Abdominal wall muscle quality in patients with severe obesity undergoing bariatric surgery and the risk of post-incisional hernia

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KEYWORDS: OBESITY**,** BARIATRIC SURGERY, INCISIONAL HERNIA, SKELETAL MUSCLE REGENERATION, PHYSICAL EXERCISE

To my family,

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RESUMO

A cirurgia bariátrica (CB) é considerada como o tratamento mais eficaz para a obesidade severa; no entanto, encontra-se associada a alguns efeitos adversos, nomeadamente o risco de hérnia pós-incisional. Embora a fisiopatologia desta complicação permaneça pouco compreendida, postula-se que um dos principais mecanismos esteja relacionado com o comprometimento do processo de regeneração muscular da parede abdominal, determinado pela obesidade. Isto pode ser atribuído, pelo menos em parte, a efeitos adversos na qualidade muscular. Contudo, não existem evidências científicas robustas em humanos que associem o dano muscular estrutural induzido pela obesidade com a ocorrência de hérnia incisional após a CB. Deste modo, o objetivo deste estudo foi testar a hipótese de que a qualidade muscular é afetada em pacientes com níveis extremos de obesidade, comparativamente com pacientes obesos menos graves. Vinte pacientes submetidos a CB foram alocados para o grupo de obesidade (Ob, n=10) ou para grupo de obesidade extrema (ExOb, n=10). Foram realizadas análises histológicas e imuno-histoquímicas para avaliar a área de secção transversal (AST) das fibras musculares de amostras de musculo esquelético abdominal, posição dos núcleos, conteúdo total de colagénio e expressão das células endoteliais. A carbonilação de proteínas e a peroxidação lipídica foram determinadas pela técnica de Slot blot, e a expressão de células satélites foi avaliada por Western blotting. Em comparação com o grupo Ob, os doentes do grupo ExOb apresentaram uma diminuição significativa da AST das fibras musculares, bem como menor expressão de células endoteliais, quer em relação à área muscular total, como também em relação ao número de fibras. No entanto, relativamente à posição dos núcleos, conteúdo total de colagénio, parâmetros de dano oxidativo e expressão de células satélite, não foram observadas diferenças significativas entre os grupos. Em suma, os nossos resultados sugerem que casos extremos de obesidade podem levar a uma qualidade do músculo esquelético inferior, nomeadamente através da diminuição do tamanho das fibras musculares e menor capilarização.

Palavras-chave: Obesidade, Cirurgia bariátrica, Hérnia incisional, Regeneração músculo esquelética, Exercício físico

ABSTRACT

Bariatric surgery (BS) is regarded as the most effective treatment for severe obesity, however it is not free of adverse effects, such as the risk of post-incisional hernia. Although its physiopathology remains poorly understood, it is postulated that one of the causes of post-incisional hernia is related to the irregular wound abdominal wall muscle repair process determined by obesity itself. This could be attributed, at least in part, to detrimental effects on muscle quality. Nevertheless, there is no robust evidence on humans associating the structural and metabolic negative effects of obesity with the occurrence of incisional hernias after BS. Thus, we aimed to test the hypothesis that muscle quality is negatively affected in patients with extreme levels of obesity, compared to less severe cases of obesity. Twenty patients submitted to BS, were allocated to either obesity (Ob, n=10) or to matched extreme obesity group (ExOb, n=10). Histological and immunohistochemical analyses were performed for assessing the abdominal wall skeletal muscle fibers cross-sectional area (CSA), myonuclei position, total collagen content, and endothelial cells expression. Protein carbonylation and lipid peroxidation was determined by Slot blot, and satellite cells (SCs) expression was assessed by Western blotting analysis of PAX7. Compared to the Ob group, the ExOb group presented significantly lower muscle fibers CSA, as well as lower expression of endothelial cells, both related to total muscle area and number of fibers. However, regarding the muscle nuclei position, total collagen content, oxidative damage parameters and SCs expression, no significant differences were observed between groups. In conclusion, the extreme cases of obesity may lead to a hampered skeletal muscle quality, namely through decreased muscle fibers size and lower capillarization.

Key Words: Obesity**,** Bariatric surgery, Incisional hernia, Skeletal muscle regeneration, Physical exercise

LIST OF ABBREVIATIONS

4-HNE	[4-Hydroxynonenal]
8-oxo-dG	[8-oxo-2'deoxyguanosine]
Acyl CoA	[Acyl-coenzyme A]
AGB	[Adjustable gastric banding]
AMPK	[AMP-activated protein kinase]
ASCs	[Adipose tissue derived stem cells]
ATP	[Adenosine triphosphate]
BMI	[Body mass index]
BM-MSCs	[Bone marrow mesenchymal stromal cells]
BPD-DS	[Biliopancreatic diversion with duodenal switch]
BS	[Bariatric surgery]
BSA	[Bovine serum albumin]
CD133+	[Cluster of Differentiation 133]
CD31+	[Cluster of differentiation 31]
CD34+	[Cluster of differentiation 34]
CSA	[Coss-sectional area]
CT	[Computed tomography]
DAB	[3,3'-diaminobenzidine tetrahydrochloride]
DLK1	[Delta like non-canonical notch ligand 1]
DNA	[Deoxyribonucleic acid]
DNPH	[2,4-Dinitrophenylhydrazine]
DPX	[Dibutyl xylene phthalate]
Drp1	[Dynamin-related protein 1]
ECM	[Extracellular matrix]

FGF-2	[Fibroblast growth factor 2]
Fis1	[Mitochondrial fission protein 1]
FPG	[Fasting plasma glucose]
GDF-8	[Growth/differentiation factor-8]
GLP-1	[Glucagon-like peptide-1]
H&E	[Hematoxylin and eosin]
H_2O_2	[Hydrogen peroxide]
HbA1c	[Glycated hemoglobin]
HFD	[High-fat diet]
HGF	[Hepatocyte growth factor]
IAT	[Intermuscular adipose tissue]
IFNγ	[Interferon-gamma]
IGF-1	[Insulin-like growth 1]
IGF-2	[Insulin-like growth 2]
IL-6	[Interleukin-6]
MCK	[Muscle creatine kinase]
MDSCs	[Muscle-derived stem cells]
MHC	[Myosin heavy]
MMP-1	[Matrix metalloproteinase-1]
MMP-2	[Matrix metalloproteinase-2]
MMP-9	[Matrix metalloproteinase-9]
MMPs	[Matrix metalloproteinases]
MMPs	[Matrix metalloproteinases]
Mrf4	[Muscle-specific regulatory factor 4]
MRFs	[Myogenic regulatory factors]
mtH2O2	[Mitochondrial Hydrogen peroxide]

Myf5	[Myogenic factor 5]
MyoD	[Myogenic differentiation 1]
MyoG	[Myogenin]
NAFLD	[Alcoholic fatty liver disease]
Notch-1	[Neurogenic locus notch homolog protein-1]
NP	[Myonuclei position]
$O2^{\bullet-}$	[Superoxide ion]
Opa1	[Optic atrophy 1]
PAX3	[Paired box 3]
PAX7	[Paired box 7]
PCNA	[Proliferating cell nuclear antigen]
PCNA	[Proliferating cell nuclear antigen]
PI3K/Akt	[Phosphatidylinositol 3-kinase/protein kinase B]
PROM1 gene	[Prominin 1]
PTEN	[Phosphatase and tensin homolog]
PYY	[Peptide yy]
ROS	[Reactive oxygen species]
RYGB	[Roux-en-Y gastric bypass]
SCs	[Satellite cells]
SG	[Sleeve gastrectomy]
SMR	[Skeletal muscle repair]
SMReg	[Skeletal muscle regeneration]
T2DM	[Type 2 diabetes mellitus]
TBS	[Tris buffered saline]
TBS-T	[Tris buffered saline – Tween]
TGF-β	[Transforming growth factor-β]

Th1	[T helper 1]
TNFα	[Tumor necrosis factor-alpha]
TSH	[Trocar site hernia]
Wnt7a	[Wnt Family Member 7a]

CHAPTER I

GENERAL INTRODUCTION

General Introduction

The worldwide prevalence of obesity has risen drastically over the past four decades and approximately a third of the population is currently classified as having overweight or obesity [1]. This is extremely concerning since obesity has severe deleterious health effects and is associated with the development of several other long-term medical comorbidities, with entails high costs for the individual patient and healthcare systems [2]. In addition, obesity, and in particular its most severe grades, promotes a significant decrease in life expectancy and deterioration of the quality of life [3].

Obesity treatment options include non-surgical interventions and bariatric surgery (BS). The non-surgical interventions, based on lifestyle and behavioral changes with diet and exercise, play an important role in obesity management [4]; however, it has been demonstrated that these strategies are, to a large extent, unable to induce substantial and durable weight losses in most patients [5]. In turn, bariatric surgery has emerged as the most effective treatment strategy for successful and sustained weight loss in patients with severe obesity [4]. Moreover, BS is also effective in the treatment of several obesity-associated comorbidities such as type 2 diabetes, arterial hypertension, cardiovascular disease and, thereby, significantly contributes to a reduction in overall premature mortality in patients with severe obesity [6, 7]. Despite its success in weight loss and metabolic benefits, BS is associated with some drawbacks, namely the increased risk of postoperative incisional hernias [8, 9].

Incisional hernias – also referred to as trocar site hernia (TSH) – are a common abdominal wall complication at the site of a bariatric surgical incision [10]. Considering the high number of incisional hernia repair surgeries performed annually [11], the real prevalence of this complication reported in post-BS studies seems to be substantially underestimated. This is particularly concerning given the huge TSH-associated treatment costs, which represent a substantial source of healthcare resources worldwide [12]. Moreover, this abdominal wall complication is a persistent cause of morbidity and harmfully impacts the quality of life of post-BS patients [13].

Although post-incisional hernia pathophysiology is currently not full-

established, it seems to be a multifactorial complication [14-16], with numerous technical and patient-related risk factors being described. Technical difficulties in patients with obesity are associated to the complexity of attaining a full-thickness incisional wound closure [17, 18], due to the higher intraabdominal pressure, thicker peritoneum, and larger preperitoneal space [19]. However, it is hypothesized that the propensity for developing incisional hernia after BS may be aggravated by the erratic wound muscle repair process determined by obesity itself.

In experiments in animals with obesity, a compromised skeletal muscle regenerative capacity following damage has been correlated to the presence of several muscle quality parameters [20-22]. Indeed, intramuscular adipose tissue deposit, which is a typical feature in patients with severe obesity, has been strongly and positively associated with altered skeletal muscle stem cells function [23-26], defective collagen metabolism [27-30], vascular alterations [31, 32], and higher oxidative stress damage [33-35]. Considering this evidence, it seems logical to speculate that skeletal muscle tissue quality is possibly impaired in patients with severe obesity that undergo BS, which could be related, at least partially, to a decreased skeletal muscle regeneration capacity after injury, namely after surgery. Nevertheless, there is no robust scientific direct evidence on humans assessing the structural skeletal muscle features in patients with severe obesity. Thus, we aimed to test the hypothesis that skeletal muscle quality is negatively affected in patients with severe obesity, which could help to explain these patients' higher propensity for development of post-BS incisional hernia.

The aim of this dissertation was to assess the quality of abdominal wall skeletal muscles in severely obese patients undergoing BS in order to identify features that could explain a lower regenerative capacity and therefore a higher risk of incisional hernia. These results would help us to identify potential treatment targets for improving abdominal muscle walls regenerative capacity and, thereby, to reduce the risk of post incisional hernia, namely through the application of pre-surgery rehabilitation exercise protocols. For this purpose, a literature review and experimental study were elaborated to test the working hypothesis that patients with obesity, in particular those with the most severe forms of obesity, have a

substantial impairment of some muscle quality parameters which could compromise the abdominal wall muscle regenerative capacity after surgery, and thereby explain the elevated risk of post-BS incisional hernia in these patients.

This dissertation is divided into five main chapters. Chapter I comprises a general introduction of the problem as well as of the aims and hypothesis to be studied. In Chapter II, an enlarged review of the literature on the most relevant topics related to the experimental study, namely regarding bariatric surgery and post-incisional hernia risk, the mechanisms of skeletal muscle regeneration and the effect of obesity on this process, as well as the possible contribution of physical exercise on muscle regeneration capacity, are presented. Chapter III encompasses the experimental study that was performed, followed by a general and comprehensive discussion of the results obtained. Chapter IV lists the main conclusions. Last, chapter V presents the complete list of references cited throughout chapters I , II, and III.

CHAPTER II

STATE-OF-ART

1. Bariatric Surgery

Obesity is, at present, one of the main worldwide public health concerns, and almost a third of the population is presently classified as having overweight or obesity [1]. Obesity is a complex, multifactorial and relapsing chronic disease, characterized by an ectopic or excessive body fat (adiposity), which impairs health and significantly decreases life expectancy [3]. Excessive adiposity predisposes the patients to the development of many long-term medical complications, such as type 2 diabetes mellitus (T2DM), osteoarthritis, non-alcoholic fatty liver disease (NAFLD) and chronic kidney disease, among other disorders [2]. Ectopic body fat increases adipokines and inflammatory mediators levels, leading to a high risk of cardiovascular disease and cancer [2].

Obesity treatment alternatives include non-surgical interventions and bariatric surgery (BS). Non-surgical treatment traditionally comprises a multi-component approach that involves behavioral counseling, dietary adjustments, increased levels of physical activity, and several pharmacotherapies [4]. Although non-surgical interventions can lead to weight reduction, patients with overweight and obesity are commonly unable to maintain this loss in the long term [5]. There is substantial evidence that, in response to weight loss induced by dieting, several compensatory biological changes occur, e.g. decreased circulating concentrations of leptin, glucagon-like peptide-1 (GLP-1), and peptide YY (PYY), as well as enhanced ghrelin levels [36]. Moreover, modifications in gut microbiota, altered brain neural responses to food stimulus, reduced vagal nerve signaling, and impaired secretion of bile acids are also described [5]. These hormonal changes induce increased hunger, reduce satiety, and lead to a higher appetite for energy-dense foods [36]. Moreover, according to changes in body composition, estimated energy expenditure following weight reduction is lower than predicted [37]. This difference between observed and expected energy expenditure after weight loss is known as metabolic adaptation and provides a strong physiological basis that explains, at least in part, the high failure rate of dietary interventions in weight management programs [5, 38].

In contrast, changes in intestinal hormone secretion reported after bariatric

surgery have been indicated as important mediators of increased satiety and reduced postoperative food intake [39]. There is compelling evidence suggesting that Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) bariatric procedures induce increased postprandial secretion of satiety hormones (i.e., GLP-1 and PYY) that reduce food intake after surgery [39]. Moreover, postprandial alterations in glicentin and oxyntomodulin, which occur during the immediate postoperative period following bariatric surgery, are also correlated with weight reduction [40]. The discordant variations observed in intestinal hormones secretion between bariatric surgery and dietary interventions result in different weight adjustments, i.e., weight relapse after diet-induced weight loss and maintenance of surgery-associated weight reduction. This evidence forms the basis to suggest that bariatric surgery can treat defective appetite regulation in obese patients, the etiological disturbance at the base of obesity [36]. Furthermore, recent findings indicate that obese patients undergoing pharmacotherapy describe health outcomes as significantly poor. Even aggressive pharmacotherapy induces a small effect on obesity-related cardiovascular risk [41].

During the last decade, bariatric surgery emerged as an effective alternative strategy in the long-term treatment of severe obesity, which is defined by a body mass index (BMI) equal to or greater than 35 kg/m^2 [4]. Bariatric surgery involves changes in the anatomy of the gastrointestinal tract, which consequently induces effects on its physiology and alterations in the flow of nutrients [42]. The concept of BS emerged in the 1950's, based on the perception that interventions requiring the resection of the small intestine resulted in weight loss [43]. Originally, bariatric techniques were specifically proposed for inducing weight loss through pure malabsorption and, consequently, severe nutritional deficiencies and also metabolic negative implications were associated with these procedures. In the following decade, it was performed for the first time a gastric bypass procedure that integrated a restriction in the stomach capacity that lead to a decrease in the amount of food ingested, laying the groundwork for mixed malabsorptive and restrictive approaches [43].

Bariatric surgery has become one of the most frequently implemented surgical procedures worldwide, with an exponential increase in the number of surgeries performed in the last years [44]. Data released by the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO) reported that in 2018 approximately 696,191 bariatric surgery procedures were conducted worldwide. Particularly in Portugal, a total of 1.397 bariatric surgeries were performed. Techniques such as adjustable gastric banding (AGB), jejunoileal bypass, and vertical banded gastroplasty are currently less considered, due to its severe side effects, high incidence of reoperation, and low long-term efficacy. In contrast, SG (55.4%) and RYGB (39.6%) represent, respectively, the first and second most frequently performed bariatric techniques globally, possibly due to the several reported advantages [45].

1.1. Roux-en-Y gastric bypass

Roux-en-Y gastric bypass is a common bariatric surgery procedure performed worldwide that leads to a long-term average weight loss of approximately 28,4% of baseline body weight [44, 46]. This procedure alters the anatomy of the gastrointestinal tract by creating first a small proximal gastric pouch of approximately 20 to 30 mL through which food circulates. The remaining part of the stomach is therefore isolated from the proximal gastrointestinal tract and no longer contacts with the nutrients ingested. The small gastric pouch, formed from the upper part of the stomach, is then anastomosed to the middle jejunum, creating a roux or alimentary branch [47]. Consequently, food and liquids bypass most of the stomach and upper small bowel (duodenum and proximal jejunum) and directly reach the mid-jejunum, accelerating the delivery of nutrients to the distal intestine. Thus, nutrients only contact pancreatic enzymes and bile acids, which pass through the biliopancreatic branch that is connected to the distal stomach, in the common channel in the distal jejunum [47].

This bariatric technique combines two mechanisms responsible for weight loss, that is, a restriction of the amount of food ingested and poor absorption of

nutrients [48]. The restriction is due to the creation of the small gastric pouch and consequent reduction of the stomach size, which favors a feeling of early satiety with a smaller volume of food ingested. On the other hand, the direct bypass between the stomach and the distal jejunum, by decreasing the contact time between nutrients and the intestinal epithelium, decreases nutrient absorption [48].

1.2. Sleeve gastrectomy

Sleeve gastrectomy, also referred to as gastric sleeve, has been widely performed as a treatment for patients with severe obesity [44]. Originally, this bariatric surgery technique was described as an initial step procedure, followed by a biliopancreatic diversion-duodenal switch to add a malabsorptive component for increasing weight reduction [49]. Due to the significant prevalence of complications in individuals with high surgical risk or morbid obesity, SG was also adopted in a two-step RYGB approach. In the first stage, SG was implemented to improve the pre surgical health status condition and ensure sufficient weight loss to safely perform the surgery and allow some co-morbid conditions to go into remission. Over time, it was found that many of these patients achieved significant weight loss and decreased obesity-related comorbidities after isolated SG, commonly without requiring a second intervention [49]. Given the sustained effectiveness in weight reduction and the metabolic benefits associated with this procedure, SG became an autonomous bariatric surgery approach for patients with severe obesity [50, 51].

This bariatric procedure involves a subtotal sleeve gastrectomy with preservation of the pylorus - including longitudinal resection of the fundus, body, and antrum - creating a sleeve or tubular duct along the lesser curvature [50]. The size of the stomach is then reduced to about a third of its original volume. Consequently, gastric contents pass more quickly into the duodenum and food intake is limited. Besides that, by removing part of the stomach, including the region where most of the hunger-inducing hormone ghrelin is secreted, sleeve gastrectomy induces a decrease in appetite [51].

1.3. Metabolic and weight loss benefits after bariatric surgery

Several randomized controlled trials and retrospective observational studies have demonstrated the effectiveness of bariatric surgery in sustained long-term weight loss, as also in the treatment of obesity-associated comorbidities and in the reduction of overall mortality, compared to non-surgical interventions [6, 7]. To date, the Swedish Obese Subjects (SOS) prospective controlled trial remains the most comprehensive long-term evidence on the effects of bariatric surgery. A total of 2010 patients with obesity underwent surgery and 2,037 weight-matched controls received standard medical treatment. Data results of 2-, 10-, 15-, and 20-years demonstrated that patients undergoing RYGB surgery achieved a significantly greater body weight decrease of 23%, 17%, 16%, and 18%, compared to only 0%, 1%, 1%, and 1% in those undergoing usual medical care, respectively. At 20-years, bariatric patients were able to maintain more than 25% of their total weight loss. Moreover, BS results in a decreased incidence of T2DM, hypertriglyceridemia, low high-density lipoprotein cholesterol, arterial hypertension and several types of cancer. In addition, bariatric surgery was associated with a 53% long-term reduction in overall mortality, as also macrovascular complications, i.e., myocardial infarction, stroke, and lower limb amputation were reduced by 32% [52].

Data results reported in the SOS study are consistent with other recent large retrospective trials that investigate the relationship between bariatric surgery and incidence of major adverse cardiovascular events [6]. For instance, in a retrospective cohort study Fisher, Johnson [53] reported that at 1-, 3-, 5-, and 7-year after BS the incidence of macrovascular disease were 0.5%, 1.1%, 2.1%, and 3.2%, while in non-surgical patients incidence was of 1.1%, 2.6%, 4.3%, and 6.2%, respectively. At 5-years, 2.8% of the control patients presented coronary artery disease, compared to 1.6% in bariatric patients. Moreover, at 1-, 3-, 5-, and 7-years post-bariatric surgery all-cause mortality was 0.4%, 0.9%, 1.3%, and 2.0% in surgical patients, while in non-surgical matched controls it was 0.9%, 2.7%, 4.5%, and 6.4%, respectively [53]. Similarly, Aminian, Zajichek [54] described that 385 bariatric patients experienced a major cardiovascular event in

an 8-year follow-up study, compared to 3243 matched nonsurgical patients (cumulative incidence of 30.8% vs 47.7%). In a nationwide nested cohort study, involving 3.701 bariatric patients and 3.701 age, sex, and BMI matched controls, Moussa, Ardissino [55] reported a total of 36 fatal or non-fatal myocardial infarction events and 4 ischemic stroke events in bariatric patients, compared to 93 myocardial infarction and 13 ischemic stroke events in non-surgical patients, respectively. Moreover, only 22 bariatric patients were diagnosed with heart failure during follow-up, while 46 new cases of heart failure were observed in control patients [55].

The relationship between BS and long-term survival in patients with obesity with end-stage kidney disease was recently assessed by Sheetz, Gerhardinger [56]. In comparison with usual treatment, bariatric surgery was correlated with a lower all-cause mortality rate of 26% vs 40%. These results are consistent with the data reported in the Liakopoulos, Franzén [57] study suggesting robust benefits for outcomes related to renal function, heart failure, and mortality risk after RYGB surgery. Thus, the authors concluded that surgery-induced weight loss produces significant benefits in terms of cardiorenal risk, including slowing the progression of end-stage renal disease, regardless of the baseline status of renal function [56, 57]. Despite the lack of randomized clinical trials, these observational studies clearly demonstrate the significant benefits of bariatric surgery in outcomes related to all-cause mortality, renal function, and major cardiovascular events [6].

Similarly, several clinical trials have demonstrated that surgery-induced weight loss was associated with decreased morbidity and cardiometabolic risk factors [7]. There is increasing evidence suggesting that BS, compared to standard medical treatment, results in improved T2DM outcomes, such as glycemic control and HbA1c levels, at 1-year [58], 2-years [59], and 3-years [60] after surgery. The Surgical Treatment and Medications Potentially Eradicate Diabetes Efficiently (STAMPEDE) trial, conducted by Schauer, Bhatt [61], provides longer-term data about the potential of BS in T2DM treatment. A total of 150 patients with obesity and diabetes were randomly designated to receive

either intensive medical therapy alone or intensive medical therapy plus RYGB or SG surgery. At 5-years, only 5% of the patients receiving medical therapy achieved an HbA1c level of 6%, compared to 29% of RYGB and 23% of SG patients. Moreover, it was shown that 89% of surgical patients were no longer on insulin treatment at 5-year, compared to 61% of the nonsurgical patients that were receiving insulin treatment [61].

In another interesting study, Ikramuddin, Korner [62] assessed the robustness of the RYGB surgery added to intensive medical treatment for the control of T2DM. In an observational 5-year follow-up of a randomized controlled trial involving 120 participants, Ikramuddin, Korner [62] observed that 13 patients (23%) undergoing RYGB achieved an HbA1c <7.0%, low-density lipoprotein cholesterol <100mg/dL, and systolic blood pressure <130mmHg, compared to only 2 patients (4%) undergoing intensive medical therapy. Similarly, in a study evaluating 61 patients with obesity and T2DM during 5-years, Courcoulas, Gallagher [63] reported that 30% of RYGB and 19% of the AGB patients had partial or complete remission of T2DM, respectively, while none of the patients undergoing a lifestyle intervention achieved this outcome. Moreover, most patients undergoing RYBG (56%) and AGB (45%) controlled T2DM without requiring any medication [63]. More recently, Mingrone, Panunzi [64] conducted a large 10-year follow-up randomized controlled trial, involving 60 diabetic patients undergoing medical management or either RYGB or BPD-DS surgery. At 10 years post-bariatric surgery, T2DM remission - that was defined as being off anti-diabetic medications, FPG ≤100mg/dL, and HbA1c ≤6.5% - was achieved in 50% of the BPD-DS patients and in 25% of the RYGB group patients, compared to only 5.5% of the medical treatment group. Moreover, bariatric patients demonstrated better glycemic control and significantly fewer diabetes-related complications [64].

In addition, several systematic reviews and meta-analyses, centered mainly on observational data, have suggested that bariatric surgery induces benefits in controlling arterial hypertension leading to remission rates between 43% to 83% [65, 66]. Similarly, studies comparing the effectiveness of BS with

intensive medical interventions have also demonstrated that bariatric patients present higher remission rates for arterial hypertension and substantially lower use of antihypertensive medications [61, 65]. The GATEWAY randomized controlled trial, conducted by Schiavon, Bersch-Ferreira [67], specifically assessed the impact of BS on hypertension remission rates. At 1-year, the hypertension remission, with prior abandonment of all medications, occurred in almost 50% of bariatric patients while remission was not achieved by any non-surgical patient. Moreover, 83.7% of bariatric patients achieved a reduction in the number of antihypertensive drugs used of ≥30%, compared to only 12.8% of control patients [67].

There is also compelling evidence that bariatric surgery is correlated with significant improvements in dyslipidemia, both in the short term [68, 69] and in the long term [46]. For instance, Schauer, Bhatt [61] described a triglyceride concentration reduction of 40% in patients undergoing RYGB and 29% in those undergoing SG 5-years after surgery while reductions in non-surgical controls were only 8%. Recent meta-analyses also describe that bariatric surgery induces substantial decreases in apnea-hypopnea index scores [70, 71]. Wong, Barnes [71] observed decreased daytime sleepiness and as well in the severity of obstructive sleep apnea following bariatric surgery. There is also evidence that bariatric surgery is associated with a significantly lower cancer incidence, namely of obesity-related cancers such as breast, liver, pancreas and cancer of distal esophagus [72, 73].

2. Complications of laparoscopic bariatric surgery

The development in bariatric surgery has been exceptional in the last decades, mainly due to the technical improvement in several bariatric techniques [74]. One of the most important advances that revolutionized bariatric surgical treatment was the introduction of the laparoscopic approach, as first reported in 1994 [75]. Laparoscopy, is a minimally invasive surgical procedure that employs a laparoscope - a thin tube with a camera attached at the end - that projects images into an external screen that allows visualization of the internal structures

during surgery [19]. This allows access to the peritoneal cavity through less extensive incisions with much smaller surgical tools, greatly reducing the need to perform extensive surgical incisions [19]. Patients undergoing bariatric laparoscopic surgery benefit from less intraoperative hemorrhage and postoperative pain, as well as reduced operative time and substantially faster recovery [76]. As a result, laparoscopy emerged as a safe and cost-effective alternative approach to conventional open abdominal surgery, with 99.3% of all bariatric procedures worldwide being performed through this procedure [77].

Although extensive evidence shows that laparoscopy provides important benefits in terms of surgical outcomes [76], the incorporation of this approach into routine surgical practice has raised concerns about the potential change in the type and incidence of postoperative complications [78]. The high standardization and reproducibility of laparoscopic surgery have made trocar-related complications some of the most frequent in bariatric patients [79]. Particular attention has been provided to trocar site hernias, a common postoperative laparoscopic complication, and a major healthcare problem [9].

2.1. Epidemiology of post-incisional hernia complication

Trocar site hernia is one of the most frequently reported adverse effects related with laparoscopic bariatric surgery. Its exact global incidence however remains undetermined [8, 9]. Although several studies have reported low incidence rates of TSH after bariatric surgery, ranging from 0% to 1.6% [80-82], considering the high rates of incisional hernias repair surgeries being performed, the real prevalence of this complication seems to be substantially underestimated [11].

This underrepresentation is due, at least partially, to the fact that patients are often not routinely assessed for this complication [8]. Also, the accurate detection of TSH depends on the duration of postoperative follow-up; however, most of the controlled studies describe short-term follow-up intervals of less than 1-year [9]. For instance, a recent meta-analysis on the incidence of incisional hernias described those studies with a follow-up higher than 1-year tended to

report a significantly higher pooled TSH prevalence compared to studies describing follow-ups of less than 1-year (4.6% vs 0.8%) [9]. Similarly, another interesting study demonstrated that incisional hernia diagnosis expanded from 12.6% at 1-year of clinical follow-up to 22.4% at 3-years, a relative increase of more than 60 percent [83]. Nevertheless, the fact that available data are based only on symptomatic patients and clinically diagnosed cases seems to be the main explanation for the likely underestimation of TSH [9]. The clinical assessment of the abdominal wall might be ineffective in detecting TSH, mainly in cases where the fascial defect is small and/or the patient has obesity [84]. Particularly in the patient with obesity, clinical assessment of abdominal TSH is known to be difficult, due to the existence of an abdominal apron [85]. A hernia embedded in subcutaneous fat, even following significant weight loss after bariatric surgery, is still challenging to diagnose clinically [85, 86]. Moreover, due to the weight loss after BS, the scar associated with the skin incision might not correspond to the site where the abdominal wall was perforated [85]. Findings of a large prospective study demonstrated the relatively low sensitivity (77%) of physical examination to diagnose TSH, which was more noticeable in patients with obesity (73%) [86]. It was shown that, in cases where a hernia was not palpated, the probability of a false negative was 23% overall and 31% in patients with obesity [86].

Therefore, the clinical examination has been proven to be unreliable to detect TSH in obese patients, suggesting that a more sensitive diagnostic test is required in this population [85, 86]. At present, a CT scan is considered the gold standard procedure for the diagnosis of TSH, mainly for patients requiring a complex operative repair [8]. This exam has proven to be reliable and reproducible, however, due to its associated high cost and high ionizing radiation exposure, it is not widely employed, especially in cases where the patient is asymptomatic [8]. Ultrasound assessment of the abdominal wall, as recommended by the European Hernia Society, has also been demonstrated to be a valid and accurate alternative to the CT scan, even in patients with obesity [87, 88]. This test facilitates the long-term follow-up of TSH, allowing high

sensitivity and specificity, with the additional benefits of being less expensive and of not being associated with radiation exposure [88].

2.2. Risk factors for post-incisional hernia development

Although the exact pathophysiology of incisional hernia is currently not completely established, there is growing evidence suggesting that it has a multifactorial origin [14-16]. Several risk factors have been correlated with a high incidence of TSH, namely technical and patient-related factors [89]. Technical risk factors of TSH include the material and type of suture used to close the abdominal incision [90], design and size of the trocars, long operative time [9, 91], and the trocar site placement [92].

The material and type of suture used during surgery differs widely between surgeons and seems to impact the probability of short- and long-term surgical complications [90]. Several randomized controlled trials and meta-analyses have addressed this issue, however there is no consensus about which suturing technique is the most appropriate for attaining complete wound closure and for decreasing the risk of TSH [90, 93, 94].

There is also compelling evidence that larger trocar sizes contribute to a substantially higher incidence of TSH [9, 92]. Although the exact size limit above which the risk of herniation increases remains unclear, most incisional hernias reported in the literature were observed in ports of ≥10 mm [91]. In a recent meta-analysis, Karampinis, Lion [9] assessed particular trocar characteristics in order to identify specific risk factors of TSH development, especially the size of the trocar. In a total of 25 trocar site hernias analyzed, no hernias were detected with 5-mm trocars and just one occurred with 10-mm trocar ports [9]. On the other hand, the assessment of larger trocar sites demonstrated a total of fourteen hernias associated with 11-/ 12-mm trocars, one in the 13-mm and nine with the 15-mm trocars [9]. It is also well established that the position of trocar insertion is correlated with the risk of incisional hernias. In fact, TSH is mainly located at the site of the midline trocars, and the navel region was the most common [92].

Additional risk factors for the development of incisional hernias directly related to the patient have also been described, namely increasing age, previous

laparotomies, history or operation for an abdominal aortic aneurysm [8, 16, 95], malnutrition [96], smoking [97] and female sex [98, 99]. Obesity is also an important recognized predisposing risk factor for incisional hernias after laparoscopic surgery [8, 95, 98]. It is postulated that the substantial intraabdominal fat and increased abdominal wall circumference in patients with obesity might contribute to a higher risk of TSH, as well as to the origin of other hernias in the abdominal wall [98]. The correlation between obesity and TSH is also attributed to several other factors, such as higher intraabdominal pressure, thicker peritoneum, larger preperitoneal space, impairment of connective tissue formation and of wound healing [28, 89, 91].

Besides the elevated risk of erratic wound healing determined by obesity itself, the occurrence of postoperative complications in these patients seems to be also related to technical difficulties, in particular to the complexity of attaining a full-thickness surgical wound closure [17, 18]. Excess adipose tissue impairs the performance of the appropriate surgical procedure, increasing the probability of TSH development [100]. The closure of all layers in smalls and deep wounds is a difficult and challenging procedure in patients with obesity [17, 100]. Although some bariatric surgeons routinely close the trocar insertion site wound when it is viable, most surgeons do not perform this [101, 102]. A few surgeons doubt that the effects of fascial closure are associated with TSH incidence; however, there is extensive evidence showing that leaving the fascial defect open or incompletely closed increases the risk of this complication [87, 89, 100].

3. Skeletal muscle repair after injury

Skeletal muscle repair (SMR) is an extremely coordinated process, comprising the activation of multiple cellular and molecular responses, where the synchronization between inflammation and regeneration is critical for a successful repair process following muscle injury [103]. SMR is successful when it results in the development of a beneficial skeletal muscle phenotype – in which damaged fibers are replaced with new ones instead of replacement for adipose or connective tissue – with the original structure and function preserved [104]. Skeletal muscle regeneration (SMReg) is described as the substitution of injured

myofibers or their segments by new ones, with no changes in the original tissue structure [104]. Ideally, to preserve skeletal muscle structure and function, the SMR should be accomplished through the SMReg process. However, depending on the complexity and nature of the injury, that is, type and severity of the insult, SMR can also occur through total or partial replacement of the injured skeletal muscle fibers by connective or adipose tissue [105]. The replacement of damaged muscle fibers by adipose or fibrotic tissue represents the worst post-injury phenotypic adaptation, resulting in tissue morphological and functional impairment [105, 106].

3.1. Pathobiology of skeletal muscle regeneration process

Regardless of the type or severity of the damage, the skeletal muscle regeneration process tends to follow a fairly similar pattern that is mainly composed of three phases, i.e., the inflammatory phase, satellite cell activation/differentiation phase and maturation phase, with the remodeling of the scar tissue and restoration of muscle function [107, 108]. The SMReg process has been explained in detail in numerous previous articles [104, 107-109] and, therefore, a brief overview will be presented in this literature review.

The initial phase following muscle injury is characterized by necrosis of damaged muscle fibers and, subsequently, by an inflammatory cell response [110]. Muscle degenerates after injury, starting with the rupture of the myofiber sarcolemma, which leads to an increase in its permeability [104]. As a consequence of the disruption of the myofibers sarcolemma integrity, intracellular contents are exposed to the extracellular environment [104]. This is accompanied by extracellular calcium influx towards the fiber interior and the release of calcium from the sarcoplasmic reticulum [103]. The activation of calcium-dependent proteases leads to proteolysis and degeneration of skeletal muscle structure. Myofiber necrosis is also the trigger for the infiltration of the injured muscle fiber by circulating leukocytes [111].

Neutrophils are the primary inflammatory cells recruited following injury [111]. Within the first 2h after damage, these cells start to infiltrate the wound site, promoting the elimination of cellular debris. Approximately at 6-24h post-injury,

neutrophils achieve peak concentration, rapidly declining afterwards [112, 113]. Noteworthy, the depletion of neutrophils results in severely impaired SMReg, due to its importance for modulating macrophage function [22, 114]. Signals from neutrophils are responsible for promoting the cytolytic activity of macrophages [114]. Under normal resting conditions, resident macrophages remain quiescent in the healthy muscle; however, increased muscle damage triggers their rapid activation [115]. Two distinct macrophage populations sequentially infiltrate the damaged muscle tissue, i.e., pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages [111]. Following the onset of neutrophil invasion, the early-invading M1 macrophages are recruited towards the muscle environment, reaching a peak concentration at about 2 days post-injury, and then also undergo a fast decrease in number [115]. These phagocytic macrophages are responsible for removing debris and releasing a complex milieu of prostaglandins, chemokines, and Th1 cytokines [115]. Both M1 macrophages and neutrophils are abundant sources of pro-inflammatory cytokines in damaged muscle during the proliferative stage of myogenesis [111]. Among the Th1 cytokines, interferon-gamma (IFNγ) [116], tumor necrosis factor-alpha (TNFα) [117, 118], and interleukin-6 (IL-6) [119] seem to be particularly implicated in myeloid cell activation and chemotaxis to sites of tissue damage. Together, these cytokines play a pivotal role in regulating muscle repair and regeneration [120].

The second stage of SMReg is characterized by the phagocytosis of the necrotic muscle fibers and their replacement by new ones [108]. During this stage, nerves, blood vessels, and muscle cells penetrate the site of injury. In particular, satellite cells (SCs) migrate to the damaged site and differentiate into skeletal muscle fibers, in an extremely orchestrated process much similar to prenatal skeletal muscle growth [121]. Postnatal development, repair, and regeneration have practically the same molecular mechanisms, in which several growth factors trigger quiescent SCs to adopt a myogenesis program that is precisely regulated by the sequential expression of a family of muscle-specific, basic helix-loop-helix transcription factors known as myogenic regulatory factors (MRFs), i.e., myogenic differentiation 1 (MyoD), myogenic factor 5 (Myf5), muscle-specific regulatory factor 4 (Mrf4), and myogenin (MyoG) [122]. As in the

differentiation program of embryonic myogenesis, MRFs play a pivotal role in myogenesis during postnatal life [122]. The huge myogenic potential of SCs mainly depends on the expression of Pax genes, namely PAX7 and PAX3, which regulate the expression of other myogenic regulators and also act as an anti-apoptotic factor [123]. Under normal resting conditions, SCs remain quiescent and reside in the myofiber basal lamina [124]. Quiescent SCs are characterized by specific expression of the paired box transcription factor PAX7, and, to a lesser extent, Pax3, which are upstream of MRFs [123]. Due to the ubiquitous elevated expression of these pax genes, quiescent SCs are practically negative for MRFs [123] .

While quiescent under non-stressful conditions, SCs become activated to enter the cell cycle as a result of the exposure to signals from the damaged myofibers, including signaling through Ephrin and Wnt7a, as well as in response to enhanced functional demands (fiber hypertrophy) or the necessity for routine maintenance (replacement of damaged apoptotic myonuclei). SCs activation begins primarily as the expression of Myf5 protein progressively increases in the most, but not all, of the activated SCs [125]. It has been shown that about 90% of SCs have an active Myf5 transcriptional locus, suggesting that these quiescent cells are previously directed to the myogenic pathway [126]. $PAX7^{+}/Myf5^{+}$ SCs undergo symmetrical divisions where all resulting cells are positioned close to the plasma membrane of myofibers to proceed with the myogenesis process [126]. Notably, the remaining (10%) of cells downregulate Myf5 and resist the differentiation process [127]. Due to lack of Myf5 expression, this small subset of activated SCs is described as myogenic stem cells, rather than myogenic progenitors [127]. The $PAX7^{+}/Myf5^{-}$ SCs can undergo asymmetric divisions, where descendent cells become adjacent to the basal lamina, thereby returning to a mitotically inactive state similar to quiescence, to maintain satellite cells pool [126]. In the early phases of activation, besides Myf5 and PAX7 expression, SCs begin also to express MyoD protein [128].

In the proliferation state, Myf5 and MyoD factors are also expressed; however, the finest distinguishing marker for proliferating SCs is the expression of proliferating cell nuclear antigen (PCNA) [129]. Upon proliferation, most of SCs

begin to downregulate PAX7 and maintain high levels of MyoD and PCNA [129, 130]. In contrast, the remaining (10%) cells retain PAX7 expression and suppress MyoD, to resist the differentiation and return back to quiescence, a process depending on Sprouty1 (Spry1) [130, 131]. Spry1 is expressed in quiescent PAX7+ SCs in undamaged muscle, downregulated in proliferating myogenic cells after damage, and reinduced as PAX7+ cells re-enter quiescence [131]. The activated and intra-nuclear translocated Notch-1 protein has been shown to sustain proliferation and inhibit the transition to differentiation state, by retaining MyoD expression at low levels [132].

There is also increasing evidence supporting the pivotal role of several growth factors in the SCs activation and proliferation [133]. Similar to Notch-1, the hepatocyte growth factor (HGF) has been demonstrated to stimulate SCs proliferation and to inhibit the progression towards differentiation due to its inhibitory role in the heterodimerization process of MRF [132, 134]. Fibroblast growth factor 2 (FGF-2) was shown to recruit SCs to the cell cycle, providing additional cells to the myogenesis program [135, 136]. Insulin-like growth factors (i.e., IGF-1 and IGF-2) were also shown to induce SCs proliferation [137]; however, IGF-1 seems to be significantly more effective in promoting SCs differentiation [138]. In contrast, the transition from quiescence into activation and proliferation state can be inhibited by myostatin (GDF-8), a member of the transforming growth factor β (TGF-β) superfamily that is highly expressed in skeletal muscle [139].

After the proliferation phase, SCs differentiate to give rise to myogenic progenitor cells - often referred to as myoblasts - an extremely proliferative transient amplifying cell population [140, 141]. Morphologically, myoblasts become elongated myocytes, which then fuse with other myoblasts, to develop multinucleated myotubes and form new myofibers, or fuse with the damaged myofiber allowing its repair [141]. Transition to the differentiation phase is regulated mainly by the ongoing change in the expression from Notch proteins in proliferating SCs to Wnt signaling in differentiated SCs [142]. At the level of MRFs, the progression toward differentiation is firstly evidenced by the progressive PAX7 downregulation with increased expression of Myf5 and MyoD.

It was demonstrated that MyoD expression is required for the SCs to leave the cell cycle and integrate the differentiation state. Upregulation of the secondary MRFs (i.e., myogenin and MRF4) stimulates the terminal differentiation of myoblasts into myofibers [143]. Once SCs express myogenin it can directly suppress PAX7 expression and the differentiation succeeds, suggesting a mutual inhibitory mechanism between MRFs and PAX7 [130]. Therefore, the terminally differentiated SCs can be identified by their expression of myogenin, MyoD, MRF4 and concomitant PAX7 downregulation, in order to become terminally differentiated. The new myofibers are characterized by centrally located nuclei and the expression of important genes and proteins for muscle cells, such as muscle creatine kinase (MCK) and myosin heavy chain (MHC).

The final stage of SMReg is characterized by the reorganization of the muscle fibers, remodeling of the scar tissue and regain of muscle function. This remodeling phase is a continuation of the repair process in which the new myofibers formed then mature and develop attachments to the surrounding extracellular matrix. Terminally differentiated myotubes mature to turn into myofibers filled with myofibrils, responsible for the skeletal muscle fiber contractile function. Ultimately, the extremities of the injured muscle fibers may not unite but, in its place, develop a new myotendinous junction with the interposed scar tissue [144]. The regeneration process is followed by hypertrophy. By inducing protein synthesis and suppressing muscle atrophy, IGF-1 and IGF-2 are responsible for triggering maturation and hypertrophy [145].

3.2. Myogenic progenitor cells

Regeneration of skeletal muscle requires a cell population capable of proliferation, self-renewal, myogenesis and resistance to oxidative or hypoxic stress [146]. Numerous potential myogenic progenitor cell populations have been suggested [147], however, their capacity to form new muscle tissue *in vivo* remains mostly undetermined. The most evident cell population with myogenic potential are muscle satellite cells [109, 148]. However, there is evidence that SCs are not the only population of progenitor cells that can contribute to muscle regeneration [149, 150].

There is increasing awareness and controversy about the role of other muscle-derived stem cells (MDSCs) populations and circulating progenitor cells, namely bone-marrow-derived cells [150, 151], in muscle growth either through myogenic differentiation or via secretion of paracrine factors that affect the adjacent cells [146, 152]. Indeed, several MDSCs groups have been identified, i.e. myogenic progenitor, myoendothelial and perivascular progenitor cells, as also a muscle-derived side population with similar properties to hematopoietic stem cells in the bone marrow [152]. Myogenic progenitor, myoendothelial and perivascular cells have been demonstrated to be an interconnected cell population [153]. When activated, MDSCs can exhibit long-term proliferation, high self-renewal, and multipotent differentiation. These cells differentiate by myogenic, osteogenic, chondrogenic, and adipogenic lineages *in vitro*, similarly to other mesenchymal progenitor cells [154]. Besides that, MDSCs can produce growth factors essential to tissue healing, thereby acting as long-lasting reservoirs for these molecules [154]. In response to muscle injury, the expression of MDSCs seems to raise rapidly, although it is unclear whether this boost is due to fast proliferation or recruitment from other sites [155].

Similar to MDSCs, adipose tissue derived stem cells (ASCs) have been shown to have the same myogenic differentiation ability as other mesenchymal progenitor cells [149, 156, 157]. The ASCs cells reside close to the capillaries within adipose tissue and have proved to be effective as a source of injectable progenitor cells [156, 157]. The bone marrow is also recognized as a rich source of myogenic mesenchymal stem cells, that have a pivotal role in muscle regeneration following injury. The bone marrow mesenchymal stromal cells (BM-MSCs) are derived from the bone marrow and are mainly present in the cavities of the marrow, however, they can be occasionally observed around the blood vessels or other tissues [158]. Findings from previous studies in experimental models have demonstrated that injected bone-marrow-derived progenitor cells are integrated into newly formed myofibers of muscle regeneration and contribute to the SCs pool, similarly to endogenous cells [159-162]. Furthermore, in a study that evaluated the effect of the local application of autologous BM-MSCs on normal muscle regeneration in a mouse model of blunt skeletal muscle trauma,

Matziolis, Winkler [163] showed that bone-marrow-derived cell treatment significantly increased muscle function. The BM-MSCs also seem to respond to muscle overloading with mobilization from the bone marrow and engrafting within the stimulated muscle [164]. An additional role of BM-MSCs is related to the fact that, in cases of substantial muscle damage, these multipotent cells can support the repair of other skeletal muscle components such as blood vessels and nerves [165, 166].

A population of circulating $CD133^+$ progenitor cells has also been recognized as having myogenic potential. Discovered from mouse footpads immunized with human fetal liver $CD34^+$ cells [167], CD133 is an extremely conserved stem cell glycoprotein antigen, encoded in humans by the PROM1 gene, with hematopoietic potential and capacity to differentiate into endothelial progenitor cells [168]. There is compelling evidence about the myogenic potential of human skeletal muscle-derived $CD133^+$ cells, which have been shown to have myogenic capacity *in vitro* and to be capable of contributing to the regeneration of muscle fibers *in vivo* in mouse models [169, 170]. Findings have demonstrated that $CD133^+$ transplanted cells functionally reconstituted the SCs pool, supporting the potential of these cells for long-term treatment of muscle diseases [169]. Torrente, Belicchi [171] has demonstrated that direct injection of $CD133^+$ cells into dystrophic muscle was associated with improvement of skeletal muscle structure and replenishment of the SCs pool. Similarly, Negroni, Riederer [172] have reported increased stem cell pool with the direct injection of $CD133^+$ cells, compared with human myoblasts, which seems to contribute to muscle regeneration. Thus, there is compelling evidence about the diversity of potential cell populations that may contribute to robust endogenous skeletal muscle regeneration. Nevertheless, each one of these populations evidently differs in its abundance within the human body and in its capacity to develop and maintain a myogenic phenotype.

4. Effect of obesity on skeletal muscle regeneration

4.1. Obesity and skeletal muscle lipid accumulation

Obesity is characterized by excessive adipose tissue accumulation in subcutaneous and visceral stores and non-adipose organs, which is described as ectopic adipose tissue accumulation [173]. Moreover, patients with obesity have increased levels of circulating fatty acids [174, 175] and ectopic accumulation of lipids in skeletal muscle due, in part, to the higher uptake of fatty acids from the circulation [176, 177]. Lipids within skeletal muscle tissue consist mainly of two distinct groups, i.e., intramyocellular lipids placed within muscle cells and extramyocellular lipids situated in adipose cells amongst myofibers [178, 179]. A portion of extramyocellular lipids comprises adipose tissue directly connected with the muscle, referred to as intermuscular adipose tissue (IMAT) [180]. In patients with obesity, the intermuscular adipose tissue deposit is strongly and positively correlated with insulin resistance and reduced muscle function [180, 181]. However, this adipose tissue accumulation seems to not influence muscle mass [182], and its impact on human muscle regeneration capacity remains unclear. The accumulation of diacylglycerols, ceramides and long-chain acyl CoA observed in the skeletal muscles of obese humans adversely impact cell signaling and metabolism, described as lipotoxicity [183, 184]. At the level of muscle tissue, the lipotoxic species affect insulin signaling and seem to be, at least partially, responsible for insulin resistance in obesity [185-187]. Despite this evidence, it remains largely unclear if other physiologic processes are compromised by the accumulation of these lipid metabolites in the muscle tissue of patients with obesity.

4.2. Obesity and skeletal muscle connective tissue disorders

The conventional view of adipose tissue as a passive storage source for excess energy from food intake is no longer acceptable. Adipose tissue has been recognized as a complex and highly active metabolic and endocrine organ [188]. In fact, a wide range of bioactive peptides, with pro-inflammatory activity, referred to as adipocytokines or adipokines are produced and secreted by adipose tissue

[188]. Although the exact mechanisms remain unclear, excess adiposity results in dysregulation of these adipose tissue-derived secretory factors, which negatively impact several physiological processes in distinct organs [28, 189]. Regarding skeletal muscle tissue, particular consideration has been provided to extracellular modifications, namely in the extracellular matrix (ECM), evident by the connective tissue issues [28].

Skeletal muscle connective tissue is structured by three interconnected layers (i.e., epimysium, perimysium and endomysium). Epimysium refers to the outermost layer that surrounds the entire muscle, the perimysium layer lines the muscle fascicles and the endomysium is the innermost layer that covers each muscle fiber. The ECM is composed by proteoglycans, glycoproteins, elastin, fibronectin and collagen - predominantly its type I and III isoforms – being linked to the myofibrillar sarcolemma by a dedicated basement membrane, associated with integrins that potentiate both structural integrity of the muscle fibers and the accurate distribution of forces throughout the muscle structure.

Severe obesity is associated with changes in certain components of the connective tissue such as matrix metalloproteinases. Matrix metalloproteinases perform an important function in several physiological and pathological processes, including wound healing [190]. In particular, MMP-2 and MMP-9 are directly involved in the ECM metabolism during connective tissue remodeling and repair [28, 191]. In normal tissues, MMPs are expressed at relatively low levels; however, their expression and activity can be induced as a consequence of pathogenic processes and/or during wound healing [29]. The activity of MMPs has proven to be significantly higher in patients with obesity, compared to normal weight individuals [28, 192]. For instance, in a study assessing the MMP-2 and MMP-9 levels in 163 patients with obesity compared to control subjects, it was possible to identify an increased expression of these enzymes in patients with obesity, suggesting alterations in the normal ECM metabolism [192]. More recently, Boumiza, Chahed [25] demonstrated that levels of MMP-1 and MMP-2 are positively correlated with BMI and that patients with obesity have a significantly higher expression of these enzymes. The fact that adipocytes are a source of MMPs seems to explain, at least partially, the elevated serum MMP-1,

MMP-2, and MMP-9 concentrations observed in patients with obesity [193].

There is increasing evidence that enhanced activity of MMPs in the population with obesity seems to contribute to altered collagen metabolism [29, 30]. Collagen is the main component of the ECM and plays a significant role in the maintenance of skin tensile strength and elasticity [29]. Types I and III collagen predominate, comprising 95% of the total collagen content. Type I collagen is characteristically strong and responsible for the mechanical resistance of tissues. It is widely distributed in the human body, including the skin, ligaments, fascia and fibrous tissue [30]. On the other hand, type III collagen is less cross-linked and supports less tensile strength. It is present in lesser amounts in the same tissues and during the first few days of wound healing, being then replaced by collagen I. Under normal conditions, type I collagen is approximately four times more prevalent than collagen III [29].

In patients with obesity, the excess of MMPs is responsible for changing collagen metabolism at three levels [29]. First, as MMP-2 and MMP-9 have a greater affinity for type I collagen, the increase in their activity results in higher degradation of type I collagen. As a result, the collagen I to III ratio is altered, contributing to decreases in the ECM mechanical properties [29]. The higher content of the weaker type III collagen relative to the stronger type I collagen leads to decreased fibril diameter and results in the synthesis of a mechanically weak tissue. This defective collagen metabolism contributes to decreased tensile strength and mechanical stability of connective tissues and scar tissue, which seems to have an adverse effect on wound healing. The higher activity of MMPs in patients with obesity contributes to defective collagen metabolism and, consequently to ECM damage [27, 191]. In fact, the higher incidence of TSH in this population can be supported by the systemic impairment of connective tissue formation and wound healing, proving the biological foundation for herniation pathophysiology [28].

4.3. Effect of obesity on skeletal muscle stem cells

There is evidence showing that the muscle regeneration process is compromised in animals under conditions of hyperglycemia and/or lipotoxicity [20, 21, 194, 195]. However, the detailed modifications, in particular, the satellite cell changes, differ between studies. In a study with a mouse model of insulin resistance attained by high-fat diet (HFD) feeding for 8 months, Hu, Wang [196] found that higher circulating fatty acids led to delayed myofiber maturation induced by cardiotoxin injury, thereby resulting in smaller regenerative myofibers. Moreover, a significant increase in collagen deposition was also observed. The SCs activation and proliferation remained unchanged. Instead, the impairment of muscle regeneration was mainly associated with increased phosphatase and tensin homolog (PTEN) expression, which inhibited PI3K/Akt signaling and decreased myofiber maturation [196]. In opposition, Woo, Isganaitis [197] observed that overweight mice exhibited reduced SCs content and muscle mass, as well as decreased regenerative capacity. Similarly, mice fed a HFD for 6 weeks showed a delay in myofiber regeneration due to attenuated proliferation of SCs, although the satellite cell content remained intact [24]. Furthermore, activation of SCs was also shown to be impaired in a murine diet-induced obesity model, which may be, at least in part, associated with a deficit in hepatocyte growth factor (HGF) signaling in skeletal muscle [198]. HGF stimulation of myogenic stem cells requires AMP-activated protein kinase (AMPK), a protein crucial to sustain SCs number and stimulate myotube development. There is evidence suggesting that SCs isolated from damaged muscle of diet-induced obese mice display reduced AMPK activity and impaired muscle regeneration [194]. For instance, Fu, Zhu [194] reported inhibition of SCs activation and proliferation, as well as less formation of regenerated fibers after cardiotoxin-induced muscle injury in obese mice. Further analysis revealed that obesity inhibited AMPK activity, which was associated with both reduced proliferation and differentiation of SCs and consequent impairment of muscle regeneration [194]. There is also compelling evidence that obese Zucker rats – a genetic model of obesity resulting from homozygous missense mutation of the leptin receptor gene [199] – displays smaller skeletal muscle size in comparison to the lean Zucker

rat [23]. This defect has been attached to a substantial decline in SCs proliferative capacity, although the proportion of quiescent SCs persisted unchanged in this obese rat [23].

4.4. Obesity-induced mitochondrial dysfunction in skeletal muscle

In skeletal muscle, obesity is associated with the inhibition of glucose uptake, dysregulation of lipid metabolism, abnormal protein turnover and, particularly, mitochondrial dysfunction [33-35]. Mitochondria are dynamic organelles that regulate several cellular functions, including cellular respiration, calcium homeostasis and oxidative stress [200]. However, the main role of this organelle is related to energy metabolism, namely the production of adenosine triphosphate (ATP) via oxidative phosphorylation [200]. In addition, mitochondria are also involved in the production and removal of reactive oxygen species (ROS), a side effect of energy metabolism [201, 202]. In fact, mitochondria represents one of the main sources for the production of ROS, such as superoxide ions [203]. Mitochondrial superoxide ($O2^{\bullet-}$) can be dismutated and, thereby, converted into a main cellular source of hydrogen peroxide (H_2O_2) [203]. H_2O_2 is a non-radical ROS that diffuses both within a cell and across cell membranes and, consequently, acts as a signaling molecule [204, 205]. The rapid capacity of ROS for diffusion, in addition to its adaptable biological activities, makes them remarkable candidates for tissue injury-to-inflammatory cell signaling, which is crucial for proper wound detection and healing [206, 207]. In particular, H_2O_2 is regarded as a key signaling molecule that plays a pivotal role in the regulation of a variety of biological processes, including tissue regeneration [208], since it acts as a trigger for the fast recruitment of leukocytes to the wound site [209]. Despite that, H_2O_2 can contribute to the opposite effect, promoting chronic tissue injury and, consequently, inflammation by inducing permanent oxidative damage in numerous macromolecules such as DNA, lipids, and proteins [210, 211]. An elevated formation of ROS causes excessive oxidative stress in skeletal muscle, inducing both cellular and mitochondrial damage [212]. Mitochondrial dysfunction is typically characterized as the failure of mitochondria to produce and maintain adequate ATP levels [213] as well as the presence of

abnormalities in substrate catabolism, ion transport, calcium buffering, mutations in mitochondrial DNA, ROS production and apoptosis [214, 215]. Due to the important roles carried by mitochondria, dysfunction of this organelle is associated with several diseases, namely T2DM and obesity [216]. Decreased mitochondrial respiration and ATP production as well as increased mitochondrial ROS production is frequently observed in skeletal muscle in obesity [216, 217].

It is well-established that excessive nutrient intake negatively impacts the function of mitochondria [218]. In this regard, there is compelling evidence that obesity is strongly associated with reduced mitochondrial function and mass [219-222]. In skeletal muscle tissue, an association between obesity and mitochondrial dysfunction has already been described in several studies [219, 220]. For instance, Jheng, Tsai [223] have demonstrated that mitochondria are characteristically smaller in the skeletal muscle of obese mice. Similarly, Bonnard et al. (2008) observed that mice exposed to a prolonged high-fat, high-sucrose dietary intervention displayed impairment of mitochondrial biogenesis, structure, and function in muscle tissue, with a reduction in mitochondrial respiration. When the role of oxidative stress in the development of these mitochondrial abnormalities was assessed, it was observed that diet-induced diabetic mice had increased production of ROS in skeletal muscle. More recently, Konopka et al. (2015) showed that respiratory control ratio, an index of respiration and ADP phosphorylation coupling, was significantly reduced in women with obesity. In comparison with lean women, patients with obesity exhibited mitochondrial bioenergetics impairment, due to the lower mitochondrial coupling and phosphorylation efficacy, as well as elevated mitochondrial H_2O_2 (mtH_2O_2) production. These findings are in line with a previous study from Anderson et al. (2009) demonstrating that lipid accumulation in skeletal muscle of rodents and humans, plays a pivotal role in boosting mtH_2O_2 and changing the cellular redox environment to a more oxidized state. Approximately 2 times more mtH_2O_2 emissions were found in patients with obesity compared to lean individuals [224].

In addition to mitochondrial biogenesis, the balance between mitochondrial fusion and fission, referred to as mitochondrial dynamics, also regulates mitochondrial function [225]. Skeletal muscle mitochondria are dynamic

organelles that continuously undergo a morphological and structural cycle involving fusion and fission, which are crucial for cell survival, development, and division [226]. Mitochondrial fusion is responsible for compensating damaged mitochondria by attaching them to healthy ones. Proteins Mitofusin 1 and 2 (Mfn1 and Mfn2, respectively) are responsible for the fusion of mitochondrial external membranes, being decisive in mitochondrial metabolism through the preservation of the mitochondrial network architecture, whereas Optic atrophy 1 (Opa1) is involved in the fusion of mitochondrial internal membranes [227]. In its turn, mitochondria fission involves the separation of damaged sites from healthy mitochondria to maintain mitochondrial function, a process largely mediated by mitochondrial proteins dynamin-related protein 1 (Drp1) and mitochondrial fission protein 1 (Fis1) [227].

The regulation of mitochondrial dynamics is a complex process that provides a balance between mitochondrial fusion and fission, essential to preserve mitochondrial health in skeletal muscle [225]. In patients with obesity this balance is replaced by impaired mitochondrial dynamics, that can involve oxidative stress, mitochondrial dysfunction, and metabolic modifications [215, 225]. Albeit the complete mechanisms underlying changes in mitochondrial dynamics in obesity remains unclear, modifications in fusion/fission processes and the excess of ROS appears to be involved [215, 225]. In fact, some authors have already demonstrated that mitochondrial fission is increased in the skeletal muscle of obese mice [216, 223]. In addition, changes in the activity of the proteins involved in mitochondrial dynamics has been observed in obesity, such as reduced expression of Mfn2 and altered expression of OPA1 [219, 220, 228]. For instance, Bach et al. (2003) has demonstrated that in obesity there is repression of Mfn2 expression, resulting in decreased glucose oxidation, alterations in mitochondrial membrane potential, cell respiration and increases in mitochondrial proton leak. These findings are in line with a study from Sebastián, Hernández-Alvarez [229] showing that Mfn2 deficiency leads to enhanced H_2O_2 concentrations, increased ROS production, as well as mitochondrial dysfunction in skeletal muscle. In addition, Liu, Jin [216] reported a reduction of 20% in both Mfn1 and Mfn2 protein levels, whereas proteins Fis1 and Drp1 were enhanced in

the skeletal muscle of mice exposed to high-fat diet consumption for 40 weeks. The molecular shift from fusion to more fission was accompanied by disrupted mitochondrial respiratory function and declined ATP content [216]. Similarly, Jheng, Tsai [223] found that mitochondrial fission protein (i.e., Drp1 and Fis1) were substantially enhanced in genetically induced obese mice (ob/ob) and high fat diet-induced obese mice in comparison with lean mice, supporting the imbalance between fusion and fission associated with obesity. Moreover, authors also observed that mitochondria in the skeletal muscle of obese mice are characteristically smaller and shorter. Therefore, current evidence suggests that the shift to fission processes possibly contributes to the impairment of mitochondrial function and increased production of ROS, described in patients with obesity [219, 220, 228].

5. Role of physical exercise on the muscle regeneration process

5.1. Skeletal muscle adaptations to physical exercise

In skeletal muscle, some of the advantageous functional adaptations induced by regular physical exercise are related to the increase in muscle mass, fascicle length, total number of sarcomeres and muscle hypertrophy [230, 231]. The hypertrophy of muscle fibers is characterized by increases in muscle cross-sectional area (CSA) [231], a hallmark adaptation to resistance exercise [232-234]. Several experiments have demonstrated that physical exercise can modulate muscle hypertrophy through muscle mass development due to increased accumulation of myofibrillar muscle protein synthesis [230, 231, 235]. The increase in the number of sarcomeres in muscle fibers enables the dissipation and distribution of mechanical tension, which reduces the effort that each muscle fiber needs to perform and, as a result, possibly reduces damage risk [236, 237].

5.2. Influence of physical exercise on extracellular matrix dynamics

Regardless of the need of more experiments assessing the possible effect of physical exercise on ECM dynamics during skeletal muscle tissue

regeneration, it is established that both collagen synthesis and activity of MMPs acutely increase with exercise training [238]. In addition, local tissue expression and release of several growth factors for ECM such as IGF-1, TGF-β and IL-6 was also shown to increase following exercise. Chronically, physical training has demonstrated to enhance both collagen turnover as well as, to some degree, net collagen content [238]. These modifications induced by training can change the mechanical properties and the viscoelastic characteristics of the tissue and, as a result, reduce its susceptibility to damaging mechanical forces [238]. An experiment on humans assessing the influence of endurance training in the vastus lateralis, has revealed acute (2h post-exercise) increases in the expression of MMP-9, responsible for modulating collagen turnover and growth factor availability activity [239]. Chronically, exercise was also demonstrated to induce increases in MMP-2 levels, which cleaves the ECM and basal lamina in angiogenesis, associated with an effective tissue regeneration process [239]. Together, these findings support the notion that physical exercise, both acutely and chronically, can efficiently modulate the ECM dynamics and, consequently, determine a favorable and less fibrotic skeletal muscle phenotype during the regeneration process [238, 239].

5.3. Effect of physical exercise on skeletal muscle stem cells

There is compelling evidence suggesting that stem cells proliferate, self-renew, or differentiate into a precise phenotype according to their specific mechanical milieu (i.e., stiffness modifications of their environment) due to their ability to respond to external mechanical forces [240-242]. As with chemical factors, mechanical dynamics appear to be imperative for the effective management of the function and fate of stem cells [240, 243]. In support of this notion, physical exercise has been suggested to be able to induce chemical signals (i.e., numerous myokines secretion), in addition to the loading-derived mechanical factor, that might be essential for SCs activation and differentiation [244, 245].

In undamaged skeletal muscle fibers, about 2-7% of the fiber nuclei are SCs in a quiescent state, expressing PAX7 marker. Nevertheless, when

subjected to overloading, trauma or damaging signals, SCs activate, proliferate and either self-renew (preserving the SC pool) or differentiate into myoblasts, as described earlier in this review [246]. In a study assessing the acute effect of physical exercise on skeletal muscle fiber SCs content and activation status, Snijders, Verdijk [247] demonstrated, through immunohistochemical analysis of the middle region of the *vastus lateralis*, that a single bout of combined endurance and resistance exercise was able to induces SCs to enter cell cycle within 9h post-training.

Although physical exercise seems to be efficient in inducing the activation of SCs, the pool of these cells only increases if they can proliferate effectively. In this regard, a single bout of running exercise was shown to either induce dynamic modifications in mouse *plantaris muscle* messenger RNA (mRNA) expression, revealed by enhanced expression of cell-cycle-related genes 24h post-training, or improved cell proliferation, confirmed by immunohistochemistry analyses [248]. Similarly, a study evaluating the impact of acute high intensity exercise on SCs activation *in vivo* in human muscle has revealed that SCs from the vastus lateralis muscle increased their expression of mononuclear cells staining for the neural cell adhesion molecule and fetal antigen 1 on days 4 and 8 post-training [249]. Therefore, Crameri, Langberg [249] concluded that SCs can be stimulated to enter the cell cycle after only one bout of unusual high intensity exercise. These results are in line with a study from Cermak, Snijders [250] demonstrating a significant increase in both SCs content and activation status, as demonstrated by the increased expression of cell cycle-related components (DLK1 for activation, and PCNA for proliferation), in the *vastus lateralis* muscles 24h after an intensive eccentric exercise training protocol. Together, these findings from studies on experimental animals as well as on humans suggest that acute physical exercise can potentiate the activation and proliferation of SCs.

Nevertheless, the beneficial impact of physical exercise on the pool of SCs does not seem to be limited to its acute effects [251-255]. For instance, a study [252] evaluating the effect of 16- weeks resistance training has revealed an increment in SCs number in both type I and II fibers. In addition, an association between the modification in SCs content and the increase in the lean muscle

mass was also observed [252]. Another experiment on humans, assessing the association between myofiber hypertrophy and SCs content in the *vastus lateralis* muscle, has revealed that individuals submitted to exercise training have higher SCs pools in basal conditions [254]. Thus, trained individuals have shown to be more able to either improve SCs number, fiber nuclei addition and attain higher fiber hypertrophy, supporting the notion that a greater SCs pool is essential during a stressful event-induced on muscle [254].

Considering that physical exercise training successfully improves skeletal muscle myogenic potential, namely by stimulating SCs pool increase in both acute and chronic conditions, its therapeutic and prophylactic impacts on the muscle regeneration process has been hypothesized. For instance, Ambrosio, Ferrari [256] has conducted an experiment on animal models assessing the adjuvant effect of exercise to enhance muscle-derived stem cell transplantation during the regeneration process into the damaged muscle. Findings from this study have demonstrated that 5 weeks of treadmill running was associated with mechanically stimulated MDSCs, visible by its enhanced proliferation after injury [256]. In addition, exercise was also associated with reduced fibrosis and improved skeletal muscle vascularity after muscle damage [256]. Similarly, a previous experiment investigating bone marrow cells transplanted into mice submitted to both forced running exercise and *extensor digitorum longus* overload revealed an improved incorporation of bone marrow-derived stem cells into damaged skeletal muscle [164]. In another experiment in animal models, SCs from physical active mice revealed an improved and faster proliferation ability and enhanced differentiation [164]. Toghether, these investigations point out that, in addition to the previously mentioned increase in the SCs pool, physical exercise training could be an efficient strategy for enhancing the skeletal muscle regeneration process by stem cell chemotactic attraction.

5.4. Effect of physical exercise on obesity-induced mitochondrial dysfunction

As previously described, there is compelling evidence supporting that obesity is strongly associated with impaired mitochondrial function in skeletal

muscle. Physical exercise, in turn, has been increasingly suggested as an effective treatment for obesity-induced mitochondrial dysfunction [33]. In support of this notion, Konopka, Asante [257] has demonstrated that 12 weeks of aerobic exercise training can reverse obesity-related mitochondrial disfucntion as evidenced by enhanced mitochondrial bioenergetics efficiency and reduced mtH_2O_2 production. Furthermore, 8-oxo-dG, a marker of DNA oxidative damage, has also been shown to be reduced by aerobic exercise training [257]. Similarly, physical exercise also seems to display a beneficial role in obesity-induced mitochondrial dynamics [33]. For instance, Greene, Lee [258] has demonstrated that physical activity ameliorates obesity-induced imbalances between mitochondrial fusion and fission, by increasing mitochondrial fusion proteins and by reducing or sustaining mitochondrial fission protein levels. Notably, expression levels of Opa1 and Mfn2, which are promoters of mitochondrial fusion, were shown to be enhanced with physical activity, while mitochondrial fission proteins expression remained unchanged, indicating a shift towards mitochondrial fusion [258]. Accordantly, physical activity appears to preserve healthy mitochondria under conditions of obesity [258], however, further investigations are needed to clarify this notion.

CHAPTER III

EXPERIMENTAL STUDY

EXPERIMENTAL STUDY

1. Materials and methods

1.1. Experimental design and ethical approval

This was an experimental cross-sectional study performed in a convenience sample of adult patients with obesity that underwent bariatric surgery in the São João Medical Hospital Center between October 2020 and February 2022.

Skeletal muscle samples were collected form patients undergoing primary bariatric surgery that were invited to participate in a study with the purpose of identifying mitochondrial dysfunction indicators in patients with severe obesity and mitochondrial indicators of weight loss prognosis after bariatric surgery. For this purpose, a biobank of several biological samples, including abdominal wall skeletal muscle samples was gathered during bariatric surgery. The inclusion criteria for participation in the study were: i) age ≥ 18 years and ii) undergoing primary bariatric surgery irrespectively of the specific surgical procedure. Exclusion criteria were: i) presence of active known neoplastic or autoimmune diseases (such as rheumatoid arthritis or lupus), ii) undergoing revisional bariatric surgery and iii) nor agreeing with the study terms or not willing to participate in the study. All patients were informed beforehand about the study aims and purposes and about the possibility that biological samples collected during surgery could be used for other scientific purposes other than the specific purpose of the initial study. Patients who agreed with these conditions, gave formal approval for participation in the study by signing a written informed consent document. The protocol of the study was revised and approved by the São João Medical Hospital Center ethics committee (**ethical approval ref: 146/19**).

1.2. Skeletal muscle samples collection

Skeletal muscle tissue samples were collected from the abdominal wall muscles during laparoscopic BS, regardless of the bariatric surgical procedure. Samples were collected at the beginning of the surgery immediately after the surgeon gained access to the peritoneal cavity and the initial inspection was considered

compatible with the continuation of the surgical procedure. Skeletal muscle tissue samples of about 200mg were collected from the interior of the abdominal cavity at the region immediately bellow the left diaphragmatic cupula, anteriorly. Samples were collected with a harmonic scalpel (Ethicon; USA) which, unlike electrosurgical instruments, uses ultrasonic vibrations to cut and cauterize tissue, minimizing thereby the possibility of thermally damaging the tissue sample during collection.

After collection, abdominal wall muscle tissue samples were stored in a closed container in ice until they were further processed for biochemical and histological analysis. Samples were stored in ice usually between 2 and 4 hours before processing. The Biobank construction phase occurred between October 2020 and February 2022, during which samples from about 210 bariatric patients were collected.

1.3. Demographic and medical information

The medical records of the patients included in the Biobank were analyzed for collecting relevant demographic and medical information namely: patients age, sex, weight, height, body mass index (BMI) waist and hip circumference, blood pressure, alcohol intake, smoking status, associated medical conditions and usual medication.

1.4. Patients group allocation

Considering the available samples in the Biobank and the characteristics of the patients included two groups of patients were defined, representing either samples from patients with the most extreme cases of severe obesity or samples from patients with milder forms of obesity. For this, n=10 skeletal muscle samples were selected from patients with BMI ≥54 $kg.m^{-2}$ [extreme obesity group (ExOb); n=10]. Ten skeletal muscle samples were also selected from patients with BMI <37 $kg.m^{-2}$ [obese group (Ob); n=10] and that were matched with the ExOb group regarding potentially confounding variables such as: age, sex, smoking status and comorbidities such as diabetes, arterial hypertension and dyslipidemia. The

total number of skeletal muscle samples included in the study was therefore n=20. This number was defined based on pragmatic criteria, namely the number of patients in the Biobank with BMI ≥55 kg.m^{-2} and resources available (reagents and laboratory consumables) necessary to perform the experiments.

1.5. Tissue processing for light microscopy

For histological analysis tissue samples were fixed overnight in 4% paraformaldehyde (P6148; Sigma-Aldrich) at 4ºC. After fixation, tissues were washed in cold phosphate buffer saline (PBS) solution for 60 min, dehydrated through graded ethanol solutions (70%, 80%, 95%, 100%) for 60 min each, cleared in xylene, embedded in melted paraffin and allowed to cure at room temperature for the final preparation of tissue sample blocks. Transverse 5µm thick sections from each skeletal muscle sample were prepared with a Leica 2125 rotary microtome (Leica Microsystems Inc) and the resulting slides were then used for muscle histomorphology and immunohistochemistry analysis after adequate drying at room temperature.

1.6. Histological analysis of the skeletal muscle tissue

1.6.1. Skeletal muscle fibers cross-sectional area and skeletal muscle fibers nucleus position analysis

Muscle sections were stained with hematoxylin and eosin (H&E). For H&E staining, sections were cleared in xylene for 10 min, rehydrated in graded ethanol solutions (100%, 95%, 85%, 75%) for 5 min each, immersed in Mayer's hematoxylin solution for 5 min, followed by immersion in 1% eosin for 5 min, followed by washing with running water, dehydration in ethanol and clearing in xylene. Slides were permanently mounted with glass coverslip and DPX (dibutyl xylene phthalate; Shandon EZ-Mount, Thermo Electron Corporation, USA). After drying, sections were examined under a light microscope (Axio Imager A1, Carl Zeiss; Germany) with a 40x magnification objective. Photomicrographs (between 11 to 17 non-overlapping images of each muscle sample) were acquired by a

coupled digital camera with Axio Vision 4.7 software. Then, photomicrographs were examined with ImageJ software (NIH, Bethesda, MD) to quantify skeletal muscle fibers' cross-sectional area (CSA) and myonuclei position (NP). Muscle fibers CSA was defined as the average cross-sectional size (μm^2) of all individual muscle fibers analyzed in each subject's skeletal muscle sample. About 14 muscle fibers were analyzed per photomicrographs.

To evaluate the number and position of myonuclei, all the fibers contained in each of the images captured were analyzed. The number of either centrally or peripherally located myonuclei was analyzed and expressed relative to total skeletal muscle cross sectional area (nº myonuclei/μm^2skeletal muscle) and relative to the number of skeletal muscle fibers (nº myonuclei/skeletal muscle fiber). A total of 3544 muscle fibers were analyzed, specifically 1806 fibers in the Ob group and 1738 fibers in the ExOb group.

1.6.2. Total collagen content analysis

To assess the expression of intramuscular connective tissue, skeletal muscle sections were stained with Picrosirius Red according to the method of Sweat, Puchtler [259] by incubation in 0.1% Sirius red picric acid for 90 minutes after deparaffinization and rehydration. Them, sections were washed in 0.5% acetic acid, dehydrated in graded ethanol solutions and cleared in xylene. Slides were permanently mounted with DPX and glass coverslip. In this histological coloration technique, collagen stains in bright red while the remaining skeletal muscle tissue stains in yellow creating a clear distinction between connective and muscle tissue. The software Image-Pro Plus 6.0 (Media Cybernetics, Inc) was used to quantify the percentage of tissue area corresponding to either skeletal muscle (yellow) or connective tissue (red). A total of 381 photomicrographs were analyzed, with an average 19 images per subject.

1.7. Immunohistochemistry analysis

Immunohistochemistry was performed for the detection of capillaries in skeletal muscle samples, as described by others Padrão, Oliveira [260]. Endothelial cells present in the muscle tissue samples were targeted as a surrogate of

capillarization. Paraffin-embedded skeletal muscle slides were deparaffinized with xylene, rehydrated in graded ethanol solutions and washed in running tap water. Antigen retrieval was performed by incubating the slides for 10 minutes in boiling 10mM Sodium Citrate Buffer, pH 6.0, in a pressure cooker. After cooling, slides were washed in water and Tris Buffered Saline (TBS) solution for 5 min. Tissue samples were then outlined with a hydrophobic marker and incubated for 2h in TBS with 0.05% (v/v) Tween 20 (TBS-T) and 3% (w/v) bovine serum albumin (BSA) at room temperature in a humid chamber for blocking of nonspecific binding sites. Slides were then incubated in anti-CD31 antibody (ab281583; Abcam) diluted 1:1000 in TBS-T with 1% (w/v) BSA overnight at 4°C in a humid chamber. Each slide had 3 skeletal muscle samples, one of which was incubated with TBS-T and not with the primary antibody for serving as a negative control. After adequate washing with TBS-T, the tissue endogenous peroxidase activity was blocked by incubation in a freshly prepared 0.3% hydrogen peroxide (H_2O_2) in TBS solution for 30 min at room temperature.

Sections were then washed in TBS-T and incubated with goat anti-rabbit IgG horseradish peroxidase secondary antibody (ab97051; Abcam) diluted 1:200 in TBS-T with 1% BSA for 1h at room temperature in a humid chamber. Thereafter, sections were washed three times under gentle stirring with TBS-T for 5 min each and incubated with freshly prepared 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution (D4293; Sigma-Aldrich) for about 3 min. After washing, slides were then counterstained with a solution of hematoxylin diluted in water (1:3; v/v) for 3 min. Finally, slides were dehydrated in graded ethanol solutions, cleared in xylene and permanently mounted with DPX and glass coverslips and analyzed under a light microscope. Negative controls were performed as described, by the omission of the primary antibody incubation step.

1.8. Skeletal muscle homogenate preparation

Skeletal muscle sections were homogenized in ice-cold RIPA lysis buffer (cat#20-188, Millipore, Burlington, Massachusetts, EUA) supplemented with a cocktail of protease inhibitors (P2850, Sigma, St. Louis, MO, USA) for biochemical analysis. Homogenates were then centrifuged at 10,000g for 20 min at 4°C, supernatants

collected, aliquoted and kept at −80°C until further biochemical analysis. Protein concentration was determined by the Bio-Rad DC Protein assay (Bio-Rad, CA, USA) following the manufacturer instructions, using bovine serum albumin (BSA) as standard.

1.9. Protein carbonylation and lipid peroxidation levels assessment by Slot Blot

Protein carbonylation, an index of protein oxidation, was determined in the skeletal muscle samples using monoclonal mouse anti-DNP (MAB2223, Sigma, Darmstadt, Germany) as primary antibody. Samples containing 20 µg of protein were then processed as previously described by Barbosa, Capela [261]. Briefly, for the protein carbonyl derivates assay, a given volume (V) of sample containing 20 µg protein was derivatized with 2,4-dinitrophenilhydrazine (DNPH). The sample was mixed with 1 v of 12% sodium dodecyl sulfate, 2V of 2mM DNPH/10 trifluoroacetic acid, followed by 1 hour of incubation in the dark, after which 1,5V of 2M Tris-base/19,3% mercaptoethanol was added for neutralization. After diluting the derivatized proteins in TBS to obtain a final concentration of 0.001µg/µL, a 100µL volume was slot blotted into nitrocellulose membrane. Lipid peroxidation was measured by slot blot using monoclonal rabbit anti-Hydroxynonenal (4-HNE) (ab46545, abcam, Cambridge, UK). Briefly, skeletal muscle samples were diluted in TBS to obtain a final concentration of 0.001 µg/µL and a volume of 100µL was slot blotted into nitrocellulose membrane.

The Slot blot membranes were blocked with 5% (w/v) non-fat dry milk in TBS-T (100mM Tris, 1.5mM NaCl, pH 8.0 and 0.5% Tween 20) for 1h at RT and then incubated for 1h with the primary antibody (anti-DNP and anti-4-HNE) diluted 1:2000 in 5% (w/v) non-fat dry milk in TBS-T. The membranes were then washed three times (10 min each) with TBS-T and incubated again for 1h at RT with rabbit anti-mouse IgG-HRP (ab6728) or goat anti-rabbit IgG-HRP (ab97051) secondary antibodies from abcam (Cambridg, UK) diluted 1:5000 in TBS-T. Immunoreactive bands were detected using the Clarity™ Western ECL Substrate (cat#1705060, Bio-Rad Laboratories, California, USA). according to the supplier's instructions, and digital images were obtained using a Molecular Imager® ChemiDocTM XRS + System (Bio-Rad Laboratories, California, USA). The images acquired

were analyzed with Image Lab™ software version 6.0.1 (Bio-Rad, Hercules, CA, USA). The optical densities obtained were expressed in arbitrary units.

1.10. Western Blotting analysis

Western blotting analysis was performed as described by Reis-Mendes, Dores-Sousa [262] in skeletal muscle homogenates. A certain volume of skeletal muscle sample equivalent to 40µg of protein was diluted 1:2 in loading buffer (0.2M Tris-HCl, pH 6.8; 10% SDS (w/v), 20% glycerol (v/v); 10mM β-mercaptoethanol, 0.05% bromophenol blue (w/v)) and incubated at 100ºC for 5 minutes. Equivalent amounts of sample proteins of each experimental group were eletrophoresed on 12.5% SDS-PAGE as described by Laemmli [263]. After protein separation, gels were blotted onto a 0,45µm nitrocellulose membrane (GE10600002, Amersham Protran, GE Healthcare, Germany) in a transfer buffer (25mM Tris, 192mM glycine, pH 8.3 and 20% methanol) for 2h (200mA). Then, nonspecific binding was blocked with EveryBlot Blocking Buffer (cat#12010020, Bio-Rad, Hercules, CA, USA). Membranes were incubated with mouse monoclonal anti-PAX7 antibody (ab218472; Abcam) diluted to 1:1000 in EveryBlot blocking buffer (cat#12010020, Bio-Rad, Hercules, CA, USA) at 4°C overnight. Then, membranes were washed in TBS-T and incubated with rabbit anti-mouse IgG-horseradish peroxidase (HRP) (ab6728, Cambridge, UK) diluted 1:5000 in EveryBlot blocking buffer (cat#12010020, Bio-Rad, Hercules, CA, USA). Immunoreactive bands were detected by using the Clarity™ Western ECL Substrate (cat#1705060, Bio-Rad Laboratories, California, USA). according to the manufacturer's instructions. The immunoreactive bands were automatically detected using the Molecular Imager® ChemiDocTM XRS + System (Bio-Rad Laboratories, California, USA). The images obtained were analyzed using the Image Lab software version 6.0.1 (Bio-Rad, Hercules, CA, USA). The optical densities obtained were expressed in arbitrary units. Protein loading was performed with Ponceau S staining [264].

1.11. Statistical analysis

The R statistical software (version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria) was used to perform the statistical analyses. Data distribution normality was analyzed using the Shapiro-Wilk test. Patients' characteristic comparisons between groups were performed with the Welch t-test for continuous variables and with Chi-square test for categorical variables. Between-group differences in muscle cross-sectional area, total collagen content, central myonuclei per total skeletal muscle area, central nuclei per number of muscle fibers, peripherally located myonuclei per total skeletal muscle area and peripherally located myonuclei per number of muscle fibers, nº $CD31^+$ cells/μm^2 skeletal muscle and nº $CD31^+$ cells/nº skeletal muscle cells were tested trough linear mixed models, with group used as fixed effect and subjects as random effect. The between-group differences in 4-HNE expression levels, protein carbonylation and PAX7 expression were tested through ordinary linear regression. QQ plots were used to check for the normality in the distribution of the residuals in each model. Due to non-normally distributed residuals, log transformations were applied to the following variables: muscle fiber cross-sectional area, central myonuclei per total skeletal muscle area, central nuclei per number of muscle fibers, peripherally located myonuclei per total skeletal muscle area and peripherally located myonuclei per number of muscle fibers, nº $CD31^+$ cells/um^2 skeletal muscle and nº $CD31^+$ cells/nº skeletal muscle cells and 4-HNE expression levels. For all these variables, after log transformation the residuals showed a normal distribution. Estimated mean differences with 95% confidence interval, on the original scale of each variable, were reported. A statistically significant value was set as $\alpha = 0.05$.

2. Results

2.1. Sample characteristics

Demographic, anthropometric and clinical characteristics of the Ob and ExOb groups are presented on **Table 1**. Patients from the ExOb group had a significantly higher weight, BMI, waist and hip circumference compared to the Ob group. Despite these differences, there were no statistically significant differences between groups for age, sex and smoking status nor for the presence of co-morbidities such as type 2 diabetes, arterial hypertension or dyslipidemia, showing a well-matched group allocation for these possible confounding variables.

Table 1. Demographic, anthropometric and clinical characteristics of patients allocated to the Ob and ExOb groups.

Variables	Group		*p* value*
	Ob (*n*=10)	**ExOb** (*n*=10)	
Age (years)	48.0±7.4	46.4±5.6	.595
Sex (female, %)	60%	60%	1.000
Height (m)	1.6±0.1	1.7±0.1	.582
Weight (kg)	95.5±10.0	154.7±15.9	<.001
BMI ($kg \cdot m^{-2}$)	35.7±1.18	56.3±2.2	<.001
Waist circumference (cm)	113.6±9.6	144.3±12.0	.006
Hip circumference (cm)	114.6±7.1	152.5±5.9	<.001
Type 2 diabetes (%)	33%	38%	.858
AHT (%)	33%	14%	.313
Dyslipidemia (%)	11%	43%	.116
Current smoker (%)	22%	0%	.131
Ex-smoker (%)	11%	29%	.453

Data: mean±standard deviation.

* *p*-value obtained by independent welch-test or chi-square test according to data type (continuous *vs* categorical).

Abbreviations: BMI = Body mass index; AHT = Arterial hypertension; Ob = Obesity; ExOb = Extreme obesity.

2.2. Histological analysis

2.2.1. Muscle cross-sectional area

The skeletal muscle fibers cross sectional area was, on average, significantly lower in patients from the ExOb group compared to patients from the Ob group (–866.9% [95% CI –1380.1 to –353.7]; $p < .001$) (**Figure 1**).

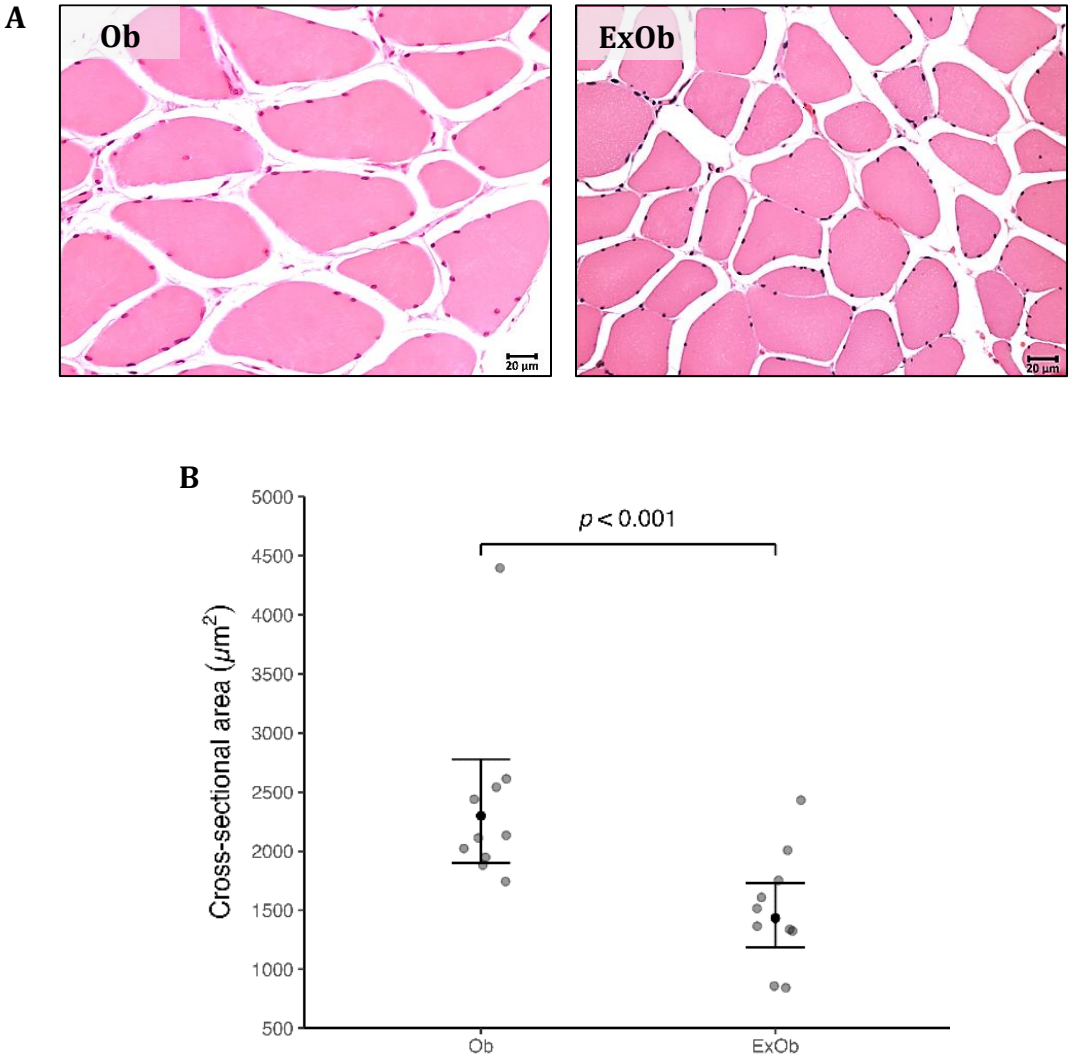


Figure 1. Representative light micrographs of abdominal wall muscles from subjects of the obesity group (Ob) and extreme obesity group (ExOb), stained with H&E, are depicted in panel **A**. Images were taken at 40× amplification. Scale bar = 20 µm. The graph with mean and respective 95% confidence interval of muscle cross-sectional area, for Ob and ExOb groups, is shown in panel **B**.

The percentage of skeletal muscle fibers according to their CSA, in each group, is also represented in **Figure 2**. The Ob group (panel **B**) showed a tendency for having skeletal muscle fibers with a higher CSA (>2500 μm^2), whereas ExOb group (panel **C**) tended to have more fibers with smaller CSA (<1500 μm^2).

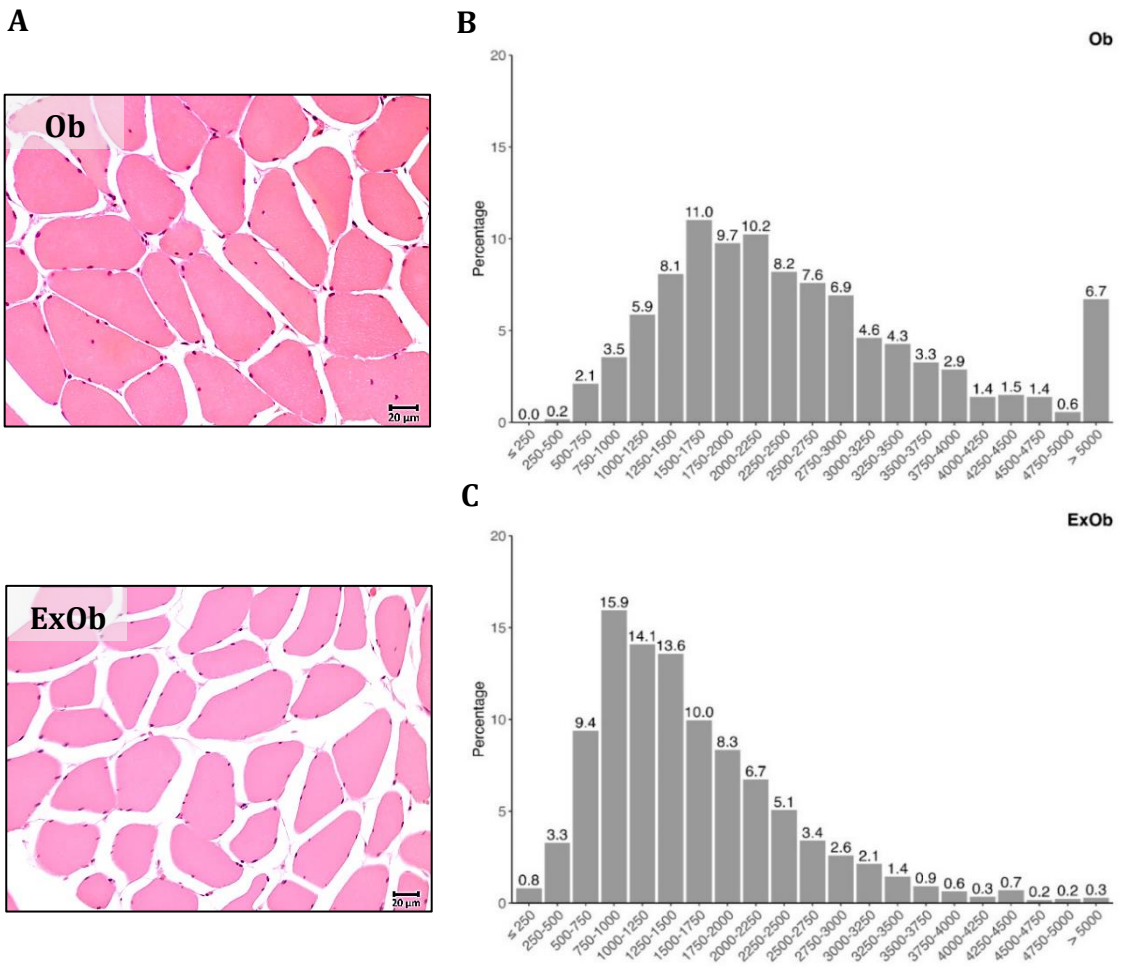


Figure 2. Representative light micrographs of abdominal wall muscles from subjects of the obesity group (Ob) and extreme obesity group (ExOb), stained with H&E, are depicted in panel **A**. Images were taken at 40× amplification. Scale bar = 20 µm. The percentage of muscle fibers according to their cross-sectional area (μm^2), for subjects in the obesity group (Ob) and extreme obesity group (ExOb), are depicted in panels **B** and **C**, respectively.

2.2.2. Muscle fibers myonuclei position

There were no significant differences on the muscle myonuclei position parameters analyzed, both for centrally and peripherally located myonuclei (**Figures 3 and 4**). Namely, there were no significant differences between groups regarding the number of myonuclei in central position per total skeletal muscle area (–0.00002 [95% CI –0.00040 to 0.00001]; *p* = .234), or per the number of skeletal muscle fibers (–0.06 [95% CI –0.12 to 0.01]; *p* = .086), as well as there were no differences regarding the number of peripherally located myonuclei per total skeletal muscle area (0.00032 [95% CI –0.00006 to 0.00069]; *p* = .116) or per number of skeletal muscle fibers (–0.68 [95% CI –1.36 to 0.00]; *p* = .067).

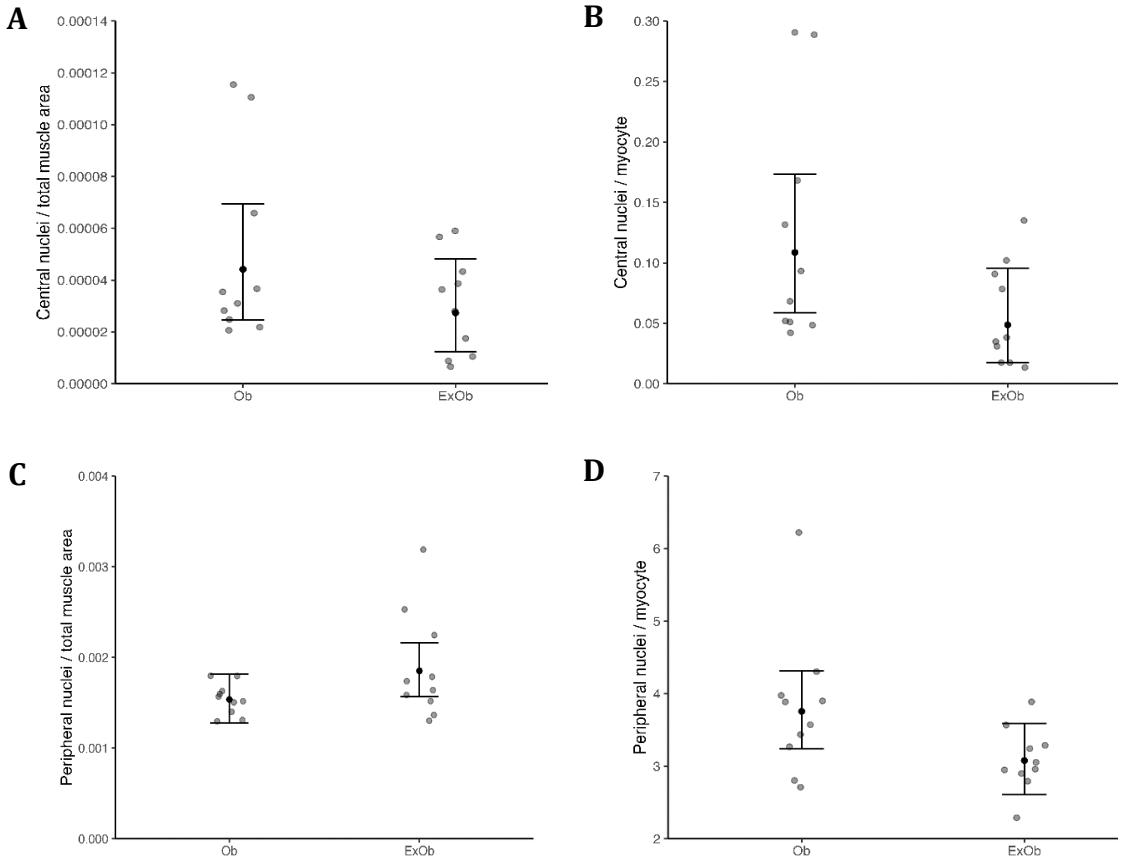


Figure 3. The graphs with mean and respective 95% confidence interval of central myonuclei per total skeletal muscle area (panel **A**) and per the number of muscle fibers (panel **B**), as well as peripherally myonuclei per total skeletal muscle area (panel **C**) and per number of muscle fibers (panel **D**), for Ob and ExOb groups.

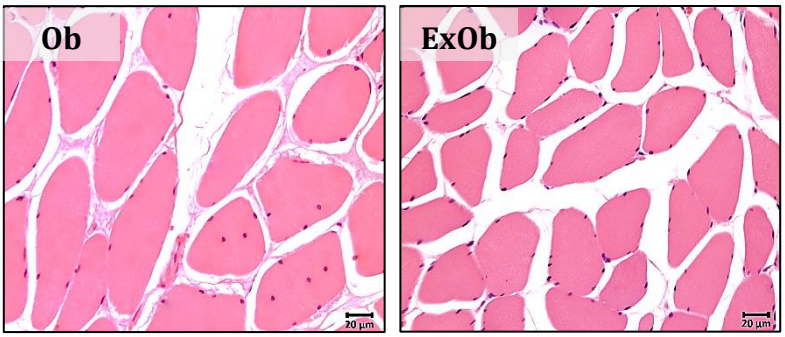


Figure 4. Representative light micrographs of abdominal wall muscle from subjects of the obesity group (Ob) and extreme obesity group (ExOb), stained with H&E. Images were taken at 40× amplification. Scale bar = 20 µm.

2.2.3. Connective tissue content

There were no significant differences between groups regarding total collagen content expressed per total skeletal muscle area (**Figure 5**). Despite that, there was a non-significant trend for the ExOb group to display a higher total collagen content compared to the Ob group (1.7 [95% CI –0.2 to 3.6]; *p* = .092).

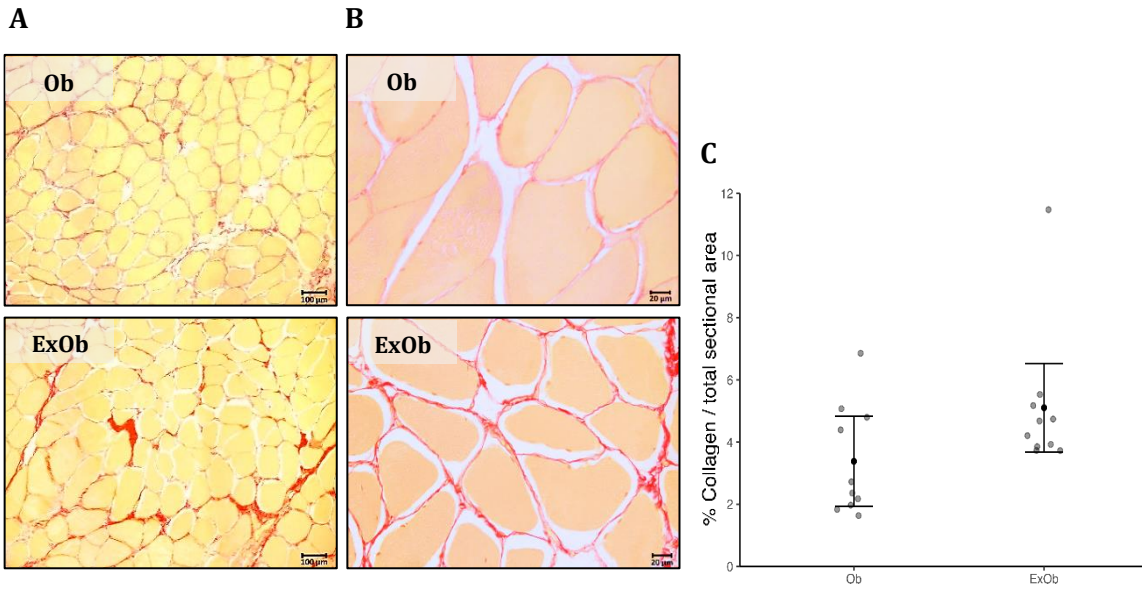


Figure 5. Representative light micrographs of abdominal wall muscle from subjects of the obesity group (Ob) and extreme obesity group (ExOb), stained with Picrosirius Red, are depicted in panels **A** and **B**. The images illustrated in panel **A** were taken at 40× magnification. Scale bar = 20 µm. The images illustrated in panel **B** were taken at 10× magnification. Scale bar = 100 µm. The graph with mean and respective 95% confidence interval of total collagen content expressed per total skeletal muscle area, is shown in panel **C**.

2.3. Immunohistochemistry analysis

2.3.1. CD31-positive cells

A statistically significant difference on the expression of $CD31^+$ cells, a marker for endothelial cells and therefore for tissue capillarity was identified between groups (**Figure 6**). Compared to the Ob group, individuals from the ExOb group presented a significant lower expression of $CD31^+$ cells, both expressed per total muscle area (–0.00013 [95% CI –0.00020 to –0.00005]; p = .004) as well as while expressed by skeletal muscle fiber number (–0.322 [95% CI –0.570 to –0.075]; p = .020).

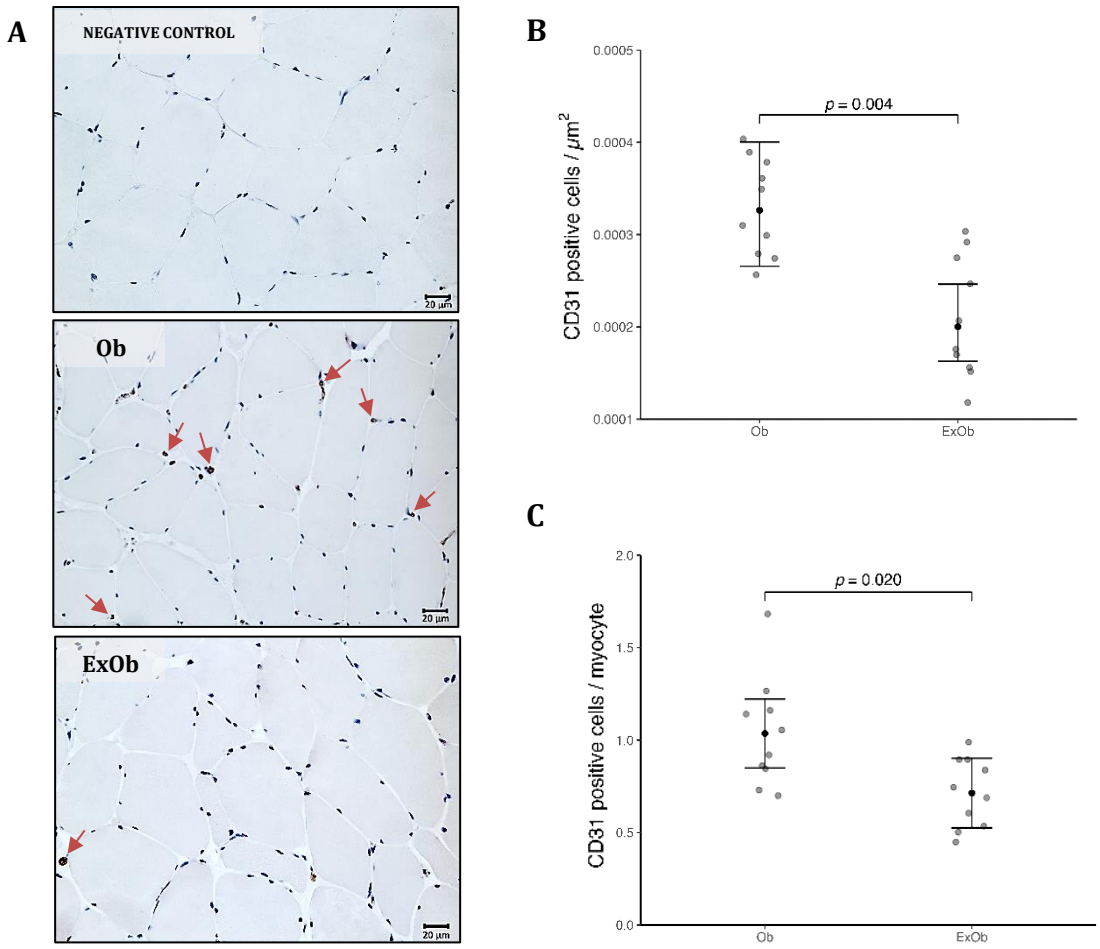
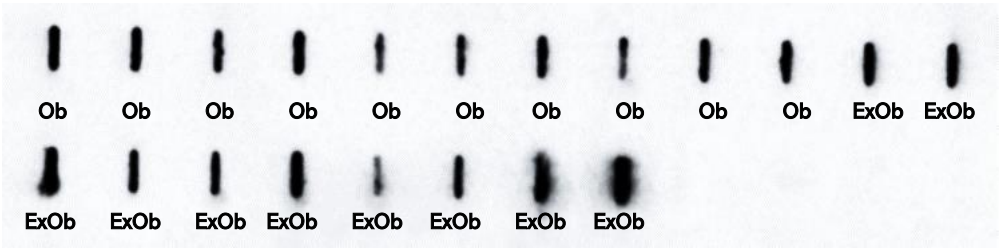


Figure 6. Representative photomicrographs of abdominal skeletal muscle samples stained by immunohistochemistry for identification of CD31-positive cells, from subjects of the obesity group (Ob) and extreme obesity group (ExOb), are depicted in panel **A**. Positive stains are indicated by the presence of a brown stain and highlighted by arrows. Scale bar = 20 µm. Images were taken at 40× amplification. The graphs with mean and respective 95% confidence interval of the number of cells staining positive ($CD31^+$) for the Ob and ExOb groups, indicative of tissue endothelial cells expressed per total muscle area (nº $CD31^+$ cells/µm² skeletal muscle; panel **B**) and per number of fibers (nº $CD31^+$ cells/nº skeletal muscle cells; panel **C**).

2.4. Slot blot analysis

2.4.1. Protein carbonylation

There were no statistically significant differences regarding protein carbonylation levels, an index of protein oxidation, between groups (–554.4 [95% CI –1544.7 to 435.9]; *p* = .287) (**Figure 7**).



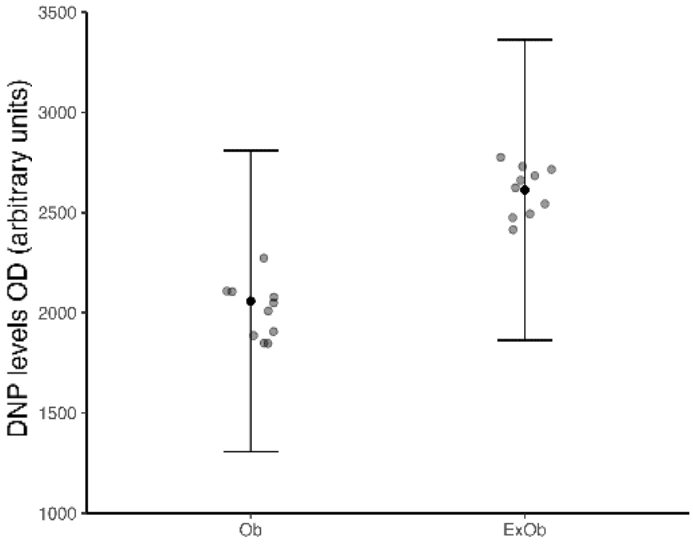


Figure 7. Slot blot analysis of abdominal wall skeletal muscle tissue homogenates, from subjects of the obesity group (Ob) and extreme obesity group (ExOb), for expression of protein carbonylation.

2.4.2. 4-HNE expression

There were no statistically significant differences on the 4-HNE expression, an index of lipid peroxidation between groups (315.1 [95% CI –548.2 to 1178.4]; *p* = .484) (**Figure 8**).

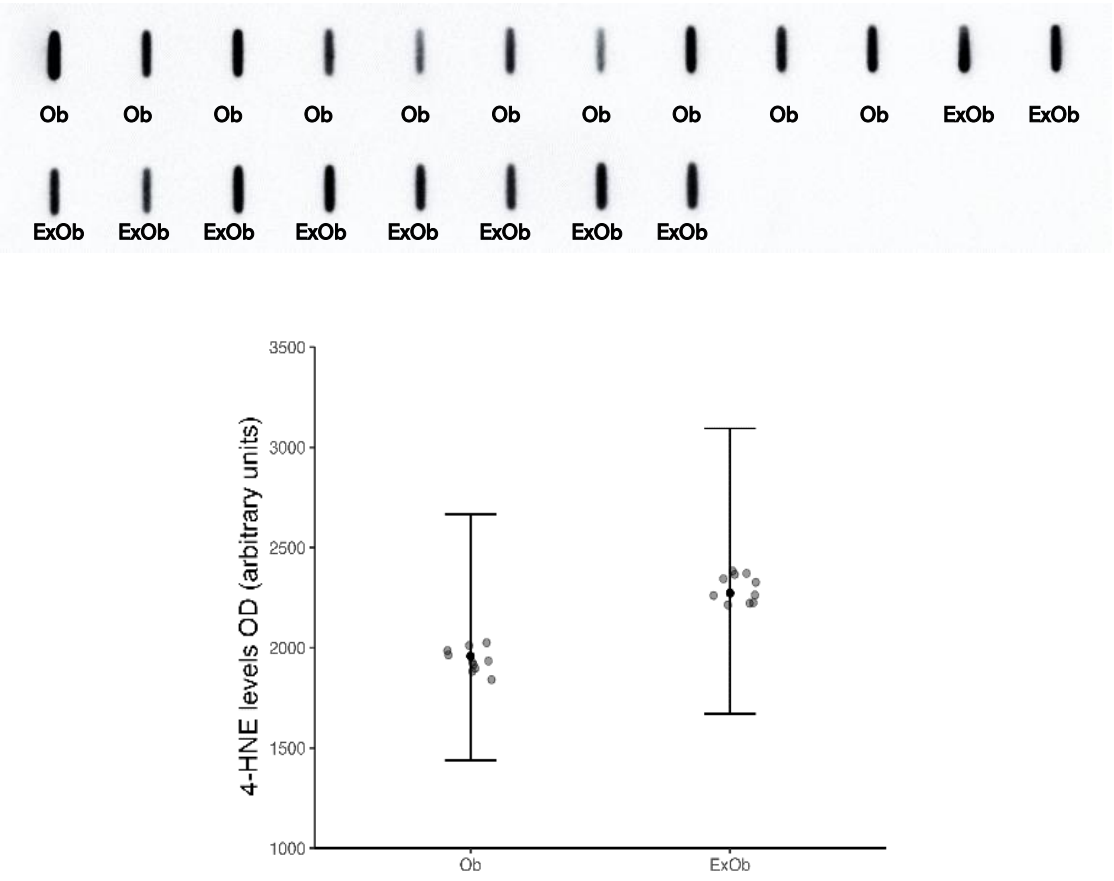


Figure 8. Slot blot analysis of abdominal wall skeletal muscle tissue homogenates, from subjects of the obesity group (Ob) and extreme obesity group (ExOb), for expression of 4-HNE.

2.5. Western blotting analysis

2.5.1. Skeletal muscle satellite cells

The comparison of skeletal muscle satellite cells expression was performed by quantification of PAX7 on both groups muscle samples by Western Blot. Results obtained showed that there was no statistically significant difference on PAX7 expression between groups (315.1 [95% CI –548.2 to 1178.4]; p = .484) (**Figure 9**).

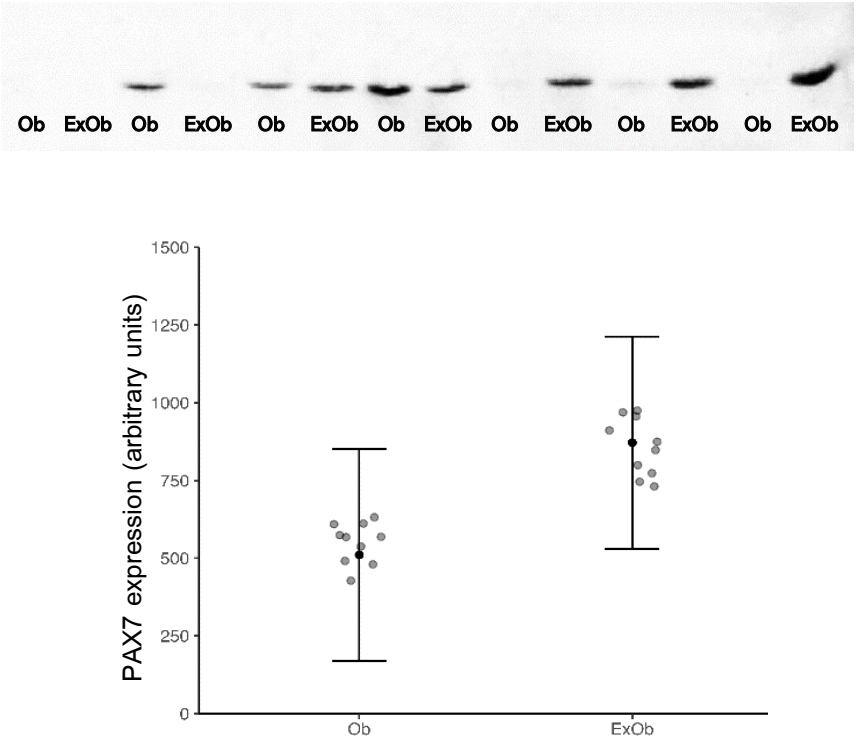


Figure 9. Western blot analysis of abdominal wall skeletal muscle tissue homogenates, from subjects of the obesity group (Ob) and extreme obesity group (ExOb), for PAX7 expression, a marker of tissue satellite cells expression.

3. Discussion

The aim of this study was to test the hypothesis that abdominal wall skeletal muscle quality was negatively affected in patients with severe obesity. To investigate this hypothesis, we collected a convenience sample from an available biobank of skeletal muscle samples and divided them into two groups: i) patients with extreme cases of severe obesity (ExOb) and ii) patients with milder forms of obesity (Ob). Skeletal muscle samples were analyzed by biochemical and histological methods to identify potential features that could mechanistically explain the higher risk of post incisional hernia typically identified in patients with obesity that undergo bariatric surgery. Compared to the Ob group, it was observed that patients from the ExOb group presented a significantly lower skeletal muscle fiber cross-sectional area, as well as a lower expression of capillary vessels, both expressed relative to total skeletal muscle area and expressed per number of skeletal muscle fibers. Regarding all the other variables assayed, namely myonuclei position (central *vs* peripheral), skeletal muscle connective tissue content, oxidative damage surrogates and satellite cells (SC) expression, no significant differences were observed between groups.

Skeletal muscle is a metabolically active tissue that requires adequate vascularization to properly respond to the need for metabolite removal and oxygen and nutrient delivery [34]. Moreover, capillarization is a key factor for adequate revascularization at the damaged site during the muscle regeneration process [265, 266]. Some experiments on animal [31, 267] and humans [268, 269] have shown that skeletal muscle capillarization is hampered by obesity, entailing implications, as example, altered diffusion capacity. Our results seem to be in line with those findings since patients with extreme cases of obesity were shown to have a lower capillary density in abdominal wall skeletal muscle compared to patients with milder cases of obesity. One of the mechanisms that might be related to the positive effect of capillarization on muscle regeneration is its association with the activity and function of stem cells, namely SC [270]. Nevertheless, despite the lower capillary density in patients with extreme obesity, our findings showed that there were no significant differences between groups

regarding SC expression.

Collagen metabolism is another important feature for skeletal muscle quality that seems to be negatively affected in patients with obesity [271]. There is compelling evidence [27, 28] supporting the notion that defective collagen metabolism could contribute to the higher propensity of patients with severe forms of obesity submitted to BS to develop post-incisional hernias, since defective collagen metabolism may decrease the tensile strength and mechanical stability of both connective and scar tissue. It is well-stablishid that the higher content of the weaker type III collagen relative to the stronger type I collagen can lead to a decreased fibril diameter and results in the synthesis of mechanically weak tissue [29]. Several experiments have demonstrated a decreased type I to type III collagen ratio, in the hernia sac and skin of patients with post-BS hernia [272-274]. For instance, Casanova, Trindade [275] has described a 6.4% reduction in type collagen III, while type I was shown to be increased by 23.7% in the *transversalis fascia* of patients with inguinal hernia compared to control patients. Recently, Szczesny, Kuligowska-Prusinska [28] has assessed the messenger RNA levels for collagen type I and III genes in the *rectus abdominalis* muscle sheath of patients with obesity and nonobese subjects. Although no significant differences were reported, an approximately twofold reduction in the relative expression level for both type I and III collagen were detected between the two patient groups [28]. These findings, which concur with most of the previous studies [25, 27, 192, 272-275], seems to be in line with our experiment demonstrating that patients with extreme cases of severe obesity have a trend to display elevated levels of total collagen per muscle section, comparatively with milder forms of obesity.

Protein carbonylation is a common type of ROS-induced protein modification [276]. Contrarily to what was initially expected, no differences in the expression of carbonylated proteins were found between both groups of skeletal muscle samples. Our initial expectation was that substantially higher levels of carbonylated proteins would be identified in patients with extreme cases of obesity because it was previously described that severe obesity is associated with increased ROS formation [33-35]. The expression of 4-HNE, another marker

of oxidative damage, namely of lipid peroxidation [277], was also similar between groups, which is in accordance with protein carbonylation results.

It has long been established that obesity is associated with enhanced absolute levels of skeletal muscle mass [278]. In the last decades, several experiments have reported that absolute levels of fat free mass [279] and muscle fibers cross sectional area (CSA) [280, 281] increases with total body mass [282]. It seems that skeletal muscle mass [278] increases with obesity due to an overload effect of the excess adipose tissue. However, it is unknown if the same pattern also occurs in severe cases of obesity. Our findings showed that patients with extreme obesity tended to have lower abdominal wall skeletal muscle fibers CSA, compared to patients with milder cases of obesity. This may seem to suggest that increases in fat mass in patients with extreme cases of obesity are detrimental for skeletal muscle mass and that above a certain obesity severity degree, the detrimental metabolic effects of obesity exceed the potentially beneficial overload effect of the additional body weight leading thereby to a decrease in skeletal muscle fibers cross sectional area.

Myonuclei are crucial for skeletal muscle homeostasis, maintenance and adaptive responses, and its assessment might help to better understand the muscle fiber responses to obesity [283]. According to the theory of myonuclear domain, each skeletal muscle nucleus is responsible for controlling an area of surrounding cytoplasm [284] and to generate adequate proteins to maintain its limited cytoplasmic area and structural proteins within that local “domain” [285]. There is increasing evidence demonstrating that myonuclei number increases as the fiber enhances in size, when muscle tissue is submitted to situations that induces increases in fiber size (i.e., muscle growth) [286-288]. Regardless that the myonuclear domain theory is well validated for the incorporation of myonuclei during muscle hypertrophy, the effect of declines on fiber size in the myonuclear number is not fully established. Some experiments have described diminished myonuclear numbers in skeletal muscle tissues experiencing a range of atrophy-inducing experimental conditions [286, 289, 290]. However, regarding the effect of obesity, there is no robust evidence assessing the myonuclear response to muscle alterations in patients with obesity. Our findings have demonstrated that

patients with extremely severe obesity, although not reaching statistical significance, tended to have a lower number of myonuclei in both in the central and peripheral position, per total number of skeletal muscle fibers, compared to less severe cases of obesity. Based on the myonuclear domain theory that hypothesizes that each myonuclei can control just a certain domain for protein synthesis and general maintenance, we hypothesized that the tendency for the decreased myonuclei number per muscle fiber area could be an indicator of a dysregulation in myonuclear domain control in muscle tissue in severe cases of obesity. Nevertheless, this hypothesis needs to be consolidated on solid observational data, to better understand the potential mechanisms controlling the lower nuclei number in patients with obesity.

The findings of the present study, although requiring further investigation to allow a more robust interpretation, seem to suggest that extreme cases of obesity might hamper some of the determinants of skeletal muscle quality and regeneration capacity, which, potentially, may contribute to the higher incisional hernia incidence typically observed in post-BS patients. Currently, it is unknown which prophylactic measures could be applied to minimize the occurrence of this prevalent post-BS complication. Considering that physical exercise successfully improves several skeletal muscle regeneration indicators [238, 239, 247, 249, 256, 291] it seems appealing to speculate that the involvement of BS patients in an exercise training program pre-surgery, focusing on strengthening the abdominal wall musculature, could attenuate the risk of post-incisional hernia.

Unfortunately, our study presents some limitations. The small sample size may have hampered the study power; therefore, precluding the detection of group differences for several of the outcomes for which a non-significant trend was observed. Also, future studies should investigate skeletal muscle quality indicators in volunteers with normal weight as a control group in order to be able to better understand the effect of obesity on skeletal muscle wall quality determinants and regeneration capacity.

CHAPTER IV

CONCLUSIONS

Conclusions

The main findings of our study suggest that patients with extreme cases of obesity tend to have a lower skeletal muscle fiber cross sectional area as well as a lower capillary density, which are indicators of lower skeletal muscle quality and could contribute to a lower regenerative capacity following injury, namely bariatric surgery.

Nevertheless, no differences were observed between groups regarding skeletal muscle nuclei position, connective tissue content, protein and lipid oxidative damage and satellite cells expression.

Further studies should consider including normal weight subjects as controls as well as investigating other potential variables that could mechanistically be involved in skeletal muscle regeneration capacity and that could be negatively affected by severe obesity.

CHAPTER V

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