefit from HVMPC intervention. Cellular senescence, lower proliferation and migration potential of anterior cruciate ligament fibroblasts might reduce its innate ability to heal (Ellis & Weiss, 2015; Waltz et al., 2023). Moreover, chondrocytes during osteoarthritis might express inflammatory and catabolic cytokines which resemble those expressed by senescent cells. Indeed, increased expression of senescence-associated beta-galactosidase, telomere shortening, and accumulation of p16 were all identified in osteoarthritis pathology (McCulloch et al., 2017). Selective clearance of senescent cells was reported to attenuate the development of post-traumatic osteoarthritis, reduce pain and enhance cartilage development (Jeon et al., 2017).

Furthermore, many of the characteristic features of wound healing and tumors are shared; the formation of a provisional stroma, with migration of macrophages, fibroblasts, and angiogenesis (Dvorak, 1986, 2015). Solid tumors, however, show functional and structural heterogeneity compared with wounds (Dvorak, 1986, 2015). Tumors are infiltrated by leucocytes that produce inflammatory cytokines and chemokines but these cells might be separated from tumors due to excessive fibrin deposition (Dvorak, 1986, 2015). Controversy surrounds the function of tumor-associated neutrophils despite their well-documented presence in tumor microenvironment (Giese et al., 2019; Shaul & Fridlender, 2017). Chronic inflammation may be considered a risk factor for tumor development; a link that was originally noted by Virchow in 1863 (Balkwill & Mantovani, 2001; Virchow, 1865). Senescence markers p16 and p21, similarly, were detected in human sarcomas (Giatromanolaki et al., 2020). Electrical stimulation using low level direct current was assessed as a possible cancer treatment (David et al., 1985; Sersa & Miklavcic, 1993). Tumor blood flow, moreover, might be modified with pulsed electrical stimulation (Parkins & Chaplin, 1999).

Chronic inflammation due to ischemia, senescence, apoptosis and aging may contribute to failed healing response. HVMPC might effectively restore healing of diseases sharing these pathological factors.

## References


Administration, D., Food, A. o., & States, D. O. o. t. U. (1959). *Appraisal of the safety of chemicals in foods, drugs, and cosmetics*: Association of Food & Drug Officials of the United States.

Balkwill, F., & Mantovani, A. (2001). Inflammation and cancer: back to Virchow? *The lancet, 357*(9255), 539-545. doi:http://dx.doi.org/10.1016/S0140-6736(00)04046-0

Chainani, P. H., Mena, M. B., Yeritsyan, D., Caro, D., Momenzadeh, K., Galloway, J. L., . . . Nazarian, A. (2024). Successive tendon injury in an in vivo rat overload model induces early damage and acute healing responses. *Frontiers in bioengineering and biotechnology, 12*. doi:http://dx.doi.org/10.3389/fbioe.2024.1327094

David, S. L., Absolom, D. R., Smith, C. R., Gams, J., & Herbert, M. A. (1985). Effect of low level direct current on in vivo tumor growth in hamsters. *Cancer research, 45*(11_Part_2), 5625-5631.

Dvorak, H. F. (1986). Tumors: wounds that do not heal. *New England Journal of Medicine, 315*(26), 1650-1659. doi:http://dx.doi.org/10.1056/NEJM198612253152606

Dvorak, H. F. (2015). Tumors: wounds that do not heal—redux. *Cancer immunology research, 3*(1), 1-11. doi:http://dx.doi.org/10.1158/2326-6066.CIR-14-0209

Ellis, B. J., & Weiss, J. A. (2015). Effects of aging on the cellular function, healing, and mechanical properties of ligaments. *Mechanical properties of aging soft tissues*, 167-185. doi:http://dx.doi.org/10.1007/978-3-319-03970-1_7

Emily, H. (2019). European pressure ulcer advisory Panel, national pressure injury advisory Panel and Pan pacific pressure injury alliance. *Prevention and treatment of pressure ulcers/injuries: clinical practice guideline. EPUAP/NPIAP/PPPIA*.

Giatromanolaki, A., Kouroupi, M., Balaska, K., & Koukourakis, M. I. (2020). Immunohistochemical detection of senescence markers in human sarcomas. *Pathology-Research and Practice, 216*(2), 152800. doi:http://dx.doi.org/10.1016/j.prp.2019.152800

Giese, M. A., Hind, L. E., & Huttenlocher, A. (2019). Neutrophil plasticity in the tumor microenvironment. *Blood, The Journal of the American Society of Hematology, 133*(20), 2159-2167. doi:http://dx.doi.org/10.1182/blood-2018-11-844548

Houghton, P. E. (2017). Electrical stimulation therapy to promote healing of chronic wounds: a review of reviews. *Chronic Wound Care Management and Research, 4*, 25-44. doi:http://dx.doi.org/10.2147/CWCMR.S101323

Jeon, O. H., Kim, C., Laberge, R.-M., Demaria, M., Rathod, S., Vasserot, A. P., . . . David, N. (2017). Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nature medicine, 23*(6), 775-781. doi:http://dx.doi.org/10.1038/nm.4324

Kawasaki, L., Mushahwar, V. K., Ho, C., Dukelow, S. P., Chan, L. L., & Chan, K. M. (2014). The mechanisms and evidence of efficacy of electrical stimulation for healing of pressure ulcer: a systematic review. *Wound Repair and Regeneration, 22*(2), 161-173. doi:http://dx.doi.org/10.1111/wrr.12134

Khouri, C., Kotzki, S., Roustit, M., Blaise, S., Gueyffier, F., & Cracowski, J.-L. (2017). Hierarchical evaluation of electrical stimulation protocols for chronic wound

healing: An effect size meta-analysis. *Wound Repair and Regeneration, 25*(5), 883-891. doi:http://dx.doi.org/10.1111/wrr.12594
Kloth, L., Polak, A., & Watson, T. (2020). Electrical Stimulation for Wounds. *Electro Physical Agents E-Book: Evidence-Based Practice*, 359.
Koel, G., & Houghton, P. E. (2014). Electrostimulation: current status, strength of evidence guidelines, and meta-analysis. *Advances in Wound Care, 3*(2), 118-126. doi:http://dx.doi.org/10.1089/wound.2013.0448
Kottner, J., Cuddigan, J., Carville, K., Balzer, K., Berlowitz, D., Law, S., . . . Pittman, J. (2019). Prevention and treatment of pressure ulcers/injuries: The protocol for the second update of the international Clinical Practice Guideline 2019. *Journal of tissue viability, 28*(2), 51-58. doi:http://dx.doi.org/10.1016/j.jtv.2019.01.001
Lala, D., Spaulding, S. J., Burke, S. M., & Houghton, P. E. (2016). Electrical stimulation therapy for the treatment of pressure ulcers in individuals with spinal cord injury: a systematic review and meta-analysis. *International wound journal, 13*(6), 1214-1226. doi:http://dx.doi.org/10.1111/iwj.12446
Mason, G., Wilson, D., Hampton, C., & Würbel, H. (2004). Non-invasively assessing disturbance and stress in laboratory rats by scoring chromodacryorrhoea. *Alternatives to Laboratory Animals, 32*(1_suppl), 153-159. doi:http://dx.doi.org/10.1177/026119290403201s25
McCulloch, K., Litherland, G. J., & Rai, T. S. (2017). Cellular senescence in osteoarthritis pathology. *Aging cell, 16*(2), 210-218. doi:http://dx.doi.org/10.1111/acel.12562
McKee, T. J., Perlman, G., Morris, M., & Komarova, S. V. (2019). Extracellular matrix composition of connective tissues: a systematic review and meta-analysis. *Scientific Reports, 9*(1), 10542. doi:http://dx.doi.org/10.1038/s41598-019-46896-0
Merry, K., MacPherson, M. M., Blazey, P., Fearon, A., Hunt, M., Morrissey, D., . . . Willy, R. W. (2024). Current practice, guideline adherence, and barriers to implementation for Achilles tendinopathy rehabilitation: a survey of physical therapists and people with Achilles tendinopathy. *BMJ open sport & exercise medicine, 10*(1), e001678. doi:http://dx.doi.org/10.1136/bmjsem-2023-001678
Parkins, C., & Chaplin, D. (1999). Tumour blood flow changes induced by application of electric pulses. *European Journal of Cancer, 35*(4), 672-677. doi:http://dx.doi.org/10.1016/s0959-8049(98)00426-2
Patricio Cordeiro, T. T., Rocha, E. A. B., & Scattone Silva, R. (2024). Effects of exercise-based interventions on gluteal tendinopathy. Systematic review with meta-analysis. *Scientific Reports, 14*(1), 3343. doi:http://dx.doi.org/10.1038/s41598-024-53283-x
Prudêncio, D. A., Maffulli, N., Migliorini, F., Serafim, T. T., Nunes, L. F., Sanada, L. S., & Okubo, R. (2023). Eccentric exercise is more effective than other exercises in the treatment of mid-portion Achilles tendinopathy: systematic review and meta-analysis. *BMC Sports Science, Medicine and Rehabilitation, 15*(1), 9. doi:http://dx.doi.org/10.1186/s13102-023-00618-2
Rees, J., Wolman, R. L., & Wilson, A. (2009). Eccentric exercises; why do they work, what are the problems and how can we improve them? *British journal of sports medicine, 43*(4), 242-246. doi:http://dx.doi.org/10.1136/bjsm.2008.052910
Rompe, J. D., Furia, J., & Maffulli, N. (2008). Eccentric loading compared with shock wave treatment for chronic insertional achilles tendinopathy: a randomized, controlled trial. *JBJS, 90*(1), 52-61. doi:http://dx.doi.org/10.2106/JBJS.F.01494

Rompe, J. D., Nafe, B., Furia, J. P., & Maffulli, N. (2007). Eccentric loading, shock-wave treatment, or a wait-and-see policy for tendinopathy of the main body of tendo Achillis: a randomized controlled trial. *The American Journal of Sports Medicine, 35*(3), 374-383. doi:http://dx.doi.org/10.1177/0363546506295940

Sersa, G., & Miklavcic, D. (1993). The feasibility of low level direct current electrotherapy for regional cancer treatment. *Regional cancer treatment, 6*, 31-31.

Shaul, M. E., & Fridlender, Z. G. (2017). Neutrophils as active regulators of the immune system in the tumor microenvironment. *Journal of leukocyte biology, 102*(2), 343-349. doi:http://dx.doi.org/10.1189/jlb.5MR1216-508R

Tomic-Canic, M., & DiPietro, L. A. (2019). Cellular senescence in diabetic wounds: when too many retirees stress the system. *Journal of Investigative Dermatology, 139*(5), 997-999. doi:http://dx.doi.org/10.1016/j.jid.2019.02.019

Virchow, R. (1865). *Die krankhaften Geschwülste: 30 Vorlesungen, geh. während d. Wintersemesters 1862-1863 an d. Univ. zu Berlin* (Vol. 2): Hirschwald.

Waltz, R. A., Whitney, K. E., Duke, V. R., Kloser, H., Huard, C., Provencher, M. T., . . . Huard, J. (2023). A Systemic and Local Comparison of Senescence in an Acute Anterior Cruciate Ligament Injury—A Pilot Case Series. *Life, 13*(7), 1567. doi:http://dx.doi.org/10.3390/life13071567

Weber, C., Thai, V., Neuheuser, K., Groover, K., & Christ, O. (2015). Efficacy of physical therapy for the treatment of lateral epicondylitis: a meta-analysis. *BMC musculoskeletal disorders, 16*(1), 223. doi:http://dx.doi.org/10.1186/s12891-015-0665-4

Wilkinson, H. N., Clowes, C., Banyard, K. L., Matteuci, P., Mace, K. A., & Hardman, M. J. (2019). Elevated local senescence in diabetic wound healing is linked to pathological repair via CXCR2. *Journal of Investigative Dermatology, 139*(5), 1171-1181. e1176. doi:http://dx.doi.org/10.1016/j.jid.2019.01.005

Yu, G. T., Monie, D. D., Khosla, S., Tchkonia, T., Kirkland, J. L., & Wyles, S. P. (2024). Mapping cellular senescence networks in human diabetic foot ulcers. *GeroScience, 46*(1), 1071-1082. doi:http://dx.doi.org/10.1007/s11357-023-00854-x

Zulbaran-Rojas, A., Park, C., Lepow, B., & Najafi, B. (2021). Effectiveness of lower-extremity electrical stimulation to improve skin perfusion. *Journal of the American Podiatric Medical Association*. doi:http://dx.doi.org/10.7547/20-172

# *Appendix*

RightsLink

BG




## Physical therapy for tendinopathy: An umbrella review of systematic reviews and meta-analyses

**Author:** Beshoy Girgis,José Alberto Duarte

**Publication:** Physical Therapy in Sport

**Publisher:** Elsevier

**Date:** November 2020

*© 2020 Elsevier Ltd. All rights reserved.*

### Journal Author Rights

Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: https://www.elsevier.com/about/our-business/policies/copyright#Author-rights

BACK

CLOSE WINDOW

© 2024 Copyright - All Rights Reserved | Copyright Clearance Center, Inc. | Privacy statement | Data Security and Privacy | For California Residents | Terms and Conditions
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

RightsLink

BG

Efficacy of physical therapy interventions for chronic lateral elbow tendinopathy: a systematic review

Author: Beshoy Girgis, José Alberto Duarte, , et al
Publication: Physical Therapy Reviews
Publisher: Taylor & Francis
Date: Dec 3, 2019

*Rights managed by Taylor & Francis*

Thesis/Dissertation Reuse Request

Taylor & Francis is pleased to offer reuses of its content for a thesis or dissertation free of charge contingent on resubmission of permission request if work is published.

BACK

CLOSE

© 2024 Copyright - All Rights Reserved | Copyright Clearance Center, Inc. | Privacy statement | Data Security and Privacy | For California Residents | Terms and Conditions
Comments? We would like to hear from you. E-mail us at customercare@copyright.com

RightsLink

Sign in/Register

High Voltage Monophasic Pulsed Current (HVMPC) for stage II-IV pressure ulcer healing. A systematic review and meta-analysis

**Author:** Beshoy Girgis,José Alberto Duarte

**Publication:** Journal of Tissue Viability

**Publisher:** Elsevier

**Date:** November 2018

*© 2018 Tissue Viability Society. Published by Elsevier Ltd. All rights reserved.*

Journal Author Rights

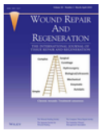
Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: https://www.elsevier.com/about/our-business/policies/copyright#Author-rights

BACK

CLOSE WINDOW

© 2024 Copyright - All Rights Reserved | Copyright Clearance Center, Inc. | Privacy statement | Data Security and Privacy | For California Residents | Terms and Conditions

Comments? We would like to hear from you. E-mail us at customercare@copyright.com



## The effect of high-voltage monophasic pulsed current on diabetic ulcers and their potential pathophysiologic factors: A systematic review and meta-analysis

**Author:** José Alberto Duarte, Davide Carvalho, Beshoy Girgis
**Publication:** Wound Repair and Regeneration
**Publisher:** John Wiley and Sons
**Date:** Jan 8, 2023

*© 2022 The Wound Healing Society.*

### Order Completed

Thank you for your order.

This Agreement between Beshoy Girgis ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

Your confirmation email will contain your order number for future reference.

| | |
|---|---|
| License Number | 5774971030156 |
| License date | Apr 23, 2024 |

Printable Details

#### Licensed Content

| | |
|---|---|
| Licensed Content Publisher | John Wiley and Sons |
| Licensed Content Publication | Wound Repair and Regeneration |
| Licensed Content Title | The effect of high-voltage monophasic pulsed current on diabetic ulcers and their potential pathophysiologic factors: A systematic review and meta-analysis |
| Licensed Content Author | José Alberto Duarte, Davide Carvalho, Beshoy Girgis |
| Licensed Content Date | Jan 8, 2023 |
| Licensed Content Volume | 31 |
| Licensed Content Issue | 2 |
| Licensed Content Pages | 16 |

#### Order Details

| | |
|---|---|
| Type of use | Dissertation/Thesis |
| Requestor type | Author of this Wiley article |
| Format | Print and electronic |
| Portion | Full article |
| Will you be translating? | No |

#### About Your Work

| | |
|---|---|
| Title of new work | The effect of high-voltage monophasic pulsed current on diabetic ulcers and their potential pathophysiologic factors: A systematic review and meta-analysis |
| Institution name | University of Porto |
| Expected presentation date | May 2024 |

#### Additional Data

| | |
|---|---|
| Order reference number | 150 |

#### Requestor Location

| | |
|---|---|
| Requestor Location | Beshoy Girgis<br>Praceta Francisco Borges, 55, 1°, Esq.°<br><br>Porto, 4200-310<br>Portugal<br>Attn: Beshoy Girgis |

#### Tax Details

| | |
|---|---|
| Publisher Tax ID | EU826007151 |

Would you like to purchase the full text of this article? If so, please continue on to the content ordering system located here: Purchase PDF
If you click on the buttons below or close this window, you will not be able to return to the content ordering system.

Total: 0.00 USD

CLOSE WINDOW ORDER MORE

© 2024 Copyright - All Rights Reserved | Copyright Clearance Center, Inc. | Privacy statement | Data Security and Privacy | For California Residents | Terms and Conditions
Comments? We would like to hear from you. E-mail us at customercare@copyright.com