THE ACUTE EFFECT OF TUINA ON HAND GRIP MUSCLE FATIGUE - PROTOCOL IN HEALTHY ADULTS

RANDOMIZED, CONTROLLED, PRELIMINARY CLINICAL STUDY

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Dissertação de Candidatura ao Grau de Mestre em Medicina Tradicional Chinesa submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto.

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DEDICATION

To my family, friends and colleagues.
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I would like to thank Prof. Dr. Henry Johannes Greten for his great help, support throughout the study period and his guidance.

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To all the participants who made this work possible.
ABSTRACT

**Background:** Gripping movements are one of the main activities of daily living (ADL). Due to its high necessity, it has a tremendous impact on daily life. Mainly power grip is influential regarding human activity, during power grip, the object is gripped so that forces are exerted on that object by the thumb, the fingers and the palm by isometric contraction. Many factors influence grip strength and its testing is of high significance.

**Methods:** We developed a randomized, controlled, preliminary clinical study. 30 healthy male and female subjects, aged between 20-30 years, were included and randomized by flip coin into two groups: experimental group and control group. The experimental group was subjected to one finger zen vibropression intervention, applied to the extra point P5`, for one minute. The control group had one minute of rest, instead. Subjects with history of trauma, ligament damage, fracture, tumor, surgery of the upper limb, diagnosed with or treated for cervical radiculopathy or herniation of intervertebral disc, were excluded.

To evaluate fatigue we measured, in the baseline, and after either tuina intervention or rest for one minute, grip strength and motor unit action potential in four stages by SS25LA hand dynamometer with clench force range 0-50 kg and by EMG unit of Biopac® MP36 respectively. During the measurements, the subjects were asked to grip the hand dynamometer as hard as they can for 6 seconds, then subjects released grip completely and waited for calibration, to normalize and re-scale, to percentage of reference value, the maximum voluntary contraction of the participant dominant arm. Then the Biopac® software determined the optimal grid display and force increments in 4 levels of strength, dividing the reference value into 4 levels. (Light, mild, moderate and maximum). After that we proceeded to the effective experience in which subjects were asked to grip the dynamometer until the generated force reaches the 1st level of strength during 6 seconds and release for 2 seconds, then they were asked to reach and hold the 2nd level of strength for 6 seconds and release for 2 seconds. Then it was asked to the subjects to reach and hold the 3rd level of grip strength for another 6 seconds and release for 2 seconds. Finally subjects were asked to reach the 4th level (The maximum level of strength) and try to sustain that level during 20 seconds. These 4 levels intended to induce fatigue in the subjects.
**Results:** The experimental group showed a non-statistically significant reduction of grip strength caused by fatigue, while the control group showed a statistically significant negative evolution of grip strength. The mean force of the experimental group showed a non-significant decrease of 2.19% (p=0.687), while the control group showed a highly significant (p<0.001) decrease of 12.52%. In maximum grip strength, the experimental group showed a non-statistically significant reduction of 4.17% (p=0.282), while the control group showed a highly statistically significant (p=0.001) reduction of 9.34%. In the experimental group, 46.7% of the subjects showed a gain of mean strength in the fourth level after tuina intervention, while in the control group, none of the subjects showed any gain of mean strength in the same level after one minute rest.

Moreover, the motor unit action potential evolution showed a reduction similar to grip strength in the fourth level of both groups. The percentage of reduction in the experimental group was 3.45% but statistically non-significant (p=0.548), while the control group showed a bigger reduction with a statistically significant percentage of 9.17% (p=0.047). In the variation of the maximum action potential, the experimental group showed a reduction of 6.35%, while the control group had an increase of 6.21%. However, both values in both groups were non-statistically significant.

**Conclusion:** The present results indicate that tuina intervention may delay fatigue through resisting the decline in force production, which may occur due to improved local blood flow, and may also improve motor unit action potential during fatigue in healthy subjects.

**Keywords:** Grip strength, power grip, fatigue, isometric contraction, tuina, one finger zen-vibropression, motor unit action potential.
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<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
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<td>ADL</td>
<td>Activities of daily living</td>
</tr>
<tr>
<td>ALT</td>
<td>Algor laedens theory</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>EMG&lt;sub&gt;rms&lt;/sub&gt;</td>
<td>Root-mean-square EMG</td>
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<tr>
<td>HRQoI</td>
<td>Health-related quality of life</td>
</tr>
<tr>
<td>MCP</td>
<td>Metacarpophalangeal joint</td>
</tr>
<tr>
<td>MVC</td>
<td>Maximal voluntary contraction</td>
</tr>
<tr>
<td>Pi</td>
<td>Inorganic phosphate</td>
</tr>
<tr>
<td>RM</td>
<td>Repetition maximum</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>TCM</td>
<td>Traditional Chinese Medicine</td>
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<tr>
<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
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CHAPTER ONE

BACKGROUND KNOWLEDGE
1. INTRODUCTION

1.1 Background

Gripping activity is one of the main activities of daily living (ADL). Due to the high frequency of usage of this activity, it has a tremendous impact on daily life.

Mainly power grip tasks are influential regarding human activity, during power grip, the object is gripped so that forces are exerted on that object by the thumb, the fingers and the palm. Isometric contraction primarily constitutes power grips [1, 2].

The fingers are laterally deviated, flexed, and laterally rotated. The flexion amount depends on the held object. The fingers function is supported by the thumb which aids in directing the force through fine adjustments. Variations of power grip include spherical grip, cylindrical grip, hook grip and lateral prehension [3].

On the other hand, manipulating an object between the fingers and the thumb without contact with the palm is considered a prehension pattern, where the primary muscle contraction is isotonic. Its varieties include tip to tip, pad to pad and pad to side prehension.

While holding an object between the thumb and the index or the middle finger, used in precision handling, is called pinching [3].

1.1.1 Muscles involved in power grip

- Extrinsic flexors of the fingers are the major source of gripping force.
- Extensor digitorum compresses the Metacarpophalangeal (MCP) joints, balancing flexors force and enhancing stability.
- Thenar muscles and adductor pollicis add to the compressive force applied to the object.
- Interossei rotate the first phalanx to the optimal alignment to compress the object.
- Only the fourth lumbrical participates in power grip [3].

The hand grip force generation demands the activity of extrinsic forearm muscles in addition to the intrinsic muscles of the hands, as reported by several studies [4, 5]. Electromyogram reports, done by these authors, showed characteristic pattern of concurrent activity of flexor digitorum superficialis and extensor digitorum.
1.1.2 Factors affecting grip strength

Grip strength represents the maximum force that a subject can voluntarily exert under normal biokinetic conditions [6]. It is the combined muscle performance that can be executed in a single muscular contraction [7].

Several factors affect grip strength, these include the following,

Hand dimensions

There is a positive correlation determined between hand dimensions, length of the fingers and anthropometric measures of the hand and grip strength [8, 9].

Static wrist position

Wrist flexion, in any degree, reduces grip strength significantly. Also, constrained grip strength is found to be significantly lower than unconstrained self-selected wrist position.

But conversely, static grip strength is not significantly altered in wrist positions between neutral and 45 degrees of extension [10]. However, at least 25 degrees of wrist extension is needed for optimum grip strength [11].

Posture and elbow position

There is a considerable variation in grip strength between subjects who did grip strength testing in sitting position with elbow flexed 90 degrees compared to standing position with the elbow in full extension [12].

These results indicated that hand grip strength testing standards should always be kept, as changes in posture or elbow position will lead to changes in grip forces that individuals can generate.

Hand Dominance

In right handed subjects, the dominant hand is stronger than the non-dominant one. But for left handed subjects, there is no significant difference between both hands [13, 14]. Grip strength should be regarded equal in both hands for left handed individuals [15].

Weight, Height, Body Surface area

Hand grip strength (of both left and right hand) is found to be positively correlated with subject’s height, weight and body surface area.
Age, Gender, Body Mass Index

Grip strength is found to be positively correlated with subjects aging from 7 to 19 years while negatively correlated with subjects aging from 20 to 73 years [14, 16].

Males hand grip strength is determined to be significantly higher than females in all groups of age. Moreover, the strength decline is found to be significant in both sexes with old age [17].

In the right hand dominant subjects, the highest grip strength is found in the 25 to 34 years subjects, while in the left hand dominant subjects, it is in the 18 to 24 years subjects. In addition, nutrition indicators (Body mass index, Mid-upper arm circumference, and arm muscle area) are found to positively correlate with grip strength in both sexes [14, 18, 19].

Occupation

Hand grip strength is considerably lower in old subjects with manual work history specifically when the subjects are stressed physically in high levels. Also, this is found to be correlated with reduced function and strength with old age [20].

Inflammation

Inflammation is found to be independently associated with hand grip strength, based on the assessment of C-reactive protein (CRP) as an inflammatory marker in non-critically ill patients.

For subjects with inflammation had reduced grip strength with values of approx. 1.6 kg in mild inflammation, 3.2 kg in moderate inflammation, and 2.6 kg in subjects with severe inflammation compared to healthy individuals [21].

1.1.3 Significance of hand grip strength testing

Physical Strength Indicator

The hand grip strength can be used as an indicator of the physical strength and health of an individual [22]. As the adequate nutritious intake and physical activity will result in improvements in muscle strength, body composition and bone mass.

The increase of forearm bones density specifically and higher levels of physical activity result in stronger grip strength.
Upper limb Strength status

Grip dynamometer can be used to indicate the general strength of the upper limb of a subject [23]. It could be used to clinically monitor levels of strength of shoulder stabilizers and also aid the rehabilitation process through identifying the current strength status and the suitable resistance loads to be prescribed to patients for strengthening goals [24].

Index of Nutritional status

Maximal voluntary contraction (MVC) of subjects was determined using a handgrip dynamometer which is found to positively correlate with the body mass index of subjects [25].

As mentioned earlier, because of this correlation, hand grip strength can be used to determine BMI of individuals and give helpful information about their general nutritive state.

Vitamin D and bone density indicator

The correlation between vitamin D and hand grip strength is significantly positive, as subjects with hip fractures, due to either deficiency of vitamin D or hyperparathyroidism, had significantly lower grip strength than healthy subjects [26].

Low density of bone has a high correlation with both the risk of hip and vertebral fractures and also reduced grip strength in women [27].

Postoperative complications, functional decline, morbidity and mortality

Hand grip dynamometry was found to be a simple way to determine the overall strength of an individual which correlates with his current functional state and risk of complications [28].

Studies generally support usage of hand grip strength testing as a morbidity predictor after surgery, as the subjects with grip strength below 15 kilograms were found to be highly susceptible to neck of femur fracture postoperative complications [29].

Furthermore, reduced grip strength was shown to be correlated with lower health-related quality of life (HRQol) in elderly men and women, which was not found to be related to age, activity, or co-morbidity [30].

While hand grip strength has been shown to be a prognostic tool for all-cause mortality among elderly people, due to its ability to indicate general muscular strength [31] and to represent an indicator of survival [32], another study has doubted the ability to use hand grip strength as a mortality prognostic factor [33].
1.2 Physiology

1.2.1 Physiologic anatomy of skeletal muscle

All skeletal muscles are constituted of muscle fibers. Each muscle fiber is composed of subunits and is usually innervated by only one nerve ending, sited around the middle of the fiber.

The sarcolemma is a membrane that covers the outer surface of muscle fibers. The surface layer of sarcolemma is fused with a tendon fiber at both ends of the muscle fiber. Therefore, the tendon fibers form bundles that are inserted into bones which are called muscle tendons.

Myofibrils in large quantities form the basic structure of a skeletal muscle. Each myofibril contains adjacent myosin and actin filaments which are the protein molecules needed for muscle contraction to occur. Cross bridges from myosin filament interact with actin filaments to produce contraction. The ends of actin filaments are attached to the Z-disks which extends crosswise across the myofibril and also from one myofibril to another.

The sarcomere is the myofibril part that exist between two successive Z disks. During contraction, the sarcomere length is about 2 micrometers. And with this length, the myosin filaments are totally overlapped by the actin filaments that just start to overlap one another. This is the length where the maximum force of a muscle can be generated.

The M line attaches the myosin filaments together so that they act as a functional unit. The sarcoplasm fills the spaces between myofibrils, which is an intracellular fluid containing large numbers of mitochondria that provides the contracting myofibrils with the needed amounts of energy in the form of adenosine triphosphate (ATP) [34].

1.2.2 Mechanism of muscle contraction

Excitation-contraction coupling

Muscle contraction is initiated through action potential that moves through a motor nerve to reach the muscle fibers. Minute amount of neurotransmitter substance called acetylcholine is secreted at the motor nerve endings.

This acetylcholine reaches the muscle fiber membrane leading to the opening of several acetylcholine-gated channels.
The opening of these channels permits the influx of large amounts of sodium ions to the interior of the muscle fiber membrane, which results in localized depolarization that subsequently causes the opening of voltage-gated sodium channels. Then the action potential starts at the muscle membrane.

The muscle membrane is depolarized due to the action potential, as most of its charge moves to the muscle fiber center, which results in the release of huge amounts of calcium ions stored at the sarcoplasmic reticulum. The contractile process is initiated afterwards as a result of sliding of actin and myosin filaments alongside due to the released calcium ions. The muscle contraction is stopped afterwards, as the calcium ions are pumped by the calcium membrane pump to the sarcoplasmic reticulum. A process that restores the muscle ability to respond to a new muscle action potential [34].

1.2.3 Energy sources for muscle contraction

Most of the muscle energy is used to activate the mechanism that pulls the actin filaments by the cross bridges, but the energy required to pump calcium ions back to the sarcoplasmic reticulum after the contraction ends and to sustain the optimal ionic medium for the muscle action potential to propagate is considerably fewer.

*Phosphocreatine* is the first energy source used in a skeletal muscle to reconstitute the used up ATP. But the general amount of phosphocreatine in the muscle fiber is considered to be small. So, a maximum muscle contraction is hard to be held more than 5 to 8 seconds without depleting both ATP and phosphocreatine storage of a muscle.

*Glycolysis* is the second major energy source, through the degradation of glycogen to pyruvic and lactic acids, reconstitution of both phosphocreatine and ATP occurs. The energy liberated from this degradation converts ADP to ATP which maintains the needed ATP energy supply to the contracting muscle and also regenerates phosphocreatine storage. In the absence of oxygen, glycolysis can still occur and supply the muscle with the needed energy for seconds up to a minute. The disadvantage is that the resulting products of this process tend to accumulate in the cells of the muscle which reduces the muscle ability to hold a maximum contraction for more than a minute. The ATP formation rate by the process of glycolysis is about 2.5 times as rapid as the ATP formed by normal cellular metabolism.

*Oxidative metabolism* is the final energy source. It is the process of combination of glycolysis end-products and cellular metabolic-substances with oxygen for the liberation of ATP.
For a long duration muscle contraction to be held, oxidative metabolism mainly supplies the muscle with the required energy. Carbohydrates, proteins or fat are the substances that muscle cells normally use to produce ATP. Fats constitute the largest share of consumed energy to maintain maximal muscle contraction over a very long-time (Numerous hours), while carbohydrates proportion is almost half of the consumed energy, if maximal contraction is to be maintained for 2 to 4 hours [34].

1.2.4 Muscle contraction characteristics

Isometric muscle contraction is a contraction of a muscle without shortening in length, while isotonic contraction is a contraction with a sustained tone but shortened length. During isometric contraction, the cross bridges are moving in a cyclic motion to produce tension without filament sliding. Although, no limb or joint movement occurs, some fibers actually shorten during contraction due to their elastic properties.

Muscles are constituted of a combination of slow, fast fibers and others with a moderate speed of contraction. In other words, muscles that are required to produce a fast response have more proportion of fast muscle fibers (Type II) compared to slow ones (Type I) and vice versa. Type I muscle fibers are relatively smaller, with small sized nerve fiber innervation, immense blood supply, higher mitochondria content, and higher myoglobin content. In contrast, Type II muscle fibers are relatively larger, with considerably larger sarcoplasmic reticulum, higher content of glycolytic enzymes, limited vascular supply and less amount of mitochondria.

Motor units are composed of the muscle fibers supplied by a one single nerve fiber. Generally, for precise muscle control to occur, as in smaller muscles that rapidly respond, fewer number of muscle fibers are supplied by a relatively larger amount of nerve fibers. On the other hand, for general rough movements, some hundred muscle fibers may exist in a single motor unit, as in the large muscles of the lower limbs for example.

1.2.5 Sequence of recruitment of motor units

Under isometric contraction circumstances, the motor units are believed to follow the recruitment order developed by Henneman (Size principle), which states that motor units are activated in a certain sequence so that the motor units with slower conduction velocity are activated first, followed by the ones with faster conduction velocities [35-37].
However, under different functional circumstances, the frequency of firing of different motor neurons may change to adapt to the desired function. Central or reflex inputs to the motor neuron pools may not equally alter the firing frequency [38].

This principle can closely relate the properties of motor neurons with muscle fiber properties, as the small sized motor neurons with long after-hyperpolarization durations and low conduction velocity axons are linked to small twitch force muscle fibers with long contraction durations, slow conduction velocity and low fatigability [39-41].

During maximal sustained contraction, the reduction of force production is mainly caused by large motor unit’s fatigue, which are the last to be recruited. Furthermore, more effort will be required to induce the last force part during maximal contraction due to the increasing thresholds of motor neurons to be activated to produce maximal force voluntarily. This process is known as threshold-spacing [42].

During fatigue, the reduction in the firing rates of motor units during maximal voluntary contraction is evident. In order to generate and maintain the same amount of power for long durations, the frequency of firing has to decline gradually. Therefore the motoneurons firing rate need to match the changing properties of contraction of muscles. A phenomena called muscular wisdom [43].

### 1.3 Realistic definition of fatigue

Many attempts were done to try to define fatigue, from a muscular perspective, it can be defined as failure of maintenance of expected or required force [44], or failure to sustain work at a certain intensity of exercise [45]. The problem with this definition is that it defines fatigue as a sudden event, while it has been shown that muscle fatigue occurs only at task failure, in other words, when exhaustion occurs. As a matter of fact, the muscle capacity to generate maximum force begins to fade once the exercise starts. So the phenomena of fatigue starts to develop almost with the start of exercise and progresses to just before the failure of the required task performance.

Exercise-induced decline in the capacity to exert muscular force or power regardless of the sustainability of the task is the definition that is more realistic in this case [46]. The peripheral ability to produce force usually deteriorates in the beginning of exercise, however, the onset of fatigue is delayed due to the changes in CNS that occur before muscle failure.
1.4 Skeletal muscle fatigue

Muscle fatigue mainly occurs due to three causes, the main one is the use-up of muscle glycogen storages, which leads to direct shortage of energy sources needed for contraction as discussed before. Also, the reduction in nerve transmission can occur with exhaustive muscle contractions.

Lastly, the lack of adequate blood supply to a contracting muscle caused by the contraction itself, which compresses the blood vessels and reduces the flow. This can result in total muscle fatigue after one to two minutes, caused by shortage of nutrients especially oxygen [34]. Fatigue is considered a multifactorial, complex phenomena. The characteristics of the performed task as the type, duration and speed of muscular contraction affect fatigue mechanisms [47].

The generation of muscle force includes many physiological processes that may extend to the whole neuromuscular system. Different factors may be involved in the fatigue phenomena. The neuromuscular system compensates for the reduced force production by regulating various muscular and nervous mechanisms.

1.5 Causes of fatigue

Several different physiological causes are involved in the reduction of force generation during submaximal or maximal muscle contraction [48]. Generally, during high intensity activities, the role of peripheral fatigue seems to be more significant than central fatigue. However, during low intensity prolonged activities, central fatigue role is more important [48]. Peripheral fatigue, which occurs distal to motoneurons stimulation point, includes branch-point failure, neuromuscular junction fatigue, reduction of muscle fiber contractile strength and modifications in the mechanisms underlying muscle action potential transmission. On the other hand, central fatigue occurs mainly due to voluntary activation failure. It includes supraspinal fatigue caused by failure of motor cortical output and reduction of rate of discharge and number of active motor units [49]. Sequence of voluntary activation is shown in figure 1. The development of central fatigue occurs either during intermittent or sustained maximal voluntary contractions (MVCs). During isometric contraction, the rate of firing of motor units is reduced, rapidly initially, and then reaches a plateau after 30 seconds. The rate of reduction may differ between muscles due to the variation in motor unit types [49].
The various factors involved in fatigue are as follows [50]:

**Central fatigue factors**
1. Primary motor cortex activation.
2. Command propagation from the central nervous system (CNS) to the motoneurons (the pyramidal pathway).

**Peripheral fatigue factors**
4. Action potential propagation from the anterior horn cells to muscle fibers including neuromuscular junction conduction.
5. Excitation contraction coupling.
6. Metabolic substrates availability.
7. Intracellular medium state.
1.5.1 Peripheral causes of fatigue

Peripheral fatigue involves changes in neuromuscular transmission, excitation-contraction coupling, propagation of muscle action potential and contractile mechanisms.

*The neuromuscular transmission* is the conversion of the action potential of the nerve into a muscle action potential and this process takes place at the neuromuscular junction. This mechanism is affected by fatigue in the following manner, the propagation of nerve action potential at the nerve ending becomes insufficient, the coupling between excitation and secretion of neurotransmitter in the synaptic gap fails to occur, the depletion of neurotransmitters is reduced, and the sensitivity of the post-synaptic acetylcholine receptors and post-synaptic membrane is decreased [51, 52].

Studies reported that neuromuscular transmission impairment seems to be a limiting factor to the excitation of muscles and contributes to force generation reduction. This appears to occur more commonly during low-intensity, long duration exercise than short-duration intense contractions [53, 54].

*Muscle action potentials* are produced when the sum total of inhibitory and excitatory presynaptic potentials exceeds or reaches the excitatory threshold of muscle cells. However, other factors may affect the nerve impulse transformation into muscular force and fatiguing exercise may impact this phenomenon [55]. Many metabolic changes accompany prolonged contraction of muscles, however, two factors seem to be the essential causes of reduced force generation capacity of myofibrils during fatigue [56]. These are the accumulation of intracellular inorganic phosphate (Pi) and hydrogen ions (H⁺).

Moreover, the increase in Pi concentration during exercise occurs due to the dissociation of phosphocreatine into Pi and creatine, and adenosine triphosphate (ATP) hydrolysis. Conversely it has been shown that medium acidification due to hydrogen ion accumulation has a very limited impact on the capacity to generate force at normal temperature of body [57]. On the other hand, it has been proven that inorganic phosphate accumulation (Pi) can impair generation of force by reducing the sensitivity of myofibrils to calcium ions by directly acting on the cross-bridges [58, 59]. Under fatiguing conditions, the increase in Pi concentrations may impair the contraction-relaxation cycles of the cross-bridges [60, 61].

These studies suggest that during the very beginning of contraction, the reduction in the force generation capacity may be caused by Pi accumulation.
While during sustained contraction, the decline in force production may be linked to the reduction in calcium ion quantities released by the sarcoplasmic reticulum. This phenomenon may occur first in fatigued muscle fibers under anaerobic conditions [62] and later on occurs in fibrils with powerful oxidative capacities [63]. The accumulation of Pi caused by exercise within muscle cells may penetrate into the sarcoplasmic reticulum and bind to calcium ions to form calcium phosphate (Ca²⁺-Pi). This process would reduce calcium ion reserves capable of being released to the sarcoplasm [64].

Moreover, the transmission of signals from transverse tubules to the sarcoplasmic reticulum and the process of calcium ion release are all dependent on ATP. Thus the reduction in ATP reserves may result in less phosphorylation in some sites related to action potential propagation which would limit calcium ions release [65].

Furthermore, during the activation of muscles, the increase in the concentration of Mg²⁺ in the sarcoplasm results in decreased amount of Ca²⁺ released by SR [66], and reduces force generation during exercise as Mg²⁺ ions bind to ATP [59], which contributes to the depletion of ATP storage of the muscles.

**Blood supply and reserves of substrates of metabolism** influence performance during sustained exercise [67, 68]. During the contraction of a muscle, an increased blood flow is needed to supply the contracting muscle with substrates, dissipate heat and evacuate metabolites. However, the contraction of muscles usually compresses the blood vessels and thus reduces their own blood supply. In fact, complete ischemia may be induced by isometric contraction.

Therefore the oxygen supply to the contracting muscle will be reduced, with the tendency to anaerobic metabolic pathways and rapid accumulation of metabolites. This will hasten the fatigue process and the reduction in force generation.

It has been shown by Sjogaard et al. that as exercise intensity increases, the blood supply drops, during continuous hand-grip between 5 and 50% of maximal voluntary contraction (MVC) [69]. Also, muscle ischemia will occur earlier with greater intensity of contraction [70]. Furthermore, it was shown by Crenshaw et al. that time of maintenance of extensor contraction of legs at 25% MVC coincided with complete ischemia time [67]. Probably the blood supply remains constant in muscle contractions of less than 15% MVC [69, 71].

The intake of additional glucose also allows the subject to sustain the exercise for longer durations [72, 73]. Therefore the availability of metabolic substrates may help maintain the required force, especially in certain types of endurance exercises.
1.5.2 Central causes of fatigue

Central fatigue involves all the spinal and supraspinal physiological changes able to reduce the amount of excitation of motoneurons. Percutaneous electrical stimulation can be used to detect the state of central fatigue [74, 75]. During maximal voluntary contraction (MVC), some motor-units do not either fire or are not recruited enough to generate maximal force. This was indicated as the peak force generated after electrical stimulation exceeded that generated after voluntary activation [76]. Loscher et al. has reported the occurrence of central fatigue during foot planter-flexors isometric contraction (30% MVC) [77]. Studies using transcranial magnetic stimulation (TMS) have shown that central fatigue can contribute for more than 25% reduction in force generated during maximal sustained contraction [49, 78, 79]. TMS is a technique used to induce muscle contractions through the stimulation of the motor cortex, which can be used to determine the status of supraspinal fatigue [80]. The reduction of motor cortex excitation may represent one of the causes of central fatigue [76].

Despite that the exact causes of central fatigue are not well known, some theories have been suggested to explain this phenomenon.

**Supraspinal fatigue** can be related to the accumulation or depletion of neurotransmitters in the brain, causing a reduction in corticospinal excitation. The neurotransmitter that is studied most in this case is serotonin (5-hydroxytryptamine 5-HT). It has been suggested that the serotoninergic activity of the brain increases with sustained exercise, which results in reduction of central impulses and the motor unit recruitment thereby [81]. The blood-brain barrier prevents the crossing of serotonin into the brain, therefore the neurons of the brain must synthesize this compound from its precursor, tryptophan (TRP). Moreover, it has been found that carbohydrates supply during maximal aerobic cycling power reduces the TRP activity and prolongs the exercise [82]. This may lead to inhibition of serotonin synthesis in the brain due to carbohydrates antagonistic effect on free TRP accumulation caused by exercise. The role of serotonin uptake and the influence of serotonin agonists and antagonists were proven to be significant in physical exercise initiation and continuation [83, 84].

**Dopamine** may affect central fatigue, as it can reduce the synthesis of serotonin through its action on TRP hydroxylase enzyme, which is considered to be the main enzyme in the synthesis of 5-HT process [85]. Also, Catecholamines (e.g. adrenaline, noradrenaline, dopamine) may affect fatigue through acting on motor action and motivation of subjects [86].
Other neurotransmitters are suggested to be involved in central fatigue process as glutamate, adenosine, acetylcholine, and gamma-aminobutyric acid (GABA) [87, 88].

**Glycogen** storage may also be considered a factor in central fatigue as the activation of the brain is linked to depletion of the storage of glycogen in the brain [89]. In other words, the brain may get easily fatigued with lack of sufficient glycogen storage, which in turn may affect serotonin activity [90]. However, the impact of glycogen depletion is more evident during prolonged exercise. Also, the reduction in blood glucose levels (hypoglycemia) caused by exercise may lead to reduced activation of the central nervous system (CNS) during sustained exercise but not during brief exercises, as it was indicated that CNS has a high capability to regenerate within seconds of rest [91].

**Muscle afferents feedback** to the brain is suggested to be influential by another hypothesis. It was shown that muscle afferents can influence supraspinal fatigue, which was concluded due to the reduction of motor-evoked potentials observed [92]. Moreover, activation from some cortical areas was found to be reduced, probably due to muscles feedback [49]. However, it was suggested that muscle fatigue mechanisms restrict voluntary activation but do not affect the excitation of the motor cortex and motoneurons [93]. Therefore, the feedback of muscle afferents may influence central supraspinal fatigue through limiting voluntary activation [76].

**Spinal fatigue** mechanism suggested that the motoneurons activation is reduced due to the inhibitory impulses of intramuscular receptors. It has been shown that the discharge rate of motoneurons may be controlled by peripheral reflexes due to metabolic changes induced by fatigue within the muscles [94]. Moreover, group III and IV muscle afferents seem to be activated by extracellular concentration of potassium and lactate [95, 96] and also by ischemia and hypoxemia [97, 98]. Activation of these muscle receptors during exhausting exercise may result in the inhibition of alpha motoneurons [99-103]. Furthermore, the effect of these receptors seem to inhibit the extensor and stimulate the flexor motoneurons [102].

**Muscle spindles** are aligned parallel to the fibers of the muscle and provide feedback to the nervous system through group Ia and II afferents. This feedback indicates the length of the muscle and the rate of change of this length [104]. Alterations in the stiffness of the muscle, due to repeated contraction, may affect the sensitivity of these spindles, the electrical activity of muscles and fatigue levels [105].

**Golgi tendon organs**, through group Ib afferents, provide feedback to the nervous system indicating the tension of the muscle [104]. These receptors are believed to inhibit the activity of motoneurons [106].
However, it is difficult to detect the impact of these receptors on fatigue as they are hard to isolate and impulses from Ia afferents affect their projecting interneurons [49].

*Renshaw cells* inhibit motoneurons, especially motoneurons in fast twitch motor units, but they are also activated by the same motoneurons [107] and by other influences. Several studies have shown that this inhibitory effect is intensified during maximal contractions [108] and is reduced during submaximal contractions of 20% MVC, when central fatigue arises [109, 110].

1.5.3 Neuromuscular adaptations to fatigue

*Motor unit adaptation* occurs through many mechanisms, the integration of increasing recruitment and modulation of motor units discharge constitute the main mechanism during submaximal levels of exercise. The discharge rate of motor units seems to drop during maximal sustained contractions [111-114]. However, the discharge rate related to submaximal exercise may increase in response to fatiguing as reported by another study [115]. The variation between subjects appears to be a factor related to differences in motor unit firing rate [116, 117]. A reduction in the firing rate of motor units will always occur during motor unit activation for certain duration [118-121].

*Potentiation* is another mechanism to adapt to fatigue, where the muscular capacity for activity is augmented through several mechanisms. These mechanisms include increased excitability of motor cortex, auxiliary stimuli generation and post-activation potentiation [122]. The cortical excitability may be increased at the beginning of a held contraction or after submaximal contractions. In spite of that, cortical inhibition may also occur during the fatigue process [123]. While post-activation potentiation mechanisms may include calcium ion release by the sarcoplasmic reticulum [124, 125], myosin light chain phosphorylation [126] and force-speed characteristics of cross bridges [127]. It appears that either repeated or sustained contractions elicit electrical and mechanical changes during the initiation of a contraction, and these mechanisms allow the development of more force and fatigue resistance. These mechanisms may allow the central nervous system to reduce the number of the motor units required to be additionally recruited.

*Muscle wisdom* is another hypothesis of a mechanism used by the body to adapt to physical fatigue [128]. This mechanism encompasses a reduction in motor unit firing rate and a decrease in speed of contraction of muscles during fatigue. Reducing the decline rate of membrane excitation and therefore Ca$^{2+}$ release may act as a defensive mechanism against muscular fatigue [103, 128].
It may also include the limitation of central activation through muscular peripheral afferent feedback, which reduces the active myofibrils ability to generate force [129]. Although the validity of the application of this theory during submaximal prolonged isometric and dynamic contractions is limited, it seems to be suitable for some muscles during maximal contractions [130].

*Group III and IV muscle afferents* also have an influential impact on delaying fatigue during exercise. As the impulses of these afferents regulate the autonomic responses to exercise, including increased ventilation, central and peripheral hemodynamic regulation to ensure sufficient blood flow to the contracting muscles. These adaptations contribute to the reduction in peripheral fatigue rate which improves the overall performance [131].

### 1.6 Rationale for manual therapy intervention

#### 1.6.1 Effects of vibration

In a systematic review by Fuller et al. [132], twenty two studies with a sum of 302 subjects were incorporated in the review of effect of vibration (mostly 3-60 HZ) on muscle perfusion. It was found that blood volume of muscle improved with vibration in five of nine studies, blood flow velocity of muscles improved with vibration in five of six studies, blood flow of muscles improved with vibration in two of three studies, arterial diameter increased with vibration in three of three studies, and vibration had no effect on muscle temperature in two of two studies. Moreover, the degree of improvement in muscle perfusion was found to be positively correlated with vibration load.

Kerschan-Schindl et al. reported that vibration exercise (26HZ) increases popliteal artery blood flow (100%) and results in erythema in the foot and calf [133]. Moreover, it was reported in another study that an intermittent vibration protocol (10-30HZ) resulted in an increase in mean blood cell velocity of the femoral artery. The greatest increase in blood flow compared to rest was recorded while using 30 HZ [134]. However, it was found that an intermittent vibration protocol (45HZ) resulted in no increase in femoral artery blood flow after three minutes by another study [135].

Several studies have analyzed the acute effect of vibration (20-150 HZ) on maximal isometric contraction [136-139]. Two of these studies examined short duration of vibration and contraction [137, 138] and the other two studied long duration contraction and vibration.
Moreover, neuromuscular performance in fatigued and unfatigued conditions was evaluated in the latter two studies [136, 139]. Only one of these studies showed a significant positive effect on maximal force, with the unfatigued neuromuscular system [137]. The total increase in maximal force was found to be increased by approximately 7.8%. But another study concluded that vibration resulted in a non-significant reduction of maximal force of approximately 5% [136].

However, in the neuromuscular fatigue condition studies, the time to fatigue after sustained maximal contraction was reduced significantly by 30% with vibration applied in comparison to control group [139]. Also, the decline of force of maximal isometric contraction was found to be significantly greater by 13% at the end of one minute maximal contraction, when vibration was applied [136]. These results suggest that vibration may delay muscle fatigue of maximal sustained contraction only during unfatigued conditions.

The acute effect of vibration was on submaximal isometric contraction was also examined by one study [140]. The activity of muscles evaluated through EMG was found to be significantly increased by vibration. However, because subjects could not maintain a constant level of contraction force, it was not possible to exactly determine whether vibration enhances submaximal isometric contraction force. This suggests that vibration probably enhances force of submaximal contraction [141].

Kin-Isler and colleagues used an electromotor to transmit vibrations to a leather belt, through an attached cable, that was placed over the biceps brachii muscle belly. An increase of 6.4% in MVC of elbow flexors was observed during a 10 second vibration exposure, using a range of frequencies of vibration (6, 12, 24 HZ) and joint angles (90°, 120°, 150°) [142]. Another study showed that the average power of biceps brachii increased by 8% following vibration application (30 HZ) of twelve international boxers that gripped a hand held vibrating device [143]. Similarly, it was reported that both non-elite and elite athlete groups showed an increase of 10.2% and 10.7% respectively in mean power and an increase of 10.4% and 7.9% respectively in peak power during vibration (Using isotonic vibrating cable of 44HZ) compared to no vibration [144].

On the other hand, other studies showed little or no effect of vibration on the power of upper-body. No significant pre-post changes in power, moment and angular velocity were found with the direct application of a custom built vibratory unit (65HZ) to the biceps brachii tendon while resistance trained males did 3 sets and 5 repetitions of (70% 1RM) biceps curls [145].
Cochrane and Hawke have reported no significant increase in power of the upper body with the application of an electric powered vibrating dumbbell (26HZ) to climbers [146].

Effect of vibration on EMG activity was evaluated using a vibrating dumbbell, it was observed that a two-fold increase of biceps brachii EMG$_{rms}$ compared to baseline occurred during intermittent vibration (30HZ), while during post-vibration, it resulted in augmented biceps power but without a corresponding increase in EMG activity [143]. Moreover, it was shown by another study that vibrating (28HZ) isometric elbow pull and push actions resulted in an increased EMG$_{rms}$ with increases in co-contraction at loads of 20% and 40% of maximum force [147]. However, vibration (65HZ), directly applied to biceps brachii tendon, did not result in an increase in EMG$_{rms}$ during lifting phase of biceps curls at 70% 1RM [145].

Low vibration frequency (5-45HZ) was shown to increase EMG activity, muscle force and power [143, 148-153], where the muscle spindle excitatory responses are speculated to play a role in enhancing muscle activation, spinal reflex mechanisms are also involved in this process. The muscle tuning response is another mechanism that may explain these responses, where the muscular system reduces the vibration stimuli to promote muscle activity that may improve muscle function subsequently. Moreover, motor unit recruitment, synchronization, and co-contraction may be responsible for force and power improvements following acute vibration [154].

Vibration training may have a positive acute and/ or chronic training effect on strength and power. However, the vibration effect, in this case, seem to be dependent upon the characteristics of vibration (application method, frequency and amplitude) and the protocols of exercise (type of training, volume, and intensity) used [155].

1.6.2 Effects of pressure

It has been shown that application of pressure on an acupuncture point, known as acupressure, may result in increased blood flow throughout a limb [156]. This effect has been attributed to De-Qi stimulation, which occurs with stimulating an acupuncture point, and this stimulation may be followed either by a dominant parasympathetic tone or a reduced sympathetic tone [157]. Moreover, the intensity of this effect was found to be positively correlated with the intensity of stimulation [158]. It has been also shown by other studies that acupressure may improve the microcirculatory blood flow, which is regarded as a proposed mechanism for acupressure therapy efficacy [159, 160].
In another study done on the effect of trigger point release through applying ischemic compression. It has been found, using microdialysis, that local blood flow (microvascular exchange), glucose and lactate levels increase up to 20 minutes following the application of trigger point release [161].

The hypothesis stating that the release of trigger points allows the tissues to be supplied by nutritive blood flow, increases perfusion of substrates and delivery of oxygen to skeletal muscles to fulfil energy demands needed to restore homeostasis, is supported by these results [161].

1.7 TCM point of view

1.7.1 The Heidelberg model of traditional Chinese medicine

The Heidelberg model of TCM [162] is a mathematical model on vegetative regulation inherent in the classical corpus medicus. Using this approach, certain technical terms like yin, yang and the phases can be translated as vegetative terms of function. This approach may be considered critical for the integration of TCM in western health care systems and research.

1.7.2 The orbes: physiological patterns

Applying the Chinese medicine model of regulation to the vegetative system results in the allocation of symptoms and signs to organ patterns or orbes (orbs). Organ patterns can be explained as vegetative physiological patterns permitting the translation of ancient Chinese physiology into terms of western knowledge [163].

The regulatory and technical aspect of yin/yang and the phases (Elements) i.e. Wood, Fire, Earth, Metal, Water, may be seen in a basic analogous example of water temperature regulation by a thermostat system. Due to the innate fluctuations, the actual temperature variation alternates around the set point approximately in a sinus wave manner (Fig. 2).
Fig. 2: Regulation as a technical process. The temperature profile of the pool is not constant (straight line), but rather sinusoidal. Temperature is on the y-axis, time on the x-axis, which corresponds to the desired temperature. (Greten 2007)

1.7.3 Sinusoidal-pattern of phases

Almost all biological systems are regulated in such a sinusoidal pattern. TCM has developed its own terminology to describe such variations relative to the set point, which could be applied to the autonomic nervous system regulation of the human body where yang-states are values above the set point; yin-states are values below the set point. The phases represent the quadrants of this sinusoidal wave.

A sinus wave may also be seen as a circular function. As in the Fou qi character which represents the basic mathematical description of the circular motion (Fig. 3).

Yang includes the phases of wood and fire while yin includes the phases of metal and water.

This model also shows clearly the basic concept of yin-deficiency and the six-stage theory of Shang Han Lun [162].
Fig. 3: Fou qi emblem: a symbol for the regulatory meaning of yin, yang and the phases. The sinusoid wave is a circular function around a shall-be value in biological systems. Yin and yang are terms of regulation that can be further differentiated in phases (wood, fire, metal, and water). The shall-be-value is associated with the central phase of earth. (Greten 2007)

1.7.4 Analogies of the phases with neurohormonal mechanisms

The Heidelberg model hypothesizes a relation between this sinusoidal-pattern of phases, the different responses of the autonomic nervous system and its main molecular effector substances (e.g. hormones, neurotransmitters, etc.) (Fig. 4).
The postulated assignment of phases of Chinese medicine into the autonomic nervous system with respective analogies between the phases and the neurohormonal mechanisms represents a base for diagnosis and treatment. Scientific evidence of efficacy of treatment based on this model has been achieved by a recent double blinded assay of evaluation in acupuncture research [162] have shown that in double blinded study design. This shows that acupuncture based on this reconstruction of the classical theory almost doubles the efficacy of current western acupuncture.

Analogue data has been shown in studies for polyneuropathy [164], congestive heart failure [165, 166], pain following sternotomy in heart surgery, respiration after heart surgery, pain after tonsillectomy [167], walking distance and peripheral arterial occlusive disease [163]. However more studies are required to support this model.
Qi is one of the fundamental concepts of TCM, usually translated as energy, life force or vital energy as the basis of everything, an immaterial form that promotes dynamism and the activity of the living being.

According to the Heidelberg model, qi is explained as the capacity for vegetative function of tissues or organs that can cause the sensation of pressure, tear or flow [168].

According to Porkert [169], qi is defined as the immaterial energy with a qualification and direction. In Chinese medicine, qi has two fundamental aspects. It designates the essence (jing), which has the function of constructing the body and the mind (shen). Also, it indicates the complex functional activities to maintain both.

The states of health and disease are related to qi and its movement. Therefore, processing and correcting the direction of movement of qi is the basis for the motion of xue (blood) transformation of essence (jing), movement of body fluids, food digestion, nutrient absorption, excretion, hydrating the skin and increasing resistance to external pathogenic factors [168].

Xue, despite having a different concept of blood in western medicine, is the functional capacity (Energy) linked to body fluids with functions of warming, moisturizing, create qi and nourishing the tissues [170].

It is driven by the qi in the system of channels (conduits). From the viewpoint of the western medical sciences, the clinical effects of xue can be compared to the effects of blood and microcirculation, including functional relationships, blood cells, plasma factors, endothelium and parenchyma [168].

Xue has a double nature: it is part of the yin and substance, and at the same time is a form of yang energy. This double nature of xue becomes obvious in the functional relation between xue and shen. Because the xue (yin) checks / or controls the shen (yang) [168].

1.7.5 Fatigue according to the Heidelberg model of TCM

The normal contractile process of the muscles follows a certain order, where the wood phase represents excitation of the muscles, the fire phase represents muscle contraction, the metal phase represents fatigue and the water phase represents regeneration as shown in figure 5.
Fatigue is the de-activation of the vegetative mechanisms animating the muscle tissue. Fatigue occurs due to the lack of qi which is caused by the contractile process that consumes qi. Localized lack of xue can cause fatigue as well. As the movement of xue is dependent on qi, lack of qi can cause xue stasis. Xue stasis will result in reduced xue reaching the contracting muscle which contributes to the fatigue process.

Moreover, metal and wood phase, respectively represented by, qi and xue deficiency in muscle tissue may cause fatigue. This may cause limbs to become weak and hypomobile as you cannot move the limbs without muscles.

![Sinus wave of muscle contraction and fatigue according to the Heidelberg model](image)

**Fig. 5: Sinus wave of muscle contraction and fatigue according to the Heidelberg model**

**1.7.6 Fatigue intervention concept according to the Heidelberg model of TCM**

The regeneration process is hastened by suppleting the pulmonary qi, through the tuina intervention on the pulmonary point P5 in this case. Moreover, because the qi moves the xue, the suppletion of qi helps the xue flow better to the contracting muscles. This helps regeneration and the maintenance of contraction.

Based on the wood-metal axis, suppleting the metal phase relaxes the wood phase, this is responsible for the arterial wall relaxation that occurs, which leads to vasodilation, increased blood flow and improved contractility.
Because the main causes of fatigue are qi and xue deficiency, the successful therapy is to supplete the qi and promote xue flow and movement. One finger zen- vibropression is considered one of the best techniques, in this case, to supplete qi, move xue and correct their lack.

1.7.7 Tuina

Tuina is the abbreviated name of the Chinese manual therapy. In fact, the original name is “tui-na, an-mo”, the Chinese names for the different techniques [173]. At present, Chinese massage therapy is widely accepted as a complementary and alternative medicine modality. There are more than 100 manipulations, many schools and names of the manipulations [171].

Tuina was developed by Chinese ancient culture, when they hurt themselves and press the affected area to allay swelling and stop pain. Over time they summarized some primitive forms of tuina [172].

The records about tuina are found in the inscriptions on tortoise shells or bones of the Shang Dynasty (16th-11th century BC). It was developed empirically and its achievements and development have been documented in a large number of classical texts. The Yellow Emperor’s Internal Classic (Huang Di Nei Jing: 475-221 BC), already included records on “An Mo”. From the Wei to the Jin dynasties (220–420 AD), tuina was used in the practice of emergency medicine. Tuina became an independent area of study in the Sui dynasty (581-618 AD) and the title of “manual therapy practitioner” began to be used during this time, when it was integrated into the highest level of the medical education system in China [173] [172].

As a medical specialty, tuina was developed during the 20th century. Currently, tuina is practiced globally. It is commonly used for the treatment of symptomatic relief of pain and neuro-musculoskeletal conditions. In some countries, it is used also with allopathic medical care, for bone fractures and joint dislocations. This therapy has been widely incorporated into practice of other clinical disciplines, such as acupuncture, internal medicine, gynecology and pediatrics [173] [172].

Consistent with the theory of TCM, the practice of tuina is guided by principles such as yin and yang phases, qi, xue and body fluids. Tuina practice involves a range of conventional diagnostic methods, such as laboratory tests, imaging, orthopedic and neurological assessments.
Tuina treatment aims to unblock the conduits, promote the circulation of qi and xue, and regulate the functions of the orbs, using various manual techniques at specified locations on the body. The therapeutic effects of tuina depend on three key factors: traditional Chinese medicine and biomedical diagnosis; selection of conduits and acupoints; effective application of the techniques [173].

1.7.7.1 Mechanisms of tuina

Simply, the curative effect of tuina can be due to a direct effect of its stress that locally promotes blood circulation, corrects xue stasis, restores and treats injured soft tissues, corrects deformities and abnormal location of bones and soft tissues in anatomic site. Moreover, it may have an indirect effect through dynamic wave signals of the manipulation which can reflexively influence the physiological function and pathological state of the body fluids, qi and xue, viscera, mind and emotion, etc. [175] [172].

The main acting principles are:

- Regulating yin and yang: Acting on the physiological functions, (yang) activating or de-activating, the organs and the different structures (yin) can be treated by promoting the flow of qi, xue and reinforcing the tendons and bones [172].

- Improving or reducing qi: The frequency and the direction of manipulations are the keys to either reinforcement or reduction of qi. The change of manipulation within a certain range means quantitative change while the change of manipulations exceeding a certain range indicate qualitative change. Generally, when we are speaking about reducing the qi, we are referring to manipulations with high frequency which can restrict the energy and work directly on the deeper regions to exert clearing, resolving and draining effects. When we perform the opposite kind of manipulations, we are reinforcing the qi [172].

- Activating xue and resolving the stasis: Xue stasis is caused by slow flow of blood due to certain pathological changes. Activating xue and reducing the resistance of blood flow, will reestablish the microcirculation and resolve the stasis [172].

- Relaxing sinews: Tuina manipulations are used to deal with the symptoms of vascular contraction, tension and spasms, regional numbness and pain by relaxing sinews [172].
Regulating sinews for repair: Tuina is commonly used to deal with dislocation of joints, transposition of soft tissues and abnormal changes in certain tissues through regulating the sinews, promoting the flow of qi and xue [172].

1.7.7.2 Application fields of tuina:

Tuina treatments have a wide applications, which treat functional rehabilitation, pain syndrome and disease in internal medicine, gynecology, pediatrics, ear nose and throat diseases. Several studies show the effectiveness of tuina in different conditions: anxiety, stiff neck, low back pain due to lumbar intervertebral disc protrusion, fatigue and others [174, 175].

Massage therapy has been shown to be particularly effective for disorders of musculoskeletal origin [176]. Tuina techniques are especially effective in treating functional disease. Mild organ diseases and acute soft tissue trauma can completely recover by multiple course treatment and self-management. Direct stimulation on the local anatomical structures can relieve local fatigue and help the body to recover from injury. The tuina mechanisms of disease prevention include the relief of fatigue [177].
CHAPTER TWO

CLINICAL RESEARCH METHODOLOGY
2. CLINICAL RESEARCH METHODOLOGY

2.1 Justification of the study
Due to the high significance of grip strength and its impact on daily living activities (ADL), attempts to increase grip strength or reduce fatigue are of high value.

The use of Chinese manual therapy (tuina) is considered a simple and potent intervention for various functional conditions, like manual workers or athletes. So, applying this technique may be an effective way to manage fatigue.

2.2. Research Team

2.2.1 Main investigator
- Beshoy Girgis
Student of the Master in Traditional Chinese Medicine – ICBAS, UP.

2.2.2 Research supervisors

Main supervisor
- Prof. Doutor Henry Johannes Greten. Director of the TCM Master Program – ICBAS, UP. Head of the Heidelberg School of Traditional Chinese Medicine, Germany.

Co-supervisors
- Prof. Doutor Jorge Machado – Director of Physiology Laboratory and Co-director of TCM Master Program, ICBAS-UP.
- Maria Joao Santos - Master in Traditional Chinese Medicine at Abel Salazar Institute for Biomedical Sciences – ICBAS-UP; TCM professional at HSCM, school of health and science – Oporto Clinic; Invited Lecturer at ICBAS-UP.
2.3 Objective of the study

To evaluate the possible benefit of using tuina intervention to delay fatigue in healthy adults.

2.4 Study hypothesis

- Hypothesis 1: Tuina delays fatigue in healthy adults.
- Hypothesis 2: Tuina improves hand grip force in healthy adults.
- Hypothesis 3: Tuina improves motor unit action potential in healthy adults.

2.5 Methods

2.5.1 Study design

An experimental, pre-test and post-test, randomized, controlled study.

2.5.2 Study population

This study focuses on student population of ICBAS-UP, aged between 20 and 30 years-old, without any pathological symptoms or complaints.

2.5.3 Eligibility criteria

**Inclusion criteria:** Healthy adult, age over 18 years, no surgery, informed consent.

**Exclusion criteria:** Subjects with history of trauma, ligament damage, fracture, tumor, or surgery of the upper limb. Subjects diagnosed with or treated for cervical radiculopathy or herniation of intervertebral disc.
2.5.4 Outcomes and measurements

2.5.4.1 Main outcome

Fatigue measured by Biopac® MP36 student lab system (Fig. 6).

2.5.4.2 Secondary outcome

Motor unit action potential measured by surface electrodes of the Electromyography (sEMG) device of the same unit Biopac MP36 student lab system.

2.5.5 Sampling and recruitment procedures

A convenient sample will be selected based on the review of files of healthy students and listed according to eligibility criteria. The main researcher will contact the potential participants, explaining the study, asking questions regarding eligibility requirements and inviting them to participate. On the first day, subjects will sign the written informed consent.

2.5.6 Randomization to groups

The selected 30 patients will be randomly assigned to the experimental (intervention) group or control (no intervention) group by the method of the coin flip.

2.5.7 Experimental procedures / design

The sample was collected in ICBAS-UP student population. After the inclusion criteria been fulfilled, 30 adult subjects (22 females, 8 males) were randomized into two groups.
The subjects of the control group (n=15; 9 females, 6 males) and experimental group (n=15; 13 females, 2 males). After one minute of baseline measurements, the individuals of the experimental group were subjected to "one finger zen vibropression" intervention for one minute, a tuina technique. Individuals of the control group, after one minute of baseline measurements, were not subject to any kind of intervention, instead they rested for 1 minute. Both groups repeated all the measurements at the end. The total time of the study for each individual was 3 minutes (Fig. 7).

Flowchart of the study:

2.5.7.1 Measurements procedures

Fatigue was measured by the SS25LA hand dynamometer with clench force range 0-50 kg. The motor unit action potential was evaluated by EMG unit of Biopac® MP36. Data from these two parameters were obtained simultaneously by following steps recommended by Biopac for measurements of muscle physiology:

Step 1 – Calibration: This step allows to take raw data, normalize and re-scale it to percentage of reference value, the maximum voluntary contraction of the participant dominant arm. Therefore, in this step, subjects were asked to grip the hand dynamometer as hard as they can for 6 seconds, then subjects released grip completely and waited for calibration.
Based on that, the software of the device determines the optimal grid display and force increments as 4 levels of strength, dividing the reference value into 4 levels. (Light, mild, moderate and maximum) [178, 179].

Step 2 – Effective experience: Subjects were asked to grip the dynamometer until the generated force reaches the 1st level of strength during 6 seconds and release for 2 seconds, then the subjects were asked to reach and hold the 2nd level of strength for 6 seconds and release for 2 seconds. Then it was asked to the subjects to reach and hold the 3rd level of grip strength for another 6 seconds and release for 2 seconds. Finally subjects were asked to reach the 4th level (the maximum level of strength) and try to sustain that level during 20 seconds (Figs. 8 and 9).

![Fig. 8: Positioning](image)

![Fig. 9: Grip strength levels](image)
2.5.7.2 Intervention

Subjects of experimental group were subjected to the classical tuina manual therapy named “one finger zen vibropression technique” for one session of one minute intervention. The selection of acupuncture point is based on potential local and segmental effects as supposed in “the Heidelberg model of traditional Chinese medicine”. Manual therapy (tuina) was performed on the selected acupoint for one minute. The experimental group was subject to one intervention for one session.

2.5.7.3 Selection of acupoint and tuina technique

(One-finger zen-vibroression, yi zhi chantui)

This technique is composed of a pressure component (2-5 kg) and a vibration component (120-160 times/min). The pressure component acts against yin agents and introduces energy, the vibration loosens conduits and muscles. Yi zhi chan tui activates qi and xue circulation, disperses qi stagnation and xue stasis [180].

Vertical vibropression with the thumb. A concentrated “energetic-posture” of the whole body with a special breathing (As in some qigong styles) is required for these effects to really work. Zen=Chan=meditation, concentrated energy. The technique is performed by pushing the tip of the thumb on the therapeutic area or acupuncture point and flexing and extending the arm the thumb following the swinging of the forearm as shown in fig.10.

Main indications of this technique are loss of movement due to blockade of the meridians, flabby paresis as a result of lack of qi and xue, and pain of joints and vertebrae [181].

Fig. 10: One Finger Zen Vibropression: Yi Zhi Chantui
The selection of the acupuncture point is based on potential location and segmental effects from the traditional Chinese medicine point of view.
Tuina technique: One-finger zen-vibropression technique (Yi zhi chantui). This technique was applied for one minute.

2.5.7.4 Acupoint P5\(^\prime\) and the pulmonary conduit

P5\(^\prime\) (Heidelberg point): Extra acupuncture point on the pulmonary conduit. P5\(^\prime\) is an extra-point of the pulmonary conduit (Lung) of the Heidelberg model, it is located 2 cun distal to P5 as shown in fig.11. By our clinical experience, it has a similar effect to P5 and P6 with both characteristics of a rimic and a conjunctory point.

P5: Conjunctory “point of conjunction” “point with an effect going inward into the yin” Extimal conduit: Earth point; Intimal conduit: Water point, strengthen the yin [182].

P6: Rimic point “cleft point”, Bottleneck of the conduit which is effective in conduit blockade [182].

The direction of the pulmonal course corresponds to that of the vector of Metal phase (▼). The pulmonal conduit starts 1 cun below the coracoid process, as an intimal conduit, it runs down on the radial inner side of the arm from proximal to distal. It ends on the radial side of the thumb [183].
Fig. 11: Pulmonary Conduit and P5 (Heidelberg Point)
Traditional Chinese unit of measurement (Cun)

Cun is a traditional measure which is the width of a person's thumb at the knuckle, whereas 1.5 cun is denoted by the width of the two forefingers and the width of four fingers side-by-side is three cuns [184]. This may be a useful tool to find acupuncture points on the human body as we used in this study. Cun measure is shown in figure 12.

Fig. 12: Chinese unit of measurement

2.5.8 Statistical plan

We analyzed the differences between groups (experimental vs. control) and inside of each group (beginning vs. end). We worked with a 95% of confidence interval.

Tests to check differences between groups (experimental vs. control):
First, we checked if there are differences between groups in the beginning, and we checked the Normality (normal distribution) to choose the kind of statistical test to use, To check the Normality, we used the Kolmogorov-Smirnov, and the variables with a P value equal or greater than 0.05 were considered with normal distribution (parametric) and the test chosen was the Student T test unpaired samples.
The variables with a P value smaller than 0.05 were considered with non-normal distribution (non-parametric) and the chosen test was the Mann-Whitney.

Tests to check the differences at the end (beginning vs. end):
We checked the groups separately, also checked the normality of each variable to choose tests. We used the Kolmogorov-Smirnov, and the variables with a P value equal or greater than 0.05 were considered with normal distribution (parametric) and the test chosen was the Student T test paired samples. The variables with a P value smaller than 0.05 were considered with non-normal distribution (non-parametric) and the chosen test was the Wilcoxon.
2.6 Ethical considerations, protection of human subjects and assessment of safety

All subjects for this study will be provided with a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study.

Subjects are informed about the goals, methods, expected benefits, and potential risks or discomforts, and have the right to decide to withdraw or continue at any moment during his/her participation. The subject is also aware that no prejudice will result if he/she refuses to participate or withdraws from the study.

This informed consent is obtained from all participants before randomization and is considered an inclusion criteria. This consent form has been approved by the EC and must be signed by the subject or legally acceptable surrogate and the investigator designated research professional obtaining the consent.
CHAPTER THREE

RESULTS
3. RESULTS

Grip strength

– Third level results:

Mean grip strength - Subjects of the experimental group showed a non-statistically significant (p=0.171) reduction of mean grip strength in the third level, with a decrease of 6.97%. The control group had a significant (p=0.008) decrease of 6.90% of mean strength (Fig. 13).

![Graph showing percentage of reduction of mean grip strength in third level](image)

**Fig. 13:** Percentage of reduction of mean grip strength in third level
Maximum grip strength - Subjects of both groups showed a decrease of maximum strength. The experimental group showed a non-statistically significant (p=0.077) decrease of 7.81%, while the control group decrease of 6.72% is statistically significant (p=0.009) (Fig. 14).

Fig. 14: Percentage of reduction of maximum grip strength in third level

– Fourth level results:

Mean grip strength - Both groups showed a decrease of mean strength. In the experimental group the 2.19% decrease is non-statistically significant (p=0.687). In the control group the 12.52% decrease is highly significant (p<0.001) (Figs. 15 and 16).
**Fig. 15:** Percentage of reduction of mean grip strength in fourth level

- **Control:** 12.52% reduction, $P < 0.001$
- **Experimental:** 2.19% reduction, $P = 0.687$

**Fig. 16:** Evolution of mean grip strength in fourth level

- **Before:** Evolution from baseline (Control: $P < 0.001$, Experimental: $P = 0.687$)
- **After:** Decrease in grip strength
Maximum grip strength – Both groups showed a decrease of maximum strength in the fourth level. The experimental group showed a non-statistically significant (p=0.282) reduction of 4.17% of maximum strength. The control group showed a highly statistically significant (p=0.001) reduction of 9.34% of maximum of strength (Fig. 17).

Fig. 17: Percentage of reduction of maximum grip strength in fourth level
Motor unit action potential

Third level – Both groups showed a decrease of mean motor unit action potential. Although the reduction of motor unit action potential of the experimental group was bigger in percentage (11.38%), it was statistically non-significant (p=0.084). The control group had a statistically significant (p=0.006) reduction of 9.53% (Fig. 18).

Fig. 18: Reduction of mean motor unit action potential in third level
Fourth level – Both groups showed a reduction of mean motor unit action potential. The percentage of reduction (3.45%), in the experimental group, is statistically non-significant (p=0.548). The control group showed a bigger reduction with a statistically significant percentage of 9.17% (p= 0.047) (Fig. 19).

Fig. 19: Reduction of mean motor unit action potential in fourth level
Fourth level – Both groups showed a variation of maximum action potential in the fourth level. The experimental group showed a reduction of 6.35%, while the control group had an increase of 6.21%. However, both values in both groups were non-statistically significant (Fig. 20).

![Variation of maximum motor unit action potential in the fourth level (before vs. after)](image_url)

Fig. 20 Variation of maximum motor unit action potential in the fourth level (before vs. after)
Percentage of subjects showing strength gain:

In the experimental group, 46.7% of the subjects showed a gain of mean strength in the fourth level after tuina intervention. However, none of the subjects in the control group showed any gain of mean strength in the same level following one minute rest (Fig. 21).

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% Subjects showing strength gain

0

% Subjects showing strength gain

46.66667

Fig. 21 Percentage of subjects showing gain of mean strength in the fourth level (before vs. after)
4. DISCUSSION

The current results indicate that the control group showed a statistically significant reduction of grip strength and motor unit action potential while the experimental group, in the same parameters, showed a non-statistically significant reduction of grip strength and motor unit action potential. These results also show that, a decrease of more than 5% of the fourth level maximum generated force, a decrease of more than 10% of mean generated force in the same level, and a total decrease of almost 6% of fourth level mean motor unit action potential have occurred in the control group compared to the experimental group.

The evolution of fatigue (mean strength in the 4th level), as a comparison between experimental and control groups, illustrates that the experimental group showed a lesser decrease of more than 10% of mean generated force in comparison to the control group. While the control group showed a statistically significant reduction of grip strength ($p<0.001$), the experimental group showed a non-statistically significant reduction of grip strength ($p=0.687$).

The current study results also suggest a more uniform, stable and high mean value of action potential peaks occurred in the experimental group compared to the control group, which may promote a relatively higher mean muscle contraction when compared to the control situation. However both values in figure 20 were non-statistically significant.

It has been shown that static wrist position, elbow position and posture affect grip strength in the following manner, where wrist flexion, in any degree, reduces grip strength significantly. Also, constrained grip strength is found to be significantly lower than unconstrained self-selected wrist position. But conversely, static grip strength is not significantly altered in wrist positions between neutral and 45 degrees of extension [10]. However, at least 25 degrees of wrist extension is needed for optimum grip strength [11].

There is a considerable variation in grip strength between subjects who did grip strength testing in sitting position with elbow flexed 90 degrees compared to standing position with the elbow in full extension [12]. These results indicated that hand grip strength testing standards should always be kept, as changes in posture or elbow position will lead to changes in grip forces that individuals can generate. Based on that, we standardized the posture, wrist and elbow positions of the subjects during measurement recording procedures according to the Biopac manual [179].
Grip strength is found to be positively correlated with subjects aging from 7 to 19 years while negatively correlated with subjects aging from 20 to 73 years [14, 16]. Males hand grip strength is determined to be significantly higher than females in all groups of age. Moreover, the strength decline is found to be significant in both sexes with old age [17]. The sample selection was done based on these results as the recruited subjects had a range of age between 20 and 30 years, so these subject’s age belongs to the same category of negative correlation with grip strength. Because simple random sampling was used in this study, the number of males and females in each group varied. However, this did not affect our final results since we compared the results within groups.

The main peripheral causes of fatigue are the accumulation of Pi at the beginning of contraction and the depletion of Ca²⁺, ATP, lack of adequate blood supply and resources for the maintenance of muscle contraction. Moreover, the role of peripheral fatigue is more significant during high intensity activities [48-73].

The central causes are the decline in firing rate of motor units and muscle afferent inhibition of alpha motoneurons [74-131]. Because the testing of fatigue in this study was done using a high intensity (maximum force) and short duration contraction (20 seconds in the fourth stage), we may assume that the role of peripheral fatigue is especially important in this study. Since the impact of the lack of adequate blood flow to the contracting muscles is significant in this case, and as the type of tested contraction is isometric contraction, which may result in a total blockade of the blood flow to the contracting muscle [34, 67, 68].

Moreover, under isometric contraction circumstances, the motor units are believed to follow the recruitment order developed by Henneman (Size principle), which states that motor units are activated in a certain sequence so that the motor units with slower conduction velocity are activated first, followed by the ones with faster conduction velocities [35-37]. During maximal sustained contraction, the reduction of force production is mainly caused by large motor unit’s fatigue, which are the last to be recruited. Furthermore, more effort will be required to induce the last force part during maximal contraction due to the increasing thresholds of motor neurons to be activated to produce maximal force voluntarily. This process is known as threshold-spacing [42].

Physiologically, there is a perfect match between large motor units with muscle fiber type II [185, 186]. Muscles are constituted of a combination of slow, fast fibers and others with a moderate speed of contraction. Type II muscle fibers are relatively larger, with considerably larger sarcoplasmic reticulum, higher content of glycolytic enzymes, limited vascular supply and less amount of mitochondria [34].
Due to the limited vascular supply of this type of muscle fibers and their higher share during maximal sustained contraction as mentioned earlier, large motoneurons and type II muscle fibers fatigue may be especially significant in this study.

We may then assume that for an intervention to be effective in delaying fatigue or increasing power, it has to act on any or all of the previously mentioned causes and/or the adaptive mechanisms. Interventions aiming to improve local blood flow may be considered of high value, in this case, since the lack of adequate blood flow to the contracting muscle, in this case, is significant as a factor of peripheral fatigue and as a factor due to the limited vascular supply of type II muscle fibers.

Vibration (3-60HZ) has been found to improve muscle blood volume, blood flow velocity, blood flow and arterial diameter. Moreover, the degree of improvement in muscle perfusion was found to be positively correlated with vibration load [132, 133].

Low vibration frequency (5-45HZ) was shown to increase EMG activity, muscle force and power [143, 148-153]. Moreover, pressure applied to an acupoint (acupressure) was found to improve blood flow throughout a limb [156], and microcirculatory blood flow [159, 160]. Trigger point release has shown improvement in local blood flow up to 20 minutes after the technique application [161].

Based on that, an additional benefit from the positive added effects of both vibration and pressure may occur by combining both of them in one technique. This may help prolong the isometric sustained contraction and may also increase maximal force under unfatigued neuromuscular conditions.

From TCM point of view, applying the one finger zen-vibropression tuina technique to the extra-acupoint P5` (Heidelberg point) in this case, based on the stated effects, aims to supplet qi and promote xue flow and movement. One of the best techniques to supplet qi and correct xue stasis is one finger zen-vibropression as it composed of both vibration and pressure components. This may improve the blood flow and provide enough substrates to the contracting muscle to maintain the contraction for longer duration, and may also hasten the recovery following contraction [172-174].

Under the aforementioned physiological concepts, we may speculate positive local effects of applying tuina technique to be due to improved local blood flow and microcirculation inducing a higher and more uniform recruitment of neuro-muscular units based on enhanced synaptic, muscle-metabolic and mechanical functions.
Since vibration training may have a positive acute and/or chronic training effect on strength and power. However, the vibration effect, in this case, seem to be dependent upon the characteristics of vibration (application method, frequency and amplitude) and the protocols of exercise (type of training, volume, and intensity) used [155]. According to that, we may speculate that combining pressure and vibration in one technique (TCM technique) may have positive results if applied to athletes, however, this is largely dependent on the type of exercise used. We suggest the application of this technique under similar conditions to healthy adults.

5. CONCLUSION AND RECOMMENDATIONS
The present results indicate that tuina intervention may delay fatigue through resisting the decline in force production, which may occur due to improved local blood flow, and may also improve motor unit action potential during fatigue in healthy subjects.

Given the short duration of the applied tuina intervention and the promising results, we recommend applying the technique for longer durations up to 5 minutes [180].

Possible future studies could study the effects of the same technique applied for longer durations and to other acupoints, study its chronic effects and help explain the mechanisms behind the positive results.

5.1 Limitations of the study
Simple random sampling process. Since the sample selection was random in this study, the recruited groups were not identical in terms of gender (Control group: 9 females, 6 males/ Experimental group: 13 females, 2 males). However, the final results were not affected by that since we compared the results within groups. To avoid this kind of deviation we suggest a stratified random sampling process.

The sample size was small. For a population of ICBAS students (±10,000 students) to be used to recruit the sample, the minimum number of subjects to recruit was found to be ± 370 subjects in order to directly compare the results between groups.
REFERENCES


CONSENTIMENTO INFORMADO, LIVRE E ESCLARECIDO PARA PARTICIPAÇÃO EM

PROJETOS DE DOCÊNCIA E/OU INVESTIGAÇÃO

de acordo com a Declaração de Helsínquia¹ e a Convenção de Oviedo²

Por favor, leia com atenção a seguinte informação. Se achar que algo está incorreto ou que não está claro, não hesite em solicitar mais informações. Se concorda com a proposta que lhe foi feita, queira assinar este documento.

Título do estudo: "Efeito agudo da técnica de tuina vibropressão na força de preensão".

Enquadramento: O estudo será realizado no Instituto Abel Salazar. No âmbito do projeto de Mestrado de Medicina Tradicional Chinesa do Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, orientado pelo Henry Johannes Greten, e supervisionado pela mestra Maria João Santos.

Explicação do estudo: Com este estudo pretende-se verificar o efeito agudo da técnica de tuina, designada por vibropressão, na força de preensão de indivíduos saudáveis. Inicialmente será aplicado um questionário sociodemográfico. Posteriormente será realizada uma avaliação na linha de base, verificando a força de preensão. Depois, será selecionado aleatoriamente, através de moeda ao ar para um dos dois grupos de estudo previstos. Um grupo será sujeito à técnica de tuina designada por vibropressão e o outro grupo não será sujeito a qualquer intervenção. A intervenção terá a duração de um minuto e todos os indivíduos serão reavaliados 2 minutos depois da primeira avaliação quanto à força de preensão. A eletromiografia consiste na recolha da energia produzida pelo músculo durante a contração. Serão colocados elétrodos adesivos sobre o local a ser estudado (braço) e esses dados serão recolhidos e gravados por um polígrafo. A técnica de preensão (Tuina) consiste em fazer pressão e vibração digital no ponto a ser estudado - P5' – que situa no antebraço. A estimulação deste ponto poderá provocar ligeiro desconforto no local da estimulação digital. Raramente, poderá causar uma ligeira equimose.

Condições e financiamento: O presente estudo será realizado sem qualquer custo para o paciente. Sendo a sua participação voluntária terá o tempo que necessitar para ponderar sobre a sua participação neste estudo. É livre de consultar a opinião dos seus familiares ou amigos. Caso decida aceitar, poderá posteriormente a qualquer momento recusar continuar no estudo, sem quaisquer tipos de prejuízos assistenciais ou outros, caso não queira continuar a participar.

² http://dre.pt/pdf1sdip/2001/01/002A00/00140036.pdf
A responsabilidade de eventuais danos ocorridos durante o estudo, será imputada à Heidelberg School of Chinese medicine, sito na Karlsruher Str. 12, 69126 Heidelberg, Germany, e cujo contacto telefónico é +49 (0) 6221 37 45 46.

Este estudo mereceu o parecer favorável da Comissão de Ética do ICBAS-UP

**Confidencialidade e anonimato:** Todos os dados recolhidos para o presente estudo asseguram uma total confidencialidade e anonimato dos participantes, os seus nomes nunca serão tornados públicos. Todos os resultados obtidos serão devidamente codificados; os dados serão apenas do conhecimento do investigador principal e dos orientadores do estudo.

Para qualquer esclarecimento poderá entrar em contacto com Beshoy Abdalla Girgis, pelo telemóvel 920478581

Eu, abaixo-assinado,
________________________________________________________BI/CC:_______

____

Declaro ter lido e compreendido este documento, bem como as informações que me foram fornecidas pela pessoa que acima assina e que considero suficientes. Foi-me garantida a possibilidade de, em qualquer altura, me retirar da participação neste estudo sem qualquer tipo de consequências. Desta forma, aceito a participação neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade e anonimato que me são dadas pelo investigador.

Porto, ____ de ________________ de 2015

Assinatura do Participante
________________________________

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Assinatura do investigador:
________________________________

____
Responsabilidade no Projeto de Investigação:

Professor Doutor Henry Greten, Docente no Mestrado em Medicina Tradicional Chinesa (MTC) no ICBAS, na qualidade de orientador do mestrando de MTC, Beshoy Girgis, declaro que concordo com os objetivos e metodologias propostas no âmbito do projeto “tuina effect in grip strength” submetido para apreciação pela Comissão de Ética do Instituto de Ciências Biomédicas de Abel Salazar. Na qualidade de Director da Clinica de MTC Heidelberg, Alemanha, declaro ainda que me responsabilizo pelas custas e qualquer dano pessoal que eventualmente possa ocorrer no percurso do estudo científico em causa.

Porto, 8 de Junho 2015

Orientador

Prof. Dr. Henry Greten

ICBAS - UP
Parecer da Comissão de Ética do ICBAS-UP

PROJETO Nº 095/2015

Título: *The acute effect of vibropression tuina technique on grip strength* (Provisional)
Investigador Responsável: Beshoy Adel Girgis (aluno do 2º ano do Mestrado em Medicina Tradicional Chinesa do ICBAS-UP)
Outros investigadores: João Ramos, Maria João Santos
Orientador: Prof. Doutor Henry Johannes Greten
Duração do Projeto: até julho de 2015

A Comissão de Ética do ICBAS-UP reuniu dia 19 de maio de 2015 no edifício do ICBAS - Sala de reuniões do Departamento de Ciências do Comportamento, na presença de Liliana de Sousa, Manuel Vilanova, Margarida Araújo, Paulo Maia e Paula Faria. Decidiu emitir *parecer favorável* à realização do projeto supracitado, por unanimidade.

Com os melhores cumprimentos,

Pela Comissão de Ética do ICBAS-UP,

Prof. Doutora Liliana de Sousa (presidente)

*The above project is in accordance with the Portuguese law and the ICBAS-UP Ethics Committee criteria.*