PhD course in:

"Biomedical science and biotechnology"

XXXII Cycle

Title of the thesis:

"Exercise Limiting factors (central vs peripheral) and the effect of HIIT on fat oxidation and V'O_2peak in obese people"

PhD student **Supervisor**

Vaccari Filippo Prof. Lazzer Stefano

ACADEMIC YEAR 2019/2020

Table of contents

List of publications

Published

1. Vaccari F, Floreani M, Tringali G, De Micheli R, Sartorio A, Lazzer S. Metabolic and muscular factors limiting aerobic exercise in obese subjects. Eur J Appl Physiol. 2019;(0123456789). doi:10.1007/s00421-019-04167-w
2. Giovanelli N, Vaccari F, Floreani M, Rejc E, Copetti J, Garra M, Biasutti L and Lazzer S. Short-term effects of rolling massage on energy cost of running and power of the lower limbs. Int J Sports Physiol Perform. 2018;13(10):1337-1343. doi:10.1123/ijspp.2018-0142

Under revision

3. Vaccari F, Giovanelli N, Lazzer S. High Intensity Decreasing Interval Training (HIDIT) increases time above 90% V'O_2peak. Submitted to: Eur J Appl Physiol.
4. Vaccari F, Passaro A, D'Amuri A, Maria Sanz J, Di Vece F, Capatti E, Magnesa B, Comelli M, Mavelli I, Grassi B, Fiori F, Bravo G, Avancini A, Parpinel M, Lazzer S. HIIT vs MICT effect on fat oxidation, V'O_2peak and mitochondrial respiration in obese. Submitted to: Eur J Appl Physiol

List of abbreviations and definition

ADP:	Adenosine diphosphate;
AMP:	Adenosine monophosphate;
AMPK:	5' AMP-activated protein kinase;
ANOVA:	Analysis of variance;
ATP:	Adenosine Triphosphate;
a-$\bar{v}O_2$ diff:	Arteriovenous oxygen difference;
BM:	Body mass
BMI:	Body Mass Index;
Ca^{2+}:	Calcium ions;
CaMKII:	$Ca^{2+/}$calmodulin-dependent protein kinase II;
CE:	Cycle ergometer
CP:	Critical Power;
CR10-Scale:	Validated scale of perceived exertion;
CTRL:	Control group;
CO:	Cardiac output;
DAP:	Diastolic Arterial Pressure;
DCP:	The difference between CP and actual power under CP in watts;
ΔMVC:	Maximal Voluntary Contraction changes in percentage;
FAT:	Lipids oxidation rate during the incremental test;
FM:	Fat mass;
FFM:	Fat-free mass;

HIDIT:	Decreasing intervals HIIT (combining High phosphocreatine intensity from 3’ to 30” and low intensity from 2’ to 20”);
HIIT:	High Intensity Interval Training;
HR:	Heart rate;
Hyperphagia:	Excessive hunger or increased appetite;
ICP:	Intermittent Critical Power;
IPAQ-SF:	International Physical Activity Questionnaire Short Form;
[La]:	Blood (capillary) lactate concentration;
LI_{HIIT}:	Long Intervals HIIT (3’ High - 2’ Low-intensity);
KE:	Knee extension exercise;
MAP:	Minimal power that elicited $V'O_2peak$;
MICT:	Moderate intensity continuous training;
MVC:	Maximal voluntary contractions of the knee extensor muscles;
MVC_end:	MVC immediately after the end of incremental exercise;
SIT:	Sprint interval training;
mRNA:	Messenger RNA;
OB:	Obese;
[PCr]:	Muscular concentration of phosphocreatine;
PGC-1α:	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha;
p38 MAPK:	P38 mitogen-activated protein kinases;
RQ:	Gas exchange ratio;
RPE:	Rate of perceived exertion;
SC:	Stage of Change Questionnaire;

SAP:	Systolic Arterial Pressure;
SF12:	Short-Form 12, questionnaire about health-related quality of life;
SF12_MI:	Short-Form 12, Mental Index;
SF12_PI:	Short-Form 12, Physical Index;
SI_{HIIT}:	Short Intervals HIIT (30” High - 20” Low-intensity);
	Tlim: (Time to exhaustion);
SVC:	Systemic Vascular Conductance;
Tlim:	Time spent above 90% $\dot{V}O_2peak$;
T>90%$\dot{V}O_2peak$:	Time spent above 90% $\dot{V}O_2peak$;
$\dot{V}CO_2$:	Pulmonary CO_2 output;
VEGF:	Vascular endothelial growth factor
$\dot{V}O_2$:	Pulmonary O_2 uptake;
$\dot{V}O_2max$:	Maximal oxygen uptake;
Vol_{TM}:	Muscle thigh volume;

Abstract

Obesity is a syndrome, widespread throughout the world and related to numerous health problems and exercise intolerance. The first purpose of the thesis was to compare obese (OB) and lean sedentary subjects (CTRL) in order to understand where the main limiting factors (either central/cardiovascular or peripheral/muscular) operating during maximal exercise tests. 15 OB and 13 CTRL participated in this study. V'O_2, HR and Cardiac Output (CO) were measured during a whole-body exercise (cycle-ergometer, CE) and a single leg knee extension exercise (KE), both incremental tests. Maximal voluntary contraction (MVCs) of knee extensor muscle were performed before and immediately after the two tests. V'O_2peak, HRpeak and COpeak were significantly higher in CE than KE, both in OB and CTRL subjects, no differences have been found between groups. Maximal work rate was lower in OB than CTRL in CE, while it was similar in the two subgroups in KE. Immediately after the end of the incremental exercises, the reduction of MVC was significantly lower in CE than KE in OB; in CTRL on the contrary, MVC decreased similarly in CE and KE tests. In conclusion this finding suggests that in obese but not in control subjects, central circulation is probably the most important limiting factor during cycling, since only in obese the periphery (thigh muscles) were relatively preserved from fatigue after CE.

Such results make think that physical training in obese should be focused on cardiovascular factors rather than on periphery. High intensity interval training (HIIT) is supposed to be more effective in improving cardiovascular function than moderate intensity continuous training (MICT). Therefore, the effects induced by a 3-month training program with either MICT or HIIT were investigated in obese adults. After the training interventions the patients were followed for further four months. Thirty-two obese patients participated in this study, at baseline (PRE), at the end of the program (POST) and after follow-up, body composition, V'O_2peak and fat oxidation rate were assessed. Vastus lateralis biopsies for the evaluation of mitochondrial respiration and blood analyses were performed only at PRE and POST and limited to a subgroup of MICT and HIIT. At POST, body and fat mass decreased equally in MICT and HIIT groups; V´O2peak increased in both groups but more in HIIT and maximal fat oxidation rate increased only after HIIT. Maximal ADP-stimulated mitochondrial respiration normalized by citrate synthase

increased in MICT and HIIT at the same extent and a reduction in total cholesterol and LDL-cholesterol was observed in both groups without differences as well. After follow-up body and fat mass were still lower compared with baseline in both groups and only after HIIT V´O_2peak and maximal fat oxidation rate were still higher. In conclusion HIIT was more effective than MICT in improving V´O_2peak and fat oxidation rate, presumably by factors “upstream” of mitochondrial function. Furthermore, only the effects of HIIT persisted even after the end of the intervention.

Finally, it has been included an appendix which tried to develop a more effective HIIT protocol. Training near V’O_2max is supposed to be the more effective way to enhance V’O_2max. HIIT is a well-known and time-efficient training method, while short HIIT bouts improve time to exhaustion (Tlim), long intervals elicit V’O_2peak quickly. The aim of this study was to evaluate time spent above 90% V’O_2peak (T>90%V’O_2peak) in three different HIIT protocols. Twelve cyclists performed three HIIT sessions (matched work and recovery power and ratio work/recovery), consisting in long intervals HIIT (LI_{HIIT}) (3 min work – 2 min recovery), short intervals HIIT (SI_{HIIT}) (30 s work – 20 s recovery) and a protocol that combine long and short intervals (HIDIT, work from 3 min to 30 s and recovery from 2 min to 20 s). Tlim, T>90%V’O_2peak, blood lactate [La] at 3rd min and at Tlim were measured. Tlim was similar in SI_{HIIT}, HIDIT and LI_{HIIT}. T>90%V’O_2 peak was significantly greater in HIDIT compared with the other protocols, and at exhaustion, no differences in [La] were found between protocols. In conclusion HIDIT showed the highest T>90%V’O_2peak, suggesting that it elicits more the V’O_2 despite a similar [La] at Tlim. In future, would be interesting to repeat the measurement with healthy obese.

CHAPTER I

General Introduction

1.1 – Obesity

1.1.1 - What it is and how to measure it.

Obesity is an excess of body fat leading to health issues. People can be defined obese relying on their Body Mass Index (BMI), calculated as weight in kilograms divided by the square person's height in meters (Canoy and Buchan 2007). BMI equal or superior than 30 $kg \cdot m^{-2}$ is considered indicative of an obesity status. Obesity is further divided in Class I with BMI comprised between 30 and 35, Class II between 35 and 40 and class III when greater than 40 $kg \cdot m^{-2}$. Also, BMI between 25 and 29.9 is indicative of pre-obesity/overweight, whilst between 18.5 and 24.9 is considered normal (Williams et al. 2015). Due to its simplicity BMI is widely used among clinician and researchers and even the World Health Organization (WHO) bases its observation by relying on BMI (WHO 2017). On the other hand, its simplicity is exactly why it is often criticized, for instance it doesn't take in account sex and races differences and requires further analysis to be standardized (Canoy and Buchan 2007). Another import issue is that BMI is not reliable in non-adults people and it need to be standardized also for age in case of children or adolescents (Cacciari et al. 2006). Finally, the muscle mass of individuals is not considered using the BMI and an athlete with a great muscle mass might be considered overweight applying just the BMI categorization despite his fat mass is low.

Category	Body mass index [c.b.]
Underweight	≤18.5
Normal weight	18.5-24.9
Overweight	25-29.9
Obesity class I	30-34.9
Obesity class II	≥35
Central obesity by abdominal circumference[*]	
Population	Cutoff
Euroamerican men	≥102 cm (40″)
Euroamerican women	≥88 cm (35″)
Asian men	≥90 cm (35″)
Asian women	≥80 cm (32″)
Central obesity by waist-to-hip ratio	
Men	>0.9
Women	>0.85

Fig 1.1.1 - Obesity classification based on different methods. (López-Jiménez and Cortés-Bergoderi 2011).

For all these reasons other methods are commonly used besides BMI such as waist circumference, waist to hip ratio, bioelectrical impedance, and skinfold thickness; nevertheless also these measurements have some limitation (Williams et al. 2015). Waist circumference and waist to hip ratio are interesting because of their simplicity in quantify central obesity, which are strongly related to cardiovascular diseases, more than general obesity (López-Jiménez and Cortés-Bergoderi 2011; Ortega B et al. 2016). Finally, dual energy X-ray absorptiometry, magnetic resonance imaging, and computerized tomography are very accurate but with limited use on diagnostic obesity due their complexity and expensiveness.

1.1.2 - Causes

Basically, the weight gain is caused by energy imbalance in which energy intake is bigger than energy expenditure (Wyatt et al. 2006; Canoy and Buchan 2007; Ng et al. 2014; Williams et al. 2015; Upadhyay et al. 2018). Sadly, physiological systems are more efficient in storing energy rather than limiting intake or increasing expenditure (Mitchell et al. 2011). In other words, the human body is designed to lean toward the side of storing fat rather than protecting against weight gain and this is why many people tend to progressively gain weight and became overweight or even obese.

Although the possible causes of this of energy intake imbalance may be several and of different nature (physiological and genetical or even behavioural and social) (Williams et al. 2015), it is the hypothalamus that regulates the homeostasis of energy intake and expenditure trough appetite control, integrating hormonal signals from periphery and transmitting them to the central nervous system (Upadhyay et al. 2018). One of the master regulators of the energy homeostasis is Leptin, an hormone made by adipose cells and some enterocytes which communicates with hypothalamus's neurons (Van Der Klaauw and Farooqi 2015). Worth of mention beside the leptin are ghrelin which is considered its complementary and insulin and glucagon, both involved also in regulating blood sugar and fat metabolism (Lucan and Dinicolantonio 2015). Fasting or losing weight drops leptin levels enhancing hunger, inhibiting energy expenditure and regulating neuroendocrine functioning to maintain homeostasis (Van Der Klaauw and Farooqi 2015). The amount of leptin seems to be related to fat mass quantity

(Van Der Klaauw and Farooqi 2015),. in fact, most obese individuals develop leptin disorders, typically presenting elevated circulating leptin levels and decreased leptin sensitivity, thus enhancing appetite (Pan et al. 2014). However, if in most cases it is obesity that causes leptin disorders, vice versa in 1%–5% of patients it has been found that it is some genetic mutations that reduce production or biological activity of leptin, thus helping to develop severe obesity (Van Der Klaauw and Farooqi 2015). However, bad behavioural habit such as excessive caloric uptake or not sufficient physical activity (energy expenditure) are by far the most common causes of weight gain (Van Der Klaauw and Farooqi 2015). Many contextual elements have an impact on weight-related behaviours and weight status; among the most important and well documented there are social economic status, geography, food preferences, physical and social environment, gender, age, cultural identity, and family composition (Williams et al. 2015). In particular, social economics advantages and level of education have been inversely related with obesity. Furthermore, family, friends and social context may contribute to the bad-eating habits, for instance having parents with bad nutrition habits increase the probability to develop obesity (Williams et al. 2015).

1.1.3 - Epidemiology

According to the World health Organization (WHO) in 2016, 39% of women and 39% of men in the world aged 18 and over were overweight (WHO 2016). With regards to Italy, the average BMI in 2014 was 26.0 $kg \cdot m^{-2}$, the percentual of overweight and obese people (BMI >25) were 64% and obese alone (BMI>30) was 23.7%. Men with BMI>25 were 68.7% and women 59.5%, whereas obese men and women were 22.5% and 24.8% respectively (WHO 2014). One of the most important studies about obesity prevalence (Ng et al. 2014), using the age-standardized BMI in men and women >20 years, found instead a prevalence of obesity of 18.6% in men and 17.7% in women in Italy and 20.5 and 21.0% in men and women, respectively, in western Europe (see Fig.1.1.3 A and B).

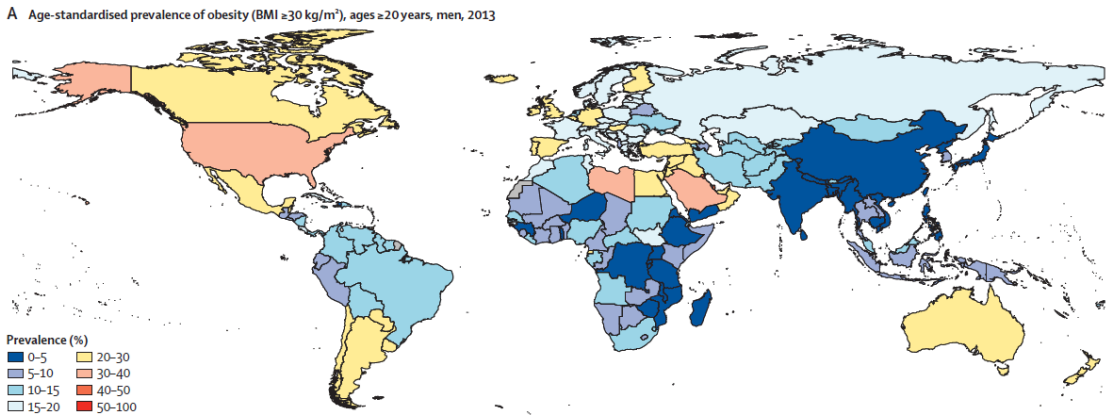


Fig.1.1.3 A – Age-standardized prevalence of obesity (BMI ≥30 kg/m²), ages ≥20 years, men, 2013 (Ng et al. 2014).

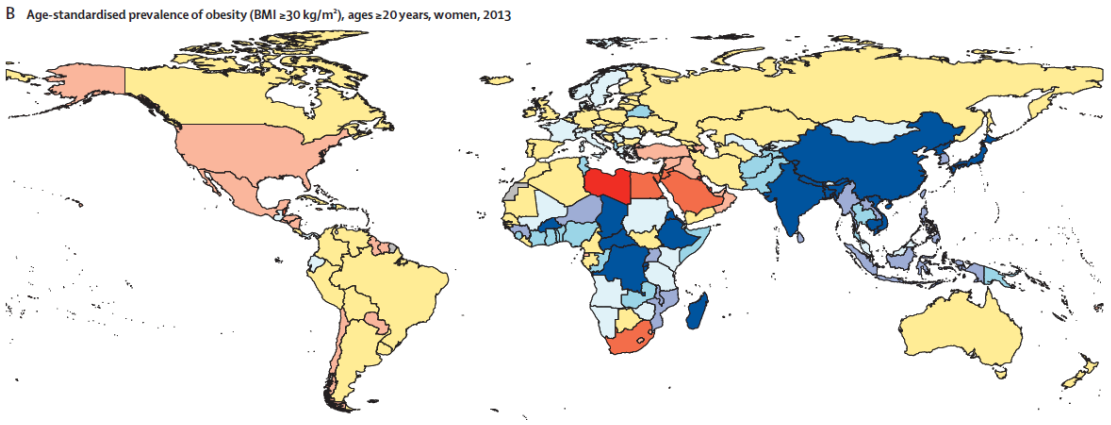


Fig.1.1.3 B – Age-standardized prevalence of obesity (BMI ≥30 kg/m²), ages ≥20 years, women, 2013 (Ng et al. 2014).

According to the previous authors (Ng et al. 2014), the prevalence of obesity and overweight has risen in the past 30 years, with distinct regional trends; in developed countries, the obesity increasing trend attenuated in the last years, but has continued to rise in developing countries (see Fig.1.1.3 C). In light of these data, obesity should be considered a major public health issue which leads to important health risks in many countries all over in the word and face this epidemy should be a priority for governs. Research on this topic might help to outreach the institutions word wide to intervene against the major determinants of obesity such as excessive caloric intake and physical inactivity.

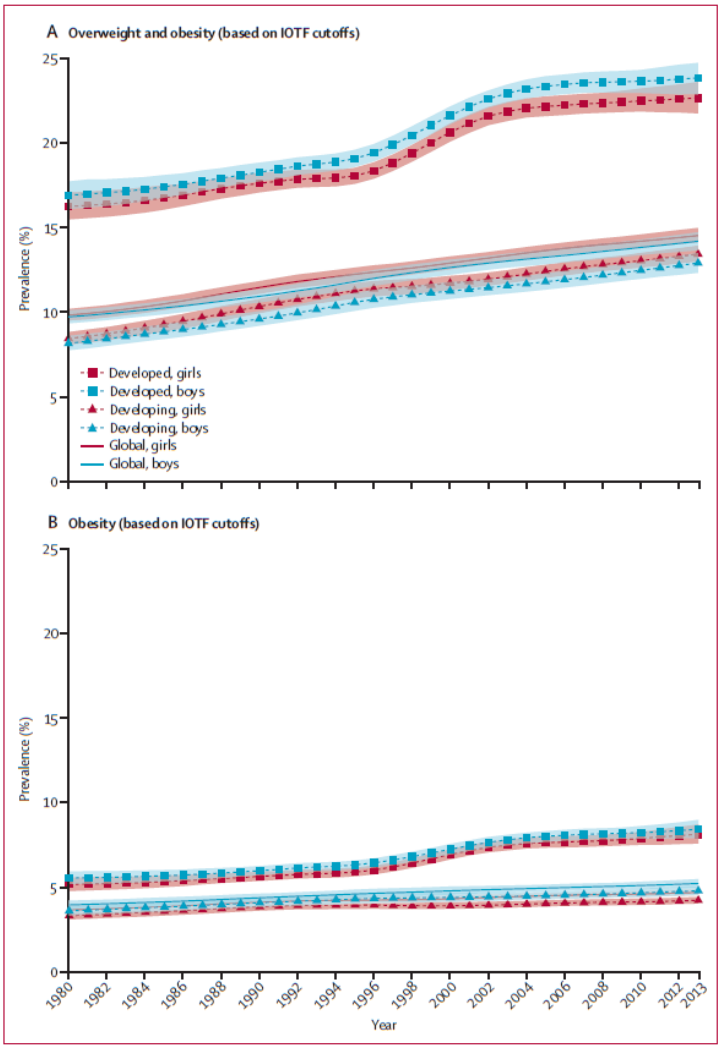


Fig.1.1.3 C – Age-standardised prevalence of overweight and obesity and obesity alone, ages ≥20 years, by sex, 1980−2013 (Ng et al. 2014).

1.1.4 - Health consequences

Obesity is a mayor risk factor for many pathologies such as premature mortality, hypertension, dyslipidaemia, insulin resistance, type 2 diabetes, cardiovascular disease, stroke, certain cancers, liver and gall bladder disease, kidney disease, sleep apnea syndrome and sedentary behaviours (contributing to a spiral of obesity) (Wyatt et al. 2006; Canoy and Buchan 2007; Williams et al. 2015; Çakmur 2017) (Fig. 1.1.4). In addition, obesity could lead to psychological and sociological consequences such as depression, emotional and behavioural disorders and low confidence. Overweight and obese children and adults are more likely subjects to stigma, teasing, and bullying which can have impact on emotional and physical health and performance (Hunger and Major 2015; Bass et al. 2017). The complexity of the causes and potential consequences of obesity

makes us understand that a successful intervention should be based on several aspects.

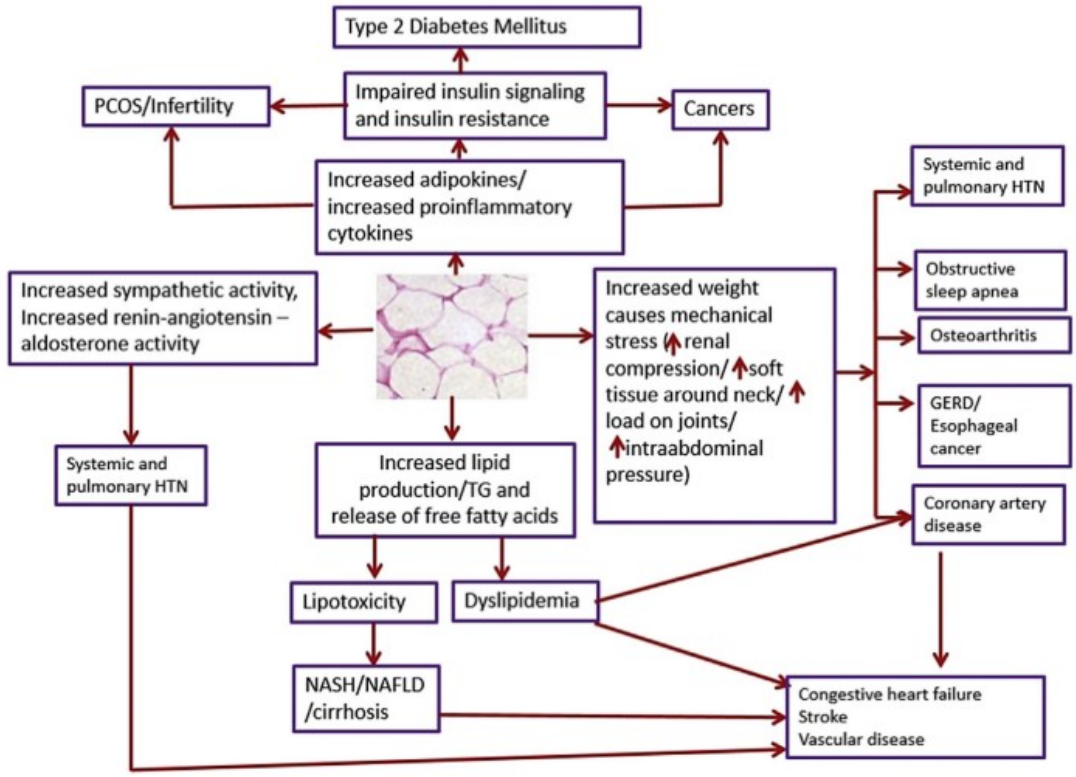


Fig. 1.1.4 - Pathways through which obesity leads to comorbidities(Upadhyay et al. 2018). GERD, Gastroesophageal re- flux disease; HTN, Hypertension; NAFLD, Nonalcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis).

1.1.5 – Metabolic limitation associated to obesity

In fasted state, the big part of lipids used for energy purpose come from adipose tissue. Fatty acids are first release from triglycerides, stored in adipose tissue, then exported into systemic circulation and finally delivered to skeletal muscle and other tissues for oxidation (Horowitz 2001). Therefore, the liberation of fatty acids from adipose tissue is limited by the rate of triglyceride lipolysis and adipose tissue blood flow (Horowitz 2001).

Epinephrine, norepinephrine and insulin regulate the lipolytic activity through a cascade of cellular signals, resulting in phosphorylation of the enzyme hormone-sensitive lipase (HSL). Catecholamines promote this process by binding to Beta-adrenergic receptors (Beta 1, Beta 2, and Beta 3), whereas on the contrary catecholamine stimulation of Alfa 2-adrenergic receptors and insulin inhibit HSL activity (Horowitz 2001). The interesting thing is that the lipolysis rate can vary in relation to regional differences in adrenergic and insulin receptor density and

function. Usually, the lipolytic sensitivity to catecholamines is high in intra-abdominal adipose tissue and the antilipolytic effect of insulin is greater in adipocytes of subcutaneous fat. Fatty acids from intra-abdominal adipose are taken up by the liver, and released in the portal circulation, and are thought to be responsible for cardiovascular and metabolic disorders such as diabetes and dyslipidaemia (Horowitz 2001). On the other side, fatty acids from sub-cutaneous adipose tissue have a more direct effect on skeletal muscle metabolism.

At rest, fatty acid circulation is by far greater and, in postprandial state despite a greater insulin response, normal suppression of lipolysis from adipose tissue is attenuated in persons with abdominal obesity than in lean subjects (Martin and Jensen 1991). On the contrary abdominal obese people have an attenuated increment of lipolysis during exercise (Horowitz 2001); beta-Adrenergic stimulation increase lipolytic rate during exercise (Arner et al. 1990) and lipolytic sensitivity to beta-agonists is compromised in people with abdominal obesity as a consequence of their lower density of beta 2-adrenergic receptors in subcutaneous adipose tissue (Reynisdottir et al. 1994). Even though it might look like counterintuitive, the lower lipolytic sensitivity to catecholamines in people with abdominal obesity is somehow “beneficial” for metabolism, helping to maintain a match between fatty acid availability and oxidation. The other side of the coin however, is that during exercise the lower fatty acid availability may impair the ability to tolerate the effort, thus alimenting the sedentary behaviour (Horowitz 2001). To counteract the low availability of fatty acid during exercise, individual with abdominal obesity tends to accumulate a big amount of intramuscular triglycerides which may facilitate fatty acids uptake to the muscle (Simoneau et al. 1999); but an excessive storage of intramuscular triglyceride is directly related to insulin resistance which can bring to metabolic syndrome or even type two diabetes (Goodpaster et al. 1997).

In addition, abdominal-obese people have been found to have low levels of carnitine palmitoyl transferase-I (CPT-I) activity as well as reduced activities of some other key mitochondrial oxidative enzymes in skeletal muscle (Simoneau et al. 1999). This may suggest that obese people with abdominal obesity have a reduced capacity to use fat as a fuel, which may further explain the accumulation of intramuscular triglycerides thus contributing to their insulin resistance (Goodpaster et al. 1997).

To sum up, we have seen that all the deleterious consequences of obesity, and in particular abdominal obesity, are the results of a series of physiological compensations that on one side allow the organism to adjust the metabolism but on the other side create another decompensation alimenting a vicious circles.

1.1.6 - Interventions

Preventing obesity allows us to avoid all these potential deleterious consequences and it is the first option of course. Therefore, it is important to outreach families and society to educate children to a proper lifestyle (Rankin et al. 2016). Medical or surgical treatments are in general considered only in case of severe obesity or particular pathological conditions; on the other hand conservative intervention, taking into account behavioural and if it is possible social aspects, is always recommended either alone or beside the medical/surgical treatment (Williams et al. 2015). According to the American College of Sport Medicine (ACSM)'s position stand (Donnelly et al. 2009), moderate-intensity physical activity between 150 and 250 $min \cdot wk^{-1}$ is effective to prevent weight gain, but it provides only modest weight loss. Greater amounts of physical activity (>250 $min \cdot wk^{-1}$) have been associated with significant weight loss without diet restriction. Intervening adding to physical activity also moderate but not severe diet restriction, only 150 to 250 $min \cdot wk^{-1}$ of moderate intensity training are enough to promote weight loss. Adding resistance training to moderate intensity endurance training can helps to further loss fat mass and enhance fat free mass (Donnelly et al. 2009). Physical activity and diet control are always recommended together (Donnelly et al. 2009), but it seems that inducing energy depletion by diet increases appetite more than the same amount of energy deficit provoked exclusively by exercise (Thivel et al. 2018).

Taking everything into consideration, it is clear that physical activity is a pillar in weight management programs, for this reason it is vital to know the exercise tolerance of obese people in order to correctly prescribe the training programs.

1.2 – Exercise tolerance in obese people

Even though obesity has been traditionally associated with impaired cardiorespiratory fitness (low $V'O_2$ max), several studies (Deforche et al. 2003; Lazzer et al. 2013; Bernhardt and Babb 2016) demonstrated that healthy obese adults did not show cardiorespiratory deconditioning. Therefore in obesity the

problem is not the absolute cardiorespiratory capacity, which is likely similar to normal-weight people, but the cardiorespiratory capacity in relation to the body weight and lean mass (Deforche et al. 2003; Lazzer et al. 2013; Bernhardt and Babb 2016). Similar conclusion has been reached in obese adolescents as well (Hansen et al. 2014), V'O_2peak seems to be reduced if expressed normalized by lean mass, but cardiopulmonary anomalies during maximal exercise testing needs further study to be confirmed. A further limiting factor in the capacity to exercise in obese, is represented by dyspnoea. Indeed, many obese subjects reported increased perception of breathlessness as a main cause of discomfort during CPET (cardio-pulmonary exercise test) and steady-state exercise measurements (Bernhardt and Babb 2016). This is likewise due to increased chest stiffness that excessively strain respiratory muscle (Dempsey et al. 2006; Salvadego et al. 2017; Alemayehu et al. 2018; Phillips and Stickland 2019).

Beside the cardiopulmonary capacity, the ability to generate force is another important factor that contribute to exercise tolerance and quality of life. Maximal absolute force is not impaired in obese, on the contrary sometime it is improved compared with lean sedentary subjects (Lazzer et al. 2013; Maffiuletti et al. 2013). Nevertheless, it has been hypothesized that excessive fat accumulation between and within muscle fibres in obese induces alterations within skeletal muscle, decreasing relative strength, relative power, and premature volitional fatigue (Bollinger 2017). It has also been hypothesized, that the above mentioned muscle contractile dysfunction contributes to alter movement patterns by increasing synergist muscles effort and altering the recruitment patterns (Bollinger 2017). This hypothetical biomechanical alteration, if confirmed, might impair CPET and steady state exercise further explaining obese exercise intolerance.

1.3 – Moderate Intensity Continuous Training *vs.* High Intensity Interval Training

Usually moderate intensity continuous training (MICT) is referred as an exercise with a constant intensity which does not overcome the gas exchange threshold (Jones et al. 2010). The exercise intensity below this threshold in fact is defined as "moderate" and describe the intensity in which the slow component of the V'O_2 is not present yet (Jones et al. 2010). In the moderate intensity domain, exercise is relatively easy and could be kept theoretically forever. Above the gas exchange

threshold but below critical power, exercise is considered heavy, this domain is characterized by a $V'O_2$ slow component that represents a progressive loss of skeletal muscle contractile efficiency and it is associated to the fatigue process (Jones et al. 2011). Between the two thresholds, the exercise could be kept very long by a healthy subject despite the accumulated fatigue. Above critical power, a constant intensity exercise will rapidly bring to exhaustion and the duration with which high intensity exercise can be performed before exhaustion is inversely proportional to power/velocity (Poole et al. 1988). However, in pathological condition as in obesity, even below the gas exchange threshold, $V'O_2$ slow components and fatigue could occur, thus leading to premature exhaustion (Alemayehu et al. 2018). Since training above critical power or even above $V'O_2$max intensities, does not permit to last the exercise for a long period (Billat et al. 1994), the recovery after first bout of exercise and further repetitions allow you to prolong the time spent at high intensity in a single training session. This method has been called High Intensity Interval Training (HIIT) and became popular after 1950s with the Olympic champion Emil Zatopek. It have been used for instance by the middle- and long-distance runners to train at intensities close to their own specific competition velocity (Billat 2001a). Whereas programming MICT is relatively easy, HIIT is more complicated due to his many factors to handle (Billat 2001a; Buchheit and Laursen 2013a). Many variables have to be taken in consideration to prescribe an HIIT session (Fig. 1.3.1), at least nine (Buchheit and Laursen 2013a). The intensity and duration of work and relief intervals are the key influencing factors. Furthermore, the number of intervals, the number of series, the between-series recovery durations and intensities contributing to determine the total work performed and the effort (Buchheit and Laursen 2013a). Last but not least, exercise modality (i.e. running vs. cycling) has so far received limited scientific interest but can deeply influence the physiological response of the training. Every manipulation of a single variable has a direct impact on metabolic, cardiopulmonary and/or neuromuscular responses and the variables have interrelated affects, so when more than one variable is manipulated contemporary, responses are more difficult to predict. An objective and relatively easy method to compare different HIIT protocols response, is represented by the time at which the athlete/subject can stay at/or close to VO_2max; HIIT compared with continuous exercises seems to be more affective

in prolong it, even until 14 minutes (Billat et al. 2001). The effect on the time at VO_2max by several HIIT modalities are listed in the review of (Buchheit and Laursen 2013a).

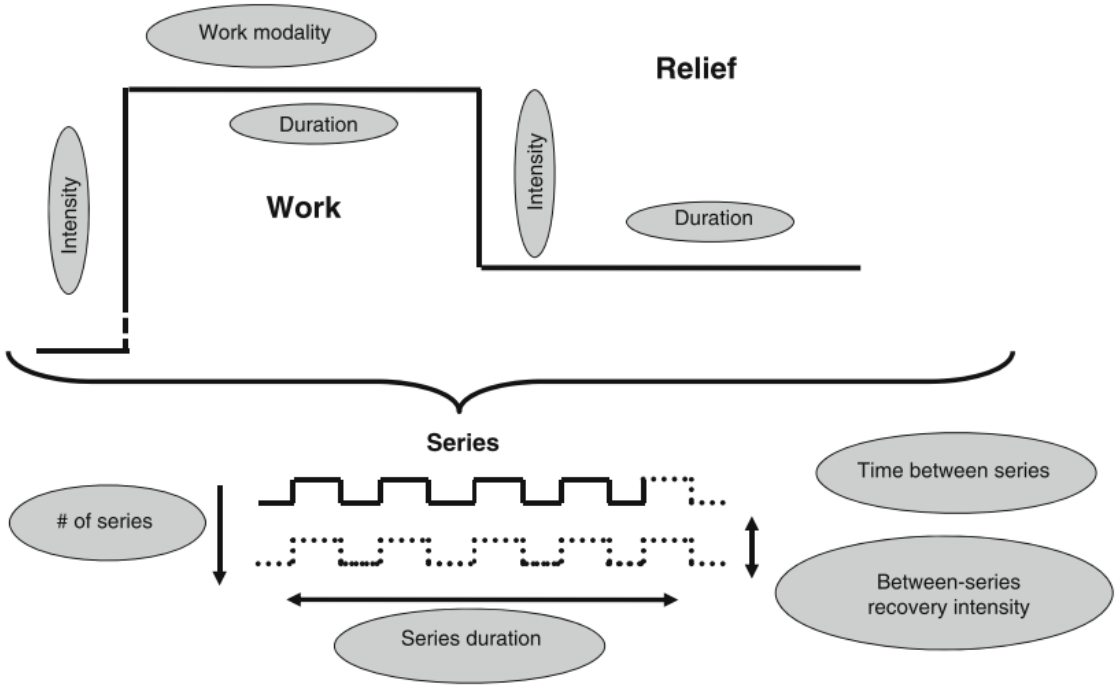


Figure 1.3.1 - Schematic illustration of the nine variables defining a HIIT session (Buchheit and Laursen 2013a).

Generally, HIIT sessions are divided in two families, short interval HIIT (bouts duration < 1 min) and long interval HIIT (bouts duration > 1 min). Short intervals are used for very high intensity, even above the VO_2max velocity/power, whereas longer intervals are more often used for lower intensity (Buchheit and Laursen 2013a). Anyway, HIIT is considered as such if the intensity is kept above the critical power (Morton and Billat 2004). Furthermore, there is also another particular but common HIIT variant which is represented by the spring interval training (SIT), a series of repeated all out efforts (Dupont et al. 2010). It is clear so, that acute physiological response/strain following HIIT is strictly dependent on HIIT set up. Short intervals interval training induce mainly metabolic strains, by eliciting large requirements from the O_2 transport and utilization systems, i.e. cardiopulmonary system and oxidative muscle fibres; it could also be present a certain degree of neuromuscular load depending on how the training is manipulated. It is also possible a large anaerobic glycolytic energy contribution maybe either prolonging a bit the intervals/series or reducing the recovery. Long intervals training is usually considered eliciting metabolic strain as well but also a

pronounced anaerobic glycolytic strain and, in some cases, a certain degree of neuromuscular load. SIT instead, along with the metabolic strain described for short and long intervals, emphasize the anaerobic glycolytic and neuromuscular strain (Billat 2001a, b; Buchheit and Laursen 2013a, b; Tschakert and Hofmann 2013).

Both exercise protocols, MICT and HIIT have been demonstrated as useful to reduce body weight in obese (De Feo 2013). However, weight loss is not the only parameter to consider in prescribing exercise in obese; seems indeed that obese but fit people are not negatively affected by their abnormal adiposity (Brown and Kuk 2015). This concept is known as “fat but fit paradox” or “obesity paradox” (Brown and Kuk 2015). High-intensity training appears to be superior in improving aerobic fitness, but on the other hand, has potentially some disadvantages. First of all, like above discussed it is more complex to prescribe, then in some case the adherence is lower compared with MICT (De Feo 2013), even though other authors claim the opposite (Stork et al. 2018).

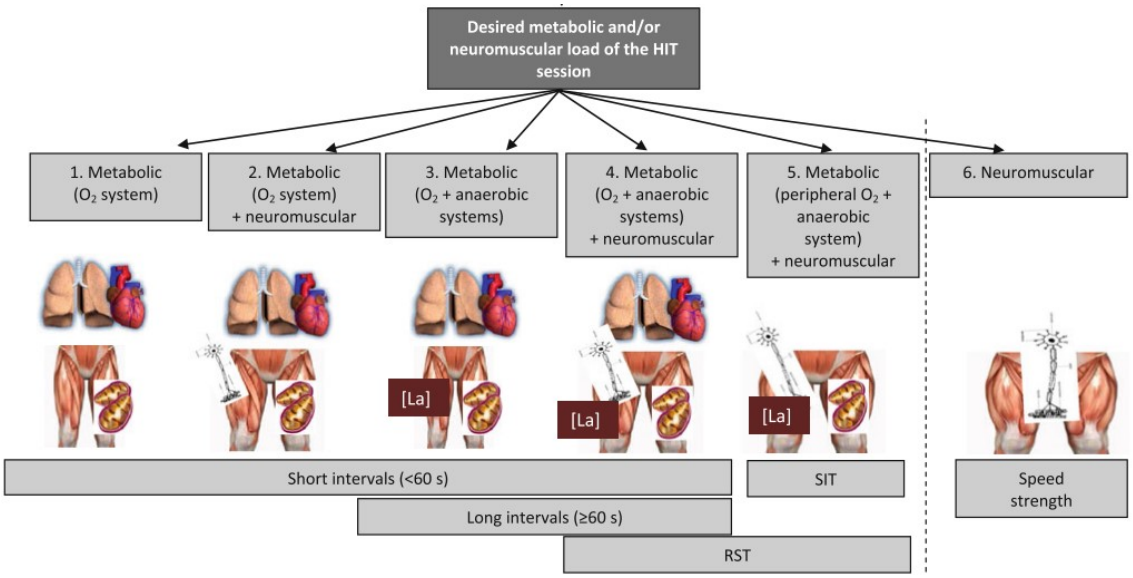


Figure 1.3.2 – Summary of HIIT acute response (Buchheit and Laursen 2013a)
HIT high-intensity interval training; [La] blood lactate accumulation, surrogate of anaerobic glycolytic energy release; RST repeated-sprint training; SIT spring interval training.

1.4 – Programming HIIT through critical power model

The critical power is the threshold that separates power outputs that can be sustained with stable physiological values (i.e. muscle phosphocreatine, blood lactate, and pulmonary oxygen uptake), from power outputs where these variables progressively increase with time until their maximum are reached and exhaustion occurs (Poole et al. 1988). The amount of work that can be done during the exercise above CP (the so-called W') is constant and determine the time to exhaustion, the higher is power output the lower will be time to exhaustion and the time to exhaustion will increase by decreasing the power output following a curvilinear relationship (Poole et al. 1988).

This relationship is typically estimated through three to five separate high-intensity exercise tests on different days, during which a subject sustains a fixed power output trial for as long as possible. When the power outputs of the test done are subsequently plotted against the respective time to exhaustion (Tlim), a curvilinear relationship which tend to an asymptote on the abscissa can be appreciated (Fig.1.4.1a). This asymptote is called "Critical power (CP)", which is measured in watts (W), while the curvature of the power–time relationship represents the work capacity available above CP and it is called W' (measured in kilojoules [kJ]). Luckily, CP and W' can be expressed in a more friendly way plotting work done in each of the separate bouts against the respective Tlim resulting in a linear relationship y = mx c, where the slope m is CP and the intercept c is W' (Fig. 1.4.1b). Once built the linear relationship work-time it is possible to forecast any Tlim relative to any given power output, or vice versa it is possible to calculate the power output needed to bring to exhaustion to any given Tlim.

In the last few years the critical power concept has been applied even in not-constant exercises and so to HIIT. It was proposed first by Morton and Billat (Morton and Billat 2004), and then further developed by the research group of Jones (Chidnok et al. 2012; Skiba et al. 2012, 2014; Clarke and Skiba 2013; Jones and Vanhatalo 2017). Making a long story short, Morton and Billat proposed the new Critical power model for intermittent exercises basing their theory on the simple but brilliant intuition that while above the critical power W' is consumed with a pace given by the differences between the CP and actual power output (as explained by the classic two-parameters Cp model), on the contrary

W’ is recovered by following the same principle during the part of exercise performed below Cp.

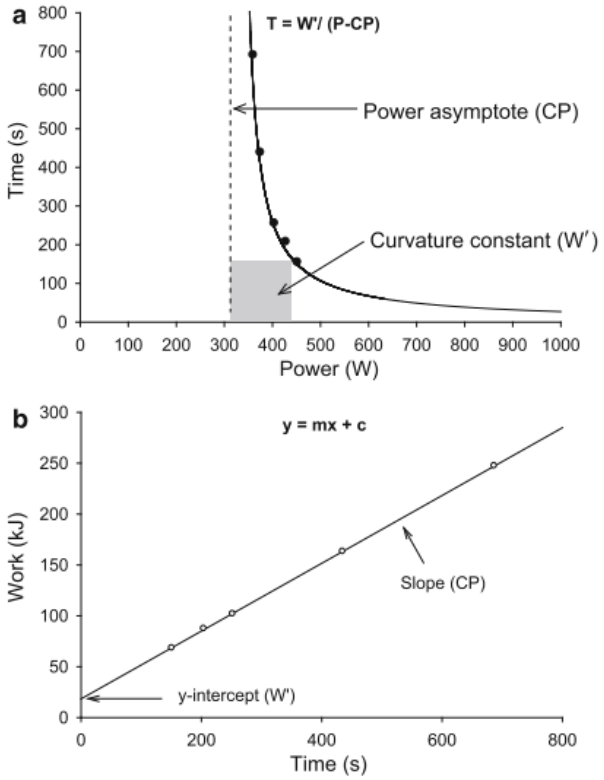


Figure 1.4.1 – a Hyperbolic relationship between power output (x-axis) and time (y-axis), where the critical power is indicated by the power- asymptote and the W’ is the curvature constant; b linearized two- parameter critical power model where total work done is plotted against time. In this permutation, the critical power is given by the slope of the regression and the W’ is the y-intercept. CP critical power, P power, T time, W’ curvature constant of power–time relationship. From (Jones and Vanhatalo 2017)

This concept was subsequently adapted by traying to take in a better account of the physiological variables underlying W’. Indeed, exercise above CP is associated with reductions in muscle phosphocreatine concentration [PCr] and pH (Meyer 1988; Chidnok et al. 2013; Jones and Vanhatalo 2017) and subsequently a reduction of the capacity to keep the exercise above CP (W’) (Ferguson et al. 2010; Skiba et al. 2012, 2014, 2015). The rate with which W’ is consumed is linear, but its recovery rate on the contrary is exponential. In fact, [PCr] increase curvilinearly following an exponential trends (Meyer 1988; Chidnok et al. 2013; Jones and Vanhatalo 2017) and W’ has a similar recovering behaviour (Ferguson et al. 2010; Skiba et al. 2012, 2014, 2015; Jones and Vanhatalo 2017). In other words, the rate with which W’ is recovered is based on

the degree with which it was consumed, the more it is consumed the faster it will be recovered and approaching the complete recovery, the recovery rate will slow down exponentially. Therefore, these researchers proposed an equation that calculates and predicts the remaining W' balance (W'_{bal}) at any time during exercise:

$$W'_{bal} = W' - \int_0^t W'_{exp} * e^{-\frac{t-u}{\tau'_W}} * du \qquad [1]$$

Where t is the time in second from the start of the high intensity exercise; W'_{exp} is the W' expenditure until that time; t-u is equal to the time(s) between segments of the exercise session that resulted in a depletion of W'; $\tau_{W'}$ is the time constant for W' reconstitution, calculated as:

$$\tau_{w'} = 546 * e^{(-0.01DCP)} + 316 \qquad [2]$$

(Skiba et al. 2012)

DCP is the difference between CP and actual power below CP in watts.

Knowing W'bal can be useful to predict the fatigue status of the subject undergoing the exercise. For example, if W'bal is close to zero, the subject will be close to exhaustion.

1.5 - Physiological adaptation to MICT and HIIT

There are several possible long terms adaptations induced by exercise, however they can be dived in two main categories: muscular and cardiovascular adaptations.

1.5.1 – Peripheral (Muscular) adaptations

There can be several kinds of Muscular adaptations as well but only the main ones will be considered in this thesis, such as mitochondrial and capillary density adaptations. Neuronal aspects will be neglected, even because apart from SIT, usually MICT and HIIT are not aimed to that (Fig. 1.3.2).

Mitochondria

Increased mitochondrial content promotes a greater reliance on fat oxidation and a proportional decrease in carbohydrate oxidation (Egan and Zierath 2013). Less glycogen degradation and lactate production at a given intensity, as result increases lactate threshold allowing individuals to exercise for longer durations and higher intensity (Joyner and Coyle 2008).

A single session of MICT or HIIT elicits mitochondrial biogenesis activating signalling pathways such as the phosphorylation of AMPK and p38 MAPK and the expression of PGC-1α mRNA (Gibala et al. 2009). Activation of these pathways, repeated regularly, leads to increase in mitochondrial content relatively rapidly (Coffey and Hawley 2007). Since cellular stress occurs in proportion to exercise intensity, there is strong evidence that higher intensities of exercise elicit greater metabolic signal related to mitochondria adaptations than moderate intensities (Egan and Zierath 2013). High intensity ATP turnover and the following greater carbohydrate oxidation are greater with high intensities, consequently to the accumulation of intracellular lactate, creatine, AMP, ADP and intracellular Ca^{2+} concentration increase proportional to exercise intensity. AMPK activity and CaMKII increase as well and are associated with greater expression of mRNA for PGC-1α, a major regulator of mitochondrial biogenesis (Egan et al. 2010). Thus, high- compared to low-intensity exercise matched for total work is more effective in improving mitochondrial biogenesis (MacInnis and Gibala 2016). Turning on chronic adaptations, few days and few training sessions are enough to appreciate improvements in mitochondrial density in healthy people or athletes and it has been suggested that training volume is an another key factor to increase mitochondrial content in humans (MacInnis and Gibala 2016). Therefore, HIIT is at least as effective as MICT in improving mitochondrial density despite less time requirement; but in addition seems to be more effective in eliciting mitochondrial function improvements (mitochondrial respiration) (Bishop et al. 2014). Actually some studies found that HIIT is more affective in improving mitochondrial density (content) as well (Daussin et al. 2008; MacInnis et al. 2016), but there is not general agreement on the issue (MacInnis and Gibala 2016). Further, some studies reported changes in mitochondrial respiratory function without concomitant changes in mitochondrial content and on the contrary other studies reported changes in mitochondrial content without consequent respiratory function improvement (Bishop et al. 2019). In any case, mitochondrial adaptations are quickly reversed following a decrement or cessation of physical activity (Bishop et al. 2014).

In obese skeletal muscle, it was reported a reduction of mitochondrial function and mass; the mitochondria are smaller and shorter and mitochondrial fission is increased (de Mello et al. 2018). Altered mitochondrial fission is associated with

insulin resistance and mitochondrial dysfunction, which in turn leads to reduced fatty acid oxidation and the inhibition of glucose transport. The only study found in literature, to our knowledge, comparing the effect of two modalities of HIIT and MICT on mitochondrial adaptations in obese, is the study of Bækkerud at al. (BÆkkerud et al. 2016). The authors found improvements in mitochondrial density following HIIT composed by 4x4 min intervals close to V'O_2max, HIIT with 10x1 min intervals at the V'O_2max intensity and MICT at 70% of HR max, without differences between training interventions. The subjects however were just overweight or class I obese. Another study (Tan et al. 2018) sought mitochondrial density adaptations following HIIT in obese but without comparison with MICT; the authors measured an ~27% improvement on mitochondrial density. On the contrary, the study of Menshikova et al 2004 shows that physical activity improves mitochondrial function in obese but it does not elicit mitochondrial proliferation (Menshikova 2004). Although there is plenty of literature comparing training adaptations following HIIT and MICT in healthy subjects, there is a lack of evidence on obese that request more research on the topic.

Capillary density

Muscle capillarization plays a central role in delivery oxygen and nutrients to the exercising muscle. Improved muscle capillarization facilitate oxygen diffusion in the peripheral muscles. Skeletal muscle capillarization requires more time than mitochondria to be manifested in response to exercise training, from weeks to months (Hoppeler et al. 1985), and changes in capillary density appear to be bigger with low intensity exercises (Gliemann 2016). Indeed, the acute response to exercise in angiogenic factors (among others VEGF), was higher following MICT than SIT (Hoier et al. 2013); and after 4 weeks of MICT, capillary density increased but remained unchanged after 4 weeks of SIT (Hoier et al. 2013). In another study (Daussin et al. 2008), following 8 weeks of HIIT and continuous training (CT), the capillary density enhancement was two-fold higher after CT. Other two studies comparing SIT and MICT found similar capillary adaptations following the two modalities (Cocks et al. 2013; Scribbans et al. 2014). Given the low abundance of literature it is hard to get a firmly conclusion, also because most of the study compare SIT and not HIIT with MICT. However, MICT seems to be superior or at least not inferior to HIIT in improving capillary density in healthy subjects.

But what about obese?

Several studies listed on the review of Pattanakuhar et al (Pattanakuhar et al. 2016) demonstrated that in obesity the concentration of oxidative-type skeletal muscle fibres are more expressed and the glycolytic-type muscle fibres are less expressed than in lean subjects. It is well known that the oxidative fibres are more densely capillaried but the study that directly or indirectly measured the capillarization in obese, are just few. Anyway, according to the study of Gavin et al., obese's muscles have lower capillary density and lower capillary-to-fibre area ratio in both type I and II fibres (Gavin 2004). HIIT can be useful to improve capillarity as seen by Tan et al. (Tan et al. 2018) following 18 sessions in 6 weeks, inducing an improvement in capillary contact per fibre and also increasing capillary-to-fibre ratio in both oxidative fibres and glycolytic fibres. Another study (Cocks et al. 2016) concludes that SIT is equally effective at improving skeletal muscle capillarization in obese, despite it requires less time than traditional MICT. A direct comparison between HIIT and MICT on capillary adaptions on obese is missing but since the study of Tan et al. and Cocks et al. have seen similar adaptation than other studies on lean subjects (Daussin et al. 2008; Cocks et al. 2013; Hoier et al. 2013; Scribbans et al. 2014), we can expect that MICT might superior or at least not inferior to HIIT in improving capillary density also in obese subjects.

1.5.2 – Central (Cardiovascular) Adaptations

Actually, muscle mitochondrial capacity to utilise oxygen exceeds maximal capacity of the cardiovascular system in delivering oxygen to the muscle in healthy people (Boushel et al. 2011). Indeed, the capacity to deliver oxygen by the cardiovascular system to the peripheral muscles, is considered the $V'O_2$ mayor limiting factor in normoxia environment (di Prampero 2003; Ferretti 2014). In humans improvements in $V'O_2max$ typically manifest in few weeks after initiating training (Andersen and Henriksson 1977) and its variation is predominately attributable to variation in stroke volume and cardiac output rather than in arteriovenous O_2 difference (Bassett and Howley 2000). The increase in maximum stroke volume observed in response to endurance training is also related to haematological adaptations (Montero et al. 2015). Blood volumes increments are faster than maximal stroke volume improvement and contributes

to increase stroke volume and decreased heart rate during submaximal exercise (Goodman et al. 2005).

In a high-cited study (Helgerud et al. 2007), HIIT was more effective than performing the same total work with MICT in improving V'O_2max. The changes in V'O_2max were strictly related to changes in stroke volume. As confirmation of these findings, the reviews of Bacon et al (Bacon et al. 2013) and Milanovic et al. (Milanović et al. 2015), reported a greater response to HIIT relative to MICT with or without matched work on V'O_2max improvement. The importance of the exercise intensity in improving V'O_2max is further evident when comparing low volume SIT (Gist et al. 2014)/HIIT (Weston et al. 2014) to MICT. SIT/HIIT performed for several weeks are more effective in improving V'O_2max than MICT despite the lower volume and less time requirement. However, the effect of intervals duration on V'O_2max is unclear. A couple of meta-analyses suggested that longer interval bouts, increased V'O_2max more than shorter (Milanović et al. 2015; Wen et al. 2019). In contrast, Helgerud et al. (Helgerud et al. 2007) didn't find significative differences between high-intensity exercise performed as short (15 s) or long (3–4 min) intervals.

If there are numerous evinces that HIIT is very effective in improving V'O_2max, less studies are available on the effects of exercise intensity and HIIT on stroke volume, cardiac output or blood volume. Maximum stroke volume increased more (Helgerud et al. 2007; Daussin et al. 2008; BÆkkerud et al. 2016) or at the same extent (Warburton 2014) following HIIT compared to MICT. After 6 weeks of training the increment in V'O_2max was related to haematological adaptations (Montero et al. 2015). However in the studies of Helgerud et al. and BÆkkerud et al. (Helgerud et al. 2007; BÆkkerud et al. 2016), changes in haematological parameters were not related to the differences in stroke volume between groups. These results demonstrate the predominant contribution of haematological adaptations to any increase in V'O_2max induced by endurance training, but it is unclear if the adaptations are influenced by the training intensity or modalities. It is tempting to suggest that, high-intensity exercise has a greater effect on central adaptations than MICT (Daussin et al. 2008), especially on maximal stroke volume and cardiac output, however, it is still into question the importance of exercise intensity for haematological adaptations.

As in lean subjects, the effect of HIIT and MICT on Obese people's $V'O_2max$, as well as lean subjects, have been widely studies. A large randomized control trial of different intensities of continuous exercise demonstrated greater $V'O_2max$ increases in response to 24 weeks of exercise performed at 75% of $V'O_2max$ compared to exercise performed at 50% of $V'O_2max$ (Ross et al. 2015). HIIT was more effective in improving $V'O_2max$ relative to MICT in several studies (Fisher et al. 2015; Lanzi et al. 2015; BÆkkerud et al. 2016; Lazzer et al. 2017). Further confirm comes from the meta-analysis García-Hermoso et al. (García-Hermoso et al. 2016) which indagated on the effect of HIIT on cardio-metabolic risk factors and aerobic capacity in obese. The meta-analysis included 6 articles published before November 10, 2015, indagating on aerobic capacity adaptations following HIIT, compared with continuous training and concluded in favour to HIIT. In contrast to the above-mentioned studies, when HIIT was carried out in real world setting, and the exercise sessions undertaken in a community park (Lunt et al. 2014) , it was only modestly effective in improving the cardiorespiratory fitness in a cohort of overweight/obese subjects. The authors hypothesized a reduced adherence to the exercise program compared with a more enjoyable walking activity (Lunt et al. 2014).

1.6 – Aims of the thesis

Obesity is associated with health issues and limited exercise tolerance. Luckily, it is enough to lose 2-3% of weight to have a significative general reduction in many risk factors and the guidelines recommend 10% weight loss to have a general improvement in cardiovascular disease(Donnelly et al. 2009). However, recent evidence suggests that weight loss does not necessarily always improve health. In some case, obese but metabolically healthy or highly fit people, are not subjects to an increased risk for health problems (Brown and Kuk 2015). Indeed it seems that obese but fit people have a lower mortality ratio compared with lean but sedentary and unfit people; this paradox is known as "Obese paradox" or "Fit but Fat" paradox (Brown and Kuk 2015). It is clear so, that sedentary behaviour is dangerous as much as obesity or even more (Same et al. 2016). Obese people as a result of their increased body weight, undergo a limitation in doing exercise; this limitation increases sedentary behaviour that turn increases body weight and impairs physical fitness, that finally further increases health problems. To break this vicious circle, it is important to lose weight but also to improve physical

fitness. Knowing the major factors limiting exercise in obese may help to implement a better training program specifically suited for this kind of population. HIIT is a nice training tool, apparently more complete than MICT since it is useful to elicit both peripheral/muscular adaptations and central/cardiovascular adaptations (MacInnis and Gibala 2016). This make it as a good candidate for the training also in obese people.

Therefore, the aim of the thesis is:

1) bringing light on the limiting factors of aerobic performance in obese subjects, with particular focus on central vs peripheral factors;
2) compare the effects of HIIT and MICT on functional characteristics such as V'O2peak (central) and fat oxidation (peripheral) and mitochondrial characteristics (peripheral) in obese;
3) evaluate the maintenance of the observed changes following the two training modalities a few months after the termination of the interventions, in order to understand which training type has potentially longer-time benefits.

It has further added an appendix to the thesis.

Design an affective HIIT protocol in improving both maximal cardiovascular and peripheral functions is not easy due to numerous variables to manage. It is important that the training session is effective as well as time efficient, since "the lack of time" is one of the major causes of physical inactivity (Stork et al. 2018). It is believed that for an optimal training stimulus it is necessary to spend at least several minutes per session in the "red zone", which generally means reaching at least 90% of maximal oxygen uptake (Midgley and Mc Naughton 2006; Buchheit and Laursen 2013a). Developing an efficient training session might help either improve effectiveness in prolonging that time or reduce the time required to achieve the set goals.
With respect to the above-mentioned reasons it has been tried to develop a new HIIT protocol called High Intensity Decreasing Interval Training (HIDIT). The aims of the appendix of this thesis was to compare the time that the oxygen uptake remained above 90% of its maximum in a single session of HIDIT and two others common HIIT modalities.

CAPTER II

Exercise limiting factors in Obesity

Adapted from:

Vaccari F, Floreani M, Tringali G, De Micheli R, Sartorio A, Lazzer S.

Metabolic and muscular factors limiting aerobic exercise in obese subjects.

Eur J Appl Physiol. 2019 Aug;119(8):1779-1788. doi: 10.1007/s00421-019-04167-w

2.1 – Introduction

As already discussed above, obesity is associated with some cardiovascular diseases, such as coronary heart disease and abnormalities in heart rate properties (Poirier et al. 2006; López-Jiménez and Cortés-Bergoderi 2011; Alpert et al. 2016; Ortega B et al. 2016). At rest, obese subjects are reported to have greater blood and systolic volumes and cardiac output than lean controls subjects (Vella et al. 2012). These alterations, which are required to supply the increased metabolic demands at rest, might lead to deleterious consequences, including the development of left ventricle hypertrophy and of abnormalities in diastolic and systolic phases (López-Jiménez and Cortés-Bergoderi 2011; Ortega, Lavie, and Blair 2016; Alpert, Omran, and Bostick 2016; Poirier et al. 2006). In severe obese people (BMI≥35 kg/m^2) this deleterious mechanisms are even more pronounced causing higher risk of cardiovascular disease and mortality (Ortega, Lavie, and Blair 2016). Blessedly, physical exercises and nutritional programs are able to reduce this risk and even reverse obesity (Donnelly et al. 2009). Physical exercises, in particular those aimed at improving the cardiorespiratory fitness level, are reported to be extremely effective (Ortega B et al. 2016). Although both sedentary obese and lean subjects have similar peak aerobic power ($V'O_2peak$) (Lazzer et al. 2013; Vella et al. 2012), sedentary obese subjects show greater peak stroke volumes (SV) and cardiac output (CO) values than the lean individuals (Vella, Paul, and Bader 2012). By contrast, obese subjects do not reach the same maximal exercise work load of their lean counterparts (Lazzer et al. 2013).

Furthermore, obese people are reported to have an increased lipids content between and within skeletal muscle fibres and between muscles (Malenfant et al. 2001; Bollinger 2017). This lipid accumulation could impair muscle quality and functions. The effect of obesity on muscle contraction properties has been investigated by several authors (Maffiuletti et al. 2013; Bollinger 2017; Tallis et al. 2018). According to these reviews, obese subjects result to have greater muscle mass, absolute force, and abundance of type II muscle fibres compared with lean controls. Moreover, obese subjects show lower abundance of type I fibres and different muscle architecture by increasing fascicle pennation angle. However, it is still controversial whether their normalized muscle force, fatigability, metabolic and recovery capacity levels result actually impaired.

In whole body exercise (e.g. running, cycling) maximal oxygen uptake is mainly limited by cardiac output rather than by the oxygen extraction and utilization capacity of the muscle (Blomqvist and Saltin 1977; di Prampero 2003). However, when the exercise is performed with small muscle mass, (e.g. single leg knee extension exercise, KE), the muscle oxygen uptake isn't limited by central circulation, but mainly by peripheral factors (i.e. at the muscle level), such as peak muscle perfusion, oxygen diffusion or mitochondrial respiratory capacity (Andersen and Saltin 1985). Indeed, greater peak muscle oxygen uptake has been found during KE than during cycle ergometer exercise(CE) (Andersen and Saltin 1985; Richardson et al. 1999). This last finding further supports the fact that central vascular factors might not represent the main source of limitation during exercises involving small muscle mass.

Taking into account all the above mentioned cardiovascular and muscular properties potentially impaired, it is not surprising that exercise tolerance is reduced in obese. The aim of the present chapter was to understand the role of central and peripheral factors in limiting the maximal aerobic performance in obese subjects. We hypothesized that obese subjects might have lower muscle fatigue (i.e. muscle ability to produce force immediately after the end of the exercise) following CE than following KE test. A similar result may help to further support the conjecture that whole body maximal exercise performance might be constrained more by central rather than peripheral factors in obese population.

2.2 – Subjects and methods

Subjects

Fifteen obese patients (OB, 12 males and 3 females) and thirteen normal weighted subjects (CTRL, 10 males and 3 females) participated in this study. Subjects were recruited from the Division of Metabolic Diseases, Italian Institute for Auxology, IRCCS, Piancavallo (VB) Italy. OB had a body mass index (BMI) above 30 $kg \cdot m^{-2}$, whereas CTRL had BMI values included between 20-25 $kg \cdot m^{2}$. OB and CTRL subjects recently included in weight management programs, or suffering from cardiovascular, respiratory, neurologic, muscular-skeletal, metabolic and/or endocrine diseases or taking any drugs known to influence energy metabolism (including beta-blockers) were excluded.

The experimental protocol was approved by the local Ethics Committee of the Italian Institute for Auxology (Milan). Before the study began, the purpose and objectives were carefully explained to each subject and written informed consent was obtained.

All subjects filled out a physical activity-related questionnaire (IPAQ-SF) (Craig et al. 2003), administered to exclude potential volunteers who were engaged in any continuous moderate or intense physical activity more than 20 minutes over than once a week, which would be indicative of a moderate physical activity level (ACSM 1991). All subjects from both subgroups were considered as “sedentary”.

Experimental protocol

Before the start of the study, the subjects were familiarized with the equipment and the procedures. Subjects were asked to avoid strenuous exercises the day before the test. The subjects came in the laboratory in two different days. The first day, after the anthropometric and body composition measurement, they approached knee extensor ergometer (KE) or Cycle ergometer (CE) and performed the incremental exercise. The two tests were administered in random order and separated by at least 2 days. Each ergometer was appropriately modified in order to perform, in the same position, the maximal voluntary contractions (MVC) trials, before and immediately after the end of the incremental test, to quantify muscle fatigue and every three minutes until 12th minute to quantify the muscle fatigue recovery.

Anthropometric characteristics and body composition

Body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board. BMI was calculated as body mass (BM, kg) stature^{-2} (m). Body composition was measured by bioelectrical impedance (BIA, Human IM Plus; DS Dietosystem, Milan, Italy), according to the method of Lukaski et al. (Lukaski et al. 1986). Fat mass (FM) and fat-free mass (FFM) were calculated with equations derived either in obese people of different ages and BMI (fat-specific formulae) or in normal weight people, by using a two-compartment model (Gray et al. 1989).

Muscle thigh volume (VolTM) was estimated by thigh length, circumference and skinfold measurements, following the Jones and Pearson's method, corrected by the equation provided by Layec et al.' work (Layec et al. 2014)

$$V = \cdot 0.866 \cdot \left\{\left(\frac{L}{12\pi}\right) \cdot (C1 + C2 + C3) - \left[\frac{S - 0.4}{2}\right] \cdot L \cdot \left[\frac{C1 + C2 + C3}{3}\right]\right\} - 1750$$

where L refers to the thigh length; C1, C2, and C3 refer to the proximal, middle, and distal circumferences, respectively; and S is skinfold thickness of the thigh. The thigh length was measured from the great trochanter to the lateral femoral epicondyle. Skinfold thickness was measured at three sites, medial anterior and lateral, at the midpoint of the tight using a Holtain Caliper (Holtain Ltd, Crymych, UK). Anthropometric characteristics of the subjects are shown in Table 1.

Maximal Voluntary Contraction and isometric fatigue.

Maximal isometric force of the knee extensor muscles of the right limb was determined on a custom-built knee extension (KE) ergometer (Salvadego et al. 2011) supplied with a force sensor. The subject was seated constrained on an adjustable seat by a safety belt with the legs hanging vertically down. A strap, connected in series to the force sensor (TSD121C, BIOPAC Systems, Inc., Goleta, CA), was tightened around the subject's right ankle. The force sensor was fixed in series to a steel frame. The position of this frame was set and blocked with a chain prior the execution of isometric knee contractions in order to obtain a knee angle of 110 degrees. The distance from the rotation center of the knee to the strap (point of force application) was measured to determine the lever arm. The force (Newton) measured by the sensor was multiply by the lever arm (meters) in order to obtain the Torque (Nm) of the MVC. The subjects performed the MVCs three times with 3-minute rest intervals. The trials that differs from the other more than 10% were excluded and repeated. After the MVCs the chain was removed, and the lever left free to allow KE exercise (see below). Immediately after the end of exercise, the lever was quickly blocked out with the chain in the previous position, and the MVC was repeated, first immediately after and then every 3 minutes during a 12 min period.

The same procedure was repeated for the cycle ergometer exercise (CE) with a modified Technogym Bike Recline (Xt Pro 600). A force sensor (TSD121C, BIOPAC Systems, Inc., Goleta, CA) with a scaffold has been added to the bike. The scaffold with the force sensor was easily fixed for the MVCs trials, afterward

set aside to avoid annoying pedaling and finally repositioned very quickly after the exercise. The subject was seated constrained on an adjustable seat by a safety belt with the legs hanging vertically down. A strap connected in series to a force sensor was tightened around the subject's right ankle. The position was set in order to obtain a knee angle of 110 degrees. In the same way previous described for KE, the subject performed three MVCs, the incremental exercise and again the MVCs immediately after and every 3 minutes during a 12 min period.

Incremental exercises

Two incremental exercise protocols were carried out under close medical supervision and 12-lead ECG monitoring; standard safety procedures were followed. The tests were carried out on a modified Technogym Bike Recline (Xt Pro 600) cycle ergometer (CE) and on a custom-built knee extension (KE) ergometer (Salvadego et al. 2011). The two tests were administered in random order and separated by 2 days. For CE, the subjects were seated constrained on an adjustable seat by a safety belt, the test comprised a 5-min warm up cycling at 60 W for 5 min at 60 revolutions·min-1; the subjects were asked to maintain this pedaling rate throughout the test. The work rate was then increased every min by 20 W starting from 0 W until volitional exhaustion.

For KE, a graded protocol was carried out as described by (Salvadego et al. 2011). Subjects were constrained on an adjustable seat by a safety belt, which anchored the angle of the hip at ~90°. Subjects pushed on a padded bar attached to a lever arm extending the lower part of the right leg from ~90 to ~170° flexion. This type of exercise confines muscle contractile activity mainly to the quadriceps femoris muscle, whereas the return to the starting position is brought about passively. After an initial 2 min of continuous KE exercise at 27 W the work rate was increased every min by 7 W until volitional exhaustion. Throughout the exercise, subjects maintained a KE frequency of about 40 min-1 with the aid of a metronome. Both CE and KE tests were terminated when the subjects were unable to continue at the required frequency despite vigorous encouragement by the operators.

"Peak" values of the investigated variables were calculated during the last ~20 s of the exhausting work rate. Heart rate (HR) was recorded by a dedicated device (Polar Electro, Oulu, Finland). O_2 uptake ($V'O_2$) and CO_2 output (VCO_2) were

determined by means of a metabolic portable unit (K5, Cosmed, Italy). Expiratory flow measurements were performed by a turbine flowmeter calibrated before each experiment by a 3 L syringe at three different flow rates. Calibration of O_2 and CO_2 analyzers was performed before each experiment by utilizing gas mixtures of known composition (16.00% O_2; 4.00% CO_2). The gas exchange ratio (R) was calculated as $V'CO_2 \cdot V'O_2$-1.

Cardiac output (CO) was monitored continuously by bioimpedance method (PhysioFlow, Manatec, France), following the procedure described in a previous study (Charloux et al. 2000). This method has been validated during maximal incremental exercises (Richard et al. 2001), and also used in overweight (Palmieri et al. 2006) and obese subjects (Vella, Ontiveros, and Zubia 2011; Vella, Paul, and Bader 2012; Charloux et al. 2000). PhysioFlow and metabolimeter values were synchronized and mediated every 10 seconds. At the end of every step, the last two measurements were taken in account and mediated. Arteriovenous oxygen difference (a-vO_2 diff.) was estimated by dividing CO (l min-1) into $V'O_2$ (l min-1) and multiplying by 100. The a-vO_2 diff. above 19 ml l00 ml-1 were considered not physiological and excluded with the correspondent CO values.

Statistical analyses

Statistical analyses were performed using SPSS 20.0 software (IBM, Chicago, USA), with significance set at $P<0.05$. All results were expressed as means and standard deviation (SD). The differences between subgroups (OB vs. CTRL) on anthropometric characteristics, body composition, MVC, Tlim (Time to exhaustion) were compared by means of a Student's t test for unpaired data. Further, Student's t test for unpaired data, was used to compare sex on MVC. The peak exercise values ($V'O_2$, CO, a-vO_2 diff, Load Peak) and MVC_end in percentage of the pre-values, that representing the muscle fatigue immediately after exercise, were analyze by two-ways analysis of variance (ANOVA) between-within factors and sex by covariate. Post hoc comparisons were made using Bonferroni procedure for significant differences. The effect of group, sex, intensity and interactions on the submaximal values ($V'O_2$, CO and a-vO_2 diff) during the two exercises were analyzed with linear mixed, multilevel, growth model, fit by maximal likelihood, taking into account groups and intensity as fixed effect and subjects and intercept as random effects. Bonferroni post hoc was applied as appropriate. For this analysis, statistical power was checked, and the results was

still reliable only if 8 or more subjects per subgroup were presented. Then the post hoc procedures were made, and the figures show, the steps until at least 8 subjects per groups were still present. The trends of the MVCs recovery (percentage of the pre-exercise values) were analyzed by ANOVA for repeated measure, within-between subjects taking in account MVC_end (MVC immediately after the exercise in percentage of pre-values) and sex by covariates.

2.3 – Results

Physical characteristics of OB and CTRL are shown in Table 2.1. OB had significantly greater BM (+90%), BMI (+95%), VolTM (+19%), FFM (+30%), FM (+309%) and %FM (+122%) than CTRL. Absolute MVC was not significantly different between the two subgroups (OB: 242±75 Nm; CTRL: 236±80 Nm; p=0.850), while MVC normalized by VolTM was lower in OB (34.84±7.26 Nm L L-1) than CTRL (43.66±10.79 Nm L-1), p=0.020.

Similarly, Tlim during incremental exercises was significantly lower in OB than CTRL for CE testing (555±108 vs. 673±113 s; p=0.009), while no differences were found for KE testing (497±155 vs. 435±143 s; p=0.27).

Table 2.1 Physical and functional characteristics of subjects.

	Obese (n = 15)	Controls (n = 13)	p
Age (y)	25.20 ± 6.76	26.54 ± 8.24	0.726
Body Mass (kg)	127.60 ± 24.67	68.00 ± 13.11	<0.001
Stature (m)	1.72 ± 0.07	1.76 ± 0.07	0.167
BMI (Kg m^{-2})	43.05 ± 7.54	21.85 ± 3.17	<0.001
Vol_{TM} (L)	6.29 ± 1.16	5.27 ± 0.99	0.020
Fat-free Mass (kg)	70.33 ± 15.42	54.01 ± 9.03	0.003
Fat Mass (kg)	57.31 ± 15.1	13.99 ± 5.38	<0.001
Fat Mass (%)	44.72 ± 6.88	20.08 ± 5.24	<0.001

All values are presented as mean ± standard deviation.
BMI: body mass index; VolTM: Muscle thigh volume.
p: Significance by means of Student's t-test for unpaired data.

Peak values of the main variables determined during CE and KE tests are given in Table 2.2. ANOVA procedures shows that V'O_2peak, HRpeak and COpeak

were not significantly different between the two subgroups, being however significantly higher in CE than KE (+126%, +33% and +46%, respectively, p<0.001).

In CE, V'O_2peak normalized by FFM was 32% lower in OB compared with CTRL (0.038±0.003 vs. 0.056±0.007 L min-1 Kg-1; p=0.001). By contrast, V'O_2peak normalized by VolTM was not significantly different between the two subgroups in KE (0.184±0.447 vs. 0.192±0.442 L min-1 L-1; p=0.657). COpeak normalized by FFM tended to be lower in OB during CE (OB: 0.312±0.100 vs. CTRL: 0.386±0.061 L min-1 Kg-1; p=0.066), while it was not significantly different between the two subgroups during KE (OB: 2.59±0.96 vs. CTRL: 2.29±0.49 L min-1 L-1; p=0.379).

A-vO_2 diff peak (Tab. 2.2) was greater in CE (+44%, p<0.001) than KE. The post hoc analysis found lower values in OB than CTRL in CE (-22%, P=0.012), no differences being found in KE (p=0.510).

Although the two subgroups reached similar V'O_2 peak in CE, workload peak was 15% lower in OB than CTRL (p=0.021), while it was not significantly different between the two subgroups in KE (Tab. 2.2).

The trends of V'O_2, HR, CO and a-vO_2 diff during the incremental tests are shown in Fig. 2.1. Only V'O_2 during CE was significantly higher in OB at each step by average (36%, p<0.001, Fig. 2.1A). No significant differences were found between the two subgroups on V'O_2, HR, CO and a-vO_2 during the incremental tests on KE (Fig. 2.1 B, D, F and H). In addition, all parameters increased significantly as a function of workload.

In OB the MVC decreased significantly immediately after the end of the incremental exercises on CE and KE (-14 and -32%, respectively, p<0.001), the reduction being significantly lower immediately after the CE than KE test (p<0.001) (Fig. 2.2). In CTRL, MVC decreased significantly immediately after the end of the incremental exercises on CE and KE tests (-26 and -30%, respectively, p<0.001), without significant differences between the two tests (Fig. 2).

In addition, the recovery trends of MVC after CE and KE incremental exercises are shown in Fig. 2.3.

MVC increased over time (time effect, p=0.003) without significant differences between the two subgroups (p=0.432), remaining 13% lower than the initial value after 12 min.

TABLE 2.2 Peak values determined during the incremental exercises on a cycle-ergometer (CE) and knee-extension ergometer (KE).

	CE		*KE*		*Significance*		
	Obese	*Controls*	*Obese*	*Controls*	*G*	*E*	*G x E.*
$V'O_2$ (L min^{-1})	2.68 ± 0.68	2.93 ± 0.65	1.26 ± 0.51	1.15 ± 0.26	0.598	<0.001	0.18
HR (bpm)	164 ± 15	177 ± 11	131 ± 22	125 ± 15	0.441	<0.001	0.031
CO (L min^{-1})	20.10 ± 5.42	20.61 ± 4.04	15.77 ± 5.89	12.60 ± 2.45	0.522	<0.001	0.017
a-vO_2 diff (mL L^{-1})	12.05 ± 3.05	15.42 ± 2.26*	9.65 ± 4.71	10.02 ± 1.26	0.040	<0.001	0.017
Load Peak (Watts)	191 ± 38	226 ± 39*	62 ± 13	58 ± 14	0.082	<0.001	0.012

All values are presented as mean ± standard deviation.
$V'O_2$: oxygen consumption; CO: Cardiac Output; a-vO_2 diff: arteriovenous oxygen difference.
Significance by two-way analysis of variance (ANOVA) between-within factors: G: group effect; E: exercise effect; G x E: group x Exercise (interaction) effect;
**: Significantly different between Obese and Control ($p<0.05$) by Bonferroni procedures*

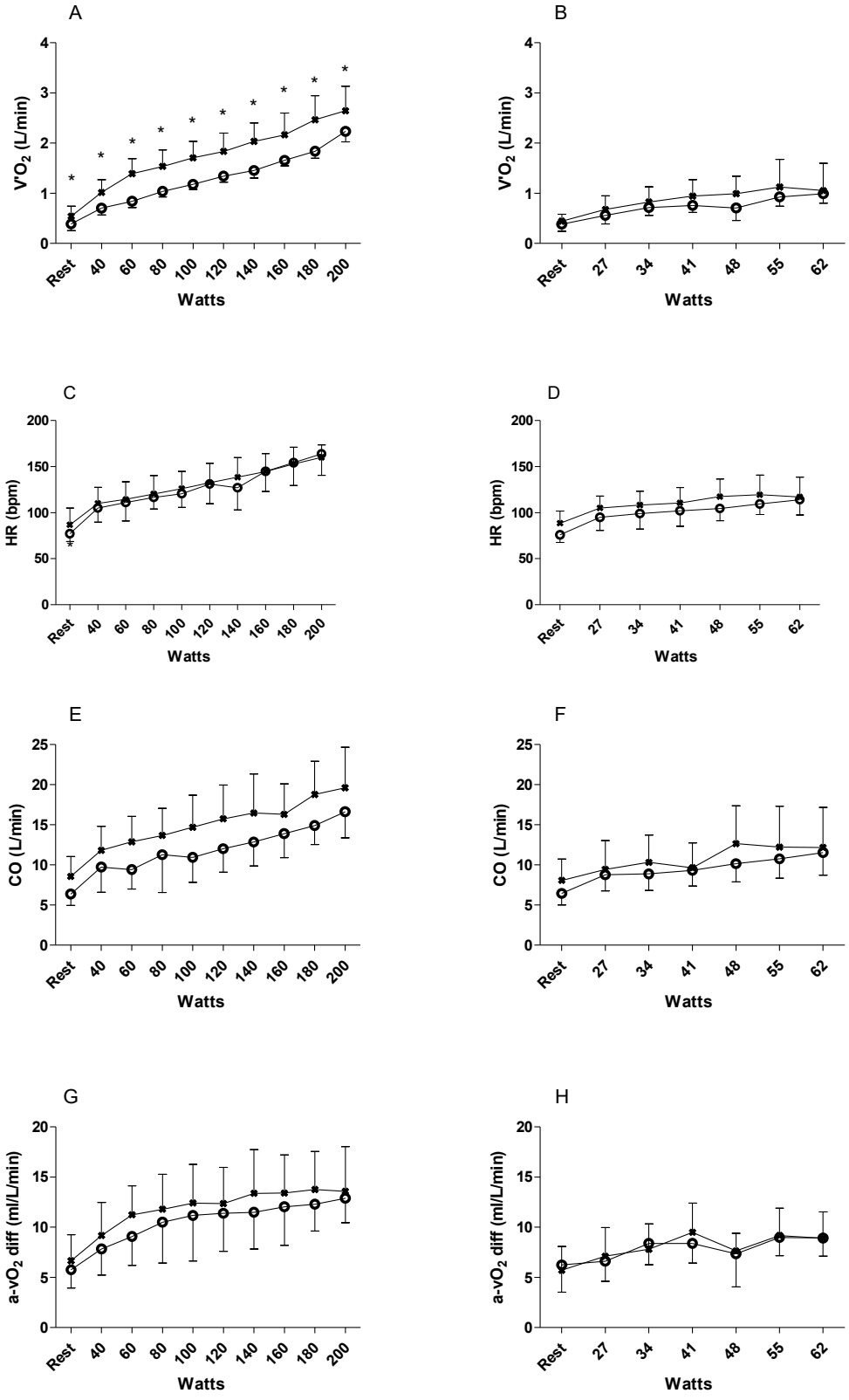


Figure 2.1 - Submaximal values during the incremental exercises on the cycle-ergometer (A, C, E and G) and knee-extension ergometer (B, D, F and H) as a function of work load (W) in OB (-x-) and CTRL (-o-) subjects.

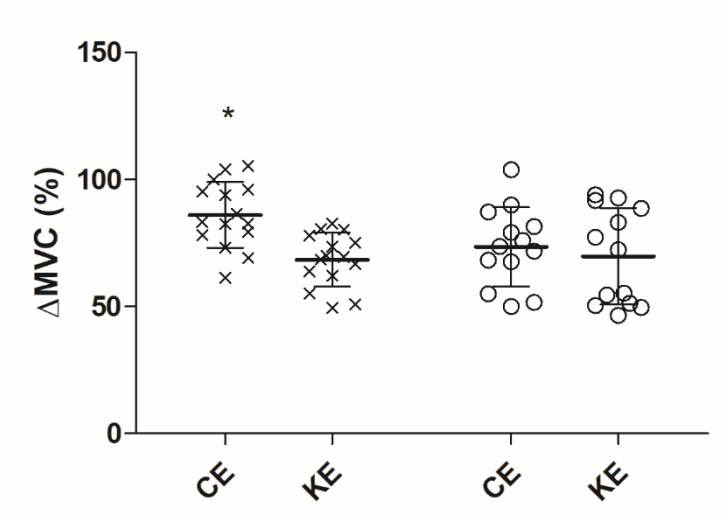


Figure 2.2- Maximal Voluntary Contraction changes in percentage (ΔMVC, %) of pre-values, obtained immediately after the end of the incremental exercises on cycle ergometer (CE) and knee-extension ergometer (KE), in OB (x) and CTRL (o) subjects.

All values are presented as mean ± standard deviation.
**: Significantly different between CE and KE exercises (p < 0.001).*

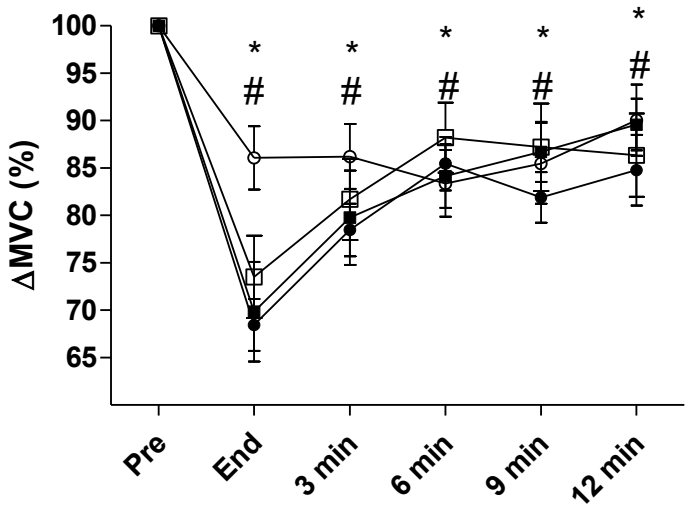


Figure 2.3 - Maximal Voluntary Contraction changes (ΔMVC, %) after 3, 6, 9 and 12 minutes the end of incremental exercises on cycle ergometer in OB (-o-) and CTRL (-□-) subjects, and on knee-extension ergometer in OB (-●-) and CTRL (-■-) subjects.

All values are presented as mean ± standard deviation.
**: Significantly different from pre values obtained after incremental exercises in obese subjects.*
#: Significantly different from pre values obtained after incremental exercises on knee in control subjects.
See statistical paragraph for more details.

2.4 - Discussion

The main results of this chapter are: 1) in OB, the reduction of MVC immediately after the end of the incremental exercise on CE was lower than on KE, while it was similar in CTRL subjects, 2) both in CE and KE, peak values of absolute $V'O_2$, HR and CO did not differ between OB and CTRL, 3) the recovery from fatigue after the end of the two incremental exercises (CE and KE) was not significantly different between OB and CTRL.

Immediately after the completion of the two incremental exercises, MVC decreased by 14 and 32% in OB (respectively after CE and KE tests, $p<0.001$), and by 26 and 30% in CTRL (respectively after CE and KE tests, $p>0.05$). These values were similar to those found by Millet and coworkers in their review about fatigue in ultra-marathon races (Millet 2011). Millet showed that the lower limb MVC decreases as a function of the duration of the running exercise: i.e. the longer the duration of the exercise, the greater the decline in knee extensor muscles force ability. However, the two variables do not follow a linear relationship. Indeed, muscle force production does not overcome the specific threshold of 30-40% MVC loss, even in extremely long race; this finding suggests that some muscle force ability level needs to be preserved, maybe to avoid neuromuscular dysfunction and mobility limitations after exercise. This strategy that is supposed to be implicated in the management of fatigue after exhausting running exercises may be also present after other types of fatiguing exercise tasks, such as KE and CE. Indeed, MVC decrements observed in the present study after the end of the two exercises in CTRL, and after the completion of KE in OB, lied on the lower margin of the fatigued threshold noticed by Millet's group. On the contrary MVC loss experienced by OB after the end of CE was lower (-14%). This finding suggests that muscle function was preserved in OB and hence other factors might have limited the performance during whole body aerobic exercise, in agreement with results from previous studies (Noakes, et al 2004; Noakes 2012; Blain and Hureau 2017). The fact that CTRL showed similar MVC decrements after the end of the two exercises might be due to the important roles that both central and peripheral factors play in the management of exercise tolerance in normal weight individuals (Blain and Hureau 2017).

During incremental exercise on CE, $V'O_2$ peak, HR peak, CO peak and a-vO_2 diff were greater than in KE in both subgroups, as previously observed (Lazzer et al.

2013; Esposito et al. 2010; Salvadego et al. 2013), thus confirming the existence of a cardiac reserve in single leg KE exercise. Blomstrand and coworkers (Blomstrand et al. 1997) showed that in the quadriceps muscle, Krebs cycle and oxygen uptake are more active and pronounced during single leg KE than during CE exercises. These findings confirm that KE exercise could be a better way to evaluate possible peripheral muscle functions avoiding central or cardiovascular constrictions.

The data show in this chapter, absolute values of $V'O_2$peak, HRpeak and COpeak, during incremental exercise on CE were not significantly different between OB and CTRL, in agreement with previous studies (Lazzer et al. 2013; Salvadori et al. 1999; Vella et al. 2012). However, submaximal $V'O_2$ values in OB were significantly higher than CTRL for the same mechanical load, as previously reported (Salvadori et al. 1992; Salvadori et al. 1999; Vella et al. 2012; Vella et al. 2011; Lazzer et al. 2013). Lafortuna and colleagues hypothesized that the greater O_2 cost in obese during cycling was mainly related to the extra work required to move legs (Lafortuna et al. 2008). In fact, although the gross mechanical efficiency was lower in obese compared with lean individuals, the net one was similar (Salvadori et al. 1992). This might explain why the exercise tolerance was impaired in obese. Nevertheless, other factors could be involved. Looking at our data, when $V'O_2$peak was normalized by FFM during CE, the maximal aerobic power in OB was lower than that observed in CTRL. This may further impair exercise tolerance, as demonstrated by the lower peak work rate described in OB during CE (Lazzer et al. 2013). Moreover, also the oxygen cost of breathing, significantly higher in resting obese individuals (Kress et al. 1999), may contribute to the increased metabolic cost of cycling in this population. Finally, the fraction of $V'O_2$ related to the work performed by respiratory muscles has been shown to rise disproportionately in obese subjects during exercise (Alemayehu et al. 2018; Salvadego et al. 2017). Indeed, these researchers (Salvadego et al. 2015; Salvadego et al. 2017; Alemayehu et al. 2018) found that respiratory muscles were overloaded during exercise in severely obese people, being associated with an increase O_2 cost and a reduced exercise tolerance (Dempsey et al. 2006).

During CE exercise, CO peak values were not significantly different between OB and CTRL. However, COpeak normalized by FFM was lower in OB than CTRL,

suggesting a lower blood and O_2 delivery at muscle level, which could limit the muscles' ability to extract O_2 in OB. These findings are partially in disagreement with the results from previous works in this field. In fact, some researchers (Ferrero et al. 1996; Salvadori et al. 1999; Vella et al. 2012; Vella et al. 2011) showed higher CO at rest in obese than lean subjects, which is probably related to the greater muscle mass owned by the former ones. While Vella and colleagues (Vella et al. 2012; Vella et al. 2011) observed higher CO peak (and submaximal) in obese compared with lean controls during CE incremental exercise; by contrast, Salvadori and colleagues (Salvadori et al. 1999) showed and opposite behavior of CO in the two subgroups. The discrepancies between these studies could be explained by the different measurement methods and the different degrees of obesity between the study populations.

To the best of our knowledge, there are very few studies measuring a-vO_2 diff in obese and it is not so clear whether the a-vO_2 diff peak in this population is a limiting factor or not. According to our data, a-vO_2 diff peak was lower in OB than CTRL on CE exercise. This finding may support the conjecture of a central limitation experienced by OB participants during the physical effort. As discussed above, for the same oxygen consumption, OB had higher O_2 cost devoted to the respiratory muscles (Salvadego et al. 2015, 2017; Alemayehu et al. 2018) compared with lean counterparts. This finding means that during exercise, the locomotory muscles of OB subjects have potentially less circulating O_2 available. So, even though the two subgroups have the same V'O_2peak and COpeak during CE, the central circulation could represent a constraining factor in OB individuals. Moreover, fatigued respiratory muscles may induce sympathetic vasoconstriction of the locomotor muscles by activation of metaboreflex pathway, and hence they might contribute to limit the performance (Dempsey et al. 2006). This mechanism, enhanced in obese (Salvadego et al. 2015, 2017; Alemayehu et al. 2018), could preserve muscle function and might explain lower muscle fatigue and a-vO_2 diff peak in OB after CE. The a-vO_2 diff values reported for OB individuals during our tests were similar to the those found by Vella and coworkers (Vella et al. 2012; Vella et al. 2011), where a-vO_2 diff was lower compared with the lean subgroup. This finding seems inconsistent with those collected by other researchers (Rowland et al. 2003) reporting the presence of a similarity between a-vO_2 diff values in the two subgroups of subjects. A-vO_2 diff depends on the capacity of

the muscle to uptake oxygen from blood circulation. This capacity might be limited by muscle blood perfusion, oxygen diffusion or mitochondrial respiratory function. Limberg and colleagues (Limberg et al. 2010) did not find differences between OB and normal weight subjects in blood flow at the muscle level, measured during single leg KE and single forearm flexion exercises. Similarly, capillary/muscle fiber ratio, which is correlated with muscle oxygen diffusion (Howlett et al. 2003, 2009), was not significantly different between OB and lean subjects (Gavin 2004). Concerning the mitochondrial respiratory function, Konopka (Konopka et al. 2015) showed that the oxidative capacity recorded in obese individuals was comparable to that observed in lean sedentary subjects, while obese exhibited a greater uncoupled mitochondrial respiratory function. Hence, obese required more oxygen to phosphorylate the same amount of ADP in ATP compared with the lean counterpart. Although insufficient to draw firm conclusion on this argument, the data from literature might support the lack of differences in a-vO_2 diff between obese and lean subjects.

In KE exercise, OB and CTRL exhibited similar maximal external workload, $V'O_2$, CO and a-vO_2 diff. Similarly, when $V'O_2$ peak was corrected for the thigh muscle volume ($V'O_2$ /Vol_{TM}), no differences have been detected in the two subgroups. This finding suggests that muscle peripheral impairments could have affected both subgroups indistinctively, once central or cardiovascular limitation was ruled out.

According to the data showed in this chapter, OB produced MVC values that were similar to those observed in CTRL subgroup. However, once normalized by the thigh muscle volume, MVC in OB resulted lower than that performed by CTRL. These results were in contrast with previous studies reporting that OB showed greater absolute muscle torque (Maffiuletti et al. 2007, 2008; Abdelmoula et al. 2012; Lazzer et al. 2013; Garcia-Vicencio et al. 2015), and, when normalized by Vol_{TM}, MVC was not significantly different (Lazzer et al. 2013) or even greater (Abdelmoula et al. 2012) in obese compared with control subjects. Discrepancies between the studies could be explained by the different measurement methods employed for the muscle mass detection. OB presented a bigger amount of lipid accumulation inside the muscle (Malenfant et al. 2001; Bollinger 2017). The method handled in the present study might have overestimated the contractile tissue included in the thigh volume by the fact that the measurements of the

circumference and the skinfold in OB took in account only the subcutaneous lipid accumulation, neglecting any intramuscular infiltrations of fat tissue. When the lipid accumulation inside the muscle was taken in account, the normalized MVC were not significantly different from non-obese subjects (Maffiuletti et al. 2013).
Finally, the capacity to recover from muscle fatigue was not affected by obesity status. This finding is in agreement with data from previous works (Maffiuletti et al. 2007; Garcia-Vicencio et al. 2015). Recovery was similar between the two subgroups across the different time points in both the exercises. MVC remained impaired even twelve minutes after the end of the exercises.
In conclusion, MVC decreased less after the end of CE than KE exercises in OB subjects. On the contrary, MVC decreased to a similar extent after the end of both exercises in CTRL. This finding might suggest the occurrence of a muscle function preservation during incremental cycling exercise to exhaustion in OB, but not in CTRL.
In KE, peak values of absolute $V'O_2$, HR and CO did not differ between OB and CTRL. This result might indicate that both subgroups reached similar metabolic status at the muscle level. Finally, similar muscle function recovery from fatigue has been observed in OB and CTRL subgroups after the end of both the exercises. Hence, it might be inferred that muscle function recovery was not impaired in obese individuals.

CHAPTER III

HIIT and MICT in Obese patients

Adapted from:

Vaccari F, Passaro A, D'Amuri A, Maria Sanz J, Di Vece F, Capatti E, Magnesa B, Comelli M, Mavelli I, Grassi B, Fiori F, Bravo G, Avancini A, Parpinel M, Lazzer S. HIIT vs MICT effect on fat oxidation, V'O_2peak and mitochondrial respiration in obese. Submitted to: European Journal of Exercise physiology

3.1 – Introduction

Beside the cardiovascular risk factor discussed in the introduction of the previous chapter, obesity is a major risk factor for type 2 diabetes mellitus, , musculoskeletal disorders and respiratory diseases as well (Williams et al. 2015). Compared to lean subjects, obese people have an impaired capacity to oxidize lipids (Lanzi et al. 2014) which is associated with low insulin sensitivity (Kelley and Simoneau 1994) and ease in gaining weight (Zurlo et al. 1990).

Optimizing fat oxidation capacity is an important objective both for performance (Hetlelid et al. 2017) and health (Achten and Jeukendrup 2004). Whole-body fat oxidation does not seems to be related with intrinsic mitochondrial oxidative capacity (Nordby et al. 2006; Dandanell et al. 2018), but rather to the amount of lean body mass (Nordby et al. 2006), mitochondrial content (Dandanell et al. 2018) and maximal oxygen uptake ($V'O_2max$) (Nordby et al. 2006; Hetlelid et al. 2017; Dandanell et al. 2018).

While generally during whole body exercise (i.e. running and walking uphill) $V'O_2max$ is considered to be mainly limited by central/cardiovascular factors (Blomqvist and Saltin 1977; di Prampero 2003), the capacity to oxidase fat is considered to be a limited peripherally as the peripheral muscles are the main active organ during exercise. Numerous studies have demonstrated that skeletal muscle metabolism and mitochondrial content/function are impaired in obesity, but there is not a general agreement about the issue (Ara et al. 2011; Nair et al. 2011; Fisher-Wellman et al. 2014). Mitochondrial content (Ritov et al. 2005; Larsen et al. 2011), levels of mitochondrial proteins (Wijngaarden et al. 2013) and their (predominantly nuclear) genes (Mootha et al. 2003; Patti et al. 2003; Ritov et al. 2009; Hwang et al. 2010), have been shown to be reduced in the skeletal muscle of obese individuals, as well as in type 2 diabetes individuals, compared to lean controls. Mitochondrial dysfunction and impaired enzymatic activity of oxidative phosphorylation complexes have been confirmed more recently in obese skeletal muscle (Devarshi et al. 2017; Formentini et al. 2017; de Mello et al. 2018).

Compared to other methods, high-resolution respirometry in permeabilized muscle fibres, the approach which was utilized for the experiment presented in this chapter, allows us a more “physiological” evaluation of mitochondrial function *ex vivo*. In studies carried out by this method in obese subjects an impaired

muscle mitochondrial metabolism was reported in terms of mitochondrial respiratory capacity (State 3 respiration) (Vijgen et al. 2013). In addition, in obese sedentary adults moderate-intensity physical activity combined with weight loss was reported to increase the enzymatic activities of the electron transport chain, which occurred without a significant increase in mitochondrial DNA, and were ascribed to an increase in mitochondrial cristae (Menshikova et al. 2007).

According to our results in the previous chapter, obese have a more pronounced central limitation to physical efforts, but as above discussed even muscles are somehow affected by obesity status. HIIT seems to be a suitable training modality for this type of population since it is more effective in improving the cardiovascular system capacity to deliver O_2 as well as periphery capacity to uptake and oxidase it. Therefore, the aim of the chapter was to compare two types of exercises: moderate-intensity prolonged exercise training (MCIT) (Keating et al. 2017) and high-intensity interval exercise training (HIIT) (Buchheit and Laursen 2013a), and in obese subjects, evaluate whether the improvement in $V'O_2peak$ (central) are accompanied by whole-body fat oxidation (peripheral) and by improvements in mitochondrial respiration (peripheral). Since to our knowledge there are no exhaustive studies on the role of the intensity and training modality on mitochondrial respiration in the obese, we hypothesize greater responses for all variables following HIIT vs. MICT. An evaluation was also performed a few months after the termination of the interventions, in order to evaluate the maintenance of the observed changes, considering that the weight maintenance after its reduction is a major issue (Donnelly et al. 2009). During the experimental period and follow-up, nutritional advices has been provided identical for both groups in order to avoid confounding nutritional variables on the outcomes.

3.2 – Materials and methods

Subjects

Thirty-two obese volunteers (17 males and 15 females) were recruited from the Exercise Physiology Laboratory of the University of Udine, where they underwent a medical and dietetic evaluation. The inclusion criteria were age between 18 and 50 years and body mass index (BMI) ≥ 30 $kg \cdot m^{-2}$. Subjects who had previously participated in weight management programs, had cardiovascular, respiratory, neurologic, muscular-skeletal, metabolic and/or endocrine diseases or those who were taking any drugs known to influence energy metabolism and

cardiorespiratory adjustments to exercise, were excluded. No subject was taking beta-blockers. The Ethics Committee of the Friuli-Venezia-Giulia Region approved the study (protocol number 1764). Before the study began, the purpose and objectives were carefully explained to each subject and written informed consent was obtained. A physical activity questionnaire was administered to exclude potential volunteers who engaged in any continuous activity longer than 20 minutes more than once a week, indicative of a moderate physical activity level (IPAQ-SF) (Craig et al. 2003).

Study protocol

After the first inclusion visit, subjects were admitted to 3 months of multidisciplinary weight-management program including lifestyle education, physical activity and dietary follow-up.

Control tests including assessment of body composition, physical capacities, fat oxidation rate, physical activities and dietary habits were performed during two-weeks before the beginning and immediately after completion the weight-management program. At the same time, blood samples and skeletal muscle biopsies of the *vastus lateralis* muscle were taken for measurement of *ex-vivo* mitochondrial respiration by high-resolution respirometry. In addition, anthropometrics indexes and physical performance were monitored monthly during the program, in order to adjust food allowances and physical activities individually. Four months after the end of the weight-management program, control tests including assessment of body composition, physical capacities, fat oxidation rate, physical activities and dietary habits were performed.

Physical activity

During the 3 month-weight-management period, subjects followed a physical training program including three training sessions per week under supervision. The subjects were splitted randomly in two groups, one group following a moderate-intensity continuous training (MICT, n=16) and the second group following high intensity interval training (HIIT, n=16).

All subjects completed 34 □□0.14 sessions of physical training. The intensity of MICT on the treadmill was set at a heart rate (HR) corresponding to 60% of the initial V'O_2peak, the duration of the training session was 44 ± 8 min. HIIT consisted of 10 min of warm up (50 % of V′O_2peak) followed by 3 to 7 repetitions

of 3 min bouts of high-intensity walking (100 % of $V'O_2$peak), interspersed by 1.5 min walking at low intensity (50% of $V'O_2$peak) and followed by 5 min of cool down (50% of $V'O_2$peak); the duration of the training session was 33 ± 4 min. Exercise intensity was set up by adjusting the slope of the treadmill. The amounts of energy expended during the training sessions were similar for both groups: 20 kJ per kg of fat-free mass (FFM), which corresponds to about 1.5 MJ per session.

Research assistants and physical trainers were responsible for verifying that each subject participated to each training session, performed the exercises correctly and completed at least 90 % of the exercise sessions. At the end of each month, aerobic tests were performed to assess physical capacities and to adjust physical training intensity individually. All subjects were also advised to practice leisure physical activities during the weekend and holidays.

During the 4-months follow-up the same training suggestions were given to all subjects. The suggestions consisted of three training session per week covering the full intensities range: one high intensity (90% HRpeak and less than 30 min), one medium intensity (~70-80% HRpeak and 30-50 min) and one low intensity (<70% HRpeak and more than 60 min). Training during the follow-up period was not supervised and compliance was checked by a questionnaire(Craig et al. 2003).

Diet and nutritional education

During the intervention period the patients followed personalized diets formulated according to the Italian recommended dietary allowances (SIO-ADI 2016). Energy supply was about 1.3 times the initial basal metabolic rate (BMR) estimated using the Harris-Benedict equation (Harris and Benedict 1918), as suggested by the Italian guidelines for obesity treatment (SIO-ADI 2016). Carbohydrates, lipids and protein supplied were 56, 27 and 17% of energy intake, respectively. Six weeks after the beginning the weight-management program food allowances were further reduced. The reduction ranged between 2610 and 3766 kJ as suggested by SIO-ADI (SIO-ADI 2016), based on their prior diet and personal feedbacks. During the weight-management period, subjects had dietetics lessons including choice of foods, and they were instructed to maintain their food habits after the end of the weight-management period.

Measurements

Anthropometric characteristics and body composition

The medical history and a physical examination of subjects were taken at the time of admission to the weight-management program. Body mass (BM) was measured to the nearest 0.1 kg with a manual weighting scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board. BMI was calculated as BM (kg) x stature^{-2} (m). Body composition was measured by bioelectrical impedance (BIA, Human IM Plus; DS Dietosystem, Milan, Italy). Fat mass (FM) and fat-free mass (FFM) were calculated with equations derived either in obese people of different ages and BMI (fat-specific formulae) by utilizing a two-compartment model (Gray et al. 1989).

Blood sampling

Blood samples were collected after an overnight fasting, before (PRE) and at the end of the weight-management program (POST) and were centrifuged in absence or presence of EDTA to obtain serum and plasma, respectively. Aliquots were stored at -80°C. HDL cholesterol after precipitation of the apo-B containing lipoproteins (Burstein et al. 1970), total cholesterol and triglycerides (TG) levels were assayed in serum by the Trinder method. The coefficient of variation was < 2% for Total and HDL cholesterol and < 5% for TG for intra- and inter-batch, respectively. LDL-cholesterol plasma levels were calculated by the Friedewald's formula (Friedewald et al. 1972). Plasma glucose was measured using standard enzymatic methods (FAR S.R.L., Italy). The coefficient of variation was < 3% for intra-assay. Fasting insulin levels were assayed using an ultrasensitive insulin ELISA kit manufactured by Mercodia AB (Sweden). The coefficient of variation was <3% for intra-assay. Fasting insulin resistance was evaluated calculating Homeostasis Model Assessment (HOMA-IR) (Matthews et al. 2009).

Dietary and physical activity habits

Dietary data were collected using a 4-day dietary (4-DD) record (food diary) given to the subjects in 3 different occasions in order to analyse their eating behavior before the beginning of the intervention, at the end of the intervention and after the follow-up period. The diaries were given together with instructions on how to record type, quantity, and mode of consumption of foods over a 24-hour period on four separate days, including one during the weekend. Data extracted from

food diaries were analysed using the Microdiet software (V2.8.6, Downlee Systems Ltd., High, Peak, UK) containing the Italian food composition database for epidemiological studies (Gnagnarella et al. 2015), integrated with information from nutritional labels when data were missing, data and the brand were specified in the diary.

To assess physical activity levels we used the validated International Physical Activity Questionnaire Short Form (IPAQ-SF) (Craig et al. 2003). The questionnaire records vigorous-intensity, moderate intensity, walking activities and the sitting time spent during the previous 7 days. The IPAQ-SF scores were converted into Metabolic Equivalent minutes per week (MET-min·week^{-1}) using the "Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ)"(International Physical Activity Questionnaire 2005).

Further, all the participants completed the Short-Form 12 (SF12) questionnaire to investigate the health-related quality of life. The questionnaire is composed by 12 items from which a physical (SF12_PI) and mental health (SF12_MI) index are obtained (Ware et al. 1996).

The current behavior change was explored by the Stage of Change Questionnaire (SC). This allows to classify people into 5 stages: pre-contemplation (not exercising and not intending to increase the physical activity), contemplation (not exercising but intending increase the physical activity), preparation (not regularly exercising), action (exercising in the past 6 months but not regularly) and maintenance (regularly exercising in the past 6 months) (Marcus et al. 1996).

Physical capacities and maximal fat oxidation rate

Peak oxygen uptake ($V'O_2peak$) and maximal fat oxidation rate were determined by using a graded exercise test on a motorized treadmill (H/P/Cosmos Sports & Medical Gmbh, Germany), under medical supervision. Subjects were asked to avoid strenuous exercise the day before the test and came to the laboratory after a 12-h fasting. Before the beginning of the study, subjects were familiarized with the equipment and the procedures. Each test was undertaken at the same time of the day in the different periods of the study, and comprised a 5-min rest period followed by walking in stages of 4-min duration until voluntary exhaustion. The rates in m·s^{-1} and incline in % followed a sequence: 1.11 (0 %), 1.11 (3 %), 1.39

(3 %), 1.39 (6 %), 1.53 (6 %), 1.53 (9 %), 1.53 (12 %), 1.53 (13 %), 1.53 (15%), 1.53 (18%), 1.53(21%) and 1.53 (24%) (Lazzer et al. 2017). During the experiment, ventilatory and gas exchange responses were measured continuously by indirect calorimetry (CPET, Cosmed, Italy). The flowmeter and gas analysers of the system were calibrated using, respectively, a 3-L calibration syringe and calibration gas (16.00 % O_2; 4.00 % CO_2). During the exercise test, an electrocardiogram was recorded continuously and displayed online for visual monitoring, and heart rate (HR) was measured with a dedicated monitor device (Garmin, US). V′O_2peak was estimated for each subject considering the last 20s of the graded exercise tests.

The substrate oxidation rate was calculated from V′O_2 and V'CO_2 values determined during the last minute of each workload level, according to the protocol of Achten (Achten et al. 2003) and using the following equations:

$$\textit{Fat oxidation rate (g min}^{-1}\textit{)} = 1.67 \cdot V'O_2\ l \cdot min^{-1} - 1.67 \cdot V'CO_2\ l \cdot min^{-1} - 0.307 \cdot P_{oxi}$$

$$\textit{Carbohydrate oxidation rate (g min}^{-1}\textit{)} = 4.55 \cdot V'CO_2\ l \cdot min^{-1} - 3.21\ V'O_2\ l \cdot min^{-1} - 0.459 \cdot P_{oxi}$$

where P_{oxi} is the protein oxidation rate. P_{oxi} was estimated by assuming that protein oxidation contributed approximately 12% of resting energy expenditure:

$$\textit{Protein oxidation rate (g min}^{-1}\textit{)} = [\textit{energy expenditure (kJ min}^{-1}\textit{)} \cdot 0.12] \cdot 16.74^{-1}\ (kJ\ g^{-1})$$

For each subject, the results of the graded exercise test were used to compute the relationship between fat oxidation rate as a function of exercise intensity, expressed as %V′O_2peak. The best fit was obtained with a polynomial relationship of the second order. The graded exercise test on the motorized treadmill was performed in the same conditions (speed and incline) in the three different periods.

Cardiovascular function.

During the graded exercise test on the motorized treadmill, at rest and during the first step at 1.11 $m \cdot s^{-1}$ and 0% slope, stroke volume (SV) and cardiac output (CO) were monitored continuously by bioimpedance method (PhysioFlow, Manatec, France), following a procedure validated during maximal incremental exercises (Richard et al. 2001). PhysioFlow and metabolimeter values were synchronized and mean values were calculated every 10 seconds. Data obtained during the last 20 s of rest and of the first step were taken in account for further analysis.

Arteriovenous oxygen difference (a-$\bar{v}O_2$ diff.) was estimated by the following equation:

a-$\bar{v}O_2$ diff. ($mlO_2 \cdot 100\ ml^{-1}$) = $V'O_2$ ($ml \cdot min^{-1}$) · CO^{-1}($ml \cdot min^{-1}$) · 100

Systolic (SAP) and diastolic (DAP) arterial blood pressures were measured twice using an inflatable cuff and mean values were calculated the last minute of rest and of the first step. Mean arterial pressure (MAP) was calculated as [2 · diastolic blood pressure + systolic blood pressure] · 3^{-1}. Measures of arterial pressure were synchronized with the CO signal to calculate systemic vascular conductance (SVC), as the ratio between CO and MAP.

Biopsies and mitochondrial respiration ex vivo

Biopsies were obtained from the *vastus lateralis* muscle by percutaneous excision after an overnight fasting. A microneedle (Tru-cut Histocore, 12 G, Biomed Instrument & product GmbH, Germany) was used to collect the specimens; after anaesthesia of the skin using lidocaine (2%) a small incision was made to penetrate skin and fascia. Respirometric analyses were performed in duplicate using, for each subject, two specimens which were put in BIOPS solution (BIOPS: 10 mM EGTA-calcium buffer [free Ca^{2+} concentration 100 $nmol \cdot l^{-1}$], 20 mM imidazole, 20 mM taurine, 50 mM K^+/4 morpholinoethanesulfonic acid, 0.5 mM dithiothreitol, 6.56 mM $MgCl_2$, 5.77 mM ATP, and 15 mM phosphocreatine, pH 7.1), containing 10 % ($wt \cdot vol^{-1}$) fatty acid-free BSA and 30 % ($vol \cdot vol^{-1}$) DMSO at 4°C, then immediately frozen in liquid nitrogen and stored at -80°C until the analysis.

The analyses were performed by measuring O_2 consumption polarographically by high-resolution respirometry (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) according to Pesta and Gnaiger (Pesta and Gnaiger 2012). Chemicals and reagents were from Sigma (St. Louis, MO, USA). The muscle sample (10-20 mg of wet weight) was quickly thawed and immediately placed in ice-cold BIOPS containing 2 $mg \cdot ml^{-1}$ BSA, to remove any residual DMSO from the tissue. Fibre bundles were cleaned as much as possible from the connective tissue and fatty tissue excesses and separated with a sharp-ended needle under magnification (MC170 HD, Leica Microsystems, Switzerland, LTD) leaving only small areas of contact. Fibres were then incubated in 2 ml of BIOPS (4°C) containing 20 $\mu g \cdot ml^{-1}$ saponin for 30 min with a continuous gentle stirring to ensure complete

permeabilization. After being rinsed twice for 10 min in respiration medium (MiR05: 0.5 mM EGTA, 60 mM potassium lactobionate, 3 mM $MgCl_2$, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose and 1 $g \cdot l^{-1}$ BSA, pH 7.1), permeabilized fibres were measured for wet weight and immediately transferred into the respirometer chambers for O_2 consumption analysis.

The instrumentation allows for O_2 consumption measurements with small amounts of samples in closed respiration chambers containing 2 ml of air-saturated respiration medium at 37°C; 2–4 mg of muscle fibres were used for the analysis. Standardized instrumental and chemical calibrations were performed to correct for back-diffusion of O_2 into the chamber from the various components, leak from the exterior, O_2 consumption by the chemical medium, and by the sensor O_2 (Pesta and Gnaiger 2012). The O_2 concentration in the chamber was maintained between 300 and 450 □M (average O_2 partial pressure 250 mmHg) to avoid O_2 limitation of respiration. Intermittent re-oxygenation steps were performed during the experiments by adding 0.3 mM hydrogen peroxide into the chambers (MiR05 was added with 280 $U \cdot ml^{-1}$ catalase before the measurements). A substrate-uncoupler-inhibitor-titration protocol was applied (Pesta and Gnaiger 2012). Measurements were run in the presence of 25 µM blebbistatin to prevent ADP-induced contraction (rigor), particularly evident in small length fibres such as those obtained from biopsy by microneedles (Hughes et al. 2015). Non-phosphorylating resting mitochondrial respiration was measured in the presence of malate (4 mM) and glutamate (10 mM), and in the absence of adenylates so that O_2 consumption was mainly driven by the back leakage of protons through the inner mitochondrial membrane (Complex I state 2, or “leak” respiration). Saturating ADP (5 mM) was then added to measure Complex I respiration in phosphorylating condition (Complex I state 3 respiration). Succinate (10 mM) was added to support convergent electron flow into the Q-junction through Complexes I and II, thereby achieving the maximal ADP-stimulated mitochondrial respiration sustained by Complex I and Complex II (Complex I+II state 3 respiration), as verified by further addition of 5 mM ADP. Cytochrome C (10 µM) was then added to test for mitochondrial outer membrane integrity, and only samples demonstrating < 10% increase in respiration were taken in consideration (i.e. MICT PRE n=6 and MICT POST n=8; HIIT PRE n=6

and HIIT POST n=7). The main mean characteristics of these subgroups were not significantly different from whole group of subjects.

Maximal electron transport system (ETS) capacity was then evaluated by stepwise addition of the chemical uncoupler protonophore carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP). Afterward, Rotenone (1 μM) was added to inhibit Complex I and to evaluate ETS sustained by Complex II (rotenone-insensitive) and by Complex I (rotenone-inhibited). Finally, antimycin A (2.5 μM) was added to inhibit also Complex III, providing a measure of residual O_2 consumption (ROX), indicative of non-mitochondrial O_2 consumption. Mitochondrial respiration was corrected for O_2 flux due to the ROX. Data were digitally recorded using DatLab4 software (Oroboros Instruments).

The respiration parameters were normalized by citrate synthase (CS) activity (see below) and expressed as (pmol $O_2 \cdot s^{-1} \cdot mU^{-1}$).

The degree of oxidative phosphorylation coupling for a specific substrate supply (glutamate and malate in this case) was determined by calculating the ratio between Complex I+II state 3 respiration minus Complex I leak respiration and Complex I+II state 3 respiration [(state 3 – leak)/state 3] (Pesta and Gnaiger 2012).

Citrate synthase activity (CS)

CS activity, was assayed spectrophotometrically (Srere 1969) by an EnSpire 2300 Multilabel Reader (PerkinElmer). After completion of the respirometer measurements, muscle fibres were recovered and processed as in Spinazzi et al (Spinazzi et al. 2012). In MICT and HIIT groups, 13 and 14 subjects were considered respectively. Briefly, muscle fibre was suspended in1:20 $wt \cdot vol^{-1}$ in a homogenization buffer containing 250 mM sucrose, 20 mM Tris, 40 mM KCl and 2 mM EGTA with 1:50 $vol \cdot vol^{-1}$ protease inhibitors (Sigma-Aldrich), and submitted to a motor driven homogenization in a pre-cooled 1 ml glass-glass potter (Wheaton, USA). The homogenate was centrifuged at 600 x g for 10 minutes and the clarified homogenate was assayed for protein concentration (Lowry et al. 1951). For CS assay 10-20 μg of protein were added to each well of a 96-well-microplate along with 100 μl of 200 mM Tris-Triton X-100 (0.2 % $vol \cdot vol^{-1}$), 20 μl of 1 mM 5,5'-dithiobis-2-nitrobenzoate freshly prepared, 6 μl of 10 mM acetyl-coenzyme A and mQ water to a final volume of 190 μl. Finally, 10 μl were added

of 10 mM oxalacetic acid that started the reaction. All assays were performed at 25 °C in triplicate. Activity was expressed as mU (nmole/min) per mg of protein

Statistical analyses

Statistical analyses were performed using SPSS 20.0 software (IBM, Chicago, USA), with significance set at $p<0.05$. All results were expressed as means and standard error (SE). Normal distribution of the data was tested using the Shapiro–Wilk test. Sphericity was verified by Mauchly's test. When the assumption of sphericity was not met, the significance of the F-ratios was adjusted according to the Greenhouse–Geisser procedure.

The differences on the training adherence between the two groups were analysed by student's test for unpaired data. Anthropometric characteristics, body composition, $V'O_2peak$ cardiovascular parameters, data derived from questionnaire and food diary, glycolipid metabolism, CS and mitochondrial function parameters were analysed with a generalized linear mixed, multilevel, growth model, fit by maximal likelihood, which accounts for random effect due to subjects and intercept and fixed effects due to group (MICT *vs* HIIT), gender, time, time^2 and interaction Group x Time, taking in account the correlation of the data. For glycolipid metabolism, CS and mitochondrial function parameters, it was not necessary add the fixed effect "time^2" since there has been only two time-points. The same analyses were applied to the fat oxidation during the exercise, adding further the % of $V'O_2peak$ as fixed effect. Since the gender distribution of the subjects was balanced in the two groups, and no gender differences and no interaction between groups were found in the parameters studied, then male and female subjects were considered together.

3.3 - Results

Adherence to the training program

During the training period, subjects were involved in 34.4±0.2 and 34.8±0.3 training sessions for MICT and HIIT groups, respectively (P=0.811), without adverse events. The average of energy expended during the training sessions were 23.8±4.1 and 22.4±2.5 $kJ \cdot kg^{-1}$ of FFM for MICT and HIIT groups, respectively (P=0.256). The duration of each session was greater for MICT (44.3±7.6 min) than for HIIT (33.6±3.6 min, P<0.001).

Anthropometric characteristics and body composition

At PRE, no significant differences were found between MICT and HIIT for age (37.3±0. 6 and 40.1±0.4 y, P=0.334), stature (1.72±0.11 and 1.71±0.07 m, P=0.895), anthropometric characteristic and body composition (Table 3.1).

At POST, mean weight loss was 5.94±0.34 and 5.75±0.28 kg (P<0.001), BMI decreased by 2.12±0.12 and 1.89±0.09 $kg \cdot m^{-2}$ (P<0.001), waist circumference decreased by 3.67±0.40 and 5.37±0.25 cm (P<0.001), hip circumference decreased by 5.05±0.41 and 4.46±0.20 cm (P<0.001), FM decreased by 5.27±0.30 and 5.45±0.33 kg (P<0.001) and FFM did not change significantly in MICT and HIIT groups, without differences between groups.

After 4-months of follow-up (Table 3.1), body mass increased by 2.96±0.37 and 1.20±0.22 kg (P<0.015) and BMI increased by 0.93±0.12 and 0.46±0.12 $kg \cdot m^{-2}$ (P<0.003), in MICT and HIIT, respectively, without differences between groups; the values, however, were still significantly lower than at PRE (P<0.003). Waist and hip circumference and FM did not change significantly during the follow-up period remaining significantly lower than at PRE (P<0.001). FFM increased significantly during the follow-up period, by 3.61±0.24 and 2.70±0.43 kg (P<0.001) in MICT and HIIT groups, respectively, and were not significantly different than at PRE (Table 3.1).

Blood parameters

Blood parameters are presented in Table 3.2. At PRE, no significant differences were found between MICT and HIIT groups for Total-, LDL- and HDL- cholesterol, triglycerides, fasting plasma glucose, fasting plasma insulin, and HOMA IR index.

After 3-months (POST) of weight-management program, Total and LDL cholesterol decreased significantly (P<0.001) by 11 and 13% in MICT and by 6 and 11% in HIIT group, without differences between groups. After the training period (POST), HDL Cholesterol, Triglycerides, Fasting Glucose, Fasting Insulin, HOMA IR index did not change significantly in either groups (Table 3.2).

Table 3.1 - Anthropometric characteristic, physical capacities and Physical activity habits before (PRE) and after 3-months (POST) of weight-management program, and after 4 months of follow-up in Moderate Intensity Continuous Training (MICT) and High Intensity Interval Training (HIIT) groups.

	MICT			HIIT			P		
	PRE (n:16)	POST (n:16)	FOLLOW-UP (n:14)	PRE (n:16)	POST (n:16)	FOLLOW-UP (n:12)	Gr	T	Gr x T
Body mass (Kg)	107.1 ± 4.4	101.2 ± 4.5*	103.8 ± 5.4*†	103.5 ± 2.7	97.8 ± 2.5*	98.0 ± 3.0*†	0.790	0.001	0.331
BMI (kg·m-2)	36.1 ± 1.3	33.9 ± 1.2*	35.2 ± 1.5*†	35.1 ± 0.9	33.2 ± 1.0*	32.9 ± 0.9*†	0.638	0.001	0.587
Waist (cm)	113.0 ± 3.5	109.4 ± 4.0*	110.5 ± 4.4*	114.1 ± 2.2	108.8 ± 2.1*	107.4 ± 2.2*	0.406	0.001	0.590
Hip (cm)	123.1 ± 2.8	118.0 ± 2.9*	119.9 ± 3.1*	120.5 ± 1.8	116.1 ± 2.2*	113.9 ± 1.5*	0.875	0.001	0.500
FFM (kg)	69.4 ± 3.9	68.6 ± 4.1	71.0 ± 5.0†	65.1 ± 2.9	64.7 ± 2.7	69.3 ± 3.9†	0.386	0.001	0.767
FM (Kg)	37.7 ± 2.7	32.4 ± 2.3*	32.5 ± 3.0*	38.4 ± 2.1	32.9 ± 2.5*	28.8 ± 2.0*	0.970	0.002	0.433
HRpeak (bpm)	180.1 ± 0.9	177.2 ± 1.1*	173.0 ± 1.3*	181.0 ± 0.9	176.0 ± 0.5*	178.2 ± 0.83*	0.141	0.001	0.149
V'O2peak ($L \cdot min^{-1}$)	3.02 ± 0.05	3.19 ± 0.05*	2.95 ± 0.06*†	2.88 ± 0.04	3.35 ± 0.05*	3.32 ± 0.06*†	0.288	0.001	0.001
V'O2peak ($mL \cdot min^{-1} \cdot Kg^{-1}$ FFM)	43.58 ± 0.39	46.80 ± 0.39*	42.02 ± 0.51*†	44.26 ± 0.45	51.51 ± 0.40*	48.04 ± 0.56*†	0.093	0.001	0.001
IPAQ_TOT (MET-min $week^{-1}$)	818 ± 220	1481 ± 292*	1965 ± 552*	766 ± 253	1257 ± 208*	2223 ± 507*	0.954	0.001	0.635
IPAQ_VIG (MET-min $week^{-1}$)	190 ± 135	547 ± 281*	928 ± 372*	45 ± 45	310 ± 138*	660.0 ± 285*	0.264	0.003	0.779
IPAQ_MOD (MET-min $week^{-1}$)	247 ± 102	596 ± 204	384 ± 136	435 ± 243	431 ± 94	1060 ± 423	0.259	0.090	0.275
IPAQ_WALK (MET-min $week^{-1}$)	380 ± 127	337 ± 94	653 ± 294	286 ± 99	517 ± 162	503 ± 121	0.948	0.080	0.936
SF12_PI (pt)	504 ± 1.9	51.4 ± 2.3	52.4 ± 1.8	50.0 ± 2.2	53.1 ± 1.7	52.6 ± 1.6	0.888	0.161	0.976
SF12_MI (pt)	46.3 ± 2.7	49.1 ± 2.2	45.2 ± 2.6	52.4 ± 1.8	50.8 ± 2.2	51.7 ± 2.4	0.282	0.611	0.984
SC (pt)	2.3 ± 0.1	3.4 ± 0.2*	3.1 ± 0.4*	2.1 ± 0.1	3.4 ± 0.2*	3.1 ± 0.3*	0.772	0.001	0.536

All values are presented as mean ± standard error.

BMI: body mass index; FM: Fat Mass; FFM: Fat free Mass; IPAQ-TOT: International Physical Activity Questionnaire; IPAQ-VIG: vigorous activity; IPAQ-MOD: moderate intensity activity; IPAQ-WALK: physical activity derived from walking; SF12_PI: Short-Form 12, questionnaire about health-related quality of life concerning physical index; SF12_MI: Short-Form 12, questionnaire about health-related quality of life concerning mental index; SC: Stage of Change questionnaire; Pt: points.

**: significantly different from PRE, P<0.05; †: significantly different from POST, P<0.05;*

Gr: group effect; T: time effect; Gr x T: groups x time effect.

Table 3.2 - Biological characteristics before (PRE) and after 3-months (POST) of weight-management program, in Moderate Intensity Continuous Training (MICT) and High Intensity Interval Training (HIIT) groups.

	MICT		HIIT		Gr	T	Gr x T
	PRE (16)	POST (16)	PRE (16)	POST (16)	P	P	P
Total Cholesterol ($mg \cdot dL^{-1}$)	220.90 ± 10.12	198.21 ± 8.99*	219.11 ± 9.00	206.11 ± 10.04*	0.801	0.001	0.332
LDL Cholesterol ($mg \cdot dL^{-1}$)	142.99 ± 7.01	126.57 ± 7.86*	147.71 ± 8.52	133.03 ± 9.52*	0.610	0.001	0.839
HDL Cholesterol ($mg \cdot dL^{-1}$)	51.34 ± 3.95	47.86 ± 3.05*	48.67 ± 2.44	48.47 ± 2.49	0.806	0.172	0.221
Triglycerides ($mg \cdot dL^{-1}$)	132.84 ± 22.07	118.88 ± 16.58	113.65 ± 12.58	123.04 ± 20.91	0.754	0.837	0.297
Fasting Glucose ($mg \cdot dL^{-1}$)	99.03 ± 2.43	97.01 ± 2.30	98.26 ± 2.38	96.74 ± 2.23	0.867	0.154	0.838
Fasting Insulin ($\mu U \cdot mL^{-1}$)	10.05 ± 1.48	10.07 ± 3.28	10.27 ± 1.14	9.05 ± 1.38	0.879	0.645	0.633
HOMA IR index	2.48 ± 0.40	2.53 ± 0.92	2.54 ± 0.32	2.22 ± 0.39	0.862	0.706	0.608

All values are presented as mean ± standard error.
LDL: low density lipoprotein; HDL: high density lipoprotein; HOMA, Homeostatic model assessment.
**: significantly different from PRE, P<0.05;*

Dietary and physical activity habits

Dietary habits

At PRE, no significant differences were found between MICT and HIIT groups in energy intake (9509±763 *vs*. 7720±522 kJ, P=0.061), and carbohydrates (45±2 *vs*. 45±1 %, P=0.953), lipids (37±2 *vs*. 36±2 %, P=0.383) and proteins (16±1 *vs*. 16±1 %, P=0.663) contribution to energy intake.

At POST, mean energy intake decreased significantly by -2224±186 and -1136±146 $kJ \cdot day^{-1}$ (P<0.001) in MICT and HIIT respectively, without difference between groups (P=0.244). Carbohydrates contribution to energy intake did not change significantly, but lipids decreased by -3.52±0.55 and -5.02±0.61 % (P < 0.05) and protein increased by +3.26±0.29 and +3.66±0.31 % (P < 0.001) in MICT and HIIT groups, respectively.

After the follow-up period, mean energy intake increased significantly by +1483±155 and +360±220 $kJ \cdot day^{-1}$ (P < 0.05) in MICT and HIIT respectively, without differences between groups (P=0.215); the values returned similar to those described in PRE (P=0.098). Carbohydrates, lipids and proteins

contribution to energy intake did not change significantly from POST to follow-up, but lipids remained lower than at PRE (-4 %, P=0.028) and proteins tended to remain higher than at PRE (+2%, P=0.061).

Physical activity habits

Physical activity habits, evaluated by the IPAQ questionnaire, were similar between the two groups (MICT and HIIT) at all three investigated time points (P values ranging from 0.259 to 0.954; Table 3.1).

After the training period (POST, Table 3.3), total (IPAQ_TOT) and vigorous (IPAQ_VIG) physical activities increased by 72 and 264 % (P <0.05) respectively, in both groups. However, moderate activity (IPAQ_MOD) and physical activity derived from walking (IPAQ_WALK) did not change significantly (respectively P=0.090 and P=0.080) in both groups.

After the follow-up period (Table 3.3), total (IPAQ_TOT) and vigorous (IPAQ_VIG) physical activities did not change significantly and remained higher than at PRE by 100 and 168 % (P <0.05) in MICT and HIIT groups, respectively. Also, moderate intensity activity (IPAQ_MOD) and physical activity derived from walking (IPAQ_WALK) after the follow-up period did not change significantly (respectively P=0.080 and P=0.090) in both groups.

The quality of life assessed by the SF12 questionnaire concerning physical and mental index showed no differences over time in both groups (Table 3.3).

Stage of change questionnaire showed (Table 3.3) an increase, after the weight management program, by 54% (P=0.001) in MICT and HIIT groups. After the follow-up period, stage of change did not change and remained significantly higher than at PRE by 25% (P=0.001) in both groups

Physical capacities

Cardiovascular parameters

At rest, $V'O_2$, CO, SV, SAP, DAP, SVC and a-$\bar{v}O_2$ diff. values were similar between the two groups (MICT and HIIT) at all three investigated time points (P values ranging from 0.132 to 0.748; Table 3.3).

After the training period (POST), SAP decreased significantly at rest by mean -5% (P<0.001) in MICT and HIIT groups, without significantly changes for the other parameters (Table 3.3).

After the subsequent follow-up period, SAP did not change significantly and remained significantly lower than at PRE by mean -5% (P=0.003) in MICT and HIIT groups. Also, DAP decreased significantly compared to POST, by 4% (P=0.033) in MICT and HIIT groups; finally, $V'O_2$ increased significantly compared to PRE, by 19% (P=0.039) in MICT and HIIT groups (Table 3.3).

During walking at 1.11 $m \cdot s^{-1}$ (4 $km \cdot h^{-1}$) and 0% slope (~40% of $V'O_2peak$), $V'O_2$, CO, SV, SAP, DAP, SVC and a-$\bar{v}O_2$ diff values were similar between the two groups (MICT and HIIT) at all three investigated time points (P values ranging from 0.195 to 0.951; Table 3.3). Physical training caused a significantly decrease in $V'O_2$ by -7% (P=0.039) in MICT and HIIT groups, and in SAP by -11% (P<0.001) in MICT and HIIT groups. After the follow-up period, $V'O_2$ did not change significantly and remained significantly lower than at PRE by 12% (P=0.011) in MICT and HIIT groups. SAP increased significantly but remained significantly lower than at PRE by 6% (P=0.007) in MICT and HIIT groups. Finally, both CO and SVC were significantly lower than at PRE by 19% (P<0.001) in MICT and HIIT groups (Table 3.3).

.

Table 3.3 - Cardiovascular parameters before (PRE) and after 3-months (POST) of weight management program, and after 4 months of follow-up in Moderate Intensity Continuous Training (MICT) and High Intensity Interval Training (HIIT) groups.

	MICT			HIIT			P		
	PRE (16)	POST (16)	FOLLOW-UP (14)	PRE (16)	POST (16)	FOLLOW-UP (12)	Gr	T	Gr x T
At rest									
$V'O_2$ ($L \cdot min^{-1}$)	0.35 ± 0.02	0.37 ± 0.02	0.39 ± 0.03*	0.37 ± 0.01	0.37 ± 0.02	0.49 ± 0.08*	0.132	0.012	0.318
CO ($L \cdot min^{-1}$)	6.71 ± 0.33	5.95 ± 0.31	5.97 ± 0.30	7.16 ± 0.30	7.15 ± 0.30	7.09 ± 0.51	0.345	0.113	0.595
SV (ml)	88.82 ± 4.86	84.10 ± 5.63	84.54 ± 4.35	92.98 ± 4.49	96.05 ± 5.76	88.64 ± 6.69	0.158	0.393	0.817
SAP (mmHg)	132.06 ± 2.66	126.13 ± 2.20*	126.15 ± 1.95*	139.56 ± 3.46	131.56 ± 2.08*	128.75 ± 2.47*	0.620	0.002	0.339
DAP (mmHg)	83.88 ± 2.10	82.69 ± 1.36	80.00 ± 2.21†	87.50 ± 2.42	90.38 ± 1.73	86.08 ± 2.03†	0.439	0.002	0.383
SVC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	67.08 ± 2.71	60.68 ± 3.06	62.76 ± 3.44	69.71 ± 3.34	69.28 ± 3.74	70.85 ± 5.26	0.410	0.118	0.463
a-vO2 diff. (mL·100mL-1)	5.42 ± 0.32	6.05 ± 0.35	5.66 ± 0.59	5.28 ± 0.30	5.45 ± 0.30	6.52 ± 0.60	0.748	0.039	0.866
Walking at 1.11 $m \cdot s^{-1}$, 0% slope									
$V'O_2$ ($L \cdot min^{-1}$)	1.27 ± 0.06	1.17 ± 0.06*	1.15 ± 0.05*	1.23 ± 0.05	1.15 ± 0.06*	1.08 ± 0.06*	0.431	0.001	0.384
CO ($L \cdot min^{-1}$)	13.77 ± 0.61	12.08 ± 0.74	10.85 ± 0.37*†	13.31 ± 0.83	14.23 ± 0.72	11.06 ± 0.44*†	0.951	0.001	0.717
SV (ml)	136.60 ± 5.73	134.50 ± 8.92	120.18 ± 7.81	135.11 ± 8.05	147.38 ± 7.73	123.75 ± 6.88	0.611	0.077	0.781
SAP (mmHg)	143.57 ± 3.76	130.00 ± 1.64*	137.86 ± 3.80*†	149.69 ± 4.24	132.50 ± 1.64*	138.75 ± 3.14*†	0.195	0.001	0.354
DAP (mmHg)	85.00 ± 2.01	82.50 ± 1.65	81.57 ± 2.12	87.50 ± 2.54	88.44 ± 1.56	85.00 ± 1.51	0.426	0.443	0.777
SVC ($ml \cdot min^{-1} \cdot mmHg^{-1}$)	133.11 ± 5.09	122.66 ± 7.78	108.90 ± 4.24*†	126.83 ± 7.43	139.30 ± 7.44	109.47 ± 5.56*†	0.498	0.012	0.762
a-vO_2 diff. (mL·100mL-1)	9.58 ± 0.65	10.17 ± 0.65	10.33 ± 0.51	9.57 ± 0.46	8.65 ± 0.73	10.47 ± 0.83	0.893	0.073	0.481

All values are presented as mean ± standard error.
V'O2: oxygen consumption, CO: cardiac output; SV: stroke volume, SAP: systolic arterial pressure; DAP: diastolic arterial pressure, a-$\bar{v}$O2 diff.: arteriovenous difference of O2; SVC: systemic vascular conductance.
Significance by generalized linear mixed model (see statistical paragraph):
**: significantly different from PRE, P<0.05;*
†: significantly different from POST, P<0.05;
Gr: group effect; T: time effect; Gr x T: groups x time effect

Peak oxygen uptake

At PRE, no significant differences were found between MICT and HIIT for HRpeak and V'O_2peak (Table 3.1).

At POST, HRpeak decreased by 2.9±0.4 and 4.9±0.3 bpm (P<0.001) in MICT and HIIT groups, without difference between groups (Table 3.1). Absolute V'O_2peak increased by 0.17±0.01 and by 0.46±0.20 L·min^{-1} (P<0.001) in MICT and HIIT groups, respectively; with a significantly lower increase in MICT than HIIT (+6% and 16%, P<0.001, Table 3.1 and Fig. 3.1A. V'O_2peak normalized by FFM increased by 3.3±0.2 and by 7.2±0.3 mL·min^{-1}·kg^{-1} FFM (P<0.001) in MICT and HIIT groups, respectively, with a significantly lower increase in MICT than HIIT (+8%, and +16%, P<0.001, Table 3.1 and Fig. 3.1B).

HRpeak did not change significantly during the follow-up period and remained significantly lower than at PRE (Table 3.1, P<0.001). Absolute V'O_2peak decreased by 0.19±0.02 and by 0.21±0.01 L·min^{-1} (P<0.001) in MICT and HIIT groups, returning at PRE values in MICT but remaining higher in HIIT (+ 8%, P<0.001, Table 1 and Fig. 3.1A). V'O_2peak normalized by FFM decreased by 4.83±0.24 and by 4.76±0.32 mL·min^{-1} kg^{-1}FFM (P<0.001) in MICT and HIIT groups, returning at PRE values in MICT but remaining higher in HIIT (+5%, P<0.001, Table 1 and Fig. 3.1B).

Fat oxidation rate

Before the training period (PRE), fat oxidation rate during the incremental test was not significantly different between groups (P=0.110). Maximal fat oxidation rate was observed at 41 ± 1% of V'O_2peak in MICT (0.41 ± 0.01 g·min^{-1}, Fig. 3.2A) and at 43 ± 1% of V'O_2peak in HIIT (0.43 ± 0.1 g·min^{-1}, Fig. 2B) groups. At exercise intensities above 60 ± 1 % of V'O_2peak, fat oxidation rate decreased markedly and the contribution of fat oxidation to energy supply became negligible above 80 ± 1 % of V'O_2peak.

After the training period (POST), in the MICT group fat oxidation rates were not significantly different from those at PRE at all the exercise intensities (Fig. 3.2A). On the other hand, the HIIT group exhibited a greater absolute rate of fat oxidation at 60 (+45%, P:0.019), 70 (+119%, P:<0.001) and 80 (+104%, P: 0.076) % of V'O_2peak (Fig. 3.2B), whereas at 40 and 50 % of V'O_2peak the values were not significantly different from those at PRE.

After 4-months of follow-up, in the MICT group fat oxidation rates were still not significantly different from those at PRE at all the exercise intensities (Fig. 3.2A). On the other hand, the HIIT group exhibited a greater absolute rate of fat oxidation at 60 (+32%, P:0.031), 70 (+28%, P:<0.001) and 80 (+80%, P: 0.047) % of V'O_2peak (Fig. 3.2B), whereas at 40 and 50 % of V'O_2peak the values remained not significantly different from those at PRE.

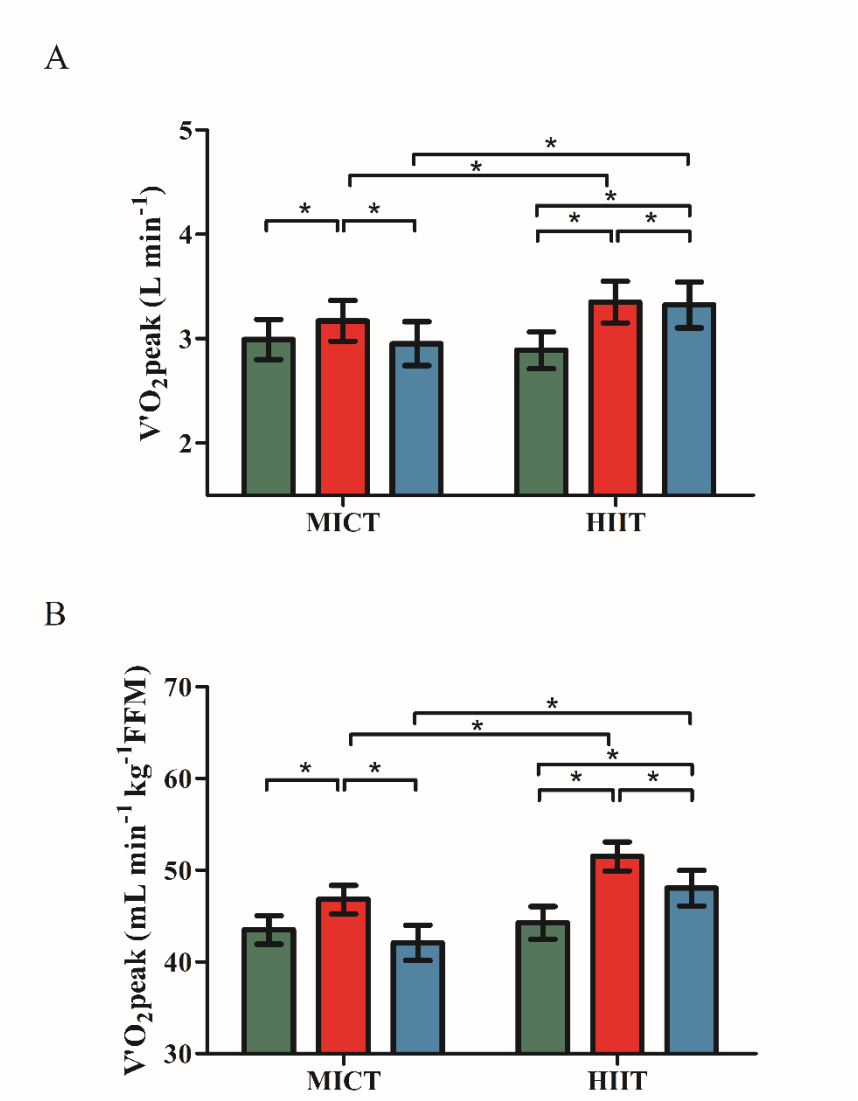


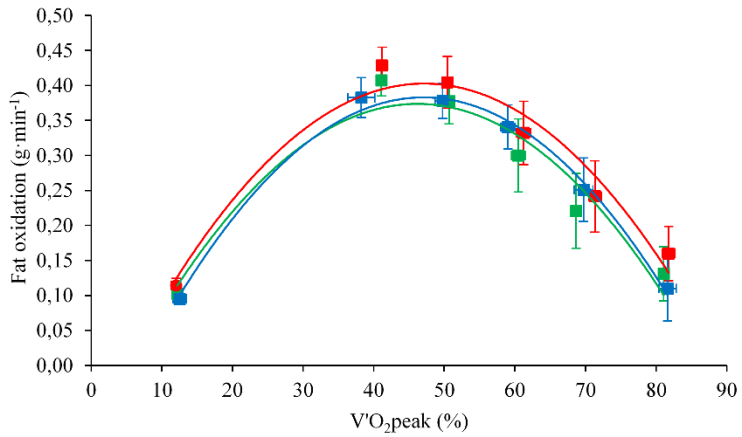
Figure 3.1 -. Absolute peak oxygen uptake (V'O_2peak, panel A) and peak oxygen uptake normalized by Fat Free Mass (V'O2peak·FFM-1, panel B) measured before (PRE, ■) and after 3-months (POST, ■) of weight-management program, and after 4 months of follow-up (■), in Moderate Intensity Continuous Training (MICT) and High Intensity Interval Training (HIIT) groups.

All values are presented as mean ± standard error.

Significance by generalized linear mixed model (see statistical paragraph):

**: Significantly different, P<0.05.*

A.



B.

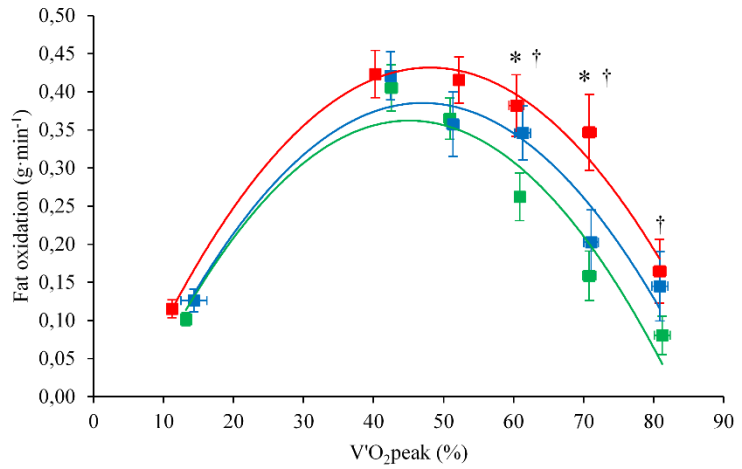


Figure 3.2 - Fat oxidation rate as a function of exercise intensity expressed as percent of peak oxygen uptake (V'O2peak) before (PRE, ■) and after 3-months (POST, ■) of weight-management program, and after 4 months of follow-up (■), in Moderate Intensity Continuous Training (MICT, panel A) and high intensity interval training (HIIT, panel B) groups.

All values are presented as mean ± standard error.

**: significantly different PRE vs. POST, P<0.05*

† : significantly different PRE vs. follow-up, P<0.05

Mitochondrial respiration ex vivo and citrate synthase activity.

Results of citrate synthase (CS) activity assays are reported in Figure 3.3C. No significant differences were observed after the weight-management program compared to the baseline values in both MICT and HIIT groups. Thus, none of the two protocols of exercise training affected CS activity

Conversely, the weight-management program affected significantly the intrinsic oxidative phosphorylation capacity. Data are reported in Figure 3.3. In particular,

maximal ADP-stimulated mitochondrial respiration (CI+II state 3 respiration) increased significantly (P 0.042) with respect to the baseline values after the weight-management program (POST) in both MICT and HIIT groups. The increase was +67% for MICT and +36% for HIIT, without significant difference between the two groups (Fig. 3.3A)

The data dealing with oxidative phosphorylation coupling at a specific substrate supply (glutamate and malate), calculated as ratio [(State 3 – Leak)/State 3] and reported in Fig. 3.3B, show that they were not affected by the two training protocols. At baseline, the values of the ratio were within 0.77-0.80 and did not change significantly after the weight-management program, for both MICT and HIIT. Finally, the maximal capacity of the electron transport system (ETS) uncoupled from the phosphorylating system, determined by addition the chemical protonophore FCCP, augmented after the weight-management program (Fig. 3D). Specifically, ETS sustained by glutamate/malate and succinate (complex I+II ETS) increased significantly ($P<0.05$) as compared with baseline in MICT (+45%) and HIIT (+61%), without significant difference between them. In addition, either rotenone-sensitive (complex I ETS) or -insensitive (complex II ETS) electron transport system exhibited a pattern similar to that of complex I+II ETS, indicating that both complex I and complex II were upregulated, although their increases *vs.* the baseline values did not reached the statistical significance.

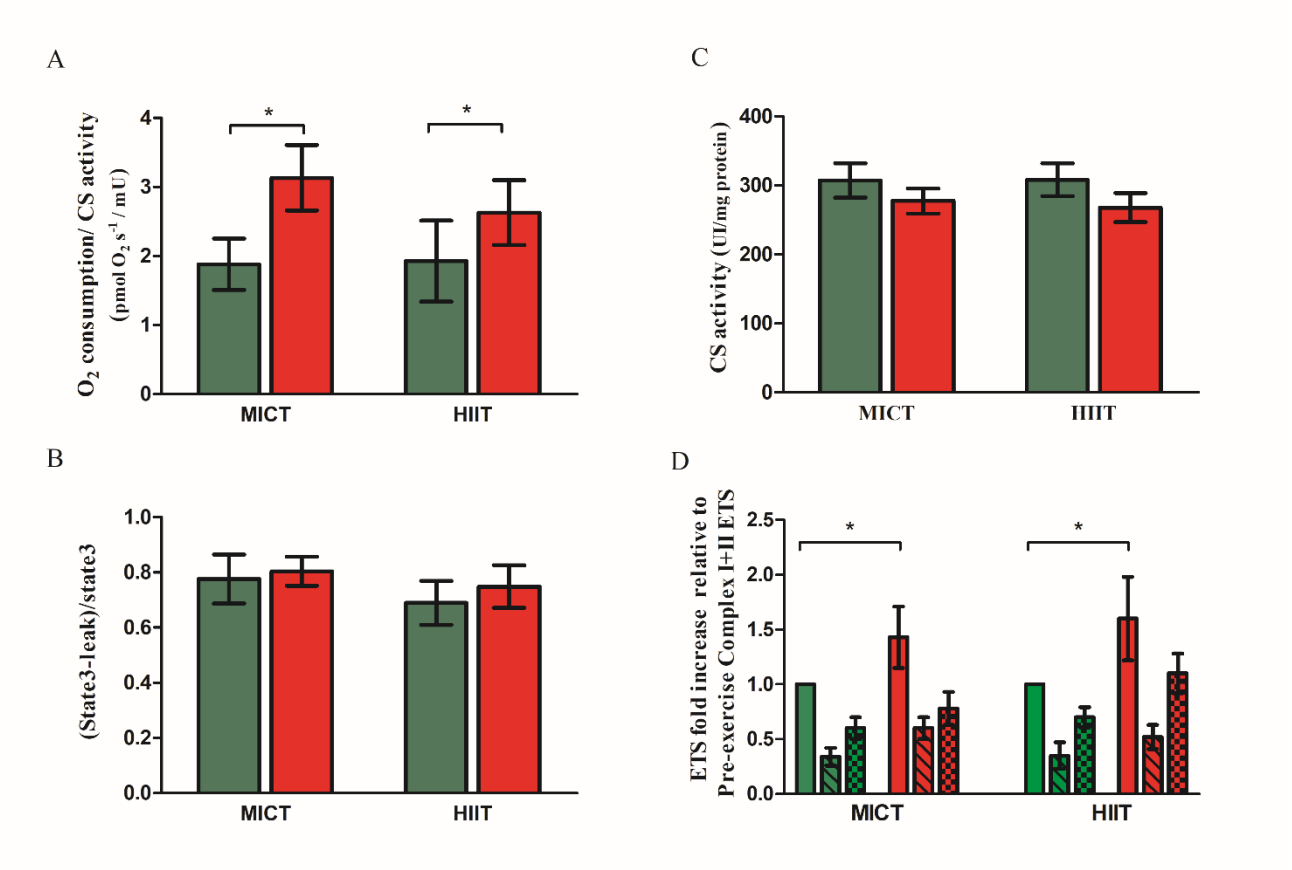


Figure 3.3 - Maximal ADP-stimulated mitochondrial respiration, i.e. CI+II state 3 respiration normalized for citrate synthase activity (panel A), and oxidative phosphorylation coupling, i.e (state3-leak)/state3 ratio (panel B), Citrate synthase activity (panel C) and in panel D, ETS sustained (rotenone-sensitive ETS) by Complex I (stripes) and by Complex II (squares) along with the ETS sustained by both Complex I and II (full coloured); all measured before (PRE, ■) and after the weight-management program (POST, ■) in Moderate Intensity Continuous Training (MICT) and High Intensity Interval Training (HIIT) groups.

All values are presented as mean ± standard error of N=6 for PRE and N=8 for POST in MICT group, N=6 for PRE and N=7 for POST in HIIT group for panel A, B and D. The values of CS (Panel C) refer to N=13 for MICT and N=14 for HIIT group. In panel D for each group the values are normalized to the respective values of PRE-exercise Complex I+II ETS.

**: significantly different, P<0.05*

3.4 Discussion

In obese patients the 3-month weight-management program entailing, in terms of exercise, MICT or HIIT, resulted in: 1) significant improvement of V′O_2peak in both groups, although more pronounced in HIIT; 2) significant increase in fat oxidation rate during submaximal exercise, only in the HIIT group; 3) similar increases, in the two groups, of maximal ADP-stimulated mitochondrial respiration. and 4) elevated adherence without adverse events in both groups. After 4 months of follow-up, only in the HIIT group V′O_2peak and fat oxidation rate were still greater compared to the baseline measurements.

HIIT program improve V'O_2peak and fat oxidation rate

Both MICT and HIIT increased the V'O_2peak, although a greater improvement was observed for HIIT, confirming previous research conducted in the general population and in obese people (Lazzer et al. 2017). HIIT would elicit V'O_2max improvements through both central and peripheral adaptations, whereas MICT would elicit V'O_2max improvements mainly through peripheral adaptation (Daussin et al. 2007). In our study, we measured a V'O_2peak increment in the MICT group and mitochondrial respiration (peripheral factor) improved as well. It must be said also that despite mitochondrial respiration improved in MICT, fat oxidation (peripheral factor) did not. This might suggest that mitochondrial respiration improvement in obese subjects do not contributed to improve also fat oxidation and the V'O_2peak increment is occurred independently from fat oxidation following MICT. The intensity that elicits maximal whole-body fat oxidation is approximately 60% of V'O_2max in well trained athletes (Achten et al. 2003), 50% in the general population (Achten et al. 2003) and 40% in obese patients (Lazzer et al. 2017). During HIIT the most part of the energy comes from carbohydrates (Hetlelid et al. 2017), since above of 80% of V'O_2max the contribution of the fat oxidation is almost negligible (Achten et al. 2003; Lazzer et al. 2017). Nevertheless, Hetlelid et al (Hetlelid et al. 2017) found that, by comparing well trained athletes and moderately active people, the higher performance by the former group during HIIT was mainly explained by their nearly threefold higher rates of fat oxidation capacity, that allows them to keep fat oxidation rate by far higher compared with moderately active people, both during high intensity exercise and during recovery. In accordance with the Hetlelid results (Hetlelid et al. 2017), the fundamental role of fat oxidation during HIIT might explain why this type of training is more efficient than MICT in improving the fat oxidation rate. Furthermore, previous studies showed that maximal fat oxidation rate was related to V'O_2max (Nordby et al. 2006; Hetlelid et al. 2017), suggesting that a training suited for improving V'O_2max might improve fat oxidation capacity as well. Our data regarding HIIT group supports this hypothesis but on the contrary the aerobic fitness improvement following MICT seems mainly due to central factors since mitochondrial respiration enhancements are not accompanied by an improvement of the capacity to utilize fats during the exercise, maybe due to the more pronounced central limitation in

obese highlighted in the previous chapter (Vaccari et al. 2019). On the other hand, carbohydrate utilization during HIIT is not different between well trained athletes and moderately active people (Hetlelid et al. 2017); moreover, it does not change following HIIT training (Lazzer et al. 2017).

$V'O_2$ and SAP at rest and during walking at submaximal intensity, decreased in both training groups, with no differences. It is noteworthy that HIIT can improve a clinically relevant parameter such as SAP, by an amount similar to that described following MICT, despite a lower training time requirement. A previous meta-analysis described a better ability by HIIT in reducing blood pressure in obese patients and in general, HIIT seems more effective in reducing markers correlated with cardiometabolic risk (García-Hermoso et al. 2016). According to the literature (Sawyer et al. 2016) HIIT might induce different vascular adaptations than MICT; given a similar $V'O_2max$ increment, HIIT would increase arterial dilatation, whereas MICT would increase the resting arterial diameter (Sawyer et al. 2016). Our data showed a slightly increment on SVC and a-$\bar{v}O_2$ diff during submaximal exercise, without differences between groups, suggesting an improvement of vascular function. According to previous research conducted on non-obese sedentary subjects (Daussin et al. 2007), HIIT improves the maximal performance of the cardiovascular system, whereas improvements obtained by MICT would mainly manifest at submaximal intensities.

<u>MICT and HIIT improve oxidative phosphorylation capacity but not efficiency</u>

Looking at the a-$\bar{v}O_2$ diff improvements it is reasonable to expect a corresponding improvement in mitochondrial respiration. Our results revealed indeed an improvement of maximal ADP-stimulated respiration and maximal ETS capacity after both MICT and HIIT, without changes in oxidative phosphorylation coupling, suggesting that oxidative phosphorylation capacity but not efficiency was enhanced by exercise training. Furthermore, we observed no improvement of mitochondrial content, as estimated by CS activity, after both training. In contrast, in non-obese subjects a study (Wallman et al. 2009) documented that some markers of mitochondrial biogenesis increase in high-intensity interval running more than in moderate-intensity continuous running, and other convincing studies claim that HIIT and so high intensity is more effective in improving markers associated with mitochondrial contents (MacInnis and Gibala 2016; MacInnis et al. 2016). On the contrary Granata et al. (Granata et al. 2016), didn't find any

changes in CS and other mitochondrial content markers following three training interventions at different intensities. So, the training's determinant factors for the improvement of mitochondrial content are not yet completely clear (Bishop et al. 2019), according to some studies the key factor may be the volume (Granata et al. 2018), but MacInnis et al (MacInnis et al. 2016) reported that sprint interval training increases mitochondrial content to a similar extent to MICT despite a reduced exercise volume. However, it is important to consider that all the above-mentioned studies refer to normal weight people though; Boyd et al (2013) (Boyd et al. 2013) instead, sought into this topic in obese/overweight subjects and didn't find difference in skeletal muscle oxidative capacity and mitochondrial content following low intensity low volume training compared to high intensity high volume training. Thus, we may infer that the increase of mitochondrial function we observed in obese subjects after the weight-management program was due to activity/assembly regulation of oxidative phosphorylation complexes or remodelling of mitochondrial inner membrane. Menshikova et al. (Menshikova et al. 2007) suggested a similar hypothesis in sedentary obese individuals undergoing moderate-intensity physical activity combined with weight loss. In particular, the authors observed an improved enzymatic capacity for oxidative phosphorylation without a significant change in mtDNA content, hypothesizing a mitochondrial cristae remodelling. Our data showing an increase in maximal capacity of both Complex I and Complex II may be in accordance to such hypothesis.

Overall, our results prompt us to propose exercise training, irrespective of the differences between the two training interventions investigated in the present study, as a good strategy to counteract the alteration of the mitochondrial proteome recently observed in skeletal muscle of subjects with obesity (Kras et al. 2018). Indeed, such proteomic profile, with proteins forming the TCA cycle increased and those forming the oxidative phosphorylation complexes decreased, has increased capacity to produce reducing equivalents of NADH and FADH2 in an impaired electron transport chain, thereby generating oxidative stress (Kras et al. 2018).

Despite both training modalities improved oxidative mitochondrial function, only after HIIT the capacity to oxidize lipids during exercise improved. It should be considered that mitochondrial oxidative capacity widely exceeds systemic O_2

delivery (Boushel et al. 2011), and does not seem to be related with total body fat oxidation (Nordby et al. 2006). Looking at our results, the improvement in fat oxidation in HIIT was not associated with changes in CS activity. This suggests that at least for 3 months of training, the improvement of fat oxidation is not due to mitochondrial adaptations, but to other factors, like improvements in O_2 muscle supply, capillary density and O_2 diffusion. Indeed, endurance athletes, compared with untrained individuals, have higher whole body maximal fat oxidation which however do not correlate with mitochondrial fat oxidation (Nordby et al. 2006); this further suggests that higher O_2 availability provided by the central cardiovascular system might be the main factor increasing whole body fat oxidation in obese subjects.

Metabolic parameters

After 3-months of weight-management program, Total and LDL cholesterol decreased significantly in both groups, while no significant effects on HDL cholesterol, triglycerides, glucose and insulin were observed in both groups. Most of the studies in obese and non-obese subjects comparing HIIT and MICT, are in agreement with our results, showing similar effects of the two training modalities on total, LDL, HDL cholesterol, and triglycerides, fasting plasma glucose and insulin, and estimated insulin sensitivity (Wallman et al. 2009).

Training Adherence

During the weight-management programs no differences in training adherence between MICT and HIIT and no adverse events were observed, in agreement with previous studies (Jung et al. 2015), but in contrast with Lunt et al (Lunt et al. 2014). Lunt et al. (2014) showed that, despite the greater potential efficiency of HIIT in improving aerobic capacity compared to MICT, the effectiveness of HIIT may be reduced due to the lower adherence to training prescriptions. In the present study we did not notice a lower adherence to the HIIT program, despite the training period was quite long (3 months): the number of training sessions (about 35) were the same in the two groups and the actual intensities were quite close to the programmed ones. HIIT was reported to be more enjoyable compared to MICT, at least inside a laboratory setting (Bartlett et al. 2011); our work showed that this is true even outside the laboratory setting and for a relatively long period of time. Given that "lack of time" remains one of the most

commonly cited barriers to regular exercise participation (Gillen and Gibala 2014), HIIT could be a time-efficient exercise strategy that warrants consideration for training prescription also in the obese population.

Follow-up

After 4 months of follow-up, V'O_2peak and fat oxidation rate decreased in both groups compared with POST. Only the HIIT group, however, maintained higher values of V'O_2peak and fat oxidation rate than at PRE, even though other cardiovascular and anthropometric characteristics were equally improved in both groups. This suggests a greater long-term efficiency of HIIT in maintaining the cardiovascular fitness and metabolic health, and so a greater ability to reduce cardiovascular risk and insulin sensitivity (Robinson et al. 2015).

Mean BM after follow-up was slightly increased in both groups compared with POST (although being still lower than at PRE). Interestingly, fat mass and waist and hip circumferences were unchanged between the end of the supervised training and the end of the follow-up period; FFM, on the other hand, increased. This suggests that physical activity, particularly vigorous physical activity, maintained after the training period, induced an increase in FFM. State of Change questionnaire rate remained higher after the follow-up period, suggesting that physical activity had positive effects in adopting and maintaining an active lifestyle (Marcus et al. 1992) independently from weight gain.

Along with the increase in FFM, improvements were observed in arterial pressure, SVC and a-$\bar{v}O_2$ diff, suggesting a positive effect of the lean mass increase on the cardiovascular system parameters (Pedersen and Febbraio 2012). Further, the hip and waist circumferences did not increase after the follow-up period despite the BM increment, suggesting a further reduction in cardiovascular risk (O'Donovan et al. 2009).

CO decreased compared to POST and a-$\bar{v}O_2$ diff tended to increase both at rest and during walking, suggesting an improved oxygen extraction of the peripheral tissues and an improvement in muscle oxidative function (Daussin et al. 2007), as confirmed by the mitochondrial data. On the other hand, in the follow-up SAP during walking increased compared to POST and SVC decreased, suggesting an increased arterial stiffness (Saladini and Palatini 2017).

Limitations

High-resolution respirometry measurements could not be carried out immediately on fresh biopsies but were performed on rapidly frozen muscle samples (see methods). Thus, it cannot be excluded that the freeze-thaw procedure led to some underestimation of maximal ADP-stimulated mitochondrial respiration, as remarked by some authors (Larsen et al. 2012; Meyer et al. 2014). Nevertheless, the accurately controlled cryopreservation procedure proposed by Kuznetsov et al. (Kuznetsov et al. 2003) was used in the present study, as in previous studies by separate laboratories (Cannavino et al. 2015; Wüst et al. 2011; Salvadego et al. 2016). Moreover, we verified the intactness of the outer mitochondrial membrane and excluded from our analyses the samples exhibiting a substantial increase in respiration following administration (in the measurement chamber) of cytochrome *c*. Thus, we considered for our analyses only the samples showing an increase in mitochondrial respiration following administration of cytochrome *c* within the limits allowing the exclusion of significant damage of the outer mitochondrial membrane (<10% increase of cyt.C-induced respiration).

Finally, we have not mitochondrial respiration data for all the subjects recruited in the present study because of the fibres fragility consequent to muscle microneedle biopsy and sample handling. Nevertheless, numerically homogeneous populations of samples (n=6-8) were analysed successfully for both the ET and HIIT group, and the main physiological mean characteristics in these subgroups were not significantly different from whole group of subjects, even though they were not paired.

Conclusions

In conclusion, MICT and HIIT improved the anthropometric measures, some cardiovascular markers and mitochondria intrinsic function. However, HIIT was more efficient in improving V'O2peak and fat oxidation capacity. The improvements of these variables were maintained also after 4 months of follow-up. These results encourage to utilize HIIT in the obese people given their more pronounced cardiovascular functional limitation, impaired capacity to oxidase fats during the exercise and tendency to loss all the improvements gained with a training period.

CHAPTER IV

CONCLUSIONS and PRATICAL APPLICATIONS

The principle aims of the studies undertaken for this thesis were to bright new light on physical training in obese.

The main results of chapter II suggest that whole body maximal exercise performance in obese might be constrained more markedly than lean subjects by central rather than peripheral factors. In fact, they have a less compromise maximal voluntary contraction after the whole-body exercise despite a similar aerobic capacity in whole body exercise, similar maximal parameter in the single leg knee extension exercise and similar recovery from fatigue.

The benefits of weight loss are not in dispute, but, due to the low success-rate for obese adults in attempting and then maintain weight loss (McGuire et al. 1999), shall be recommended that activities entailing physical fitness improvements would be included within the weight management programs.

In this respect, HIIT could be introduced even in the training routine of obese people. We have shown that HIIT is at least as effective as MICT in improving BMI and body composition, with less time requirement and similar training adherence. Furthermore, the main advantage is that HIIT is more effective in improving aerobic fitness after a relatively long training period (3 months) and in improving fat oxidation capacity.

After four months of follow-up the body composition was equally improved in the two groups, since during the follow-up period the physical activity of the two groups, as reported by the questionnaire, was not different. However, the $V'O_2max$ and fat oxidation remained improved only in HIIT group.

An inability to oxidize lipids seems to be an important factor associated with insulin resistance (Kelley and Simoneau 1994), as well as with a high rate of weight gain (Zurlo et al. 1990). HIIT effectively improving fat oxidation may help this type of patients in improving their situation, both enhancing metabolic status and preventing from long-term weight regain, since it was able to keep improved fat oxidation even after four months of follow-up. Furthermore, some of the benefits typically attributed to exercise, such as lower insulin resistance, hypertension and plasma concentration of LDL, are presumably related to the improved fat oxidation (Achten and Jeukendrup 2004). Beside the more heal-related aspects, improving fat oxidation is beneficial for exercise performance and endurance capacity (Hawley et al. 1998). Helping obese people to be comfortable even with moderate intensity activities, may encourage them to break the vicious

circle of sedentary thus improving their quality of life. Further, a better cardio-respiratory fitness is a valuable outcome in accordance with the results of several prospective studies that have shown a significant inverse relationship between cardio-respiratory fitness and mortality in obese (Kokkinos et al. 2009). In addition, for some patients introducing intensity variation in the exercise schedule might represent a mental stimulus (Stork et al. 2018) augmenting long- term adherence to regular exercise. Being aware that it is only a speculation, we can expect that HIIT might be useful for the long-time results in obese training.

In normal-weight people and rats, training intensity may be decisive in improving mitochondrial respiration. In contrast, it appears that training volume, rather than training intensity, may be the key-factor for improvements in mitochondrial content (Bishop et al. 2014). Despite our data showed an increment in mitochondrial respiration, there was not difference between the two groups and no increment in mitochondrial content at all. This was one of the first studies comparing mitochondrial function adaptations following either HIIT or MICT in obese; therefore, it is premature to give practical insights on exercise training prescription to maximise improvements in mitochondria function and content. However increasing training volume, mitochondrial content would increase as well, as seen by previous works (BÆkkerud et al. 2016; Tan et al. 2018), but, there is no other works either confirming or refuting our results on mitochondrial respiration following HIIT and MICT in obese.

HIIT might have some contra arguments though. It has to be consider that sedentary and obese people usually view exercise as a “sacrifice” or “something impossible” (De Feo 2013). So, in some subject prescribing high intensity exercises could be counterproductive.

In practical terms, the general advice may be to start with exercise program at a moderate intensity and increase progressively within the first sessions. Then after the first months, once patients have significantly improved their aerobic capacity and skills, it is useful to variate the stimulus inserting even HIIT and high intensity exercises (De Feo 2013).

APPENDIX

New HIIT method proposal: HIDIT

Adapted from:

Vaccari F, Giovanelli N, Lazzer S. High Intensity Decreasing Interval Training (HIDIT) increases time above 90% V'O_2peak. Submitted to Eur J Appl Physiol.

4.1 – Abstract of the appendix

Maximal oxygen uptake ($V'O_2max$) is defined as the highest rate at which oxygen can be uptake from the environmental air and used by cell metabolism during physical activity (Hill and Lupton 1923), it is a relevant parameter of the cardiorespiratory capacity, important for endurance athletes (di Prampero 2003) and patients (Poole et al. 2012). It has been shown that, in order to improve the $V'O_2max$, a training protocol should prolong the time in which the oxygen uptake remains close to the maximum (within 5-10% of $V'O_2max$) for as long as possible (Wenger and Bell 1986; Midgley and Mc Naughton 2006). High intensity interval training (HIIT) is very effective in maintaining the metabolic rate near $V'O_2max$ (Buchheit and Laursen 2013a), more than continuous endurance training (Midgley and Mc Naughton 2006) and can be composed by either short or long bouts at high-intensity (work) alternated by recovery periods (recovery) at low intensity (or rest) (Buchheit and Laursen 2013a).

Comparing matched work HIIT protocols, those with longer interval results in higher $V'O_2$, HR, RPE and faster [La] kinetics at the end of the exercise (Turner et al. 2006), and longer time above 90% of VO_2max if the total exercise time is fixed (Millet et al. 2003). On the other hand, if the exercise is carried to exhaustion, HIIT with shorter interval duration leads to a longer Tlim (Rønnestad and Hansen 2016). Given these features, it is not easy to handle HIIT if the aim is to prolong time near $V'O_2max$. In this regards, according to two complete reviews (Midgley and Mc Naughton 2006; Buchheit and Laursen 2013a, b), the intensity should be set between ~90% and ~105% of the $V'O_2max$ power/velocity with long (>1-2 min) intervals and between ~100 and ~120% of the $V'O_2max$ power/velocity with short (<45 s) intervals. The effect of recovery intensity on the time at $V'O_2max$ instead is not straightforward (Buchheit and Laursen 2013a), Midgley and Naughton (Midgley and Mc Naughton 2006) recommended setting the intensity between 50% of the $V'O_2max$ velocity and the lactate threshold. Indeed, active recovery is preferable since it can increase the contribution of aerobic metabolism in HIIT performance (Dorado et al. 2004).

To further optimize the performance, a “Fast start pacing strategy” has been proposed in continuous exercises (Jones et al. 2008; Bailey et al. 2011; Dekerle et al. 2015; Leprêtre et al. 2016). Starting faster and then decreasing the intensity

allows $V'O_2$ kinetics to speeds up increasing the oxidative contribution to energy turnover, thus improving the performance (Jones et al. 2008; Bailey et al. 2011; Leprêtre et al. 2016) and prolonging the time close to $V'O_2max$ (Billat et al. 2013). Thereafter, the fast start pacing strategy has been successfully applied to HIIT too (De Aguiar et al. 2013; Lisbôa et al. 2015; Rønnestad et al. 2019).

We hypothesized that similarly to the fast start pacing strategy, an HIIT protocol that aims to keep the $V'O_2$ as longer as possible close to $V'O_2max$, could start with long bouts (2-4 min), to increase the $V'O_2$ quickly (Millet et al. 2003; Turner et al. 2006); then, both work and recovery bouts should be reduced when the subject is approaching the exhaustion. to increase Tlim (Rønnestad and Hansen 2016).

Therefore, the aim of our study was to compare the time above 90% of $V'O_2peak$ ($T>90\%V'O_2peak$) in three different HIIT protocols. The proposed HIIT exercises had the same intensity and work/recovery ratio and were structured as follow: 1) constant short intervals (SI_{HIIT}), 2) decreasing length of the intervals from long to short (HIDIT), and 3) constant long intervals (LI_{HIIT}).

Based on the characteristics described above (Millet et al. 2003; Turner et al. 2006; Rønnestad and Hansen 2016), we hypothesized that in HIDIT the $T>90\%V'O_2peak$ will be longer. A major physiological determinant which can explain the variability between subjects in the Tlim during interval and continuous exercises, is the differences among the lactate threshold intensity and the $V'O_2max$ intensity (Midgley et al. 2007). We tried to establish the HIIT intensity based on the critical power (CP), in order to minimise the between-subjects variability and to verify whether the Tlim of HIDIT is correlated with the difference CP-load peak.

Twelve cyclists performed three HIIT sessions. Every protocol had the same work and recovery power and ratio work/recovery. The three protocols consisted in long intervals HIIT (LI_{HIIT}) (3 min work – 2 min recovery), short intervals HIIT (SI_{HIIT}) (30 s work – 20 s recovery) and a protocol that combine long and short intervals (HIDIT, work from 3 min to 30 s and recovery from 2 min to 20 s). Tlim, $T>90\%V'O_2peak$, blood lactate [La] at 3^{rd} min and at Tlim were measured.

The results have shown that HIDIT applied to cycling exercise in well-trained amateur cyclist may enhance $T>90\%V'O_2peak$ without reduction of Tlim, ratio $T>90\%V'O_2peak \cdot Tlim^{-1}$ or average $V'O_2$. The average $V'O_2$ is higher in HIDIT

compared with LI_{HIIT}. Finally, despite the higher stimulation of the $V'O_2$, the rate of perceived exertion and the other physiological parameters at the end of the exercise were not different compared with long or short intervals HIIT suggesting that HIDIT was not more demanding.

4.2 – Materials and methods

Subjects

We enrolled twelve middle age, no smokers, amateur cyclists (41±11 y; 76±10 Kg; $V'O_2$peak 4.32±0.47 $L \cdot min^{-1}$), Table 1. They reported at least 3 training sessions per week in the previous six months. None of the subjects had evidence of significant diseases or took regular medications.

Study protocol

The Ethics Committee of the Friuli-Venezia-Giulia (Comitato Etico Unico Regionale C.E.U.R.) approved the study (protocol number 9626). During the first visit to the laboratory, an operator explained the purposes and objectives of the study to each subject and obtained a written informed consent. Then, participants underwent medical examination and performed a maximal ramp-incremental exercise test on cycle ergometer to measure the $V'O_2$peak. Although the objectives were explained to the subjects, we did not reveal the hypothesis that HIDIT could prolong T>90%$V'O_2$peak to do not affect the results. After the first visit, the participants were examined three or four times to determine the critical power, and three times for performing the SI_{HIIT}, HIDIT and LI_{HIIT} tests. Every visit was separated from the previous one by two days and the participants were instructed to avoid consumption of caffeinated beverages for at least 8 h before each test and to abstain from vigorous physical activity in the 24-h preceding each testing session. Every subject concluded the entire protocol within four weeks from the first visit. The critical power parameters were used to program the HIIT tests. Subsequently, during the three HIIT tests we measured time to exhaustion (Tlim), T>90%$V'O_2$peak, blood lactate concentration [La], rate of perceived exertion using the Borg CR10-Scale (Borg et al. 2010) and $V'O_2$ at the 3rd minute and at the end of exercise.

Incremental exercise

The incremental exercise was performed under medical supervision and standard safety procedures were followed. During the first visit, an operator instructed the subjects to correctly report the rate of perceived exertion on the CR10 scale (Borg et al. 2010). Incremental exercise, critical power trials and HIIT tests protocols were performed by utilizing a cycle ergometer (CE) (Monark Ergomedic 839E). Every test was preceded by the same warm up procedure: 10-min cycling at 100 W followed by 2-min resting. During the first warm up, subjects chose their preferred pedalling cadence (~ 90 rpm). The steady ramp-incremental test started from 100 W and the power continued to increase with a pace of 25 $W \cdot min^{-1}$ throughout the test until volitional exhaustion. Throughout all the tests (incremental and HIITs), the exhaustion was defined as the inability to maintain the assigned cadence within 10 rpm longer than 5 s, despite the strong operator's encouragement.

We measured $V'O_2$ and $V'CO_2$ breath-by-breath by using a metabolic unit (Quark CPET, Cosmed, Italy). We measured the ventilation by a turbine calibrated before each experiment by a 3 L syringe at three different flow rates. Calibration of O_2 and CO_2 analysers was performed before each experiment by utilizing gas mixtures of known composition (16.00% O_2; 4.00% CO_2). $V'O_2$ peak corresponded to the highest $V'O_2$ average obtained in 30 s.

Power duration relationship

The same warm-up and the same cadence were used for the critical power (CP) test as well. CP and the amount of work that can be done during exercise above CP (W') (Jones and Vanhatalo 2017; Burnley and Jones 2018) were estimated from three to four high intensity trials at exhaustion from 80% to 100% of the peak power detected during the incremental test and adopted to result in 'exhaustion' in a minimum of ~2 min and a maximum of ~15 min (Jones and Vanhatalo 2017). The work done in each of the separate exercise bouts has been plotted against sustainable time. So, the following work (W) – time (t) linear regression was used to find CP and W' (Moritani et al. 1981; Hill 1993; Jones and Vanhatalo 2017).

$$W = CPt + W' \qquad [1]$$

According to the equation, the CP is given by the slope of the regression and the W' is the y-intercept.

HIIT tests

After the incremental test and the critical power trials, subjects performed three HIIT tests in a randomised order. The power of the work and recovery bouts and the work/recovery ratio were the same between the trials and we only adjusted the duration of the intervals (see Table 1 for average values). We set the ratio work/recovery time at 3/2 for all the training tests. The high intensity bouts power has been customised for every subject and corresponded to the power that was supposed bringing to exhaustion in five minutes according to the following equation (Jones et al. 2010):

$$Power = \frac{W'}{t=300s} + CP; \qquad [2]$$

it was about 117% of CP. The low intensity was mirrored below CP (about 83% of CP), in this way the CP threshold was exactly in the middle between high and low intensity.

The three tests were structured as follow:

- Short Intervals (SI_{HIIT}): 30 seconds at high intensity and 20 seconds at low intensity repeated until exhaustion;
- High Intensity Decremental Intervals Training (HIDIT): 3 minutes high intensity and 2 minutes low intensity, 2 minutes high intensity and 1 min and 20 sec low intensity, 1 min high intensity and 40 sec low intensity, 45 sec high intensity and 30 sec low intensity, then 30 sec high intensity and 20 sec low intensity repeated until the exhaustion. The ratio high-low intensity time was always (3/2). It has been adopted this protocol to quickly increase $V'O_2$ during the first long intervals bringing the subjects close to exhaustion and then the interval duration has been decreased rely on W'bal model (introduced in this thesis at page 16) trying to avoid exhaustion but keeping the subject always close to it. The model developed by Skiba and the research group of Jones (Chidnok et al. 2012; Skiba et al. 2012, 2014, 2015; Jones and Vanhatalo 2017), propose an equation that calculates and predicts the remaining W' (W'_{bal}) at a given time during the exercise:

$$W'_{bal} = W' - \int_0^t W'_{exp} * e^{-\frac{t-u}{\tau'_W}} * du \quad [1]$$

Where *t* is the time in seconds from the start of the high intensity exercise; *W'exp* is W' expenditure until that time; *t-u* is equal to the time (s) between segments of the exercise session that resulted in a depletion of W'; $\tau_{W'}$ is the time constant for *W'* reconstitution, calculated as:

$$\tau_{w'} = 546 * e^{(-0.01DCP)} + 316 \quad [2]$$

(Skiba et al. 2012)

DCP is the difference between CP and actual power below CP in watts.

$\tau_{W'}$ was keep the same proposed by Skiba (Skiba et al. 2012) for all the subjects. Then, the *equation 3* was applied as follow:

$$'W'_{bal} = W' + \frac{W'_{exp}}{\tau_{W'}}\left[1 - e^{\frac{t}{\tau_{W'}}}\right] \quad [3]$$

Where *t* is the time from the start of the HIIT test.

With these equations it is possible to forecast W'bal at any moment, therefore, the duration of the intervals has been chosen applying the *W'bal* model (*equation 3)* to prolong the exercise up to ~12 minutes.

- Long Intervals (LI_{HIIT}): 3 minutes at high intensity and 2 minutes at low intensity repeated until exhaustion.

Throughout the HIIT protocols, we measured the ventilatory parameters by using a breath-by-breath metabolic unit (CPET, Cosmed, Italy) and then we obtained the averaged values every 5 seconds. Before, after three minutes and at the end of exercise, we measured $V'O_2$, HR, [La], RPE and respiratory quotient (RQ). An operator collected a capillary blood sample from earlobe to measure the [La] with a dedicated device (Lactate Pro 2, Arkaray.inc., Japan), at the same time the subjects reported RPE consulting the CR10 scale positioned in front of them. Finally, the total time spent above 90 % of VO_2peak was determined as the sum of each averaged 5-s when the $V'O_2$ was equal or higher than 90 % of $V'O_2$peak.

Statistical analyses

In light of the data of Aguilar et al. (De Aguiar et al. 2013) that implemented a procedure similar to ours, for our purposes we calculated a sample size of 12 subjects to have a statistical power of 80% in order to refute the null hypothesis

and to obtain an ES of 0.88, with an alpha error of 0.05 and a beta error of 0.20 by using a one-way ANOVA with Bonferroni Correction.

Statistical analysis was performed using SPSS 20.0 software (IBM, Chicago, USA), with significance set at $P<0.05$. All results were expressed as means and standard deviations (SD). We investigated the differences between HIIT trainings protocol on: Tlim, T>90%V'O_2peak, T>90%V'O_2peak - $Tlim^{-1}$, work above CP (calculated as the total time in seconds above CP multiply by the difference between the high intensity power and CP, in Watts), average V'O_2 and finally the values at third minute and at Tlim (V'O_2, HR, [La], CR10-scale and RQ). All the parameters were analysed by one-way repeated-measures analysis of variance (ANOVA). Where the analysis found a significant difference, planned contrast between HIDIT and SI_{HIIT} and between HIDIT and LI_{HIIT} were used with Bonferroni correction to determine the origin of such effects. Confidence Interval of the differences (CI) and Effect size (ES) have been calculated by using the Cohen's d ($0<d<0.20$ small; $0.20<d<0.50$, medium; $0.50<d$, large). Correlations between the percentage of CP with respect to load Peak (%CP-Load Peak) and HIIT's Tlim were determined using two-tailed Pearson's product moment correlation coefficients.

4.3 – Results

Incremental test and CP trials

The Peak values attained during the incremental test are shown in TABLE 4.1 together with the power corresponding to the CP, the total work above CP (W') and the power imposed for the high and low intensity bouts. Even though the attainment of V'O_2peak was not set as a priori criteria for the constant work rate tests of the power-duration relationship, V'O_2peak has been always reached by the subjects.

Table 4.1 - Age, body mass and functional characteristic of the subjects (n:12).

	Mean ± SD	Min - Max
Age (year)	41 ± 11	29 - 62
Body mass (kg)	76 ± 10	66 - 95
HR peak (b min^{-1})	174 ± 10	155 - 193
$V'O_2$ peak (L min^{-1})	4.32 ± 0.47	3.66 - 5.10
Load Peak (W)	356 ± 40	295 - 436
CP (W)	254 ± 30	212 - 320
W' (KJ)	12.8 ± 4.1	8.5 - 22.7
High Intensity (W)	297 ± 35	249 - 364
Low Intensity (W)	212 ± 30	172 - 275

All values are mean and standard deviation (SD). HR: heart rate; V'O2peak: peak oxygen consumption; CP: critical power; W': total work sustainable above the critical power; High and Low intensity: the average intensity sustained during the HIIT tests.

Table 4.2 - Main results of the HIIT tests and selected physiological variable at 3rd minute and at the end of the tests.

	SI_{HIIT}	*HIDIT*	LI_{HIIT}	P
T lim (s)	714 ± 265	798 ± 185	664 ± 282	0.144
T>90%V'O_2peak (s)	183 ± 225	312 ± 207 [a,b]	179 ± 145	0.029
T>90%V'O_2peak·Tlim^{-1}	0.25 ± 0.29	0.39 ± 0.24	0.26 ± 0.21	0.070
Work > CP (KJ)	18.74 ± 8.95	22.01 ± 10.40	19.28 ± 11.06	0.136
Average V'O_2 (% peak)	82.00 ± 6.61	84.16 ± 4.00	79.56 ± 7.08	0.044
Values at 3rd minute				
V'O_2 (% peak)	85.33 ± 7.11	90.75 ± 5.94 [a]	89.58 ± 6.52	0.004
HR (% peak)	89.00 ± 4.00	91.00 ± 3.91 [a]	92.60 ± 3.60	0.003
[La] (mmol·L^{-1})	5.69 ± 1.62	8.03 ± 2.69 [a]	7.85 ± 3.01	0.007
CR10-scale	5.29 ± 1.57	6.67 ± 2.12 [a]	6.52 ± 2.03	0.008
QR	1.04 ± 0.06	1.10 ± 0.09 [a]	1.11 ± 0.08	>0.001
End Values:				
V'O_2 (% peak)	99.75 ± 8.62	100.17 ± 5.27	99.83 ± 8.36	0.981
HR (% peak)	97.80 ± 3.99	97.40 ± 2.99	97.50 ± 3.98	0.802
[La] (mmol·L^{-1})	10.75 ± 2.04	10.71 ± 4.72	10.83 ± 3.58	0.991
CR10-scale	9.48 ± 0.70	9.25 ± 1.78	9.56 ± 1.08	0.701
QR	0.97 ± 0.05	0.95 ± 0.05	1.00 ± 0.10	0.113

All values are mean and standard deviation (SD).

SI_{HIIT}: Short Intervals HIIT; HIDIT: High Intensity Decremental Intervals training; LI_{HIIT}: Long intervals HIIT; Tlim: time to exhaustion; T>90% V'O_2peak: time spent above 90% V'O_2peak; V'O2: oxygen uptake; mean V'O_2: mean V'O_2 maintained during the HIIT tests; HR heart rate; [La] blood lactate concentration; CR10-scale: perceived exertion; RQ: respiratory quotient.

Significance by one-way repeated-measure ANOVA. When p<0.05, planned contrasts with Bonferroni correction.

a: p<0.05 in post hoc HIDIT vs SIHIIT;

b: p<0.05 in post hoc HIDIT vs LIHIIT;

HIIT tests

The power corresponding to high intensity bouts was 297±35 W (117±6% of CP) and the low intensity power was 212±30.4 W (83±6% of the CP) (Tab. 4.2).

T>90%V'O_2peak was significantly longer in HIDIT (312±207 s) (P=0.026) compared with SI_{HIIT} (183±225 s; P= 0.036; CI: 255 to 3s; ES: 0.62) and LI_{HIIT} (179 ±145 s; P= 0.027; CI: 258 to 7s; ES: 0.64) (Tab. 4.2, Fig. 4.2).

There were not differences in Tlim (P=0.144), in the ratio T>90%V'O_2peak - $Tlim^{-1}$ (P=0.070) and in Work>CP (P= 0.136) between the three protocols (Tab. 4.2).

The average V'O_2 maintained during the HIDIT test (84.16±4.00%V'O_2peak) was significantly higher (ANOVA P=0.044) compared with LI_{HIIT} (80.16±7%V'O_2peak; P= 0.022; CI: 8.70 to 0.46%; ES: 0.17), but not significantly different than SI_{HIIT} (79.56±7.08% V'O_2peak; P= 0.106; CI: 6.79 to -1.46%; ES: 0.10).

The ANOVA procedure found a significant difference in V'O_2 % of V'O_2peak after three minutes (P=0.028) but it was similar in HIDIT (91±6%) and LI_{HIIT} (90±7%) (P= 0.339; CI: 4.77 to -2.43%; ES 0.18) and significantly higher in HIDIT compared with SI_{HIIT} (85±7% of V'O_2max) (P=0.006; CI: 9.02 to 1.81%; ES: 0.83). There was a significant difference also in HR% of HR peak after three minutes (0.028) and it was similar in HIDIT and LI_{HIIT} (91±4 vs 93±4%, respectively; P= 0.160; CI: -4.26 to 1.05%; ES: 0.37) but significantly higher in HIDIT compared with SI_{HIIT} (89±4%) (P= 0.019; CI: 5.35 to 0.04%; ES: 0.61). Similarly, ANOVA found significant difference after three minutes in CR10-Scale reports (P=0.008) and RQ (P=0.006). CR10 after three minutes was similar in HIDIT and LI_{HIIT} (6.7±2.1 vs 6.5±2.0, respectively; P= 0.824 CI:1.31 to -1.03; ES: 0.05) and significantly higher than SI_{HIIT} (5.3±1.6) (P= 0.031; CI 2.53 to 0.20; ES: 0.55). RQ after 3 minutes was not different in HIDIT and LI_{HIIT} (1.10±0.09 vs 1.11±0.08, respectively; P= 0.410; CI 0.05 to -0.02; ES: 0.05) and significantly higher than in SI_{HIIT} (1.04±0.06) (P=0.031; CI: 0.10 to 0.02; ES: 0.25) (Table 4.2).

There were not significant differences in [La] at rest before the three tests (SI_{HIIT}, HIDIT and LI_{HIIT}) (respectively: 1.13±0.20; 1.19±0.26 and 1.17±0.27 $mmol \cdot L^{-1}$, P>0.05). However after 3 minutes ANOVA found a difference (P<0.001) and [La] was higher in HIDIT (8.03±2.69 $mmol \cdot L^{-1}$) compared with SI_{HIIT} (5.69±1.62 $mmol \cdot L^{-1}$, P= 0.003; CI: 4.29 to 0.69 $mmol \cdot L^{-1}$; ES: 0.78), and similar to LI_{HIIT} (7.85±3.01 $mmol \cdot L^{-1}$, P= 0.007; CI 2.71 to -1.41mmol L^{-1}; ES: 0.05). At Tlim [La],

$V'O_2$, HR and RPE were not significantly different in the three tests (see Table 4.2).

Finally, the 59% of the Tlim variance in SI_{HIIT} could be explained by %CP-Load Peak, but there was not any significant correlation between %CP-Load Peak and Tlim in HIDIT and LI_{HIIT} (Fig. 4.3).

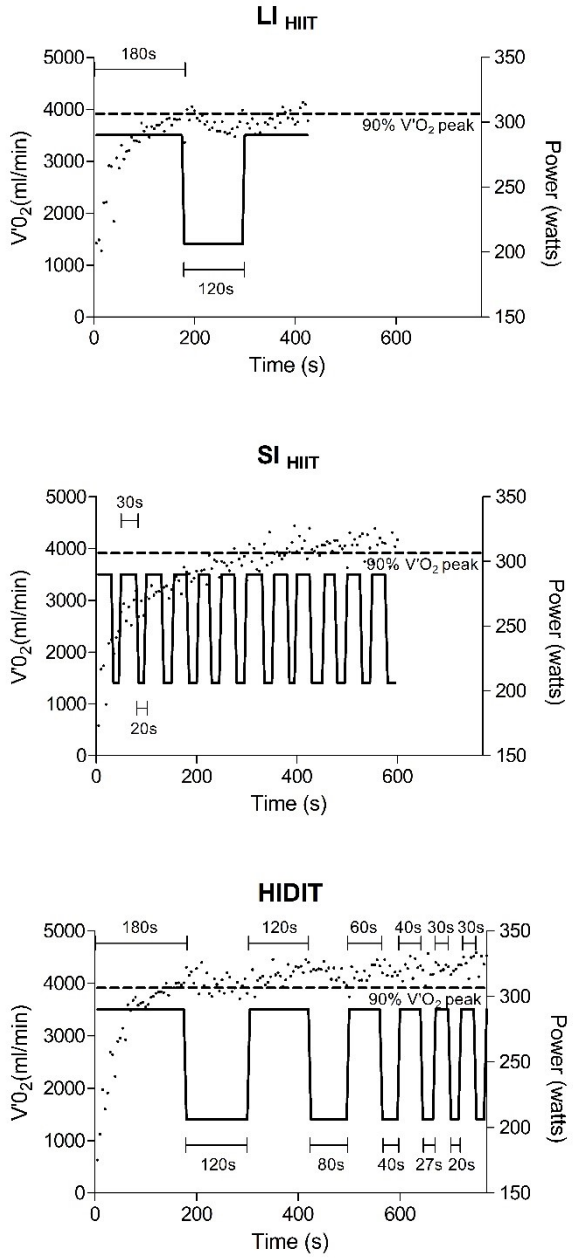


Figure 4.1

LI_{HIIT}: Long Intervals HIIT (3' High - 2' Low-intensity); SI_{HIIT}: Short Intervals HIIT (30" High - 20" Low-intensity); HIDIT: decreasing intervals HIIT (combining High intensity from 3' to 30" and low intensity from 2' to 20")

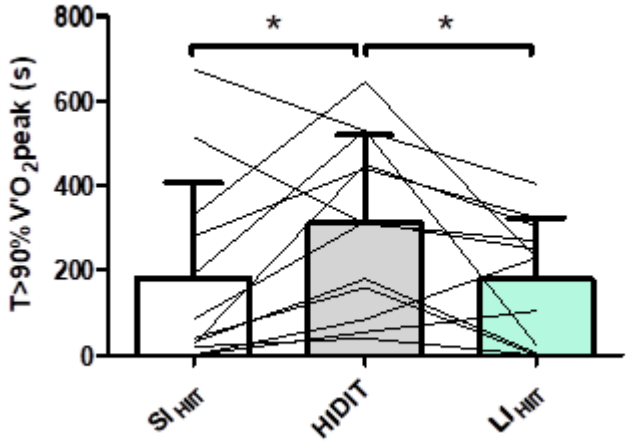


Figure 4.2

**: Significance by one-way repeated-measures ANOVA and planned contrast with Bonferroni correction between HIDIT and SI_{HIIT} and between HIDIT and LI_{HIIT} were used post hoc comparison, p<0.05.*

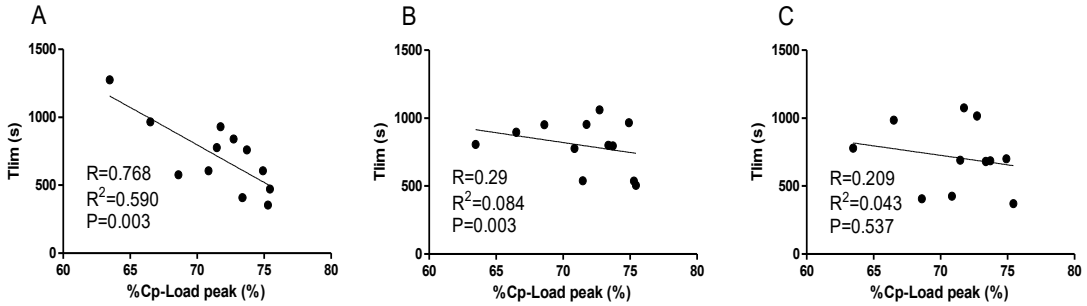


Figure 4.3.

Relationship between Tlim and the difference among percentage of CP with respect to Load Peak (A. SI_{HIIT}, B. HIDIT and C. LI_{HIIT}).

4.4 – Discussion

The main finding of the present study was that HIDIT enhances the time above 90% of $V'O_2$peak compared with the other HIIT protocols, despite having similar Tlim, [La], HR, RPE and $V'O_2$ at exhaustion. In addition, there was a relationship between Tlim and the percentage of Cp with respect to load Peak exclusively in SI_{HIIT}.

In HIDIT (and LI_{HIIT}) the protocol started with 3 min of high intensity as opposed to just 30 seconds in SI_{HIIT} and this resulted in a larger $V'O_2$, HR, [La], CR10 scale

and RQ at the 3rd minute. This is in agreement with the observations of Millet et al.(Millet et al. 2003) and Turner et al.(Turner et al. 2006), in which during long interval HIIT, a faster metabolic stimulation occurred. However, at Tlim there weren´t any difference in any of the above-mentioned parameters, suggesting that independently from the protocols adopted, the participants reached their personal maximal performances. Indeed, $V'O_2$ and HR were close to the peak values (100% and 97%, respectively) and the Borg scale was near 10, while [La] was above 10 $mmol \cdot L^{-1}$. It is worth to note that HIDIT led to longer T>90%$V'O_2$peak with the same RPE at the end of the exercise. In other words, HIDIT has potential bigger training benefits, despite the same perceived effort. But on the other hand, even though Tlim in HIDIT (798 s) seems longer than LI_{HIIT} (664 s) and similar to SI_{HIIT} (714 s), the ANOVA did not show any significant difference (p=0.144). Our results seems to be in contrast with previous studies (Millet et al. 2003; Turner et al. 2006; Rønnestad and Hansen 2016). Millet et al (Millet et al. 2003) showed that comparing some matched work HIIT protocols, those with shorter intervals elicited lower $V'O_2$, HR and RPE at the end of the exercise, suggesting the possibility to last more with shorter intervals. Similarly, Turner et al (Turner et al. 2006) compared four HIIT protocols with the same intensity (work and recovery) and ratio work/recovery, reporting that in HIIT with shorter intervals, the [La] was lower after 30 minutes of exercise compared with longer intervals. In particular, in the HIIT protocol with shorter intervals (work 10s/ recovery 20s), the [La] was even at steady state after 30 minutes of exercise, whereas the one with longer intervals (work 90s/ recovery 180s) the subjects lasted less than 10 minutes before exhaustion.

Surprisingly, the specific effect of the duration of the interval, maintaining a fixed work / rest ratio in the same group of subjects, has not been strongly studied apart from the study already cited (Millet et al. 2003; Turner et al. 2006; Rønnestad and Hansen 2016). It is known that increasing the work interval duration, while keeping the other variables constant, increases the time close to $V'O_2$max (Rozenek et al. 2007; Wakefield and Glaister 2009) and on the country longer recovery interval duration decreases the time close to $V'O_2$max (Smilios et al. 2017). However, to our knowledge the only study that measured the time close to $V'O_2$max and Tlim in HIIT, matching work rate and ratio work/ recovery and isolating the interval duration variable, was performed by Rønnestad and

Hansen (Rønnestad and Hansen 2016). They compared three cycling HIIT protocols in which the work bouts were set at maximal aerobic power ($V'O_2max$ power), the recovery at 50% of the $V'O_2max$ power and the work/ recovery ratio was 2/1 and concluded that HIIT with shorter interval duration (30 s) leads to a longer Tlim (~1400 s), Time>90%$V'O_2peak$ (~680 s) and higher ratio Time>90%$V'O_2peak \cdot Tlim^{-1}$ (0.55) compared with HIIT with longer bouts. Although the protocol (Rønnestad and Hansen 2016) was quite different, Tlim, Time>90%$V'O_2peak$ and their ratio were higher than ours in all the protocols. This discrepancy may be ascribed to the higher fitness level ($V'O_2peak$= ~66 $mL \cdot kg^{-1} \cdot min^{-1}$) and the younger age of the participants in the previous study (Rønnestad and Hansen 2016). Another possible explanation might be the intensity at which our protocol has been set (on average ~83% of load peak). However in the current study, we used a ramp test in order to obtain some others (unpublish) parameters of the aerobic function (Whipp et al. 1981). Incremental ramp tests compared with step tests tend to overestimate the load peak (Revill et al. 2002; Bentley and McNaughton 2003; Zuniga et al. 2012). In the case of Bentley et al. 2003, the ramp modality overestimates the peak power by more than 10-15% compared with the step modality (424.0 Watts ±24,6 vs 355.7 Watts ±16,7 respectively). However, in our paper we used the incremental test to determine $V'O_2peak$, since its determination is not affected by the test modality (Revill et al. 2002; Bentley and McNaughton 2003; Zuniga et al. 2012), and the intensity of the HIIT tests was set exclusively by using CP as described above. For this reason, we can expect that the real power during the high intensity phase was actually above 90% of $V'O_2peak$.

Trying to benefit from faster $V'O_2$ kinetic at the beginning of exercise, we proposed to prolong the firsts intervals while other authors proposed a fast start strategy (De Aguiar et al. 2013; Lisbôa et al. 2015; Rønnestad et al. 2019). De Aguiar et al. 2013 (De Aguiar et al. 2013), showed that fast start HIIT protocol (Starting work rate from 125 decreased until 105% of the intermittent CP, ICP) enhanced the time above 95% of $V'O_2max$ compared to other two protocols with constant work rate at 125% ICP, and constant work rate at 105% ICP. Nevertheless, the protocol that used lower intensity (105% ICP) allowed longer Tlim, and the protocol that adopted higher intensity bouts (125% ICP) showed a bigger ratio Tlim - time above 95% of $V'O_2max^{-1}$. In addition, the recent work of

Rønnestad et al. 2019 (Rønnestad et al. 2019) confirmed that the fast start pacing strategy can be a good strategy to accumulate more time at a high percentage of $V'O_2$peak and despite a similar mean speed induced a lower RPE compared with the traditional HIIT. Even when the decrement is repeated every bout of the HIIT (every bout starts and finish with the same work rate of the previous ones), time near $V'O_2$max could be increased compared to a classical HIIT protocol (Lisbôa et al. 2015). Fast start strategy is a useful tool to improve Time near/at $V'O_2$max and could be successfully applied to HIIT. On the other side, fast start HIIT impairs Tlim in comparison with protocols with the same final exercise work rate (De Aguiar et al. 2013). Compared with a fast start protocol, HIDIT has the advantage to quickly stimulate the oxygen uptake at the beginning without negatively affecting Tlim. Moreover, fast start HIIT but not HIDIT protocol impairs the ratio T>90%$V'O_2$peak - $Tlim^{-1}$ (De Aguiar et al. 2013). The HIDIT protocol that we proposed combines the advantages of different protocols previously studied (short intervals, long intervals and fast start) and can be used during the training sessions that aim to accumulate time at VO_2max.

During HIIT, over the recovery phase below CP, [PCr] and W' recover following an exponential trend: when the exercise generates a depletion of [PCr] and W', the recovery rate is faster when the depletion is wide and becomes slower approaching the complete recovery (Meyer 1988; Ferguson et al. 2010; Skiba et al. 2012, 2014; Jones and Vanhatalo 2017; Vinetti et al. 2017). According to our hypothesis, HIIT with short intervals, was supposed to benefits from this physiological feature, thus improving Tlim as show above (Rønnestad and Hansen 2016). Recovery after few seconds (i.e. 30s) from the start of the exercise, is not necessary as the depletion of [PCr] and W' is minor and the rate of recovery slow (Vinetti et al. 2017). However, close to the exhaustion, the depletion of [PCr] and W' are considerable and the rate of recovery higher, in that moment few seconds of recovery (i.e 20s) are more effective than in the beginning (Vinetti et al. 2017) thus allowing to improve Tlim. As discussed above, the ANOVA procedure failed to find differences between the three HIIT protocols in Tlim, perhaps due to the wide age range (29-62y) of subjects involved in the present study, even though their training history and status were uniform. Since we were aware of the heterogenicity of the subjects, we set the high intensity relying on CP and W' as the intensity that allowed every subject to last 5 minutes

before exhaustion. In this way, we smooth the individual differences, as much as possible. Furthermore, we did not found correlation among age/HR peak and the main outcomes, the $V'O_2$ kinetics during the first three minutes of HIDIT and LI_{HIIT} (unpublished) and we did not found relationships between $V'O_2$peak-CP and the main outcomes as well. The lack of relationship among age and other variables suggests that age did not influence our main results. From another point of view, our data may even support the idea that HIDIT could be applied in well trained male adults with a wide range of age. However, it is interesting to note that only in SI_{HIIT} there is a relationship between Tlim and %CP-Load Peak, confirming what was found by Midgley et al (Midgley et al. 2007), so future research that aims to indagate on Tlim in HIIT may benefit by selecting subjects with homogeneous %CP-Load Peak, although Tlim in HIIT with longer interval seem not to be correlated with it. In light of this, it is then tempting to suggest that individuals with a wide gap among the CP and the Load Peak could benefit more by Short interval HIIT to prolong Tlim.

Further research is needed to verify whether T>90%$V'O_2$peak could be enhance with HIDIT in different HIIT protocols and different populations. However, HIDIT might be useful in sport training in particular when the aim is to improve $V'O_2$max and/or keep as long as possible a specific power or velocity. An example could be the training for the track cycling races. If the aim is to bring the athlete to conclude the race at a given time, the most specific training is to pedal at the velocity for that race time for a distance as near as possible to the distance of the race. Afterward, after the recovery, repeat for a shorter distance and so on. Starting with short interval would not be that specific and keeping going with the first interval distance would not possible for the fatigued athlete.

Furthermore, HIDIT could be useful for patients or for wellness purposes setting a lower percentage of $V'O_2$max or other physiological parameters. For example, if an exercise is intended to avoid exceeding a given [La] cut-off, it can start with a longer interval to save time and then decreases the length of the interval to avoid exceeding the [La] cut-off. However, we suggest adopting this protocol to athletes and patients who aim to training near/at $V'O_2$max, thereby improving it.

Conclusion

In conclusion, HIDIT applied to cycling exercise in well-trained amateur cyclist may enhance T>90%V'O_2peak without reduction of Tlim, ratio T>90%V'O_2peak - $Tlim^{-1}$ or average V'O_2. The average V'O_2 is higher in HIDIT compared with LI_{HIIT}. Finally, despite the higher stimulation of the V'O_2, the rate of perceived exertion and the other physiological parameters at the end of the exercise were not different compared with long or short intervals HIIT suggesting that HIDIT was not more demanding.

ADDITIONAL PUBLICATION

From:

Nicola Giovanelli, Filippo Vaccari, Mirco Floreani, Enrico Rejc, Jasmine Copetti, Marco Garra, Lea Biasutti and Stefano Lazzer

Short-term effects of rolling massage on energy cost of running and power of the lower limbs

Int J Sports Physiol Perform. 2018;13(10):1337-1343. doi:10.1123/ijspp.2018-0142

ACKNOWLEDGEMENTS

I would like to thank the patients and control subjects who agreed to participate to the study nr 1. I would also thank the subjects who agreed to participate to the study nr 4, and the nursing staff at the Department of Public Health - A.A.S. n. 3 “Alto Friuli-Collinare-Medio Friuli” Gemona del Friuli – Udine (Italy), for their qualified assistance. Finally, it has to be thanked the participants to the study nr 3 for their time and effort to ensure the success of the project, in particular the “Pedale Gemonese” association (Gemona del Friuli, Udine, Italia).

Then I want to thank all the co-authors, my supervisor Prof. Stefano Lazzer for his good leading role, my colleagues of the Exercise Physiology Lab for their support and the Prof. Bruno Grassi for his precious advises.

The study nr 1 was supported by Progetti di Ricerca Corrente, Istituto Auxologico Italiano (Milan); and by Fondazione Pietro Pittini (Italy).

The study nr 3 was supported by Fondazione Pietro Pittini (Italy).

The study nr 4 was supported by the University of Udine research funds.

REFERENCES

Abdelmoula A, Martin V, Bouchant A, et al (2012) Knee extension strength in obese and nonobese male adolescents. Appl Physiol Nutr Metab 37:269–275

Achten J, Gleeson M, Jeukendrup AE (2003) Determination of the exercise intensity that elicits maximal fat oxidation. Med Sci Sports Exerc 24:603–608

Achten J, Jeukendrup AE (2004) Optimizing fat oxidation through exercise and diet. Nutrition 20:716–727. doi: 10.1016/j.nut.2004.04.005

Alemayehu HK, Salvadego D, Isola M, et al (2018) Three weeks of respiratory muscle endurance training improve the O2 cost of walking and exercise tolerance in obese adolescents. Physiol Rep 6:e13888

Alpert MA, Omran J, Bostick BP (2016) Effects of Obesity on Cardiovascular Hemodynamics, Cardiac Morphology, and Ventricular Function. Curr Obes Rep 5:424–434

Andersen BYPER, Henriksson JAN (1977) Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. J Physiol 270:677–690

Andersen P, Saltin B (1985) Maximal perfusion of skeletal muscle in man. J Physiol 366:233–249

Ara I, Larsen S, Stallknecht B, et al (2011) Normal mitochondrial function and increased fat oxidation capacity in leg and arm muscles in obese humans. Int J Obes 35:99–108

Arner P, Kriegholm E, Engfeldt P, Bolinder J (1990) Adrenergic regulation of lipolysis in situ at rest and during exercise. J Clin Invest 85:893–898. doi: 10.1172/JCI114516

Bacon AP, Carter RE, Ogle EA, Joyner MJ (2013) VO2max Trainability and High Intensity Interval Training in Humans: A Meta-Analysis. PLoS One 8:. doi: 10.1371/journal.pone.0073182

BÆkkerud FH, Solberg F, Leinan IM, et al (2016) Comparison of Three Popular

Exercise Modalities on VO2max in Overweight and Obese. Med Sci Sports Exerc 48:491–498

Bailey SJ, Vanhatalo A, Dimenna FJ, et al (2011) Fast-start strategy improves V̇O2 kinetics and high-intensity exercise performance. Med Sci Sports Exerc 43:457–467

Bartlett JD, Close GL, Maclaren DPM, et al (2011) High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: Implications for exercise adherence. J Sports Sci 29:547–553. doi: 10.1080/02640414.2010.545427

Bass J, Wilkinson D, Rankin D, et al (2017) An overview of technical considerations for Western blotting applications to physiological research. Scand J Med Sci Sport 27:4–25. doi: 10.1111/sms.12702.An

Bassett D, Howley ET (2000) Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med Sci Sport Exerc 70. doi: 10.1097/00005768-200001000-00012

Bentley DJ, McNaughton LR (2003) Comparison of Wpeak, VO2peak and the ventilation threshold from two different incremental exercise tests: Relationship to endurance performance. J Sci Med Sport 6:422–435. doi: 10.1016/S1440-2440(03)80268-2

Bernhardt V, Babb TG (2016) Exertional dyspnoea in obesity. Eur Respir Rev 25:487–495. doi: 10.1183/16000617.0081-2016

Billat LV (2001a) Interval training for performance: A scientific and empirical practice. Special recommendations for middle- and long-distance running. Part I: Aerobic interval training. Sport Med 31:13–31. doi: 10.2165/00007256-200131010-00002

Billat L V (2001b) Interval training for performance: a scientific and empirical practice. Special recommendations for middle- and long-distance running. Part I: aerobic interval training. Sport Med 31:13–31

Billat V, Petot H, Karp JR, et al (2013) The sustainability of VO2max: Effect of decreasing the workload. Eur J Appl Physiol 113:385–394

Billat VL, Renoux JC, Pinoteau J, et al (1994) Reproducibility of running time to exhaustion at V'O2max in subelite runners. Med Sci Sport Exerc 26:254–257

Billat VL, Slawinksi J, Bocquet V, et al (2001) Very short (15s - 15s) interval-training around the critical velocity allows middle-aged runners to maintain V??O2 max for 14 minutes. Int J Sports Med 22:201–208

Bishop DJ, Botella J, Genders AJ, et al (2019) High-Intensity Exercise and Mitochondrial Biogenesis: Current Controversies and Future Research Directions. Physiology 34:56–70. doi: 10.1152/physiol.00038.2018

Bishop DJ, Granata C, Eynon N (2014) Can we optimise the exercise training prescription to maximise improvements in mitochondria function and content? Table of contents: 1. Mitochondria 2. Mitochondrial biogenesis 3. Overview of exercise-induced mitochondrial biogenesis 4. Can we optimise ex. Biochim Biophys Acta 1840:1266–1275

Blain GM, Hureau TJ (2017) Limitation of fatigue and performance during exercise: the brain–muscle interaction. Exp Physiol 102:3–4

Blomqvist GC., Saltin B (1977) Cardio vascular adaptations to physical training. Annu Rev Physiol 39:221–231

Blomstrand E, Rådegran G, Saltin B (1997) Maximum rate of oxygen uptake by human skeletal muscle in relation to maximal activities of enzymes in the Krebs cycle. J Physiol 501:455–460

Bollinger LM (2017) Potential contributions of skeletal muscle contractile dysfunction to altered biomechanics in obesity. Gait Posture 56:100–107

Borg E, Borg G, Larsson K, et al (2010) An index for breathlessness and leg fatigue. Scand J Med Sci Sport 20:644–650

Boushel R, Gnaiger E, Calbet JAL, et al (2011) Muscle mitochondrial capacity exceeds maximal oxygen delivery in humans. Mitochondrion 11:303–307. doi: 10.1016/j.mito.2010.12.006

Boyd JC, Simpson CA, Jung ME, Gurd BJ (2013) Reducing the Intensity and Volume of Interval Training Diminishes Cardiovascular Adaptation but Not

Mitochondrial Biogenesis in Overweight/Obese Men. PLoS One 8:1–8

Brown RE, Kuk JL (2015) Consequences of obesity and weight loss: A devil's advocate position. Obes Rev 16:77–87. doi: 10.1111/obr.12232

Buchheit M, Laursen PB (2013a) High-intensity interval training, solutions to the programming puzzle: Part I: Cardiopulmonary emphasis. Sport Med 43:313–338

Buchheit M, Laursen PB (2013b) High-intensity interval training, solutions to the programming puzzle: Part II: Anaerobic energy, neuromuscular load and practical applications. Sport Med 43:927–954

Burnley M, Jones AM (2018) Power–duration relationship: Physiology, fatigue, and the limits of human performance. Eur J Sport Sci 18:1–12. doi: 10.1080/17461391.2016.1249524

Burstein M, Scholnick HR, Morfin R (1970) Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 11:583–95

Cacciari E, Milani S, Balsamo A, et al (2006) Italian Cross Sectional Growth Charts for Height Weight and " - BMI (2 to 20 yr). J Endocrinol Invest 29:581–593

Çakmur H (2017) Obesity as a Growing Public Health Problem. Adiposity - Epidemiol Treat Modalities. doi: 10.5772/65718

Canoy D, Buchan I (2007) Challenges in obesity epidemiology. Obes Rev 8:1–11. doi: 10.1111/j.1467-789X.2007.00310.x

Charloux A, Lonsdorfer-Wolf E, Richard R, et al (2000) A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: Comparison with the ″direct″ Fick method. Eur J Appl Physiol 82:313–320

Chidnok W, Dimenna FJ, Bailey SJ, et al (2012) Exercise tolerance in intermittent cycling: Application of the critical power concept. Med Sci Sports Exerc 44:966–976

Chidnok W, DiMenna FJ, Fulford J, et al (2013) Muscle metabolic responses

during high-intensity intermittent exercise measured by 31P-MRS: relationship to the critical power concept. Am J Physiol Regul Integr Comp Physiol 305:R1085-92

Clarke DC, Skiba PF (2013) Rationale and resources for teaching the mathematical modeling of athletic training and performance. Adv Physiol Educ 37:134–152. doi: 10.1152/advan.00078.2011

Cocks M, Shaw CS, Shepherd SO, et al (2013) Sprint interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males. J Physiol 591:641–656. doi: 10.1113/jphysiol.2012.239566

Cocks M, Shaw CS, Shepherd SO, et al (2016) Sprint interval and moderate-intensity continuous training have equal benefits on aerobic capacity, insulin sensitivity, muscle capillarisation and endothelial eNOS/NAD(P)Hoxidase protein ratio in obese men. J Physiol 594:2307–2321. doi: 10.1113/jphysiol.2014.285254

Coffey VG, Hawley JA (2007) The molecular bases of training adaptation. Sport Med 37:737–763. doi: 10.2165/00007256-200737090-00001

Craig CL, Marshall AL, Sjöström M, et al (2003) International physical activity questionnaire: 12-Country reliability and validity. Med Sci Sports Exerc 35:1381–1395

Dandanell S, Meinild-Lundby A-K, Andersen AB, et al (2018) Determinants of maximal whole body fat oxidation in elite cross-country skiers: role of skeletal muscle mitochondria. Scand J Med Sci Sports 3–5. doi: 10.1111/sms.13298

Daussin FN, Ponsot E, Dufour SP, et al (2007) Improvement of V̇O2maxby cardiac output and oxygen extraction adaptation during intermittent versus continuous endurance training. Eur J Appl Physiol 101:377–383

Daussin FN, Zoll J, Dufour SP, et al (2008) Effect of interval versus continuous training on cardiorespiratory and mitochondrial functions: relationship to aerobic performance improvements in sedentary subjects. AJP Regul Integr Comp Physiol 295:R264–R272

De Aguiar RA, Turnes T, De Oliveira Cruz RS, Caputo F (2013) Fast-start strategy increases the time spent above 95 %VO2max during severe-intensity intermittent running exercise. Eur J Appl Physiol 113:941–949

De Feo P (2013) Is high-intensity exercise better than moderate-intensity exercise for weight loss? Nutr Metab Cardiovasc Dis 23:1037–1042. doi: 10.1016/j.numecd.2013.06.002

de Mello AH, Costa AB, Engel JDG, Rezin GT (2018) Mitochondrial dysfunction in obesity. Life Sci 192:26–32

Deforche B, Lefevre J, De Bourdeaudhuij I, et al (2003) Physical Fitness and Physical Activity in Obese and Nonobese Flemish Youth. Obes Res 11:434–441

Dekerle J, De Souza KM, De Lucas RD, et al (2015) Exercise tolerance can be enhanced through a change in work rate within the severe intensity domain: Work above critical power is not constant. PLoS One 10:1–15. doi: 10.1371/journal.pone.0138428

Dempsey JA, Romer L, Rodman J, et al (2006) Consequences of exercise-induced respiratory muscle work. Respir Physiol Neurobiol 151:242–250

Devarshi PP, McNabney SM, Henagan TM (2017) Skeletal muscle nucleo-mitochondrial crosstalk in obesity and type 2 diabetes. Int J Mol Sci 18:

di Prampero PE (2003) Factors limiting maximal performance in humans. Eur. J. Appl. Physiol.

di Prampero PE, Margaria R (1968) Relationship between O2 consumption, high energy phosphates and the kinetics of the O2 debt in exercise. Pflugers Arch 304:11–9

Donnelly JE, Blair SN, Jakicic JM, et al (2009) Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc 41:459–471

Dorado C, Sanchis-Moysi J, Calbet JAL (2004) Effects of recovery mode on performance, O2 uptake, and O 2 deficit during high-intensity intermittent exercise. Can J Appl Physiol 29:227–244. doi: 10.1139/h04-016

Dupont G, McCall A, Prieur F, et al (2010) Faster oxygen uptake kinetics during recovery is related to better repeated sprinting ability. Eur J Appl Physiol 110:627–634

Egan B, Carson BP, Garcia-Roves PM, et al (2010) Exercise intensity-dependent regulation of peroxisome proliferator-activated receptor γ coactivator-1α mRNA abundance is associated with differential activation of upstream signalling kinases in human skeletal muscle. J Physiol 588:1779–1790. doi: 10.1113/jphysiol.2010.188011

Egan B, Zierath JR (2013) Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell Metab 17:162–184. doi: 10.1016/j.cmet.2012.12.012

Esposito F, Mathieu-Costello O, Shabetai R, et al (2010) Limited maximal exercise capacity in patients with chronic heart failure: Partitioning the contributors. J Am Coll Cardiol 55:1945–1954

Ferguson C, Rossiter HB, Whipp BJ, et al (2010) Effect of recovery duration from prior exhaustive exercise on the parameters of the power-duration relationship. J Appl Physiol 108:866–74

Ferrero S, Perrone-Filardi P, Desiderio A, et al (1996) Left Ventricular Systolic and Diastolic Function in Severe Obesity: a Radionuclide Study. Cardiology 87:347–353

Ferretti G (2014) Maximal oxygen consumption in healthy humans: theories and facts. Eur J Appl Physiol 114:2007–2036. doi: 10.1007/s00421-014-2911-0

Fisher-Wellman KH, Weber TM, Cathey BL, et al (2014) Mitochondrial respiratory capacity and content are normal in young insulin-resistant obese humans. Diabetes 63:132–141

Fisher G, Brown AW, Bohan Brown MM, et al (2015) High intensity interval- vs moderate intensity- training for improving cardiometabolic health in overweight or obese males: A Randomized controlled trial. PLoS One 10:1–15. doi: 10.1371/journal.pone.0138853

Formentini L, Ryan AJ, Gálvez-Santisteban M, et al (2017) Mitochondrial H+-ATP

synthase in human skeletal muscle: contribution to dyslipidaemia and insulin resistance. Diabetologia 60:2052–2065. doi: 10.1007/s00125-017-4379-z

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clin Chem 18:499–502. doi: 10.1177/107424840501000106

García-Hermoso A, Cerrillo-Urbina AJ, Herrera-Valenzuela T, et al (2016) Is high-intensity interval training more effective on improving cardiometabolic risk and aerobic capacity than other forms of exercise in overweight and obese youth? A meta-analysis. Obes Rev 17:531–540

Garcia-Vicencio S, Martin V, Kluka V, et al (2015) Obesity-related differences in neuromuscular fatigue in adolescent girls. Eur J Appl Physiol 115:2421–2432

Gavin TP (2004) Lower capillary density but no difference in VEGF expression in obese vs. lean young skeletal muscle in humans. J Appl Physiol 98:315–321

Gibala MJ, McGee SL, Garnham AP, et al (2009) Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1α in human skeletal muscle. J Appl Physiol 106:929–934

Gillen JB, Gibala MJ (2014) High-intensity interval training: a time-efficient exercise strategy to improve health and fitness? Appl Physiol Nutr Metab 39:409–412

Gist NH, Fedewa M V., Dishman RK, Cureton KJ (2014) Sprint interval training effects on aerobic capacity: A systematic review and meta-analysis. Sport Med 44:269–279. doi: 10.1007/s40279-013-0115-0

Gliemann L (2016) Training for skeletal muscle capillarization: a Janus-faced role of exercise intensity? Eur J Appl Physiol 116:1443–1444. doi: 10.1007/s00421-016-3419-6

Gnagnarella P, Salvini S, Parpinel M (2015) Food Composition Database for Epidemiological Studies in Italy. In: http//www.bda-ieo.it/. Version 1

Goodman JM, Liu PP, Green HJ (2005) Left ventricular adaptations following

short-term endurance training. J Appl Physiol 98:454–460. doi: 10.1152/japplphysiol.00258.2004

Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE (1997) Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. Diabetes 46:1579–1585. doi: 10.2337/diacare.46.10.1579

Granata C, Jamnick NA, Bishop DJ (2018) Training-Induced Changes in Mitochondrial Content and Respiratory Function in Human Skeletal Muscle. Sport Med 48:1809–1828. doi: 10.1007/s40279-018-0936-y

Granata C, Oliveira RSF, Little JP, et al (2016) Training intensity modulates changes in PGC-1α and p53 protein content and mitochondrial respiration, but not markers of mitochondrial content in human skeletal muscle. FASEB J 30:959–970. doi: 10.1096/fj.15-276907

Gray DS, Bray GA, Gemayel N, Karl. K (1989) Effect of obesity on bioelectrical impedance. Am J Clin Nutr 50:255–260

Hansen D, Marinus N, Remans M, et al (2014) Exercise tolerance in obese vs. lean adolescents: A systematic review and meta-analysis. Obes Rev 15:894–904. doi: 10.1111/obr.12202

Harris J, Benedict F (1918) A Biometric Study of Human Basal Metabolism. Proc Natl Acad Sci U S A 4:370–3

Hawley JA, Brouns F, Jeukendrup A (1998) Strategies to enhance fat utilisation during exercise. Sport Med 25:241–257. doi: 10.2165/00007256-199825040-00003

Helgerud J, Høydal K, Wang E, et al (2007) Aerobic high-intensity intervals improve V'O2max more than moderate training. Med Sci Sports Exerc 39:665–671. doi: 10.1249/mss.0b013e3180304570

Hetlelid KJ, Plews DJ, Herold E, et al (2017) Rethinking the role of fat oxidation: Substrate utilisation during high-intensity interval training in well-trained and recreationally trained runners. BMJ Open Sport Exerc Med 3:1–9. doi: 10.1136/bmjsem-2015-000047

Hill DW (1993) The critical power concept. A Review. 16:237–254

Hill A V., Lupton H (1923) Muscular exercise, lactic acid, and the supply and utilization of oxygen. Qjm os-16:135–171. doi: 10.1093/qjmed/os-16.62.135

Hoier B, Passos M, Bangsbo J, Hellsten Y (2013) Intense intermittent exercise provides weak stimulus for vascular endothelial growth factor secretion and capillary growth in skeletal muscle. Exp Physiol 98:585–597. doi: 10.1113/expphysiol.2012.067967

Hoppeler H, Howald H, Conley K, et al (1985) Endurance training in humans: Aerobic capacity and structure of skeletal muscle. J Appl Physiol 59:320–327

Horowitz JF (2001) Regulation of lipid mobilization and oxidation during exercise in obesity. Exerc Sport Sci Rev 29:42–46. doi: 10.1097/00003677-200101000-00009

Howlett RA, Gonzalez NC, Wagner HE, et al (2003) Selected Contribution: Skeletal muscle capillarity and enzyme activity in rats selectively bred for running endurance. J Appl Physiol 94:1682–1688

Howlett RA, Kirkton SD, Gonzalez NC, et al (2009) Peripheral oxygen transport and utilization in rats following continued selective breeding for endurance running capacity. J Appl Physiol 106:1819–1825

Hughes MC, Ramos S V., Turnbull PC, et al (2015) Mitochondrial bioenergetics and fiber type assessments in microbiopsy vs. Bergstrom percutaneous sampling of human skeletal muscle. Front Physiol 6:1–10. doi: 10.3389/fphys.2015.00360

Hunger JM, Major B (2015) Weight Stigma Mediates the Association Between BMI and Self-Reported Health NIH Public Access Author Manuscript. Heal Psychol 34:172–175. doi: 10.1037/hea0000106

Hwang H, Bowen BP, Lefort N, et al (2010) Proteomics analysis of human skeletal muscle reveals novel abnormalities in obesity and type 2 diabetes. Diabetes 59:33–42

International Physical Activity Questionnaire (2005) Guidelines for Data

Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) – Short and Long Forms, revised on November 2005. Ipaq 1–15

Jones AM, Grassi B, Christensen PM, et al (2011) Slow Component of V˙O2 Kinetics: Mechanistic Bases and Practical Applications. Med Sci Sports Exerc 1–18

Jones AM, Vanhatalo A (2017) The “Critical Power” Concept: Applications to Sports Performance with a Focus on Intermittent High-Intensity Exercise. Sport Med

Jones AM, Vanhatalo A, Burnley M, et al (2010) Critical power: Implications for determination of V’O2max and exercise tolerance. Med Sci Sports Exerc

Jones AM, Wilkerson DP, Vanhatalo A, Burnley M (2008) Influence of pacing strategy on O2 uptake and exercise tolerance. Scand J Med Sci Sport 18:615–626

Joyner MJ, Coyle EF (2008) Endurance exercise performance: The physiology of champions. J Physiol 586:35–44. doi: 10.1113/jphysiol.2007.143834

Jung ME, Bourne JE, Beauchamp MR, et al (2015) High-intensity interval training as an efficacious alternative to moderate-intensity continuous training for adults with prediabetes. J Diabetes Res 2015:

Keating SE, Johnson NA, Mielke GI, Coombes JS (2017) A systematic review and meta-analysis of interval training versus moderate-intensity continuous training on body adiposity. Obes Rev 18:943–964. doi: 10.1111/obr.12536

Kelley DE, Simoneau JA (1994) Impaired free fatty acid utilization by skeletal muscle in non-insulin- dependent diabetes mellitus. J Clin Invest 94:2349–2356. doi: 10.1172/JCI117600

Kokkinos P, Myers J, Nylen E, et al (2009) Exercise capacity and all-cause mortality in African American and caucasian men with type 2 diabetes. Diabetes Care 32:623–628. doi: 10.2337/dc08-1876

Konopka AR, Asante A, Lanza IR, et al (2015) Defects in mitochondrial efficiency and H2O2emissions in obese women are restored to a lean phenotype with aerobic exercise training. Diabetes 64:2104–2115

Kras KA, Langlais PR, Hoffman N, et al (2018) Obesity modifies the stoichiometry of mitochondrial proteins in a way that is distinct to the subcellular localization of the mitochondria in skeletal muscle. Metabolism 89:18–26. doi: 10.1016/j.metabol.2018.09.006

Kress J, Pohlman A, Alverdy J, Hall J (1999) Obesity, respiratory function and breathlessness The impact of morbid obesity on oxygen cost of breathing (V˙O2RESP) at rest. Am J Respir Crit Care Med 160:883–886

Kuznetsov A V., Kunz WS, Saks V, et al (2003) Cryopreservation of mitochondria and mitochondrial function in cardiac and skeletal muscle fibers. Anal Biochem 319:296–303

Lafortuna CL, Agosti F, Galli R, et al (2008) The energetic and cardiovascular response to treadmill walking and cycle ergometer exercise in obese women. Eur J Appl Physiol 103:707–717

Lanzi S, Codecasa F, Cornacchia M, et al (2015) Short-term HIIT and Fatmax training increase aerobic and metabolic fitness in men with class II and III obesity. Obesity 23:1987–1994

Lanzi S, Codecasa F, Cornacchia M, et al (2014) Fat oxidation, hormonal and plasma metabolite kinetics during a submaximal incremental test in lean and obese adults. PLoS One 9:. doi: 10.1371/journal.pone.0088707

Larsen FJ, Schiffer TA, Borniquel S, et al (2011) Dietary inorganic nitrate improves mitochondrial efficiency in humans. Cell Metab 13:149–159. doi: 10.1016/j.cmet.2011.01.004

Larsen S, Wright-Paradis C, Gnaiger E, et al (2012) Cryopreservation of human skeletal muscle impairs mitochondrial function. Cryo Letters 33:170–6

Layec G, Venturelli M, Jeong E, Richardson RS (2014) The validity of anthropometric leg muscle volume estimation across a wide spectrum : From able-bodied adults to individuals with a spinal cord injury. J Appl Physiol 116:1142–1147

Lazzer S, Salvadego D, Porcelli S, et al (2013) Skeletal muscle oxygen uptake in obese patients: Functional evaluation by knee-extension exercise. Eur J

Appl Physiol 113:2125–2132

Lazzer S, Tringali G, Caccavale M, et al (2017) Effects of high-intensity interval training on physical capacities and substrate oxidation rate in obese adolescents. J Endocrinol Invest. doi: 10.1007/s40618-016-0551-4

Leprêtre PM, Lopes P, Koralzstein JP, Billat V (2016) Heavy cycling exercise at fixed heart rate prevent the decline of stroke volume and delay time to exhaustion in trained adolescents. Sci Sport

Limberg JK, De Vita MD, Blain GM, Schrage WG (2010) Muscle blood flow responses to dynamic exercise in young obese humans. J Appl Physiol 108:349–355

Lisbôa FD, Salvador AF, Raimundo JAG, et al (2015) Decreasing power output increases aerobic controbution during low-volume severe-intensity intermittent exercise. 2434–2440

López-Jiménez F, Cortés-Bergoderi M (2011) Obesity and the heart. Rev Esp Cardiol 64:140–149

Lowry OH, Rosebrough NJ, Farr LA, Randall RJ (1951) Protein Measurement with the Folin Phenol Reagent*. Anal Biochem 217:220–230

Lucan SC, Dinicolantonio JJ (2015) How calorie-focused thinking about obesity and related diseases may mislead and harm public health. An alternative. Public Health Nutr 18:571–581. doi: 10.1017/S1368980014002559

Lukaski HC, Bolonchuk WW, Hall CB, Siders WA (1986) Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol 60:1327–1332

Lunt H, Draper N, Marshall HC, et al (2014) High Intensity Interval Training in a Real World Setting: A Randomized Controlled Feasibility Study in Overweight Inactive Adults, Measuring Change in Maximal Oxygen Uptake. PLoS One 9:e83256

MacInnis MJ, Gibala MJ (2016) Physiological adaptations to interval training and the role of exercise intensity. J Physiol 00:1–16. doi: 10.1113/JP273196

MacInnis MJ, Zacharewicz E, Martin BJ, et al (2016) Superior mitochondrial

adaptations in human skeletal muscle after interval compared to continuous single-leg cycling matched for total work. J Physiol 00:1–14. doi: 10.1113/JP272570

Maffiuletti NA, Jubeau M, Agosti F, et al (2008) Quadriceps muscle function characteristics in severely obese and nonobese adolescents. Eur J Appl Physiol 103:481–484

Maffiuletti NA, Jubeau M, Munzinger U, et al (2007) Differences in quadriceps muscle strength and fatigue between lean and obese subjects. Eur J Appl Physiol 101:51–59

Maffiuletti NA, Ratel S, Sartorio A, Martin V (2013) The Impact of Obesity on In Vivo Human Skeletal Muscle Function. Curr Obes Rep 2:251–260

Malenfant P, Joanisse DR, Thériault R, et al (2001) Fat content in individual muscle fibers of lean and obese subjects. Int J Obes 25:1316–1321

Marcus BH, Bock BC, Pinto BM, Clark MM (1996) Exercise initiation, adoption, and maintenance. Explor Sport Exerc Psychol (eds VanRaalte J Brew BW) 133–158

Marcus BH, Rossi JS, Selby VC, et al (1992) The stages and processes of exercise adoption and maintenance in a worksite sample. Heal Psychol 11:386–395

Martin ML, Jensen MD (1991) Effects of body fat distribution on regional lipolysis in obesity. J Clin Invest 88:609–613. doi: 10.1172/JCI115345

Matthews VB, Åström MB, Chan MHS, et al (2009) Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. Diabetologia 52:1409–1418. doi: 10.1007/s00125-009-1364-1

McGuire MT, Wing RR, Hill JO (1999) The prevalence of weight loss maintenance among American adults. Int J Obes 23:1314–1319. doi: 10.1038/sj.ijo.0801075

Menshikova E V. (2004) Effects of weight loss and physical activity on skeletal muscle mitochondrial function in obesity. AJP Endocrinol Metab 288:E818–

E825

Menshikova E V., Ritov VB, Ferrell RE, et al (2007) Characteristics of skeletal muscle mitochondrial biogenesis induced by moderate-intensity exercise and weight loss in obesity. J Appl Physiol 103:21–27

Meyer A, Charles AL, Zoll J, et al (2014) Cryopreservation with dimethyl sulfoxide prevents accurate analysis of skinned skeletal muscle fibers mitochondrial respiration. Biochimie 100:227–233. doi: 10.1016/j.biochi.2014.01.014

Meyer RA (1988) A linear model of muscle respiration explains monoexponential phosphocreatine changes. Am J Physiol 254:548–53

Midgley A, Mc Naughton L (2006) Time at or near VO2max during continuous and intermittent running. J Sports Med Phys Fitness 46:1–14

Midgley AW, McNaughton LR, Carroll S (2007) Physiological determinants of time to exhaustion during intermittent treadmill running at vV'O2max. Int J Sports Med 28:273–280. doi: 10.1055/s-2006-924336

Milanović Z, Sporiš G, Weston M (2015) Effectiveness of High-Intensity Interval Training (HIT) and Continuous Endurance Training for VO2max Improvements: A Systematic Review and Meta-Analysis of Controlled Trials. Sport Med 45:1469–1481. doi: 10.1007/s40279-015-0365-0

Millet GP, Candau R, Fattori P, et al (2003) Responses to Different Intermittent Runs at Velocity Associated With. Can J Appl Physiol 28:410–423

Millet GY (2011) Can Neuromuscular Fatigue Explain Running Strategies and Performance in Ultra-Marathons ? The Flush Model. Sport Med 41:489–506

Mitchell NS, Catenacci VA, Wyatt HR, Hill JO (2011) Obesity: Overview of an epidemic. Psychiatr Clin North Am 34:717–732. doi: 10.1016/j.psc.2011.08.005

Montero D, Cathomen A, Jacobs RA, et al (2015) Haematological rather than skeletal muscle adaptations contribute to the increase in peak oxygen uptake induced by moderate endurance training. J Physiol 593:4677–4688. doi: 10.1113/JP270250

Mootha VK, Lindgren CM, Eriksson KF, et al (2003) PGC-1α-responsive genes

involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34:267–273. doi: 10.1038/ng1180

Moritani T, Nagata A, Devries HA, Muro M (1981) Critical power as a measure of physical work capacity and anaerobic threshold. IR 24:339–350

Morton RH, Billat VL (2004) The critical power model for intermittent exercise. Eur J Appl Physiol 91:303–307

Nair KS, Irving BA, Lanza IR (2011) Can dietary nitrates enhance the efficiency of mitochondria? Cell Metab 13:117–118

Ng M, Fleming T, Robinson M, et al (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet 384:766–781. doi: 10.1016/S0140-6736(14)60460-8

Noakes TD (2012) Fatigue is a brain-derived emotion that regulates the exercise behavior to ensure the protection of whole body homeostasis. Front Physiol 3 APR:1–13

Noakes TD, St. Clair Gibson A, Lambert E V. (2004) From catastrophe to complexity: A novel model of integrative central neural regulation of effort and fatigue during exercise in humans. Br J Sports Med 38:511–514

Nordby P, Saltin B, Helge JW (2006) Whole-body fat oxidation determined by graded exercise and indirect calorimetry: A role for muscle oxidative capacity? Scand J Med Sci Sport 16:209–214. doi: 10.1111/j.1600-0838.2005.00480.x

O'Donovan G, Thomas EL, McCarthy JP, et al (2009) Fat distribution in men of different waist girth, fitness level and exercise habit. Int J Obes 33:1356–1362

Ortega B F, Lavie J C, Blair N S (2016) Obesity and Cardiovascular Disease. Circ Res 118:1752–1770

Palmieri V, Russo C, Palmieri EA, et al (2006) Isolated Left Ventricular Diastolic Dysfunction: Implications for Exercise Left Ventricular Performance in Patients Without Congestive Heart Failure. J Am Soc Echocardiogr 19:491–

498. doi: 10.1016/j.echo.2005.12.011

Pan H, Guo J, Su Z (2014) Advances in understanding the interrelations between leptin resistance and obesity. Physiol Behav 130:157–169. doi: 10.1016/j.physbeh.2014.04.003

Pattanakuhar S, Pongchaidecha A, Chattipakorn N, Chattipakorn SC (2016) The effect of exercise on skeletal muscle fibre type distribution in obesity: From cellular levels to clinical application. Obes Res Clin Pract 11:112–132. doi: 10.1016/j.orcp.2016.09.012

Patti ME, Butte AJ, Crunkhorn S, et al (2003) Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc Natl Acad Sci U S A 100:8466–8471. doi: 10.1073/pnas.1032913100

Pedersen BK, Febbraio MA (2012) Muscles, exercise and obesity: Skeletal muscle as a secretory organ. Nat Rev Endocrinol 8:457–465

Pesta D, Gnaiger E (2012) High-Resolution Respirometry: OXPHOS Protocols for Human Cells and Permeabilized Fibers from Small Biopsies of Human Muscle. In: Methods in molecular biology. pp 25–58

Phillips DB, Stickland MK (2019) Respiratory limitations to exercise in health: a brief review. Curr Opin Physiol 10:173–179. doi: 10.1016/j.cophys.2019.05.012

Poirier P, Giles TD, Bray GA, et al (2006) Obesity and cardiovascular disease: Pathophysiology, evaluation, and effect of weight loss. Arterioscler Thromb Vasc Biol 26:968–976

Poole DC, Hirai DM, Copp SW, Musch TI (2012) Muscle oxygen transport and utilization in heart failure: implications for exercise (in)tolerance. AJP Hear Circ Physiol 302:H1050–H1063

Poole DC, Ward S a, Gardner GW, Whipp BJ (1988) Metabolic and respiratory profile of the upper limit for prolonged exercise in man. Ergonomics 31:1265–1279. doi: 10.1080/00140138808966766

Rankin J, Matthews L, Cobley S, et al (2016) Psychological consequences of

childhood obesity: psychiatric comorbidity and prevention. Adolesc Health Med Ther 7:125–146

Revill SM, Beck KE, Morgan MDL (2002) Comparison of the peak exercise response measured by the ramp and 1-min step cycle exercise protocols in patients with exertional dyspnea. Chest 121:1099–1105. doi: 10.1378/chest.121.4.1099

Reynisdottir S, Wahrenberg H, Carlstrom K, et al (1994) Catecholamine resistance in fat cells of women with upper-body obesity due to decreased expression of beta2-adrenoceptors. Diabetologia 37:428–435

Richard R, Lonsdorfer-Wolf E, Charloux A, et al (2001) Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. Eur J Appl Physiol 85:202–207

Richardson RS, Grassi B, Gavin TP, et al (1999) Evidence of O2 supply-dependent VO2 max in the exercise-trained human quadriceps. J Appl Physiol 86:1048–1053

Ritov VB, Menshikova E V., Azuma K, et al (2009) Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. Am J Physiol Metab 298:E49–E58

Ritov VB, Menshikova E V, He J, et al (2005) Deficiency of Subsarcolemmal Mitochondria in Obesity and Type 2 Diabetes. Diabetes 54:8–14. doi: 10.2337/diabetes.54.1.8

Robinson SL, Hattersley J, Frost GS, et al (2015) Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. J Appl Physiol 118:1415–1422. doi: 10.1152/japplphysiol.00058.2015

Rønnestad BR, Hansen J (2016) Optimizing Interval Training at Power Output Associated with Peak Oxygen Uptake in Well-Trained Cyclists. J Strength Cond Res 30:999–1006

Rønnestad BR, Rømer T, Hansen J (2019) Increasing Oxygen Uptake in Well-Trained Cross-Country Skiers During Work Intervals With a Fast Start. Int J

Sports Physiol Perform 1–7. doi: 10.1123/ijspp.2018-0360

Ross R, Stotz PJ, Lam M (2015) Effects of exercise amount and intensity on abdominal obesity and glucose tolerance in obese adults: A randomized trial. Ann Intern Med 162:325–334. doi: 10.7326/M14-1189

Rowland T, Bhargava R, Parslow D, Heptulla RA (2003) Cardiac response to progressive cycle exercise in moderately obese adolescent females. J Adolesc Heal 32:422–427

Rozenek R, Funato K, Kubo J, et al (2007) Physiological responses to interval training sessions at velocities associated with V'O2max. J Strength Cond Res 21:188–192

Saladini F, Palatini P (2017) Isolated Systolic Hypertension in Young Individuals: Pathophysiological Mechanisms, Prognostic Significance, and Clinical Implications. High Blood Press Cardiovasc Prev 24:133–139. doi: 10.1007/s40292-017-0199-y

Salvadego D, Domenis R, Lazzer S, et al (2013) Skeletal muscle oxidative function in vivo and ex vivo in athletes with marked hypertrophy from resistance training. J Appl Physiol 114:1527–1535

Salvadego D, Keramidas ME, Brocca L, et al (2016) Separate and combined effects of a 10-d exposure to hypoxia and inactivity on oxidative function in vivo and mitochondrial respiration ex vivo in humans. J Appl Physiol 121:154–163. doi: 10.1152/japplphysiol.00832.2015

Salvadego D, S., Lazzer, et al (2011) Functional impairment of skeletal muscle oxidative metabolism during knee extension exercise after bed rest. J Appl Physiol 111:1719–1726

Salvadego D, Sartorio A, Agosti F, et al (2017) Respiratory muscle endurance training reduces the O 2 cost of cycling and perceived exertion in obese adolescents. Am J Physiol - Regul Integr Comp Physiol

Salvadego D, Sartorio A, Agosti F, et al (2015) Acute respiratory muscle unloading by normoxic helium-O2 breathing reduces the O 2 cost of cycling and perceived exertion in obese adolescents. Eur J Appl Physiol 115:99–

109

Salvadori A, Fanari P, Fontana M, et al (1999) Oxygen uptake and cardiac performance in obese and normal subjects during exercise. Respiration 66:25–33

Salvadori A, Fanari P, Mazza P, et al (1992) Work capacity and cardiopulmonary adaptation of the obese subject during exercise testing. Chest 101:674–679

Same R V., Feldman DI, Shah N, et al (2016) Relationship Between Sedentary Behavior and Cardiovascular Risk. Curr Cardiol Rep 18:1–7. doi: 10.1007/s11886-015-0678-5

Sawyer BJ, Tucker WJ, Bhammar DM, et al (2016) Effects of high-intensity interval training and moderate-intensity continuous training on endothelial function and cardiometabolic risk markers in obese adults. J Appl Physiol 121:279–88

Scribbans TD, Edgett BA, Vorobej K, et al (2014) Fibre-specific responses to endurance and low volume high intensity interval training: Striking similarities in acute and chronic adaptation. PLoS One 9:. doi: 10.1371/journal.pone.0098119

Simoneau JA, Veerkamp JH, Turcotte LP, Kelley DE (1999) Markers of capacity to utilize fatty acids in human skeletal muscle: Relation to insulin resistance and obesity and effects of weight loss. FASEB J 13:2051–2060. doi: 10.1096/fasebj.13.14.2051

SIO-ADI (2016) Italian's Standard for the treatment of obesity. In: Soc. Ital. di Nutr. Um. http://www.sio-obesita.org/

Skiba PF, Chidnok W, Vanhatalo A, Jones AM (2012) Modeling the expenditure and reconstitution of work capacity above critical power. Med Sci Sports Exerc 44:1526–1532

Skiba PF, Clarke D, Vanhatalo A, Jones AM (2014) Validation of a Novel Intermittent W′ Model for Cycling Using Field Data. wwwIJSPP-Journal.com Orig Investig Int J Sport Physiol Perform 9:900–904

Skiba PF, Fulford J, Clarke DC, et al (2015) Intramuscular determinants of the

ability to recover work capacity above critical power. Eur J Appl Physiol 115:703–713

Smilios I, Myrkos A, Zafeiridis A, et al (2017) The effects of recovery duration during high-intensity interval exercise on time spent at high rates of oxygen consumption, oxygen kinetics and blood lactate. J Strength Cond Res 1. doi: 10.1519/JSC.0000000000001904

Spinazzi M, Casarin A, Pertegato V, et al (2012) Assessment of mitochondrial respiratory chain enzymatic activities on tissues and cultured cells. Nat Protoc 7:1235–1246

Srere PA (1969) Citrate Synthase. Methods Enzym 3–5

Stork MJ, Gibala MJ, Martin Ginis KA (2018) Psychological and Behavioral Responses to Interval and Continuous Exercise. Med Sci Sports Exerc 50:2110–2121

Tallis J, James RS, Seebacher F (2018) The effects of obesity on skeletal muscle contractile function. J Exp Biol 221:

Tan R, Nederveen JP, Gillen JB, et al (2018) Skeletal muscle fiber-type-specific changes in markers of capillary and mitochondrial content after low-volume interval training in overweight women. Physiol Rep 6:1–8. doi: 10.14814/phy2.13597

Thivel D, Finlayson G, Miguet M, et al (2018) Energy depletion by 24-h fast leads to compensatory appetite responses compared with matched energy depletion by exercise in healthy young males. Br J Nutr 120:583–592. doi: 10.1017/S0007114518001873

Tschakert G, Hofmann P (2013) High-intensity intermittent exercise: Methodological and physiological aspects. Int J Sports Physiol Perform 8:600–610. doi: 10.1123/ijspp.8.6.600

Turner AP, Cathcart AJ, Parker ME, et al (2006) Oxygen uptake and muscle desaturation kinetics during intermittent cycling. Med Sci Sports Exerc

Upadhyay J, Farr O, Perakakis N, et al (2018) Obesity as a Disease. Med Clin North Am 102:13–33. doi: 10.1016/j.mcna.2017.08.004

Vaccari F, Floreani M, Tringali G, et al (2019) Metabolic and muscular factors limiting aerobic exercise in obese subjects. Eur J Appl Physiol. doi: 10.1007/s00421-019-04167-w

Van Der Klaauw AA, Farooqi IS (2015) The hunger genes: Pathways to obesity. Cell 161:119–132. doi: 10.1016/j.cell.2015.03.008

Vella CA, Ontiveros D, Zubia RY (2011) Cardiac function and arteriovenous oxygen difference during exercise in obese adults. Eur J Appl Physiol 111:915–923

Vella CA, Paul DR, Bader J (2012) Cardiac response to exercise in normal-weight and obese, Hispanic men and women: Implications for exercise prescription. Acta Physiol 205:113–123

Vijgen GHEJ, Bouvy ND, Hoeks J, et al (2013) Impaired skeletal muscle mitochondrial function in morbidly obese patients is normalized one year after bariatric surgery. Surg Obes Relat Dis 9:936–941

Vinetti G, Fagoni N, Taboni A, et al (2017) Effects of recovery interval duration on the parameters of the critical power model for incremental exercise. Eur J Appl Physiol 117:1859–1867

Wakefield BR, Glaister M (2009) Influence of work-interval intensity and duration on time spent at a high percentage of V'O2 max during intermittent supramaximal exercise. J Strength Cond Res 23:2548–2554

Wallman K, Plant LA, Rakimov B, Maiorana AJ (2009) The effects of two modes of exercise on aerobic fitness and fat mass in an overweight Population. Res Sport Med 17:156–170. doi: 10.1080/15438620903120215

Warburton DE (2014) Blood volume expansion and cardiorespiratory function: effects of training modality. Med Sci Sports Exerc 36:991–1000

Ware JE, Kosinski M, Keller SD (1996) A 12-Item Short-Form Health Survey: Construction of Scales and Preliminary Tests of Reliability and Validity. Med Care 34:220–233

Wen D, Utesch T, Wu J, et al (2019) Effects of Different Protocols of High Intensity Interval Training for VO2max Improvements in Adults: A Meta-Analysis of

Randomised Controlled Trials. J Sci Med Sport 1–7. doi: 10.1016/j.jsams.2019.01.013

Wenger HA, Bell GJ (1986) The Interactions of Intensity, Frequency and Duration of. Sport Med 3:346–356

Weston M, Taylor KL, Batterham AM, Hopkins WG (2014) Effects of low-volume high-intensity interval training (HIT) on fitness in adults: A meta-analysis of controlled and non-controlled trials. Sport Med 44:1005–1017. doi: 10.1007/s40279-014-0180-z

Whipp BJ, Davis JA, Torres F, Wasserman K (1981) A test to determine parameters of aerobic function during exercise. J Appl Physiol Respir Environ Exerc Physiol 50:217–221. doi: 10.1152/jappl.1981.50.1.217

WHO (2017) Overweight and obesity. In: World Heal. Organ. Glob. Heal. Obs. https//www.who.int/gho/en/

WHO (2016) Overweight and obesity. In: https://www.who.int/gho/ncd/risk_factors/overweight_obesity/obesity_adults/en/

WHO (2014) Global status report on noncommunicable diseases 2014

Wijngaarden MA, van der Zon GC, van Dijk KW, et al (2013) Effects of prolonged fasting on AMPK signaling, gene expression, and mitochondrial respiratory chain content in skeletal muscle from lean and obese individuals. Am J Physiol - Endocrinol Metab 304:. doi: 10.1152/ajpendo.00008.2013

Williams EP, Mesidor M, Winters K, et al (2015) Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem. Curr Obes Rep 4:363–370. doi: 10.1007/s13679-015-0169-4

Wüst RCI, Myers DS, Stones R, et al (2011) Regional skeletal muscle remodeling and mitochondrial dysfunction in right ventricular heart failure. Am J Physiol Circ Physiol 302:H402–H411. doi: 10.1152/ajpheart.00653.2011

Wyatt SB, Winters K, Dubbert PM (2006) Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem. Am J Med Sci 331:166–174

Zuniga JM, Housh TJ, Camic CL, et al (2012) Metabolic parameters for ramp versus step incremental cycle ergometer tests. Appl Physiol Nutr Metab 37:1110–1117. doi: 10.1139/H2012-098

Zurlo F, Lillioja S, Esposito-Del Puente A, et al (1990) Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. Am J Physiol Metab 259:E650–E657. doi: 10.1152/ajpendo.1990.259.5.e650