Theoretical foundations and state of the art

Obesity

Obesity is a global health problem, which its prevalence has steadily increase in the last decades. As consequences of obesity several impairments have been described involving the cardiovascular, metabolic, cardiac, respiratory and cognitive systems, making it a key public health challenge in the present and near future. Locally, Chile is the second OECD country with the highest obesity prevalence with 34.4% of its population (1), putting obesity as one of the major concerns for Chilean public health.
The classical description of the etiology of obesity resides in the combination of an increase in energy intake combined with low levels of physical activity/energy expenditure. This process derives in an increase in white adipose tissue depots, ultimately increasing body weight. Therefore, lifestyle modifications such as changes in diet and increases in physical activity levels (which can be in the form of prescribed physical exercise) are highly recommended in the management of obesity. However, and considering the multifactorial nature of obesity, these interventions on its own are not enough for successful body weight loss and restorage of metabolic health (2).

Tissue-specific impairments related to obesity

Adipose tissue is known to play an important role as an endocrine organ, where adiponectin is described as one of the most abundant adipokine in the circulation (3). Adiponectin also has autocrine/paracrine actions in adipose tissue, where its high-molecular-weight (HMW) isoform has been described as the most bioactive complex (4). During obesity, lower levels of HMW adiponectin along with decreased activity/quantity of its most abundant receptor, adiponectin-receptor 1 (ADIPOR1), are claimed to partially mediate the development of insulin resistance by several mechanisms (5). For instance, considering that adiponectin can activate 5' adenosine monophosphate-activated protein kinase (AMPK) by increasing the pAMPK/AMPK ratio, which promotes glucose transporter type 4 (GLUT4) vesicle formation and translocation to the adipocyte's membrane, an anti-hyperglycemic feature has been attributed to this hormone (6), feature that during obesity is decreased (5). In terms of mitochondrial function, adipocytes produce >95% of its ATP through oxidative phosphorylation (7), energy that is mainly used for triglyceride synthesis and adipokine release. Impairments on this aspect of adipocyte physiology have been described in subjects with obesity (8). In the context of obesity, these mitochondrial changes are particularly relevant because one purposed mechanism for futile energy consumption is mitochondrial uncoupling, process where the proton motion dissipation that is normally used to produce ATP in the mitochondria is release in the form of heat. Proteins associated with uncoupling are Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and uncoupling protein 1 (UCP1, mainly present in brown adipocytes) (9). However, under certain circumstances, white adipose tissue can exhibit brown areas (i.e. browning) where the presence of these mitochondrial proteins is particularly high. However, in animal models of obesity, it has been described that the content of these proteins is decreased (10, 11), suggesting that obesity disrupts this feature, further predisposing this tissue to continue its expansion rather than its dissipation.
Another common consequence of obesity, and particularly ectopic fat deposition, is the development of non-alcoholic fatty liver disease (NAFLD). However, if this excessive fat accumulation is chronic, liver damage and dysfunction can be developed through several mechanisms, including inflammation and fibrosis, characterized by increases in growth factors such as transforming growth factor β (TGFβ) and collagen (12, 13). Moreover, impairments in liver glucose metabolism can be observed during obesity, given that decreases in pAMPK/AMPK ratios along with lower levels of mitochondrial proteins (e.g.: peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)) have been described in mice and humans (14, 15), changes that overall impair glucose metabolism and promotes hyperglycaemic states.
Skeletal muscles are described as the main glucose disposal organs in the human body. Therefore, impairments in its biology is highly linked with the development of insulin resistance. Recently, it has been described that skeletal muscle produces adiponectin, which acts in an autocrine/paracrine manner (16). However, it has been proposed that, during obesity, this response becomes dysfunctional given that quadriceps from high-fat-fed mice have shown lower levels of HMW adiponectin and ADIPOR1 (16). These impairments have been associated with decreases in AMPK activation pathways which in turn reduces GLUT4 vesicles formation and translocation impairing glucose uptake and metabolism (17). On the other hand, since adiponectin has shown training-related effects in skeletal muscle given by increases in mitochondrial proteins (e.g., PGC-1α), which are associated with increases in exercise capacity (18), obesity-related decreases of these mediators have been associated, in part, with skeletal muscle adiponectin disturbances (16).

To summarize the interaction of the metabolic impairments derived from obesity, figure 1 illustrates the described effects of obesity on insulin-sensitive tissues and plasma proteome (e.g., inflammatory mediators and hepato-adipo-myokines).

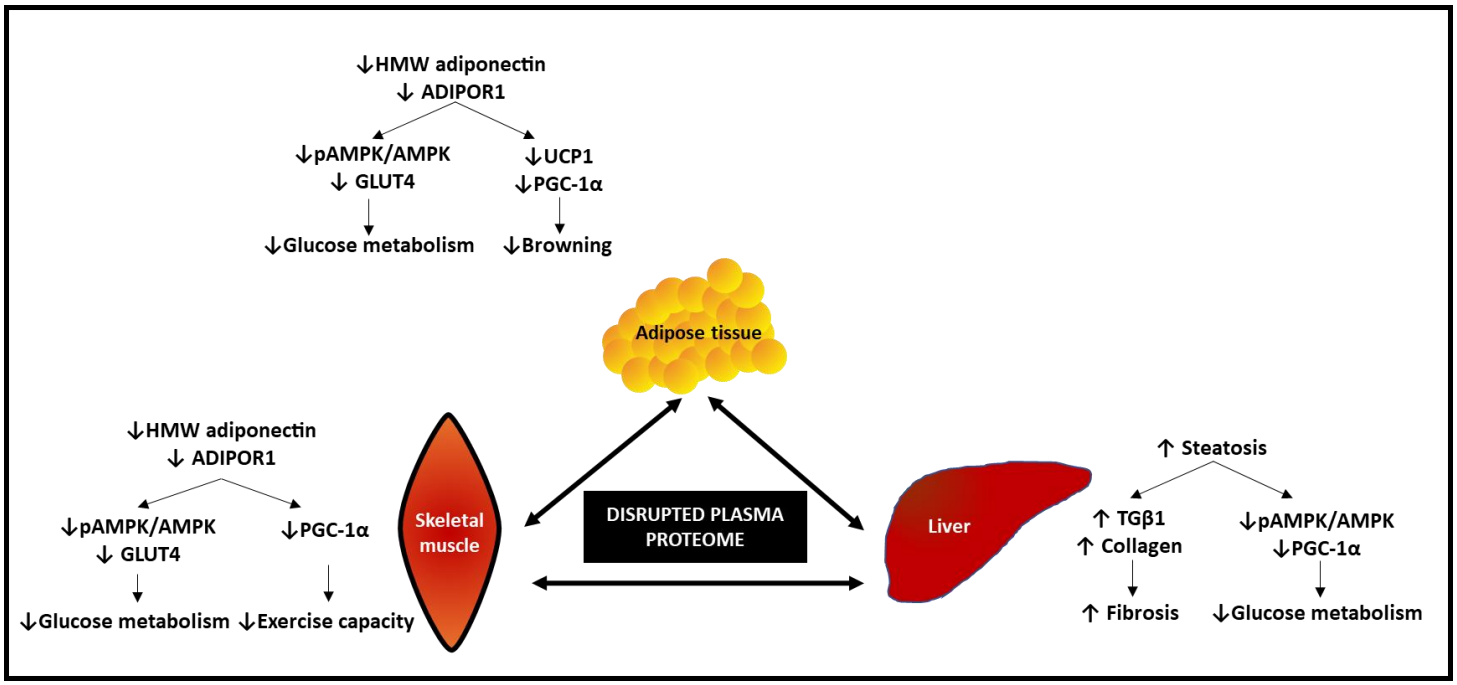

Figure 1. Summary of obesity-related metabolic impairments in insulin-sensitive tissues. In the context of obesity, insulin-sensitive tissues such as white adipose tissue, skeletal muscle, and liver undergo metabolic impairments. Decreases in the phosphorylation levels of AMPK, and in the glucose transporter GLUT4 negatively impacts glucose metabolism. Tissue-specific changes are also seen, where lower levels of brite adipocytes (browning), impaired exercise capacity in skeletal muscles, and higher levels of fibrosis in the liver have been described.

Bariatric surgery

Because of the previously described metabolic impairments associated with obesity, bariatric surgeries have gain popularity among health professionals in the management of severe obesity (body mass index (BMI) ≥ 35kg/m^2. This surgery aims to reduce the stomach space, so with the consequent decrease in food intake, rapid weight loss is achieved. In Chile, bariatric surgeries are an increasingly selected therapeutic alternative, considering that in 2011 nearly 3000 patients underwent this surgery (19). Its low mortality levels (0.32%) (20) makes it a very safe procedure with postoperative complications in the range of 0 to 10.6% (20). However, considering the abrupt change in energy intake, pre- and post-operative interventions are included in the candidate to undergo bariatric surgery management, where supervised physical exercise is one of the most frequent.
Interestingly, most of the literature on the matter is focused on exercise prescription during the post-operative period where a recently published systematic review and meta-analysis gathered the information of 20 studies on the issue. The main findings were that exercise during the post-operative period was successful in optimizing body weight loss, fat mass loss, and improving physical fitness (21). As a response to these findings, a consensus document written by the Bariatric and Metabolic Surgery Chilean Society gave directions in terms of how exercise should be prescribed during the pre- and postoperative period (22). However, the recommendations for the pre-operative period are unspecific where aerobic exercise was suggested. This is without question a reflection of the lack of studies exploring the potential benefits that pre-operative interventions, particularly in the form of programmed exercise could confer (23). An intriguing situation considering the well-known metabolic benefits of exercise in people with obesity and diabetes (24).

Exercise and obesity

In terms of physical exercise, it has different characteristics that are known to exert differential physiological adaptations. For instance, exercise can be prescribed with a particular modality, frequency, duration, and intensity, where the relevance of the latter has been thoroughly described (25), given that vigorous exercises (where a limited number of repetitions can be performed) activates anaerobic pathways to extract energy

by skeletal muscles, which are mainly dependent of glycolysis; whereas exercise at which the intensity allows the person to sustain the effort for several minutes activates pathways dependent of nutrient oxidation in the form of carbohydrates and lipids (26). Considering the previous, aerobic exercise is frequently described in the context of obesity, specifically for its positive effects on glucose transport and insulin sensitivity in normal and pathological conditions (i.e. type 2 diabetes). This is how Kieran et al. described decreases in fasting insulin levels and higher levels of insulin sensitivity and glucose disposal rates with only 7 days of running/cycling exercise in adults with obesity and type 2 diabetes (27).

Exercise and bariatric surgery

Considering the clear benefits of performing exercise in an obesity context, a barrier that the clinician has to face during the management of candidates to undergo bariatric surgery are the very low levels of spontaneous physical activity that they describe, which are reported as low as that only a 10% of them performed the recommended levels of physical activity (28), the factor that predisposed them to present lower cardiorespiratory fitness levels, which in turn is associated with longer operating room times and Intensive Care Unit days (29). On top of this, the recommended physical activity levels for these patients are higher than the ones recommended for the global population (150 min/week) (25), where for candidates to undergo bariatric surgery 200 min/week were required to observe higher levels of weight loss post-surgery (30). These findings take higher relevancy considering that medically supervised weight loss programs (without exercise) during the preoperative period have shown little to no effect on this outcome (31). Because of these barriers, recent efforts have been focused on finding new exercise modalities to unsure adherence during the pre-operative period.

As a response to the classical moderate-intensity continuous exercise (MICT), high-intensity interval training (HIIT) has been proposed and developed more intensely during the last two decades (32). The reported advantages of HIIT are to be more enjoyable and time-efficient than more classical exercise prescriptions, such as MICT (33). Moreover, some authors have suggested that HIIT confer higher physiological adaptations than MICT (32), even under an obesity context; however, recent studies have questioned this statement. Is the case of the study of Jung et al. where after two weeks of MICT and HIIT, adults with prediabetes showed similar improvements in cardiorespiratory fitness and systolic blood pressure (32). To complement these findings recent studies and systematic reviews/meta-analysis have found comparable results in people with obesity in terms of aerobic capacity, body weight loss, total fat mass and cholesterol (34, 35). Moreover, Keating et al. reported that in terms of fat distribution, MICT exhibits better results reducing the android fat percentage in overweight adults compared to HIIT (36), highlighting that depending on the outcome studies, exercise intensity might have a specific effect.

No studies comparing MICT vs. HIIT in candidates to undergo bariatric surgery are known. However, studies describing combined exercise training (aerobic + anaerobic) (37-40) or only strengthening exercises (41) are available. The aerobic component was always of moderate-intensity and, as expected, physiological outcomes such as aerobic capacity, and body fat mass improved after exercise training.

Even when the previously reported results are promising, their findings are sustained mainly by clinical/physiological measurements, where systemic (e.g., fasting glucose, insulin, oral tolerance glucose test, lipid profile), and specific metabolic outcomes (e.g., tissue markers of insulin action and glucose transport) are hugely lacking. This is particularly concerning knowing that obesity-related insulin resistance is far to be a stereotypical process, where variation in its presentation is described and seems to be independent of body weight and fat mass among individuals with obesity (42). These results might find an explanation in that obesity-associated insulin resistance is a process that depends on the function of several organs that are known to be insulin-sensitive (e.g. skeletal muscle, white adipose tissue, and liver). This point is particularly relevant during bariatric surgery considering that some patients exhibit certain resistance to this procedure, a phenomenon that is defined as the lack of metabolic health improvements after the surgery (43), where the metabolic/endocrine function of insulin-sensitive tissues is proposed as main etiology of this phenomenon. Therefore, interventions that aim to restore insulin sensitivity and metabolic function during obesity, and particularly during the preoperative period of candidates to undergo bariatric surgery must consider these factors.

Studies that have explored these exercise regimes and tissue-specific metabolic effects of exercise during obesity are scarce and have been mainly done in animal models. However, these studies have reported after comparing MICT vs HIIT seems to be tissue dependent. For instance, 10 weeks of HIIT showed improvements in body insulin sensitivity, increases in the glucose transport protein GLUT4 in the gastrocnemius muscle of db/db mice, changes not seen after MICT (44). In complement, even when similar liver insulin-sensitizing effects from MICT and HIIT have been described in diet-induced obese rats, the latter reduced inflammatory mediators such as NF-κB (45), and in white adipose tissue improvement in insulin signaling have been

described in high-fat-fed mice (11). Considering these results, HIIT seems to be metabolically preferable over MICT during obesity in terms of metabolic benefits; however, limitations in terms of exercise prescription in the previous studies hinders the possibility of making fair comparisons between HIIT and MICT, given that differences in exercise intensities are usually normalized by exercise volume (11) instead of equalizing intensities between programs. This is relevant because when HIIT and MICT are comparable in terms of energy expenditure the metabolic effects on humans with overweight or obesity trends to be comparable in terms of decreases in intrahepatic fat levels and circulating insulin (46).
In this context, in previous experiments from our group we have shown tissue-specific effects of HIIT and MICT on high-fat-fed mice (Figure 2), where the average intensities, session duration, and distance covered per session was comparable between training programs. Findings that drove us to hypothesize that exercise intensity confers specific effects on insulin-sensitive tissues during obesity; however, no clinical studies have been performed to corroborate these findings, and more importantly, no studies on candidates to undergo bariatric surgery are known to investigate if there is a specific type of exercise program that exerts the most beneficial metabolic effects on this population. In complement and considering the relevance of the maintenance of metabolic benefits post-surgery on these patients, no studies have compared if the metabolic effects of HIIT or MICT during the preoperative period are preserved during the post-surgery period.

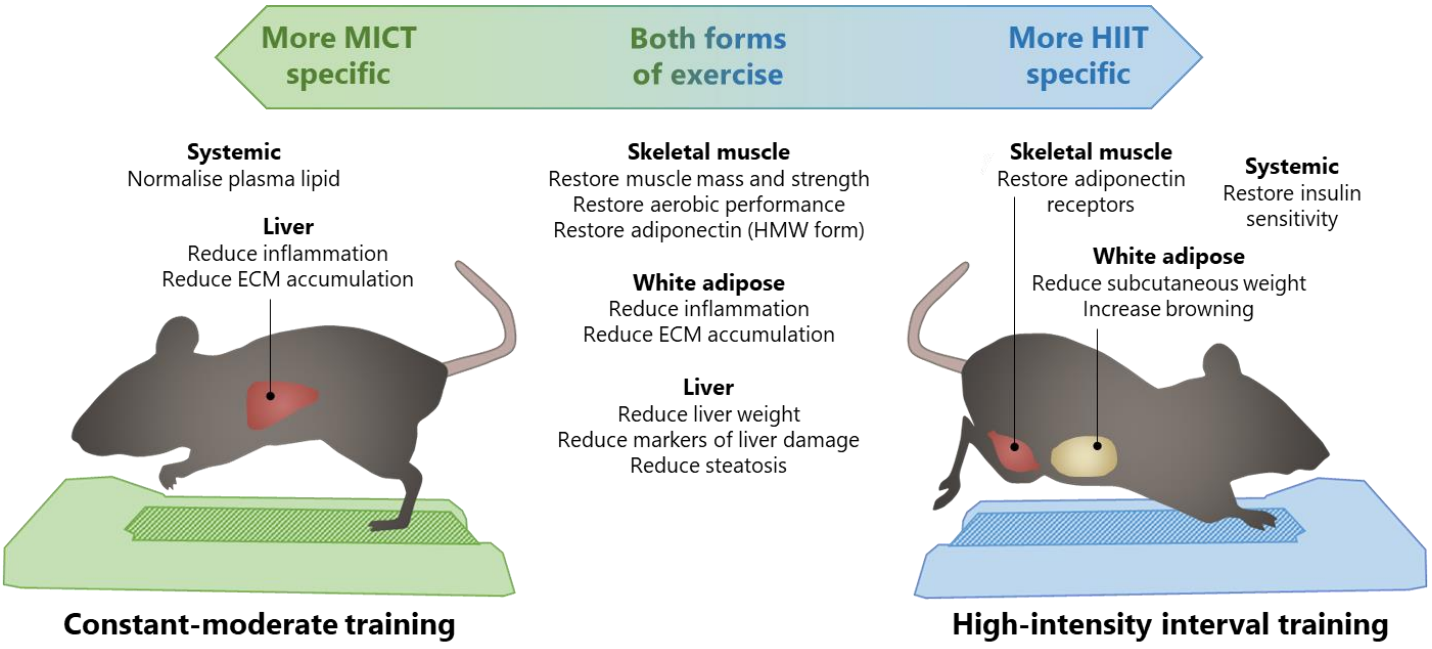

Figure 2. Differential metabolic effects of MICT and HIIT on skeletal muscle, white adipose tissue, and liver of high-fat fed mice. Adapted from: Martinez-Huenchullan SF, Ban LA, Olaya-Agudo LF, et al. Constant-Moderate and High-Intensity Interval Training Have Differential Benefits on Insulin Sensitive Tissues in High-Fat Fed Mice. Front Physiol. 2019;10:459. doi:10.3389/fphys.2019.00459 (47)

Objectives, hypothesis and research questions

Our research questions are:

1. Do exercise training programs with different intensities (MICT and HIIT) exert differential physical and metabolic effects on insulin-sensitive tissues (skeletal muscle, white adipose tissue, and liver) in candidates to undergo bariatric surgery?
2. Are there potential circulatory and tissue-specific metabolic mediators that can explained the expected differential effect of preoperative MICT and HIIT in candidates to undergo bariatric surgery?
3. Do the preoperative effects of HIIT and MICT favor the post-surgery expected improvements in physical and metabolic function of patients that underwent bariatric surgery?

Our hypothesis is:

1. Preoperative HIIT will induce higher physical and tissue-specific metabolic benefits in skeletal muscle, white adipose tissue, and liver, compared to MICT, of candidates to undergo bariatric surgery, and those benefits will be kept 6 months after the surgical procedure.

Considering the literature reviewed and presented in this project, along with our previous work in the field, we hypothesize that obesity will impair the metabolic function of insulin-sensitive tissues in different ways, changes that will also be reflected in by a ***disrupted plasma proteome***. However, exercise will revert some of these changes and an exercise-intensity and tissue-specific manner, as shown in figure 3.

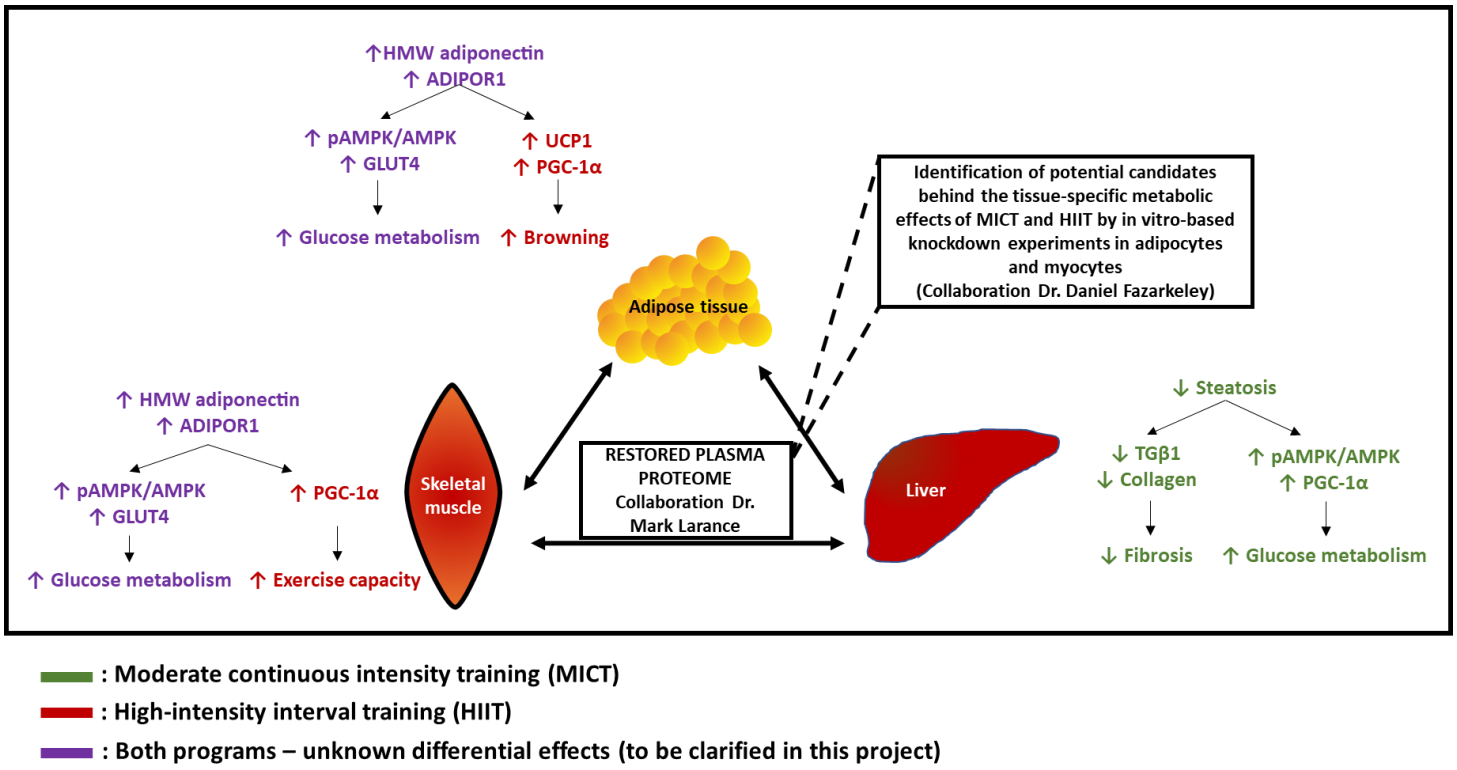

Figure 3. Expected metabolic results from this project. Colors indicate expected specific exercise effects along with unclear exercise specificity to be explored in this project. Collaborations are also indicated and are fully explained in the Methodology section.

Aims:

1. To compare the effects of preoperative MICT and HIIT on the physical function of candidates to undergo bariatric surgery.
2. To compare the effects of preoperative MICT and HIIT on the metabolic function of skeletal muscle, white adipose tissue, and liver of candidates to undergo bariatric surgery.
3. To explore the proteomic signatures after preoperative MICT and HIIT in candidates undergo bariatric surgery.
4. Identify potential metabolic candidates that could explained the metabolic effects of MICT and HIIT in candidates undergo bariatric surgery.
5. To assess the post-surgical physical and systemic metabolic function effects of preoperative MICT and HIIT on bariatric surgery patients.

Potential impact and scientific novelty of your proposal

To the extent of our knowledge, this would be the first study that aims to elucidate the tissue-specific effects of two exercise programs with differing intensities during severe obesity. The rationale behind this study resides in that metabolic impairments derived from obesity are not homogeneous between individuals, where tissue-specific insulin resistance has been described in humans (42). Therefore, strategies that lack specificity in terms of their targets for metabolic benefits will theoretically have lower levels of impact. These interactions could be the responsible of why not all subjects that perform physical exercise achieve the expected metabolic improvements (2). A similar phenomenon has been described in patients that undergo bariatric surgery, defined as resistance to this procedure (43) and its associated with the delayed or mild metabolic improvements after the surgery. Interestingly, exercise has been recommended as a coadjutant therapeutic strategy to prevent this scenario from happening, even when the lack of studies that investigate specific exercise prescriptions has been recognized. Therefore, the potential impact of this project will influence the present strategy to counteract the metabolic impairment associated with obesity, particularly in candidates to undergo bariatric surgery, so the maximum benefit from this intervention can be achieved.

Even when recent studies have shown clear results regarding the tissue-specific effects of physical exercise in an obesity context (47, 48), these findings have only been described in animal models of obesity, where clinical studies are critically lacking. Finally, considering that this project will involve candidates during the preoperative surgery, its eventual results could be applicable to the general population with obesity, given that the surgery will not influence the exercise effects on that period.

Methodology

Study design

This will be a randomized controlled single-blind clinical trial. A summary of the study design and its steps is showed in Figure 4.

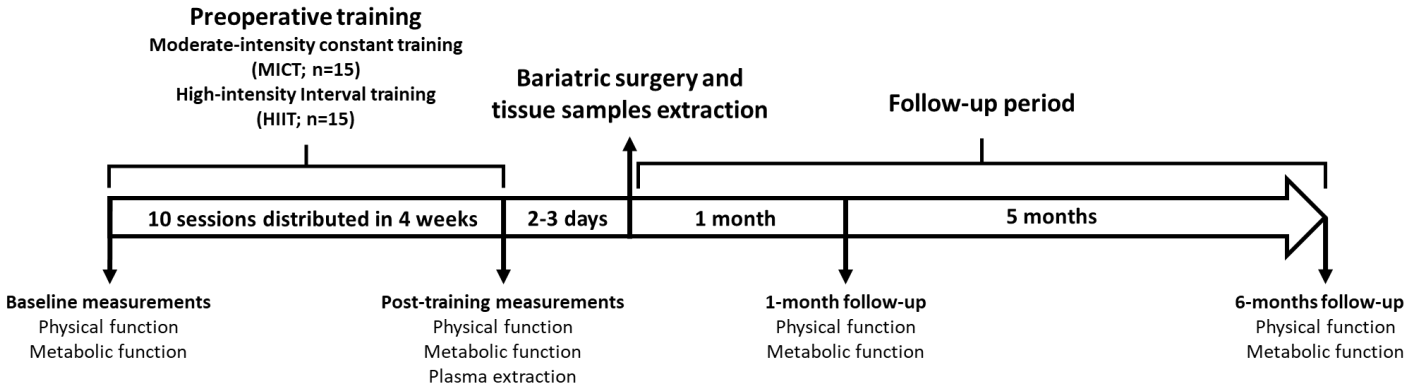

Figure 4. Study design.

To achieve Aim 1: To compare the effects of preoperative MICT and HIIT on the physical function of candidates to undergo bariatric surgery.

Candidates to undergo bariatric surgery in Clinica Alemana de Valdivia will be recruited for this experiment. As inclusion criteria, participants will have to be aged between 18 and 60 years old, and planned to undergo sleeve gastrectomy, to decrease variability in terms of surgical procedures. On the other hand, participants who are not allowed to attend supervised exercise sessions, have medical contraindications to perform physical activity, declare functional limitations which not allow them to complete a progressive cardiorespiratory fitness test and/or have uncontrolled neuropsychiatric illnesses will not be included in this study.

For sample size calculations, using the software GPower 3.1 and considering an effect size of 0.35 in terms of improvement of insulin sensitivity after MICT and HIIT (49), a power of 0.95, an α of 0.05, two groups and a correlation among repeated measures of 0.5 the total sample size required for this project is 24. For an expected dropout of 20% during the experiment, the sample size to be recruited will be of 30 participants, whom will be distributed randomly into the following groups:

- Moderate-intensity constant training (MICT) group (n=15)
- High-intensity interval training (HIIT) group (n=15)

After recruitment and randomization between groups, physical function measurements will be performed as follows: **Muscle strength (upper limbs):** This will be measured through hand dynamometry using the manufacturer's protocol (Jamar®) in kg, where the subject will be in a seated position with its shoulder in neutral position, elbow in 90° flexion, and wrist in neutral position; **muscle strength (lower limbs):** This will be measured through the maximum knee extensor isometric strength using a force gauge connected to a quadriceps chair, with the in a 90° flexion position in N (50), where the electromyography signal will be registered as well with wireless electromyography sensors (Delsys, Trigno); **cardiorespiratory fitness:** This will be measured in the Exercise Physiology Laboratory of the Austral University of Chile, through the modified Bruce protocol (25) which consists in a progressive treadmill-based test until voluntary exhaustion (34) is reached; a gas exchange analyzer (Jaeger Oxycon Mobile) will measure peak oxygen consumption can be measured (VO_2peak) in ml/kg/min; **static balance:** This will be measured in the Movement Analysis Laboratory of the Austral University of Chile, through a force platform (Bertec model FP4060-05-PT) where the center of pressure (COP) displacement will be measured in a standing position with eyes open and closed during one minute in each condition (51). COP will be measured in terms of anterior-posterior and lateral displacement, along with the area between these two measurements (higher displacement and area are

associated with poorer balance); **anthropometric measurements:** weight (kg), height (cm), waist (cm) and hip (cm) circumferences will be measured as previously described (25) in order to obtain the body mass index, waist-to-hip ratio, and waist-to-height ratio; **body composition:** This will be measured through Bioelectric Impedance Analysis with a validated instrument (52) for this purpose (InBody® 270). Measures of total fat (% of body weight) and muscle mass (% of body weight) will be obtained; **spontaneous physical activity:** This will be measured through the international physical activity questionnaire (IPAQ) in its short form as previously recommended for candidates to undergo bariatric surgery (22).

After baseline physical and metabolic function measurements are completed, physical therapy sessions will commence. Each training session will start with a warm-up period of 5-10 min with low intensity exercises in the form of walking/cycling. Afterwards, MICT and HIIT sessions will start as follows: MICT: will be defined as treadmill-running at an intensity of 50% of the heart rate reserve (%HRR) for 30 min. HIIT: will consist in six bouts of high-intensity exercise at 80%HRR of 2.5 min each, intercalated by six active rest periods at 20%HRR of 2.5 min each (Figure 5). The time of these intervals was set following the recommendations from the literature in terms of HIIT usage in an obesity context (35). %HRR will be obtained with the Karvonen formula maximum heart rate (HR max)– resting heart rate (HRrest), where the theoretical maximal heart rate will be obtained by the equation 200-(Age*0.5), which has been validated in healthy adults and in people with obesity (53, 54). Afterwards, and considering the recommendations of the Chilean Consensus regarding exercise and physical activity for adults undergoing bariatric surgery (22), both training groups (MICT and HIIT) will undergo a strengthening component consisting in counter-resistance exercises of major muscle groups in lower and upper limbs along with the abdominal region or core for 15-20 min. Each session will end with a cool-down period of 5-10 min. All sessions will be prescribed and supervised by two physical therapists (Miss Mariana Kalazich-Rosales, and Miss Camila Mautner-Molina), whom have experience managing candidates to undergo bariatric surgery **(please see support letter in the Annexes).** The participants will be blind in terms in the type of aerobic exercise performed. For ethical reasons and following the recommendations from the Bariatric and Metabolic Surgery Chilean Society, in parallel to the training sessions, similar nutritional advice will be given to both training groups by the same nutritionist, Miss Francisca Fuentes-Leal **(please see support letter in the Annexes)**, this to keep this intervention similar throughout the participants, independently of the training group that there are in.

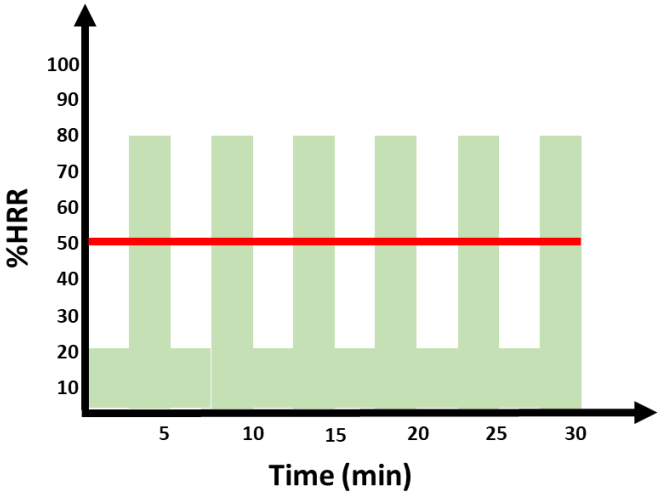

Figure 5. Moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) showed by the red line and the green figures respectively. %HRR: heart rate reserve percentage.

After training and 2-3 days before the surgery, physical function and systemic metabolic function outcome will be measured again to compare eventual changes from the baseline condition.

Pitfalls: In case an untrained group with obesity is necessary to visualize the physical improvements derived from MICT and HIIT, untrained subjects with obesity with similar phenotypic profile can be invited to participate.

To achieve Aim 2: To compare the effects of preoperative MICT and HIIT on the metabolic function of skeletal muscle, white adipose tissue, and liver of candidates to undergo bariatric surgery.

To characterize the systemic metabolic function of the participants, the following assays will be performed in the Clinical Laboratory (LABOCLIN) of the Austral University of Chile: circulating levels of thyroid hormones (TSH), transaminases (ALT and AST), lipid profile, hemogram, electrolytes (Na, Cl, P), albumin, bilirubin, creatinine, and urea nitrogen. These measurements will be performed at baseline, and 2-3 days before the surgery by trained and experienced professionals. These parameters are particularly important at baseline, given that they will allow us to determine if the two experimental groups are similar before starting the exercise intervention.

During the laparoscopic bariatric surgery, the surgeon in charge of these procedures in all participants, Dr. Carlos Cárcamo-Ibaceta **(please see support letter in the Anexus)** will extract <1 gr of skeletal muscle from the abdominal wall, white adipose tissue from the subcutaneous depot, and liver. These samples will be transferred to recipients with saline (0.9% NaCl) to be transported to the Histology, Pathology and Anatomy Laboratory if the Austral University of Chile for its processing.
To quantify protein levels, western immunoblotting will be used as follows. Plasma (0.5 µl), skeletal muscle (25-30 mg), subcutaneous adipose tissue (15-20mg) and liver (10-15 mg) will be homogenized in ice-cold radioimmunoprecipitation assay buffer (RIPA). Proteins will be extracted and quantified using a detergent compatible (DCTM) protein assay (Bio-Rad®, DCTM catalog number 500-113 and 500-114). Tissue samples containing 40 µg of protein or 0.5 µL of plasma will be run on polyacrylamide gradient gels (4-15%) (Bio-Rad®, catalog number 4568086). The proteins contained within the gel will be then transferred to a nitrocellulose membrane (Bio-Rad®, catalog number 1704158). Using specific primary antibodies for: Adiponectin (Cell Signaling, catalog number 2789S), AdipoR1 (Abcam, catalog number ab126611), Phospho-AMPK (Cell Signaling, catalog number 2535S), AMPKα (Cell Signaling, catalog number 2532S), GLUT4 (Cell Signaling, catalog number 2213S), PGC-1α (Cell Signaling, catalog number 2178S), Collagen I (Cell Signaling, catalog number 39952S), Collagen VI (Abcam, catalog number ab6588), and TGF-beta (Biolegend, catalog number 846802), proteins of interest will be detected after overnight incubation at 4°C. After 1 hr incubation at room temperature with peroxidase labelled secondary antibodies (Anti-rabbit IgG 1:10000, Sigma®, catalog number S9169; Anti-mouse IgG 1:10000, Sigma®, catalog number A9044), membranes will be washed with tris buffered saline plus tween-20 (TBST) and developed with a chemiluminescent substrate (ClarityTM Western ECL substrate, Bio-Rad®, catalog number 170-5061) and visualized. Densitometric analysis of the bands will be performed using ImageJ software. Protein loading will be confirmed, and specific band intensities will be normalized using Ponceau S staining, as previously recommended (55).
Tissue samples will also be investigated from a histological point of view. Samples immediately after extraction from the surgery, will be fixated in 10% formalin for 24 h and then they will undergo overnight processing at the Anatomy, Histology, and Pathology Laboratory of the Austral University of Chile. From paraffin-embedded blocks, 5 µm sections will be first stained with hematoxylin and eosin to investigate the effects of MICT and HIIT on global tissue structure. Also, in other 5 µm sections, after antigen retrieval (through microwave at pH 6.0 citrate buffer for 30 min), the slides will be incubated overnight at 4°C with primary antibodies against adiponectin (Cell Signaling, catalog number 2789S), GLUT4 (Cell Signaling, catalog number 2213S), UCP-1 (Abcam, catalog number ab155117), and Collagen I Cell Signaling, catalog number 39952S). After secondary antibody incubation for 30 min (1:200, Biotinylated anti-rabbit IgG, Vector Laboratories®, catalog number BA-1000) at room temperature, color was developed using avidin-biotin complex (Vectastain ABC kit, Vector Laboratories®, catalog number PK-4000) with diaminobenzidine (DAB) (Dako®, catalog number K3468). The reaction was terminated by washing the slides in ddH2O and the slides were imaged using a light microscope, with a microimaging software. Quantification of percentage positive staining in each slide was achieved using the image processing software Fiji® (56).
Pitfalls: In case an untrained group with obesity is necessary as a control group to compared the metabolic effects of MICT and HIIT on skeletal muscle, white adipose tissue, and liver, untrained subjects with obesity with an elective cholecystectomy surgery, with a similar phenotypic profile, can be invited to participate and during the laparoscopic surgery samples from the three tissues can be extracted. It is not possible to have candidate to undergo bariatric surgery without undergoing physical training, as this intervention is mandatory and by not delivered it, we could incur in ethical misbehaviors. Moreover, skeletal muscle fiber-typing and mitochondrial structural studies (MitoTracker, ThermoFisher ®) along with protein markers of mitochondrial fussion (e.g. MFN1/2) and fission (e.g. DRP1) can be incorporated, considering its major role in exercise-derived metabolic effects, particularly in obesity (57).

To achieve Aim 3: To explore the proteomic signatures after preoperative MICT and HIIT in candidates undergo bariatric surgery.

Whole blood samples (~3 mL) will be collected from arm ventral venous puncture during the baseline and post-training period. Afterwards, plasma will be extracted by centrifuging each tube at 1000g for 10 min at room temperature. From here, 50 µl will be aliquoted in eppendörf tubes and keep frozen at -80°C. Once all samples are collected, they will be shipped to Sydney, Australia, specifically to Mark Larance's laboratory located in the Charles Perkins Centre of The University of Sydney **(please see support letter in Anexus)**, for sample preparation and proteomic analysis.
Pitfalls: In case the tissue proteomics analysis take more time than expected, from the plasma proteomic analysis, several metabolic candidates can be selected for the *in vitro* experiments to be conducted by our collaborator Dr. Daniel Fazarkeley. Moreover, a directed selection of metabolic candidates can be measured

through specific ELISA-based and/or Luminex-based assays (e.g.: adiponectin, FGF21, resistin, leptin, interleukin 6).

To achieve Aim 4: Identify potential metabolic candidates that could explained the metabolic effects of MICT and HIIT in candidates undergo bariatric surgery.

After the plasma proteomic analysis is completed, as described in Aim 3, datasets will be shared with Dr. Daniel Fazarkeley and his group, to identify potential candidates that could explain the metabolic effects of MICT and HIIT in skeletal muscle and white adipose tissue. Afterwards, using adipocytes and myocytes as cell models, he will knock down the genes from where these protein candidates are transcribed in order to see if its decrease in function results in metabolic impairments, particularly from an insulin signaling and glucose transport point of view **(please see attach letter of support in the Annexes)**.

Pitfalls: In case we face delays in identifying potential candidates for the *in vitro* experiments, we can direct these trials by knocking down adiponectin and adiponectin receptor 1 genes, as we have found that they might play a role in the differential effects of MICT and HIIT in mouse models of obesity (47, 58, 59).

To achieve Aim 5: To assess the post-surgical physical and systemic metabolic function effects of preoperative MICT and HIIT on candidates to undergo bariatric surgery.

After the first and sixth month after the surgery, participants will be contacted to repeat the physical function measurements, as described in Aim 1. Moreover, venous blood samples will be collected to characterize the systemic metabolic function as described in Aim 3. The measurements in this point will be circulating levels of thyroid hormones (TSH), transaminases (ALT and AST), lipid profile, hemogram, electrolytes (Na, Cl, P), albumin, bilirubin, creatinine, and urea nitrogen.

Pitfalls: No major pitfalls are expected in this point. If the adherence falls after the surgery, we are calculating our sample size with a 20% extra than the size statistically required, in case of drop-offs.

Work plan

Activity	Year 1	Year 2	Year 3
Aim 1: To compare the effects of preoperative MICT and HIIT on the physical function of candidates to undergo bariatric surgery.			
Participants recruitment			
Baseline measurements			
Exercise training			
Data analysis			
Aim 2: To compare the effects of preoperative MICT and HIIT on the metabolic function of skeletal muscle, white adipose tissue, and liver of candidates to undergo bariatric surgery.			
Bariatric surgeries (sample extraction)			
Tissue-samples processing and analysis			
Data analysis			
Aim 3: To explore the proteomic signatures after preoperative MICT and HIIT in candidates undergo bariatric surgery.			
Sample shipping to Dr. Mark Larance at The University of Sydney			
Proteomic analysis			
Data analysis			
Aim 4: Identify potential metabolic candidates that could explained the metabolic effects of MICT and HIIT in candidates undergo bariatric surgery.			
Share proteomic dataset with Dr. Daniel Fazarkeley at the University of Cambridge			
Selection of potential candidates behind MICT and HIIT effects			
In vitro experiments in adipocytes and myocytes by knocking down potential candidates			
Data analysis			
Aim 5: To assess the post-surgical physical and systemic metabolic function effects of preoperative MICT and HIIT on candidates to undergo bariatric surgery.			
Physical and metabolic function measurement after 1-month post-surgery			
Physical and metabolic function measurement after 6-months post-surgery			
Data analysis			

Common to all aims			
Presentation of findings at conferences			
Manuscript preparation			
Final report preparation			
Final report submission			

Project's team skills

Dr. Carlos Cárcamo-Ibaceta: He is the lead surgeon of the Bariatric Patient Management Team of Clinical Alemana de Valdivia, with more than 15 years of experience conducting these procedures. **Miss Francisca Fuentes-Leal:** Nutritionist, member of the Bariatric Patient Management Team of Clinical Alemana de Valdivia, with several years of experience assisting these candidates to ensure proper nutritional intake during the pre-and postoperative periods. **Miss Mariana Kalazich-Rosales and Miss Camila Mautner-Molina:** physical therapist, members of the Bariatric Patient Management Team of Clinica Alemana de Valdivia and members of the Chilean Society of Bariatric and Metabolic Surgery, with experience conducting physical training on candidates to undergo bariatric surgery during its pre- and postoperative period. Camila has a Masters degree in Cardiorrespiratory Physical Therapy in San Sebastian University. **Mr. Mauricio San Martín-Correa:** manager of the Human Movement Analysis Laboratory of the Instituto de Aparato Locomotor y Rehabilitación de the Austral University of Chile. He has training obtaining and analysing data from force platforms, force gauges and electromyographical sensors. He is currently finalizing a master's degree in physical therapy with a mention in Biomechanics in the Catholic University of Maule. **Mr. Manuel Monrroy-Uarac:** manager of the Exercise Physiology Laboratory of the Instituto de Aparato Locomotor y Rehabilitación de the Austral University of Chile. He has training and experience measuring peak oxygen uptake in subjects with different clinical conditions, ranging from athletes to patients with cardiopulmonary dysfunctions. He is currently finalizing a master's degree in physical therapy with a mention in Exercise Biology in the Catholic University of Maule. **Dr. Pamela Ehrenfeld-Slater:** Associate Professor, Head of the Laboratory of Cellular Pathology at the Institute of Anatomy, Histology and Pathology in the Austral University of Chile, with vast experience and productivity of the molecular and cellular biology of cancer, inflammation and metabolism. We are current collaborators, where one article is already published (16) and another one under review. **Dr. Mark Larance:** His laboratory located in the Charles Perkins Centre of The University of Sydney aims to determine how lifestyle interventions (e.g. diet & exercise) can improve metabolic health in humans, for the prevention and treatment of metabolic diseases, through the usage of plasma and tissue proteomics from animal and human samples. **Dr. Daniel Fazarkeley:** His laboratory located at the Institute of Metabolic Science of the University of Cambridge, aims to understand the molecular details of insulin-stimulated glucose transport, and how this process breaks down in disease, through the development of screening platform in *in vitro* models in adipocytes and myocytes, to identify and knock down genes to see if they are involved in insulin-stimulated glucose transport.

REFERENCES

1. OECD. OECD Reviews of public health: Chile Paris: OECD Publishing; 2019 [Available from: http://www.oecd.org/publications/oecd-reviews-of-public-health-chile-9789264309593-en.htm.
2. Boutcher SH, Dunn SL. Factors that may impede the weight loss response to exercise-based interventions. Obes Rev. 2009;10(6):671-80.
3. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature. 2006;444(7121):847-53.
4. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem. 2003;278(41):40352-63.
5. Akingbemi BT. Adiponectin receptors in energy homeostasis and obesity pathogenesis. Prog Mol Biol Transl Sci. 2013;114:317-42.
6. Daval M, Foufelle F, Ferre P. Functions of AMP-activated protein kinase in adipose tissue. J Physiol. 2006;574(Pt 1):55-62.
7. Cedikova M, Kripnerova M, Dvorakova J, Pitule P, Grundmanova M, Babuska V, et al. Mitochondria in White, Brown, and Beige Adipocytes. Stem Cells Int. 2016;2016:6067349.
8. Yin X, Lanza IR, Swain JM, Sarr MG, Nair KS, Jensen MD. Adipocyte mitochondrial function is reduced in human obesity independent of fat cell size. J Clin Endocrinol Metab. 2014;99(2):E209-16.
9. Seale P, Lazar MA. Brown fat in humans: turning up the heat on obesity. Diabetes. 2009;58(7):1482-4.
10. Fromme T, Klingenspor M. Uncoupling protein 1 expression and high-fat diets. Am J Physiol Regul Integr Comp Physiol. 2011;300(1):R1-8.
11. Wang N, Liu Y, Ma Y, Wen D. High-intensity interval versus moderate-intensity continuous training: Superior metabolic benefits in diet-induced obesity mice. Life Sci. 2017;191:122-31.
12. Lo L, McLennan SV, Williams PF, Bonner J, Chowdhury S, McCaughan GW, et al. Diabetes is a progression factor for hepatic fibrosis in a high fat fed mouse obesity model of non-alcoholic steatohepatitis. J Hepatol. 2011;55(2):435-44.
13. Williams KH, Shackel NA, Gorrell MD, McLennan SV, Twigg SM. Diabetes and nonalcoholic Fatty liver disease: a pathogenic duo. Endocr Rev. 2013;34(1):84-129.
14. Aharoni-Simon M, Hann-Obercyger M, Pen S, Madar Z, Tirosh O. Fatty liver is associated with impaired activity of PPARgamma-coactivator 1alpha (PGC1alpha) and mitochondrial biogenesis in mice. Lab Invest. 2011;91(7):1018-28.
15. Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell Metab. 2015;21(5):739-46.
16. Martinez-Huenchullan SF, Tam CS, Ban LA, Ehrenfeld-Slater P, McLennan SV, Twigg SM. Skeletal muscle adiponectin induction in obesity and exercise. Metabolism. 2020;102:154008.
17. Vu V, Bui P, Eguchi M, Xu A, Sweeney G. Globular adiponectin induces LKB1/AMPK-dependent glucose uptake via actin cytoskeleton remodeling. J Mol Endocrinol. 2013;51(1):155-65.
18. Iwabu M, Yamauchi T, Okada-Iwabu M, Sato K, Nakagawa T, Funata M, et al. Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. Nature. 2010;464(7293):1313-9.
19. Csendes J A. Número total de operaciones de patología digestiva alta en Chile año 2011. Revista chilena de cirugía. 2015;67(1):61-4.
20. Csendes A, Maluenda F. Morbimortalidad de la cirugía bariátrica: Experiencia chilena en 10 instituciones de salud. Revista chilena de cirugía. 2006;58(3).
21. Bellicha A, Ciangura C, Poitou C, Portero P, Oppert JM. Effectiveness of exercise training after bariatric surgery-a systematic literature review and meta-analysis. Obes Rev. 2018;19(11):1544-56.
22. Cancino-Ramirez J, Troncoso-Ortiz EJ, Pino J, Olivares M, Escaffi MJ, Riffo A, et al. [ercise and physical activity in adults who underwent bariatric surgery. Consensus document. Rev Med Chil. 2019;147(11):1468-86.

23. Pouwels S, Wit M, Teijink JA, Nienhuijs SW. Aspects of Exercise before or after Bariatric Surgery: A Systematic Review. Obes Facts. 2015;8(2):132-46.
24. Jung ME, Bourne JE, Beauchamp MR, Robinson E, Little JP. High-intensity interval training as an efficacious alternative to moderate-intensity continuous training for adults with prediabetes. J Diabetes Res. 2015;2015:191595.
25. ACSM. ACSM's guidelines for exercise testing and prescription. Tenth ed: Lippincott Williams & Wilkins; 2016. 480 p.
26. Lieber R. Skeletal muscle structure, function, and plasticity. 3rd ed. Philadelphia, USA.: Wolters Kluwer, Lippincott Williams & Wilkins; 2010.
27. Kirwan JP, Solomon TP, Wojta DM, Staten MA, Holloszy JO. Effects of 7 days of exercise training on insulin sensitivity and responsiveness in type 2 diabetes mellitus. Am J Physiol Endocrinol Metab. 2009;297(1):E151-6.
28. Bond DS, Jakicic JM, Unick JL, Vithiananthan S, Pohl D, Roye GD, et al. Pre- to postoperative physical activity changes in bariatric surgery patients: self report vs. objective measures. Obesity (Silver Spring). 2010;18(12):2395-7.
29. Trivax JE, Gallagher MJ, Alexander DV, deJong AT, Kasturi G, Sandberg KR, et al. Poor Aerobic Fitness Predicts Complications Associated with Bariatric Surgery. Chest. 2005;128(4).
30. Kerrigan DJ, Carlin AM, Munie S, Keteyian SJ. A Cross-sectional Study of Reported Exercise and Medium-Term Weight Loss Following Laparoscopic Bariatric Surgery. Obes Surg. 2018;28(12):3923-8.
31. Parikh M, Dasari M, McMacken M, Ren C, Fielding G, Ogedegbe G. Does a preoperative medically supervised weight loss program improve bariatric surgery outcomes? A pilot randomized study. Surg Endosc. 2012;26(3):853-61.
32. Turk Y, Theel W, Kasteleyn MJ, Franssen FME, Hiemstra PS, Rudolphus A, et al. High intensity training in obesity: a Meta-analysis. Obes Sci Pract. 2017;3(3):258-71.
33. Thum JS, Parsons G, Whittle T, Astorino TA. High-Intensity Interval Training Elicits Higher Enjoyment than Moderate Intensity Continuous Exercise. PLoS One. 2017;12(1):e0166299.
34. Gerosa-Neto J, Panissa VLG, Monteiro PA, Inoue DS, Ribeiro JPJ, Figueiredo C, et al. High- or moderate-intensity training promotes change in cardiorespiratory fitness, but not visceral fat, in obese men: A randomised trial of equal energy expenditure exercise. Respir Physiol Neurobiol. 2019;266:150-5.
35. Su L, Fu J, Sun S, Zhao G, Cheng W, Dou C, et al. Effects of HIIT and MICT on cardiovascular risk factors in adults with overweight and/or obesity: A meta-analysis. PLoS One. 2019;14(1):e0210644.
36. Keating SE, Machan EA, O'Connor HT, Gerofi JA, Sainsbury A, Caterson ID, et al. Continuous exercise but not high intensity interval training improves fat distribution in overweight adults. J Obes. 2014;2014:834865.
37. Baillot A, Mampuya WM, Comeau E, Meziat-Burdin A, Langlois MF. Feasibility and impacts of supervised exercise training in subjects with obesity awaiting bariatric surgery: a pilot study. Obes Surg. 2013;23(7):882-91.
38. Baillot A, Mampuya WM, Dionne IJ, Comeau E, Meziat-Burdin A, Langlois MF. Impacts of Supervised Exercise Training in Addition to Interdisciplinary Lifestyle Management in Subjects Awaiting Bariatric Surgery: a Randomized Controlled Study. Obes Surg. 2016;26(11):2602-10.
39. Baillot A, Vallee CA, Mampuya WM, Dionne IJ, Comeau E, Meziat-Burdin A, et al. Effects of a Pre-surgery Supervised Exercise Training 1 Year After Bariatric Surgery: a Randomized Controlled Study. Obes Surg. 2018;28(4):955-62.
40. Pico-Sirvent I, Aracil-Marco A, Pastor D, Moya-Ramon M. Effects of a Combined High-Intensity Interval Training and Resistance Training Program in Patients Awaiting Bariatric Surgery: A Pilot Study. Sports (Basel). 2019;7(3).
41. Delgado Floody P, Jerez Mayorga D, Caamano Navarrete F, Osorio Poblete A, Thuillier Lepeley N, Alarcon Hormazabal M. Twelve Weeks of Physical Exercise Interval with Surcharge Improves the Anthropometric Variables of Obese Morbid and Obese with Comorbidities Candidates to Bariatric Surgery. Nutr Hosp. 2015;32(5):2007-11.
42. Chen DL, Liess C, Poljak A, Xu A, Zhang J, Thoma C, et al. Phenotypic Characterization of Insulin-Resistant and Insulin-Sensitive Obesity. J Clin Endocrinol Metab. 2015;100(11):4082-91.

43. Gilbertson NM, Paisley AS, Kranz S, Weltman A, Kirby JL, Hallowell PT, et al. Bariatric Surgery Resistance: Using Preoperative Lifestyle Medicine and/or Pharmacology for Metabolic Responsiveness. Obes Surg. 2017;27(12):3281-91.
44. Chavanelle V, Boisseau N, Otero YF, Combaret L, Dardevet D, Montaurier C, et al. Effects of high-intensity interval training and moderate-intensity continuous training on glycaemic control and skeletal muscle mitochondrial function in db/db mice. Sci Rep. 2017;7(1):204.
45. Cho J, Kim S, Lee S, Kang H. Effect of Training Intensity on Nonalcoholic Fatty Liver Disease. Med Sci Sports Exerc. 2015;47(8):1624-34.
46. Winn NC, Ying L, Rector RS, Parks EJ, Ibdah JA, Kanaley JA. Energy-matched moderate and high intensity exercise training improves nonalcoholic fatty liver disease risk independent of changes in body mass or abdominal adiposity - a randomized trial. Metabolism. 2018;78:128-40.
47. Martinez-Huenchullan SF, Ban LA, Olaya-Agudo LF, Maharjan BR, Williams PF, Tam CS, et al. Constant-Moderate and High-Intensity Interval Training Have Differential Benefits on Insulin Sensitive Tissues in High-Fat Fed Mice. Front Physiol. 2019;10:459.
48. Groussard C, Maillard F, Vazeille E, Barnich N, Sirvent P, Otero YF, et al. Tissue-Specific Oxidative Stress Modulation by Exercise: A Comparison between MICT and HIIT in an Obese Rat Model. Oxid Med Cell Longev. 2019;2019:1965364.
49. Jelleyman C, Yates T, O'Donovan G, Gray LJ, King JA, Khunti K, et al. The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis. Obes Rev. 2015;16(11):942-61.
50. Fransen M, Crosbie J, Edmonds J. Isometric muscle force measurement for clinicians treating patients with osteoarthritis of the knee. Arthritis Rheum. 2003;49(1):29-35.
51. Menegoni F, Galli M, Tacchini E, Vismara L, Cavigioli M, Capodaglio P. Gender-specific effect of obesity on balance. Obesity (Silver Spring). 2009;17(10):1951-6.
52. Czartoryski P, Garcia J, Manimaleth R, Napolitano P, Watters H, Weber C, et al. Body Composition Assessment: A Comparison of the DXA, InBody 270, and Omron. Journal of Exercise and Nutrition. 2020;3(1):1.
53. Miller WC, Wallace JP, Eggert KE. Predicting max HR and the HR-VO2 relationship for exercise prescription in obesity. Med Sci Sports Exerc. 1993;25(9):1077-81.
54. She J, Nakamura H, Makino K, Ohyama Y, Hashimoto H. Selection of suitable maximum-heart-rate formulas for use with Karvonen formula to calculate exercise intensity. International Journal of Automation and Computing. 2014;12(1):62-9.
55. Gilda JE, Gomes AV. Stain-Free total protein staining is a superior loading control to beta-actin for Western blots. Anal Biochem. 2013;440(2):186-8.
56. Gallo-Oller G, Ordonez R, Dotor J. A new background subtraction method for Western blot densitometry band quantification through image analysis software. J Immunol Methods. 2018;457:1-5.
57. Dai W, Jiang L. Dysregulated Mitochondrial Dynamics and Metabolism in Obesity, Diabetes, and Cancer. Front Endocrinol (Lausanne). 2019;10:570.
58. Martinez-Huenchullan SF, Maharjan BR, Williams PF, Tam CS, McLennan SV, Twigg SM. Differential metabolic effects of constant moderate versus high intensity interval training in high-fat fed mice: possible role of muscle adiponectin. Physiol Rep. 2018;6(4).
59. Martinez-Huenchullan SF, Maharjan BR, Williams PF, Tam CS, McLennan SV, Twigg SM. Skeletal muscle adiponectin induction depends on diet, muscle type/activity, and exercise modality in C57BL/6 mice. Physiol Rep. 2018;6(20):e13848.