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“The effects of Polarized training and High Intensity Interval training on body composition, aerobic capacities and fat oxidation in individuals with obesity.”

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List of publications

Danielewicz, A. L., Lazzer, S., Marra, A., Abbruzzese, L., **D'Alleva, M.**, Martino, M., Isola, M.,Avelar, N. C. P., Mendonça, V. A., Lacerda, A. C. R., & Sartorio, A. (2023). Prediction of resting energy expenditure in Italian older adults with severe obesity. Frontiers in endocrinology, 1 4 , 1283155. https://doi.org/10.3389/fendo.2023.1283155

D'Alleva, M., Lazzer, S., Tringali, G., De Micheli, R., Bondesan, A., Abbruzzese, L., & Sartorio, A. (2023). Effects of combined training or moderate intensity continuous training during a 3-week multidisciplinary body weight reduction program on cardiorespiratory fitness, body composition, and substrate oxidation rate in adolescents with obesity. Scientific reports, 1 3 (1), 17609. https://doi.org/10.1038/s41598-023-44953-3

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Conference proceedings

M. D'Alleva, N. Giovanelli, F. Graniero, R. Spaggiari. S.J. Molina, D. Sergi, E. Dalla Nora, A. Passaro, S. Lazzer. The influence of obesity on cardiac and muscular inflammatory biomarkers after prolonged aerobic exercise". Poster presentation at the XV national congress of the Società italiana delle scienze motorie e sportive. November 2^{nd}-4^{th}, 2023. Naples, Italy.

M. D'Alleva, N. Giovanelli, F. Graniero, L. Mos, V. Billat, F. Fiori, M. Marinoni, M. Parpinel, S. Lazzer. Using mobile health applications to enhance cardiorespiratory fitness and body composition in obese adults. Oral presentation at the internation congress of the Società italiana di telemedicina. March 10th-11th. Bologna, Italy.

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M. D'Alleva, F. Vaccari, M. Floreani, F. Fiori, M. Marinoni, M. Parpinel, S. Lazzer. Effects of 3-month polarized training vs. high intensity interval training on cardiorespiratory fitness, body composition and fat metabolism in obese adults. Oral presentation at the XIII national congress of the Società italiana delle scienze motorie e sportive. October 8^{th}-10^{th}, 2021. Padua, Italy.

List of abbreviations and definition

ACC	American College of Cardiology
ACSM	American College of Sport Medicine
AerT	aerobic threshold
AMP	adenosine monophosphate
AMPK	activates AMP-activated protein kinase
AnT	anaerobic threshold
ATP	adenosine triphosphate
a-vO2diff	arteriovenous oxygen difference
BIA	bioelectrical impedance
BM	body mass
BMI	body mass index
BMR.	basal metabolic rate
BV	blood volume
BWRP	body weight reduction program
CIM	carbohydrate-insuline model
CO	cardiac output
COMB	combined training
COPD	chronic obstructive pulmonary disease
CPET	cardiopulmonary exercise testing
CPT-1	carnitine palmitoyltransferase-1
CRF	cardiorespiratory fitness
CS	citrate synthase
CVD	cardiovascular disease
Cw	cost of walking
EBM	energy balance model
EE	energy expenditure
ELISA	enzyme-linked immunosorbent assay
FFM	fat free mass
FM	fat mass
GET	gas exchange threshold
HC	hip circumference
Hct	haematocrit
HIIT	high-intensity interval training

HC	hip circumference
HMW	high molecular weight
HR	heart rate
HVT	high volume training
IMTG	intramuscular triglycerides
IPAQ-SF	international physical activity questionnaire short form
LMW	low molecular weight
LT1	first lactate threshold
LT2	second lactate threshold
LVM	left ventricular mass
MedDiet	mediterranean diet
MET	metabolic equivalent
Mets	metabolic syndrome
MFO	maximal fat oxidation
MICT	moderate-intensity continuous training
MMW	medium molecular weight
NAFLD	non-alcoholic fatty liver disease
OSA	obstructive sleep apnoea
PA	physical activity
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PYR	pyramidal training
POL	polarized training
PV	plasma volume
RABIT®	running advisor billat training
RER	respiratory exchange ratio
RPE	rate of perceived exertion
SIT	sprint interval training
SV	stroke volume
T2D	type II diabetes
TID	training intensity distribution
TL	training load
THR	threshold training
TRIMP	training impulse
VAT	visceral adipose tissue

$V'O_2$	oxygen consumption
VT1	first ventilatory threshold
VT2	second ventilatory threshold
WC	waist circumference
WHO	world health organization

Abstract

Compared to lean people of the same age and gender, individual with obesity presented a lower exercise tolerance, lower maximum oxygen consumption (V'O$_2$max) and lower metabolic flexibility. High intensity interval training (HIIT) and moderate intensity continuous training (MICT) are the most utilized physical exercise for improving body composition and aerobic physical capacities in individuals with obesity. However, a weekly combination of a high volume of MICT (i.e., 70-80%) with a low volume of HIIT (i.e., 20-30%) leads to important improvements in body composition and endurance performance-related variables (i.e., V'O$_2$max and ventilatory thresholds) in both endurance athletes and people with obesity. Nonetheless, in the few available studies on people with obesity the weekly proportions of HIIT and MICT were not determined a priori. For this reason, the first aim of the thesis was to compare a 12-week period of a combination of MICT and HIIT, called combined training (COMB), with HIIT on body composition, endurance performance-related variables (V'O$_2$max) and fat oxidation rate in male adults with obesity. Thirty-four obese male adults participated in this study (n: 18 for COMB, n: 16 HIIT), and completed ~ 36 training sessions. COMB training consisted of 3 repetitions of 2 minutes at 95% of peak oxygen uptake (V'O$_2$ peak) (e.g., HIIT 20%), followed by 30 minutes at 60% of VO$_2$ peak (e.g., MICT 80%). The HIIT group performed 5-7 repetitions of 2 minutes at 95% of VO$_2$ peak. Body composition, V'O$_2$ peak, and the fat oxidation rate were measured at baseline (PRE) and at the end of the training period (POST). The two training programs were equivalent in caloric expenditure. At POST, body mass (BM) and fat mass (FM) decreased equally in both groups by -3kg and -2kg respectively ($P < 0.05$). V'O$_2$ peak increased in both groups by 16% ($P < 0.05$). The maximal fat oxidation (MFO) rate increased similarly in both groups from 0.32 ± 0.05 to 0.36 ± 0.06 g min^{-1}($P < 0.05$). Thus, COMB training appears to be a valid alternative to HIIT for improving anthropometric parameters, cardiorespiratory fitness (CRF) and fat oxidation in male adults with obesity.

The current scientific literature on adults with obesity showed i. little individualization of training based on individual characteristics, ii. a limited number of weeks of training (i.e. 12 weeks on average) and iii. no mention of the weekly distribution of HIIT and MICT, the aim of the second chapter was to investigate changes in body composition and physical capacities induced by a 24-week polarized POL or threshold (THR) programme in male adults with obesity providing training indications based on ventilatory thresholds rather than arbitrary percentages of V'O$_2$max or heart rate (HR). Twenty male patients participated in this study (mean age 39.8 ± 6.3 y; mean BMI 31.6 ± 2.7 kg m^{-2}) (n: 10 POL, n: 10 THR). The POL group performed more weekly training below the gas exchange threshold GET (i.e. 90%), while the THR group performed more weekly training of medium to high intensity (i.e., 30% > GET) After 24-week, BM and fat mass FM decreased by -3 kg and -4

kg ($P < 0.05$) respectively, similarly in both groups. $V'O_2$max and $V'O_2$ at respiratory compensation point (RCP) increased in the POL group (+8 and +9%, $P<0.05$) and in the THR group (+4 and +4%, $P < 0.05$), as well $V'O_2$ at GET increased similarly in both groups (+13%, $P<0.05$). Thus, individualized training with a POL and THR approach were equally effective in improving body composition and physical capacities in adults with obesity.

As obesity in children and adolescents increases, aerobic exercise is becoming an important strategy to improve body composition and physical capacities of this population. HIIT and MICT are the most widely used forms of exercise, although current international guidelines recommend a combination of the two for further cardiovascular improvements. Thus, the third chapter should therefore examine the effects of COMB (i.e. a combination of MICT and HIIT) compared to continuous MICT administered during a 3-week in-hospital body weight reduction program (BWRP) on body composition, endurance performance-related variables, and substrate oxidation in adolescents with obesity. The 3-week in-hospital BWRP included moderate energy restriction, nutritional education, psychological counselling and two different physical exercise protocols. Twenty-one male adolescents with obesity participated in this randomised control trial (mean age: 16.1 ± 1.5 years; mean BMI 37.8 ± 4.5 kg m^{-2}) (n:10 for COMB, n:11 MICT) and completed ~30 exercise sessions. The COMB group completed 3 repetitions of 2 minutes at 95% of $V'O_2$peak (e.g. HIIT $\leq$ 20%), followed by 30 minutes at 60% of $V'O_2$ (e.g. MICT $\geq$ 80%). Body composition, $V'O_2$peak, basal metabolic rate (BMR), energy expenditure and substrate oxidation rate were measured during at PRE and POST. The two training programmes were equivalent in terms of calorie consumption. At POST, BM reduction was significantly greater in the MICT group than in the COMB group ((i.e., -5 and -8 kg, respectively, $P < 0.05$), while FM decreased similarly by -4 kg in both groups ($P < 0.05$). Only in the COMB group fat-free mass (FFM) was maintained after the 3-week BWRP. The $V'O_2$peak increased only in the COMB group by an average of 0.28 ± 0.22 L min^{-1} ($P < 0.05$). The MFO increased only in the COMB group by 0.04 ± 0.03 g min^{-1} ($P < 0.05$). Thus, COMB training is a viable alternative to MICT to improve anthropometric characteristics, physical capacities, and MFO in adolescents with obesity during a 3-week inpatient BWRP.

Finally, two appendices have been included. In the first appendix, we validate a Running Advisor Billat Training Test (RABIT®) that can be used to identify the three intensity domains in individuals with obesity useful for individualized exercise prescription. Thirteen volunteers with obesity completed a graded (GRAD) and a RABIT test. The RABIT test consisted of three fixed levels of perceived exertion (RPE): 1) 10 minutes at RPE 13, 2) 5 minutes at RPE 16 and 3) 3 minutes at RPE 18. GRAD was performed in 1-minute increments, increasing the speed by 0.5 km/h every minute until voluntary exhaustion. The parameters determined during the RABIT test were compared with the parameters determined during the GRAD test. At RPE 18, $V'O_2max$, ventilation (VE), maximal heart rate (HRmax) and running speed did not differ significantly from the values measured in the GRAD test. The oxygen consumption ($V'O_2$), VE and HR measured at RPE 16 and RPE 13 of the RABIT test did not differ significantly from the GET and RCP parameters measured during the GRAD test. However, the running speed measured during RPE 16 and RPE 13 of the RABIT test was -5.03 ($P < 0.05$) and -7 % ($P < 0.05$) respectively, compared to running speed measured in the GRAD test. Thus, the RABIT test may be useful to identify the three domains of physical training and to plan training sessions for adults with obesity by integrating the data of heart rate and speed during the three trials.

The second appendix was designed to determine the effects of 24 weeks of training with two different training POL and THR on body composition, physical capacities and adiponectin expression in adults with obesity. Adiponectin, an adipokine involved in the regulation of insulin sensitivity and anti-inflammatory processes and secreted by adipose tissue, decreased during obesity. Thirteen male adults with obesity (mean age 39.8 ± 6.3 y; mean BMI 31.6 ± 2.7 kg m^{-2}) participated for 24 weeks in two different exercise programmes , POL and THR, which consisted of walking or running (or a combination of the two methods) under their normal living conditions. In PRE and POST, body composition, endurance performance-related variables and the concentration of adiponectin in saliva and serum were measured. At POST, BM decreased similarly in both groups by an average of -4kg ($P < 0.05$). FM decreased by -4kg ($P < 0.05$). $V'O_2max$ increased by an average of 0.20 L min^{-1} ($P < 0.05$). We also observed an increase in salivary and serum adiponectin concentrations at POST compared to PRE by 5 µg mL-1 and 5 ng mL-1, respectively ($P < 0.05$). Finally, we found significant correlations between serum Δadiponectin and Δhip ($R = -0.686$, $P < 0.05$) and between salivary Δadiponectin and Δwaist ($R = -0.678$, $P = 0.011$). Our results suggest that a 24-week exercise programme, regardless of intensity and volume, improves body composition and physical capacities. These improvements are associated with an increase in adiponectin expression in both saliva and serum.

Chapter 1

General Introduction

1.1 Obesity

1.1.1 Definition and classification

Obesity is a condition characterized by an excessive or abnormal accumulation of adipose tissue within the body (Canoy and Buchan 2007). A subject is considered obese if he meets one of the following criteria: body mass index (BMI), calculated as weight in kilograms divided by the square person's height in meters, equal or greater than 30 kg m^{-2} (Canoy and Buchan 2007); percentage of fat mass (FM) $\geq$ 25% for men, and 35% in women related to body mass (BM) (Canoy and Buchan, 2007). However, based on BMI values, people can be classified into different categories (Williams et al. 2015):

- Underweight (BMI < 18.5kg m^{-2}).
- Normal weight (18.5 < BMI < 24.9 kg m^{-2}).
- Overweight (25 < BMI < 29.9 kg m^{-2}).
- Grade I obesity (30 < BMI < 34.9 kg m^{-2}).
- Grade II obesity (35 < BMI < 39.9 kg m^{-2}).
- Grade III obesity (BMI > 40 kg m^{-2})

BMI is an inexpensive tool for defining the condition of overweight/obesity in many people. At the same time, it does not require any specialized healthcare personnel; it only needs the use of a balance, a meter, and a calculator. Although BMI is easy to use, it has many limitations regarding its excessive genericity. In fact, BMI does not provide any information on the amount of FM contained in the body; it does not allow to distinguish between FM and fat-free mass (FFM). Also, BMI does not differentiate between males and females, as well as between different ethnicities (Gonzalez et al. 2017). Consequently, based on the above, other methods are commonly used for obesity definition(Williams et al. 2015). Starting from less accurate methods, waist circumference, waist to hip ratio and skinfold thickness are interesting because of their simplicity in quantifyng central obesity, which is strongly related to cardiovascular diseases, more than general obesity (Ortega et al. 2016). Finally, more accurate methods are bioelectrical impedance, dual energy X-ray absorptiometry, magnetic resonance imaging, and computerized tomography (Williams et al. 2015). However, the above mentioned methods are very limited to use due their complexity and expensiveness.

1.1.2 Causes

The main cause of obesity is an energy imbalance in which energy intake is greater than energy expenditure (Williams et al. 2015). Many contextual elements affect weight-related behaviours and weight status. Among the most important and well-documented ones there are socioeconomic status, geographic location, dietary preferences, physical and social environment, gender, age, cultural identity and family composition (Williams et al. 2015). Socioeconomic advantages and educational attainment are inversely related to obesity. In addition, family, friends and social environment in general can contribute to poor eating habits. For example, having parents with poor eating habits increases the likelihood of developing obesity (Williams et al. 2015). However, the main theories on the obesity development to date are as follows:

- Energy Balance Model (EBM),
- Carbohydrate-Insuline Model (CIM)

1.1.2.1 Energy Balance Model

EBM theory states that excessive food intake, regardless of the type, creates a “positive” energy balance that leads to an increase in adipose tissue in the body (Figure 1.1.2.1) (Schwartz et al. 2017) As the hypothalamus regulates the homeostasis of energy intake and expenditure through appetite control by receiving hormonal signals from the periphery and transmitting them to the central nervous system triggering impulses such as motivation and craving, the increasing availability of energy-dense and ultra-processed products (simple sugars) on the market attracts many people and thus increases the risk of developing obesity (Hall et al. 2022). Therefore, the EBM model for weight loss suggests focusing more on the quality of food rather than just energy consumption.

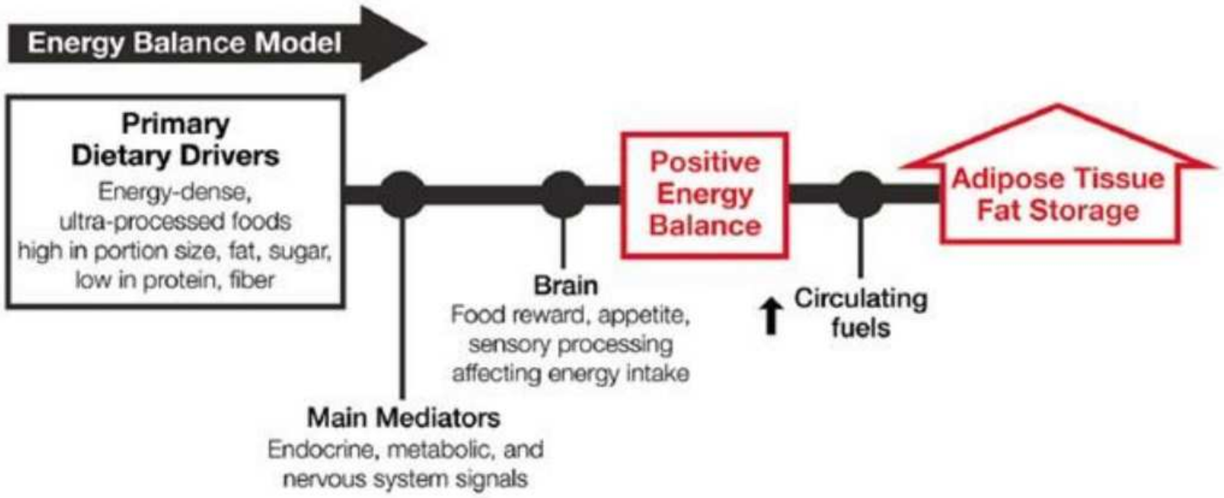


Figure 1.1.2.1 *Schematic representation of the EBM theory* (Hall et al. 2022)

1.1.2.2 Carbohydrate-Insuline Model

The CIM theory states that the consumption of foods with a high glycaemic load such as simple sugars, starches and processed cereals, when consumed over a prolonged period, triggers anabolic hormonal responses such as insulin secretion, which inhibit lipolysis processes and increase the deposition of fats in adipose tissue (Figure 1.1.2.2) (Ludwig et al. 2021). This mechanism transfers much of the energy to the processes of fat deposition and reduces the release of fatty acids in the blood, leaving little energy available to active tissues (including the brain), especially in the postprandial phase (Shimy et al. 2020). In the long term, the reduced catabolic activity, especially in adipose tissue, stimulates the brain to feel hungrier, increasing the demand for food. If the person resists the repeated urge to eat and restricts food intake, the body responds by reducing energy expenditure. This generally manifests as: fatigue, sedentary behaviour, decreased thermogenesis, increased muscle efficiency and other mechanisms that serve to conserve energy ((Lennerz et al. 2013).

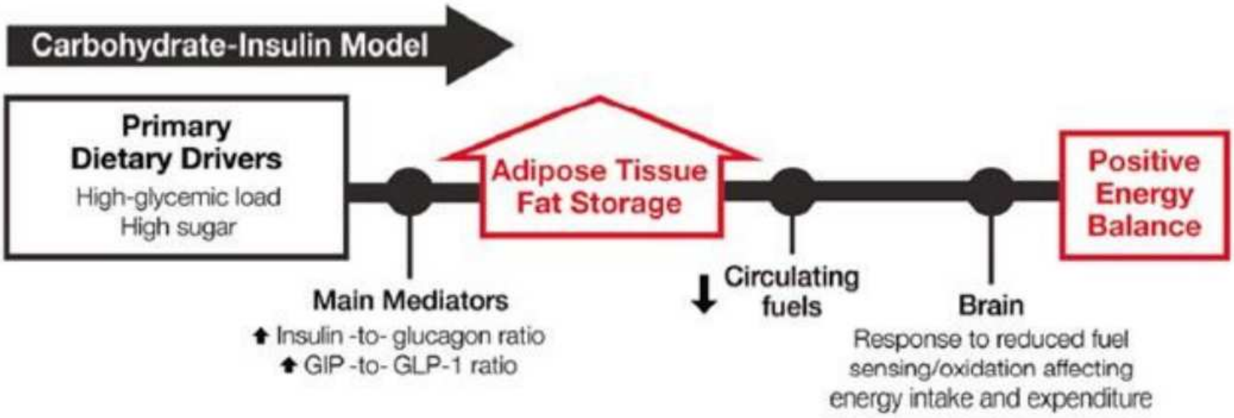


Figure 1.1.2.2 *Schematic representation of the CIM theory* (Hall et al. 2022)

1.1.3 Epidemiology

According to a recent global estimate, ~ 650 million adults are considered obese (WHO 2016). In deeper, 39% of women and 39% of men in the world aged 18 years and over were overweight (WHO, 2016). In Italy, the average BMI in 2014 was 26.0 kg m^{-2}, the percentage of overweight and obese people (BMI > 25 kg m^{-2}) was 64% and the percentage of obese people alone (BMI ≥ 30 kg m^{-2}) was 23.7%. When the population is stratified by age, the prevalence of overweight and obesity is 27% among 18-24-year-olds, it gradually increases to 54% after 50 years, it reaches 59% among 65–74-year-olds, but it progressively decreases after 75 years to 49% among those over 85. Thus, both overweight and obesity increase with age, but this becomes less common after 75, as BMI is subject to fluctuations related to biological and pathological factors. In addition, the prevalence of overweight and obesity is higher among those who are poor due to economic resources or low education, as well as among residents of southern regions (Figure 1.1.3) (Masocco et al.2023).

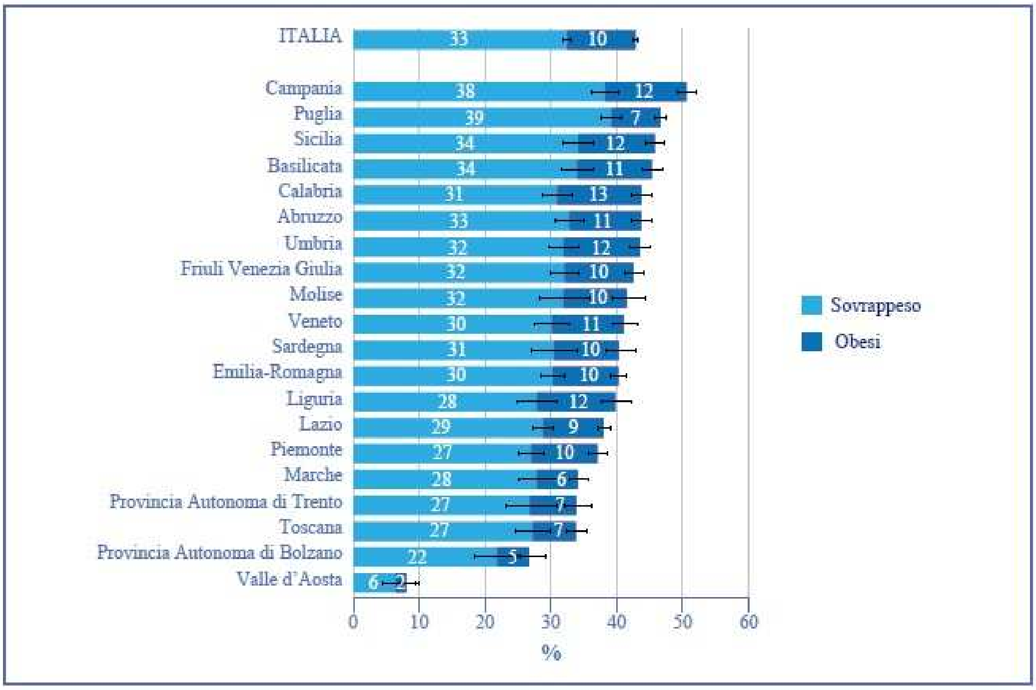


***Figure. 1.1.3** Prevalence of obesity (BMI ≥30 kg/m²) in different regions of Italy in men and women aged ≥18 years)*(Masocco et al. 2023).

1.1.4 Health consequences

The condition of obesity entails a series of structural changes that mainly affect the cardiovascular system, the respiratory system and the skeletal muscles. These effects are described in detail in the following sections.

1.1.4.1 Obesity and the cardiovascular system

Obesity has a direct impact on the cardiovascular system, particularly on the left ventricle. In most cases, the heart of a adults with obesity shows concentric hypertrophy of the left ventricle (Aurigemma et al. 2013) . There are two reasons for this condition: i. an increase in peripheral resistance, which leads to an increase in preload on the left ventricle, and ii. a higher metabolic load due to increased visceral adiposity, which requires a higher cardiac output in obese people (Bella et al. 1998). As a result, both the filling capacity and the contractility of the left ventricle are reduced without a marked change in the left ventricular ejection fraction (De Simone et al. 2005). Since the geometry of the left ventricle is directly related to the geometry of the left atrial chamber, obesity increases the size of the first chamber and thus also the size of the second. The higher the degree of obesity, the larger the left atrium becomes, even without obvious functional change (Abhayaratna et al. 2006). Finally, compared to normal weight individuals, people with obesity have fat accumulation at the level of the epicardium, which is directly involved in the production of proinflammatory cytokines, directly affecting coronary atherosclerosis (Bertaso et al. 2013). People with obesity have a 3-4 times higher risk of developing arterial hypertension, arteriosclerosis and acute myocardial infarction compared to lean people (Williams et al. 2015).

1.1.4.2 Obesity and the respiratory system

The excessive accumulation of fat in the chest and abdominal cavities have a negative impact on the mechanical function of the respiratory system and leads to compression of the chest cavity and lungs with restricted movement of the diaphragm and a consequent reduction in lung volume. In fact, the condition of obesity contributes to a significant reduction in residual functional capacity, expiratory reserve volume, residual volume, and total lung capacity (Watson et al. 2010). In addition to impairing respiratory function, obesity also contributes to a higher likelihood of developing respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD). Underlying this increased risk appears to be inflammation associated with excess visceral adipose tissue (VAT), which is exacerbated in people with metabolic syndrome (Mets) (Leone et al. 2009).

1.1.4.3 Obesity and musculoskeletal system

People with obesity show a reduction in type I muscle fibers and an increase in type IIx muscle fibers compared to lean individuals (Damer et al. 2022). In addition, obesity contributes to a reduction in both the number and functionality of mitochondria with a negative impact on aerobic metabolism (Georgiev et al. 2022). Also, people with obesity showed lower capacity to oxidize fat during moderate and high-intensity aerobic activity (Horowitz 2001; Lanzi et al. 2014). Indeed, when maximal fat oxidation (MFO) is expressed with respect to FFM, the normal weight individuals showed greater capacity to oxidize fat during exercise than did the overweight and individuals with obesity, independently of age, sex, and cardiorespiratory fitness both in males (7.67 ± 0.54 *vs.* 6.30 ± 0.48 *vs.* 6.88 ± 0.54 mg kg FFM^{-1} min^{-1}) and females (9.53 ± 0.31 *vs.* 8.39 ± 0.43 *vs.* 7.52 ± 0.74 mg kg FFM^{-1} min^{-1}), respectively (Amaro-Gahete et al. 2019). These findings support the idea that obese individuals may suffer from metabolic inflexibility (i.e., the capacity to adapt one’s nutrient oxidation balance to alterations in metabolic or energy requirements) during exercise owing to a lower fat oxidation capacity per unit of metabolically active tissue (Amaro-Gahete et al. 2019). Possible causes of this phenomenon include i. the reduction in the number and activity of mitochondria as previuolsy mentioned (Georgiev et al. 2022), ii. the reduction in the number and activity of carnitine palmitoyltransferase-1 (CPT-1) (Houmard 2008) the largest transporter of long-chain fatty acids in the mitochondria, iii. the lower density of beta-2-adrenergic receptors in subcutaneous adipose tissue (Reynisdottir et al. 1994). Thus, the excess of FM and the reduced capacity to use fat as a fuel at rest and during exercise may further explain the accumulation of intramuscular triglycerides (IMTG) in individuals with obesity (Goodpaster and Sparks 2017).

1.1.4.4 Obesity and the metabolic health

Adipose tissue is a highly metabolically active organ with a variety of functions such as fat storage, mechanical protection, thermal insulation, immune response, endocrine functions and thermogenesis (Kahn et al. 2019). Obesity leads to local hypoxia and mechanical stress in pre-existing adipocytes as well in VAT and ectopic depots (such as muscle, liver and pancreas) causing hypertrophic adipocytes (Kahn et al. 2019; Thyfault and Bergouignan 2020). Hypertrophic adipocytes increase lipolysis and the release of inflammatory cytokines, which are involved in the pathophysiology of obesity-related metabolic and cardiovascular diseases (Upadhyay et al. 2018). One of the main negative consequences of increased VAT is insulin resistance (i.e. the inability of insulin to effectively stimulate glucose uptake in the metabolic tissues of skeletal muscle, adipose tissue, and liver). The prevailing view is that obesity precedes and causes insulin resistance, whereupon the beta cells of the pancreas secrete hyper insulin to compensate for the impaired insulin signaling (Iozzo 2009). However, new evidence suggests that hyperinsulinemia is likely to contribute to metabolic dysfunction and the development of obesity (Templeman et al. 2017). Insulin resistance not only contributes to hyperglycemia in type 2 diabetes, but is also thought to play a role in inappropriate excessive (ectopic) fat storage in the liver (hepatic steatosis), which represents a negative vicious cycle (Hodson and Karpe 2019). Higher levels of ectopic storage of lipids in muscle and liver are in turn associated with insulin resistance. The liver becomes selectively insulin resistant in controlling glucose production but remains highly sensitive to the lipogenic effects of insulin (Smith et al. 2020). Finally, insulin resistance also plays a fundamental role in reduced metabolic flexibility (Goodpaster and Sparks 2017). Inflammation, oxidative stress and endoplasmic reticulum stress have also been linked to metabolic dysfunction via both lipid and non-lipid mediated pathways (Petersen and Shulman 2018).

1.1.4.5 Obesity and cardio-respiratory fitness

The maximum oxygen consumption ($V'O_2max$) is the benchmark for determining the individual cardiorespiratory fitness level (CRF) (BASSETT 2000). Individuals with obesity have a lower cardiorespiratory capacity in relation to body weight and lean mass (Amaro-Gahete et al. 2019; Gaesser and Angadi 2021). The causes could be multifactorial:

1. Some clinical studies demonstrate that muscle capillary density is lower in people with metabolic disease (i.e., including obesity) with and impaired muscle blood flow during physical activity due to endothelial disfunction (Rosenblat et al. 2022).

2. As previously mentioned, individuals with obesity showed a reduction in both mitochondrial number and functionality (Georgiev et al. 2022), as mitochondrial activity is strongly related to $V'O_2max$ in elite endurance athletes and in the clinical population (van der Zwaard et al. 2016).

3. A high percentage of FM is associated with poor exercise tolerance (Vargas et al. 2018). During aerobic exercise, individuals with obesity showed both lower availability of fatty acids and lower utilization of fat in muscle, which impaired the ability to tolerate exertion (Horowitz 2001). Indeed, MFO is related to $V'O_2max$ (Nordby et al. 2006; Hetlelid et al. 2015), suggesting that low CRF is accompanied by impairments in fat oxidation capacity also.

4. Another limiting factor for the physical performance of people with obesity is dyspnea (shortness of breath). The high proportion of FM in the trunk increases chest stiffness, leading to respiratory muscle strain (Dempsey et al. 2006; Salvadego et al. 2017; Alemayehu et al. 2018; Phillips and Stickland 2019), which subsequently increases the perception of breathlessness during cardiopulmonary exercise testing (CPET) and steady-state exercise measurements (Bernhardt and Babb 2016). Thus, excessive adipose tissue around the rib cage and in the visceral cavity increases the work of breathing during exercise (Oppenheimer et al. 2014) and the oxygen uptake of the muscles involved in breathing, which reduces the oxygen availability to the muscles involved during exercise (Alemayehu et al. 2018), reducing exercise tolerance.

5. During the walking activity, the cost of locomotion (Cw, i.e. the amount of energy spent above resting to transport 1 kg body mass (BM) over 1 meter distance) of people with obesity is ~10% higher than that of normal weight individuals (Browning et al. 2006). It could be that individuals with obesity exhibit an alternative pattern of exercise by increasing the effort of synergistic muscles and altering recruitment patterns (Tallis et al. 2018).

As a result, low cardio-respiratory fitness is therefore an independent risk factor for health, regardless of BMI levels (Gaesser and Angadi 2021). Studies haves shown that as physical activity decreases, $V'O_2$ max also decreases by approximately 0.3-0.4% day^{-1} (Narici et al. 2021) with negative effects on body weight (increases of ~1.5 kg per month) (Pellegrini et al. 2020). On the contrary, people with physically active obesity have a lower risk of mortality from all causes and heart disease compared to lean, untrained individuals (Gaesser and Angadi 2021).

1.2 Physical activity guidelines

Physical activity is recognized as an important lifestyle intervention in weight management programmes for obese individuals as it creates an energy deficit to reduce body mass (BM) (Petridou et al. 2019) improves CRF (Rugbeer et al. 2021), and optimizes fat oxidation capacity (Achten and Jeukendrup 2004a; Vaccari et al. 2020). According to physical activity guidelines shared by the American College of Sport Medicine (ACSM) (Donnelly et al. 2009), and in agreement with the American College of Cardiology (ACC) (Jensen et al. 2014), at least 150 minutes of moderate-intensity aerobic physical activity per week^{-1} is recommended for individuals with obesity to achieve the minimum level of physical activity. Subsequent studies have found that obese individuals should exercise for approximately 250 minutes per week^{-1} to achieve clinically significant weight loss (> 5% of initial weight) (Donnelly et al. 2009; Williamson et al. 2015).

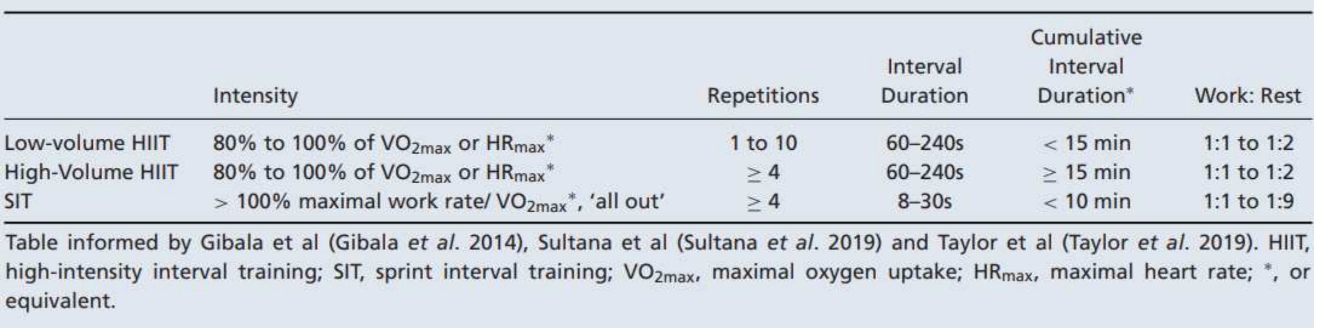
The recommendations apply equally to men and women, as it appears that there are no differences in weight loss rates between the two genders for the same amount of exercise (Wewege et al. 2017a; Keating et al. 2017). However, if you add moderate, but not strict, dietary restriction to physical activity, 150 to 250 minutes per week^{-1} of moderate-intensity exercise is sufficient to promote weight loss. Adding resistance training to moderate-intensity endurance training can help to further reduce fat mass and increase lean mass (Willis et al. 2012). Physical activity and a controlled diet are always recommended together (Donnelly et al. 2009), but it appears that inducing an energy deficit through diet stimulates appetite more than the same amount of energy deficit induced by exercise alone (Thivel et al. 2018). Recent scientific literature suggests that moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) are the most useful training methods to lose weight and improve $V'O_2$max in people with obesity (Su et al. 2019).

1.2.1 Moderate Intensity Continuous Training

MICT is as an exercise with a constant intensity which does not overcome the gas exchange threshold (GET) and without slow component of the oxygen consumption ($V'O_2$) (Poole and Jones 2012). Moderate-intensity training is characterized by a continuous and constant work of 3-6 metabolic equivalents (MET, i.e. a multiple of energy expenditure at rest), or by a training with heart rate (HR) that is between 64-76% of HRmax. Typically, the duration of MICT is between 30 and 60 minutes (Garber et al. 2011; Su et al. 2019). Most studies using MICT in adults with obesity include an average training period of 12 weeks with an average training frequency of 2 to 3 training sessions per week, with both indoor and outdoor cycling, walking, or running (Su et al. 2019). Although the benefits of MICT have been proven (Rugbeer et al. 2021), people with obesity cite "lack of time" as one of the biggest barriers to adopting an active lifestyle (Su et al. 2019). As a result, there is a need to explore other exercise methods that are effective and efficient in terms of time and results.

1.2.2 High Intensity Interval Training

$V'O_2$max intensity does not allow training over a long period of time (Billat et al. 1994b). Therefore, dividing the training program into shorter "periods" of higher intensity, separated by recovery phases, seems to extend the time spent at high intensity in a single training session. HIIT training is characterized by periods of high intensity (i.e., 8-240 seconds per step, intensity between 80-100% HRmax or ≥ 80%$V'O_2$ max) alternating with short periods of rest or low intensity exercise (i.e. ≤ 60% HRmax) in training sessions that last an average of 30 minutes (i.e. including warm-up and cool-down) (Gibala et al. 2014). HIIT sessions are divided in two categories, short interval HIIT (bouts duration < 1 min) and long interval HIIT (bouts duration > 1 min). HIIT can be further subdivided into low volume HIIT (i.e. active work duration < 15 minutes) and high volume HIIT (i.e. active work duration ≥ 15 minutes) or as "sprint interval training" (SIT) with "all-out" effort with low volume of exercise (i.e. < 10 minutes) (Taylor et al. 2019; Sabag et al. 2022).



	Intensity	Repetitions	Interval Duration	Cumulative Interval Duration*	Work: Rest
Low-volume HIIT	80% to 100% of VO_{2max} or HR_{max}*	1 to 10	60–240s	< 15 min	1:1 to 1:2
High-Volume HIIT	80% to 100% of VO_{2max} or HR_{max}*	≥ 4	60–240s	≥ 15 min	1:1 to 1:2
SIT	> 100% maximal work rate/ VO_{2max}*, 'all out'	≥ 4	8–30s	< 10 min	1:1 to 1:9

Table informed by Gibala et al (Gibala *et al.* 2014), Sultana et al (Sultana *et al.* 2019) and Taylor et al (Taylor *et al.* 2019). HIIT, high-intensity interval training; SIT, sprint interval training; VO_{2max}, maximal oxygen uptake; HR_{max}, maximal heart rate; *, or equivalent.

Figure 1.2.2 *HIIT classification according to* Taylor et al. (2019; Sabag et al. (2022).

When planning HIIT sessions, you need to consider the intensity and duration of the exercise (i.e., the main influencing factors), the number of intervals, the number of series, the recovery times and intensities between series, and the type of exercise (i.e., running or cycling) (Buchheit and Laursen 2013a). Each manipulation of a single variable has a direct effect on metabolic, cardiopulmonary and/or neuromuscular responses (Buchheit and Laursen 2013a, b). In short, SIT and short HIIT can induce primarily metabolic stress by inducing a large anaerobic glycolytic metabolism and high demands on the O_2 transport and utilization systems, as well as neuromuscular stress (Buchheit and Laursen 2013a, b). Prolonged interval training also usually results in metabolic stress, but also in marked anaerobic glycolytic stress and, in some cases, some degree of neuromuscular stress (Buchheit and Laursen 2013a). For more details, see Buchheit and Laursen (2013a, b).

HIIT has been shown to produce similar and sometimes even greater improvements in both cardiorespiratory function and body composition than MICT in people with obesity, although it requires less time and sometimes less energy expenditure (Sultana et al. 2019). In addition, HIIT is a safe and well-tolerated exercise program for people with obesity (Türk et al. 2017a). Further details are provided in the next sections.

1.3 Effects of HIIT and MICT on body composition

The current scientific literature suggests that HIIT and MICT are the best way to improve body composition in individuals with obesity compared to resistance training alone (Chen et al. 2023). To date, there is still no uniform consensus on which training is best between HIIT and MICT (Chen et al. 2023). In a study conducted by Schjerve et al. (2008), forty male individuals with obesity (BMI: 30 kg m^{-2}) underwent HIIT and MICT training programs for 3 session a week for 12 weeks. The HIIT protocol involved 4 work intervals of 4 minutes each (i.e., 85-95% of HRmax), interspersed with 3 minutes of rest at 50-60% of HRmax. In contrast, the MICT protocol involved a single session of 47 minutes performed at 60-70% of HRmax. The results showed that both the HIIT and MICT protocols were effective in reducing BM (HIIT ~2% and MICT ~3%) and FM (HIIT ~2.2% and MICT ~2.5%), with no significant differences between the two groups. Martins et al. (2016) have enrolled forty-six sedentary individuals with obesity (30 female, 16 male) (34.4 ± 8.8 years, BMI 33.3 ± 2.9 kg m^{-2}) to conducted twelve weeks of HIIT or MICT on cycle ergometers (three sessions per week) with the same total energy expenditure (i.e., 250 kcal). The HIIT protocol involved 8-second sprints interspersed with 12-second recoveries, whereas the MICT consisted of continuous cycling at 70% of HRmax. Both sessions ended with an energy expenditure of 250 kcal per session. At the end of the training interventions, the researchers found no significant differences between HIIT and MICT in reducing the percentage of BM (HIIT -1.3 ± 2.1 kg; MICT -0.8 ± 3.2 kg) and trunk FM region (HIIT -0.6 ± 1.8%; MICT -0.01 ± 2.9%). However, the average duration of HIIT session was shorter than MICT (i.e., 20 *vs.* 32 minutes, respectively) (Martins et al. 2016). Vaccari et al. (2020) engaged thirty-two volunteers with obesity (39 years; BMI 36 kg m^{-2}) to participated at 3-month either MICT or HIIT. The average duration of MICT was ~44 minutes with a HR corresponding to 60% of the initial $V'O_2$peak. HIIT consisted of 3-7 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), with a mean duration of ~33 minutes. At the end of the training interventions, mean BM loss was 5.84 ± 0.15 kg (time effect $P < 0.001$), while FM decreased by 5.37±0.16 kg (time effect $P <0.001$) in both groups, without significant differences between the two groups. As for the previous study, the duration of HIIT was ~10 minutes lower than MICT. Also, Reljic et al. (2021) investigate the effects of HIIT and moderate intensity interval training (MIIT) in a period of 12 weeks, in a group of 117 individuals with obesity (49.8 ± 13.6 years, BMI: 38.2 ± 6.2 kg m^{-2}). The HIIT protocol consisted of 5 interval bouts of 1 min at 80-95% HRmax interspersed with 1 min of low intensity recovery (total session time: 14 minutes). The MIIT protocol was designed identically with 5 interval bouts of 1 min at 65–80% HRmax interspersed with 1 min of low intensity recovery (total session time: 14 minutes). Both groups achieved a significant reduction of BM (HIIT: − 3.9 kg, $P < 0.001$; MIIT: − 2.0 kg, $P = 0.004$) without significantly differences between groups. On the contrary FM significantly decreased only in the HIIT group (-1.8 kg, $P < 0.001$). Also, recent review and meta-analysis found no significant differences between HIIT and MICT in reducing BMI and FM (Wewege et al. 2017; Keating et al. 2017; Su et al. 2019). From the analysis of the previously mentioned studiess, the average of BM reduction with HIIT and MICT is 1.67 and 1.63 kg respectively, while the reduction of FM (kg) is 1.50 and 1.35 kg respectively for HIIT and MICT.

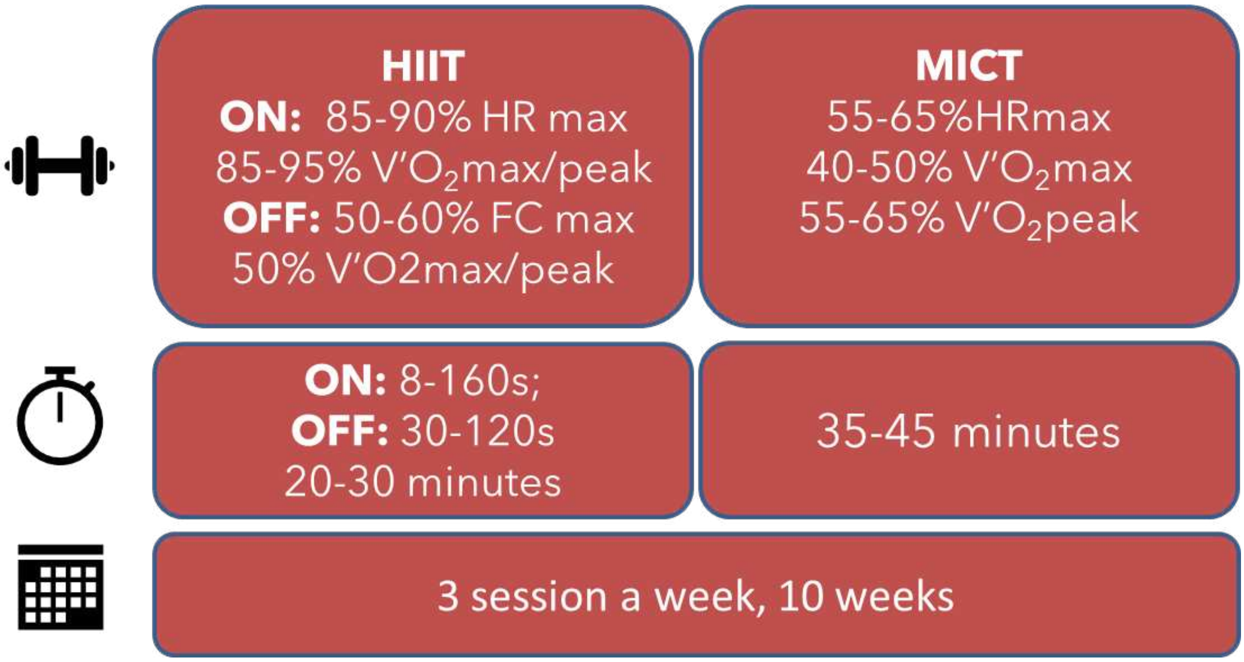
Thus, it appears that HIIT offers similar benefits to MICT in improving body composition, but in a more time-efficient manner, considering that lack of time is the main barrier to abstaining from physical activity in people with obesity (Wewege et al. 2017; Keating et al. 2017; Su et al. 2019). From a physiological perspective, it is possible that the help of MICT in reducing BM and FM is due to the improvement in the ability of skeletal muscle to increase fatty acid content and utilisation during aerobic exercise (Murray and Rosenbloom 2018; Purdom et al. 2018), which are typically impaired in obesity (Georgiev et al. 2022). In contrast, the rapid energy demand of the body during HIIT leads to ATP resynthesis to maintain exercise performance, which is mainly derived from glycolytic metabolism (Kramer et al. 2023). Acetyl-CoA carboxylase catalyses the formation of malonyl-CoA, which inhibits carnitine palmitoyl transferase (CPT-I) activity and appears to reduce β-oxidation involved in ATP resynthesis (Purdom et al. 2018), although there have been counterproductive findings on fat oxidation during HIIT (Hetlelid et al. 2015; Lazzer et al. 2017; Vaccari et al. 2020). It is possible that HIIT increases excess post-exercise oxygen consumption (EPOC) compared to MICT during the slow phase of O_2 kinetics in the recovery phase (i.e. from 30 minutes to 22 hours after the end of the exercise session) (Panissa et al. 2021). This occurs due to greater fat utilisation after exercise to maintain energy demands while glycogen resynthesis occurs (Moniz et al., 2020) through a significant increase in circulating hormones that promote fat oxidation (i.e. catecholamines and growth hormone) (Moniz et al. 2020), although the results are contradictory (Lazzer et al. 2017). In addition, in all studies mentioned so far, FFM was maintained or slightly reduced with no significant difference between the two training groups, which may be helpful for maintaining weight loss (Wewege et al. 2017; Keating et al. 2017).

Thus, considering the recent scientific literature and through different physiological mechanisms, both HIIT and MICT are useful for weight loss and FM in adults with obesity (Kramer et al. 2023). Finally, the studies conducted to date have been carried out over an average period of 12 weeks, studies with longer periods (i.e., 6 months) are needed to evaluate the differences between HIIT and MICT on body composition.

1.4 Effects of HIIT and MICT on maximal oxygen consumption

The mortality risk associated with obesity is largely attenuated or eliminated by moderate-to-high levels of CRF by increasing the level of physical activity (PA) (Gaesser and Angadi 2021). Current scientific literature suggests that aerobic exercise in the form of HIIT and MICT is the best way to improve (Gaesser and Angadi 2021). However, to date, there is still no consensus between HIIT and MCT on the most effective strategy. Some studies have attempted to compare these types of training. A recent study by (Poon et al. 2020) involving twenty-four participants (48.1 ± 5.2 years; BMI of 25.8 2.3kg m^{-2} showed that low volume HIIT (10 x 1 minute at 80-90% of HRmax) resulted in similar improvements in $V'O_2max$ (i.e., 10% increase for both groups) compared to higher volume MICT (50 min $session^{-1}$ at 65-70% of HRmax). Nevertheless, (Winding et al. 2019) have shown that 11 weeks of low-volume HIIT training performed three times a week (10 x 1 min at 95% of peak power) resulted in similar or even greater improvements in cardiorespiratory fitness, body composition and glycaemic control in adults with obesity and type 2 diabetes compared with higher-volume MICT, despite less exercise time (45% less) and lower energy expenditure (35% less) (Winding et al., 2018). In a study by Matsuo et al. 2014), it was observed that a low-volume HIIT performed five times per week (3 x 3 minutes at 80-90% of $V'O_2max$, separated by a 2-minute recovery period) resulted in significant improvements in $V'O_2max$ compared to a MICT protocol that included one session of 40 minutes at constant intensity (60-65% $V'O_2max$) within 8 weeks (HIIT: 22.5 ± 12.2% - MICT: 10.0 ± 8.9%) (Matsuo et al., 2014). Vaccari et al. (2020) have shown that after 3-months of HIIT or MICT, absolute $V'O_2peak$ increased by 6% in MICT and by 16% in the HIIT group (interaction G × T $P < 0.001$). 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 $V'O_2max$ than MICT in several studies (Fisher et al. 2015; Lanzi et al. 2015; BÆkkerud et al. 2016; Lazzer et al. 2017). Further confirmation comes from the meta-analysis of 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, investigating aerobic capacity adaptations following HIIT, compared to continuous training and concluded in favour of HIIT. In line with García-Hermoso et al. (2016) a recent review by (Su et al. 2019) have shown that although, there is no significant difference between HIIT and MICT in improving $V'O_2max$, HIIT appears to be the most effective training strategy in terms of time effectiveness for improving CFR. In contrast to the above-mentioned studies, the Rugbeer et al. (2021) meta-analysis undertaken between December 2, 2019, and April 18, 2020, that included 26 studies reported no significant difference in CRF between MICT versus HIIT in individuals being overweight or obese. Moreover, MICT was significantly better at improving CRF compared to SIT in overweight or people with obesity (Rugbeer et al. 2021). Also, when HIIT was carried out in real world setting, and the exercise sessions were undertaken in a community park (Lunt et al. 2014), it was only modestly effective in improving the cardiorespiratory fitness in a cohort of individuals overweight or with obesity. The authors hypothesized a reduced adherence to the exercise program compared to a more enjoyable walking activity (Lunt et al. 2014). Analysis of the above studies found that HIIT result in an average improvement in $V'O_2max$ or $V'O_2peak$ between 0.24-0.28 L min^{-1} and 3.40-3.84 ml kg^{-1} min^{-1}, while MICT induced an average improvement in $V'O_2max$ or $V'O_2peak$ between 0.14-0.17 L min^{-1} and 2.33-2.76 ml kg^{-1} min^{-1}. Thus, HIIT appears to be more effective than MICT for improving CRF in individuals with obesity. Medium-interval HIIT (i.e., a medium step duration of three minutes) three-times per week in a short period of time (i.e. ≤ 12 weeks) resulted in the largest improvement in CRF during weight management program (Fig. 1.4). Thus, it seems that in a short period of training HIIT was significantly better in improving CRF compared to MICT in overweight or obese people. Since 3.5 mL kg^{-1} min^{-1} improvement in $V'O_2max$ was associated with 15% decrement in the risk of cardiovascular diseases, both the training interventions are effective in improving the cardiometabolic health of individuals with obesity (Kodama et al. 2019).

Despite the widely documented increase in $V'O_2max$ reported in response to intense interval training, the mechanisms underpinning this adaptation are poorly understood. The Fick equation denotes that any change in $V'O_2max$ is determined by changes in central oxygen transport (i.e, cardiac output (CO) = heart rate * stroke volume (SV)) or oxygen extraction as represented by the arteriovenous oxygen difference ($a\text{-}vO_2diff$) (BASSETT 2000). Below is shown a brief discussion of the central and peripheral factors behind the improvements in $V'O_2max$.



	HIIT	MICT
🏋	**ON:** 85-90% HR max 85-95% V’O$_2$max/peak **OFF:** 50-60% FC max 50% V’O2max/peak	55-65%HRmax 40-50% V’O$_2$max 55-65% V’O$_2$peak
⏱	**ON:** 8-160s; **OFF:** 30-120s 20-30 minutes	35-45 minutes
📅	3 session a week, 10 weeks	

Figure 1.4 *Average characteristics of the HIIT and MICT protocols in improving body composition and physical capacities in individuals with obesity.*

1.4.1 Central (Cardiovascular) Adaptations

The ability to transport oxygen of the cardiovascular system to the peripheral muscles is considered the most important limiting factor for V'O$_2$ in a normoxic environment (di Prampero 2003; Ferretti 2014). In humans, improvements in V’O$_2$max usually manifest within a few weeks of starting exercise (Andersen and Henriksson 1977), and their fluctuations are primarily due to variations in SV and CO rather than a-vO$_2$diff (BASSETT 2000). Maximal SV increased equally or more after HIIT compared to MICT (Warburton et al. 2004; Daussin et al. 2007; BÆkkerud et al. 2016). Furthermore, the increase in V'O$_2$max after 6 weeks of training has been associated with haematological adaptations (Montero et al. 2015), although a recent systematic review and meta-analysis showed that changes in blood volume (BV), plasma volume (PV) and haematocrit (Hct) do not appear to contribute to the increase in V’O$_2$max due to the small, reported effect size (Astorino et al. 2022). Furthermore, HIIT or SIT required a minimum of 2-6 weeks to improve central oxygen delivery in the form of CO. Previous results in inactive and active adults with lower V’O$_2$max show significant (+2.0 ± 0.3 L-min^{-1}) increases in COmax after only 9 and 10 HIIT sessions, suggesting that training intensity, rather volume is crucial for increasing COmax and that COmax is in turn one of the most important determinants for increasing V’O$_2$max compared to pre-training (Astorino et al. 2022). The higher COmax and then SVmax may be associated with a greater stimulus for adaptation in cardiac morphology (i.e., changes in left ventricular mass, LVM) greater after HIIT than MICT (i.e., mean difference = 4.5%, P = 0.034) (Rosenblat et al. 2022). Thus, in the first weeks of training, the increases in V'O_2max demonstrated during intense interval training are accompanied by an increase in central O_2 supply (i.e. an increase in SV and CO), with no significant contribution from changes in BV, PV and Hct. It has been shown that interval training causes a 1-2% higher improvement in V'O_2max compared to continuous training and this with a significantly lower training volume (Milanović et al. 2015), which could be explained by the different cardiovascular adaptations induced by HIIT.

1.4.2 Peripheral (Muscular) adaptations

The a-vO_2diff depends on the ability of the working muscle to uptake O_2 from the capillaries and its subsequent utilization by the mitochondria for energy purposes (BASSETT 2000). In this regard will be considered in this thesis mitochondrial and capillary density adaptations following HIIT and MICT.

1.4.2.1 Mitochondria

One of the main goals of chronic training is to increase the ventilatory threshold and V'O_2max to be able to train longer and at a higher intensity. This is achieved through increased fat oxidation and a proportional decrease in carbohydrate oxidation with less lactate at a given intensity (Joyner and Coyle 2008; Egan and Zierath 2013). Regular MICT or HIIT training triggers mitochondrial biogenesis (i.e. the formation of new components of the mitochondrial reticulum) (Granata et al. 2018) and activates the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), an important regulator of mitochondrial biogenesis, albeit via different pathways (Gibala et al. 2009). A high volume of MICT increases intramuscular calcium, which activates a messenger of mitochondrial biogenesis, calcium–calmodulin kinase (Laursen 2010) (Fig. 1.4.2.1). At the same time, HIIT leads to a reduction in adenosine triphosphate (ATP) concentration and a simultaneous sharp increase in adenosine monophosphate (AMP), which activates AMP-activated protein kinase (AMPK) (Laursen 2010) (Fig. 1.3.1.1). In terms of chronic adaptations, a few days and a few training sessions are sufficient to see improvements in mitochondrial density (content) in healthy individuals or athletes, and it has been suggested that training volume is key factor in increasing mitochondrial content in humans (MacInnis and Gibala 2017). Indeed, recent evidence has shown that exercise volume (i.e. MICT) appears to be an important determinant of exercise-induces rises in mitochondrial content (an effect potentially influenced by exercise duration) (Granata et al. 2018; Bishop et al. 2019), while exercise intensity appears to be a key factor in exercise-induced increases in mitochondrial respiration (MacInnis and Gibala 2017; Granata et al. 2018). Exercise-induced changes in mitochondrial content and respiratory function appear to be regulated differently and are not necessarily linked (Granata et al. 2018; Bishop et al. 2019). Indeed, Granata et al. (2016) showed that after 20 days of high-volume training (HVT), mitochondrial respiration and citrate synthase (CS) activity increased by ~40-50%. However, reducing exercise volume by ~50% of pre-exercise volume was effective in maintaining all measured mitochondrial respiratory parameters except CS activity (~36% above baseline). Therefore, combining MICT and HIIT in a polarized approach (i.e. see above for more details) may be the optimal training strategy for mitochondrial adaptations (Laursen 2010; Bishop et al. 2019). Supplementing the high-volume programme with high-intensity training can lead to an improvement in the ability of skeletal muscle to generate ATP aerobically in athletes and non-athletes. To our knowledge, the only study found in the literature comparing the effects of both HIIT and MICT modalities on mitochondrial adaptations in individuals with obesity is the study by BÆkkerud et al. (2016). The authors found improvements in mitochondrial density after HIIT with 4x4-minute intervals close to V'O_2max, HIIT with 10x1-minute intervals at V'O_2max intensity and MICT at 70% of HR max, with no differences between the training interventions. Another study (Tan et al. 2018) investigated mitochondrial density adaptations following HIIT in overweight individuals, but without comparison to MICT; the authors measured a ~27% improvement in mitochondrial density. In contrast, some authors suggested that physical activity improves mitochondrial function in individuals with obesity but does not induce mitochondrial proliferation (Menshikova et al. 2005). Finally, in a cohort of people with obesity and type 2 diabetes, Van Ryckeghem et al. (2022) showed that O_2 extraction during exercise increased earlier in the HIIT group during the first 12 weeks, whereas in the MICT group O_2 extraction increased after 24 weeks of training, suggesting an increased capacity for oxidative ATP production, even though mitochondrial content and function were not directly measured. Although there is much literature comparing exercise adaptations following HIIT and MICT in healthy subjects, there is a lack of evidence in individuals with obesity, so more research on this topic is required to highlight the differences between HIIT and MICT or a combination of HIIT and MICT over a long training period to outline the effects on peripheral adaptation of V'O_2max (i.e. ≥ 12 weeks).

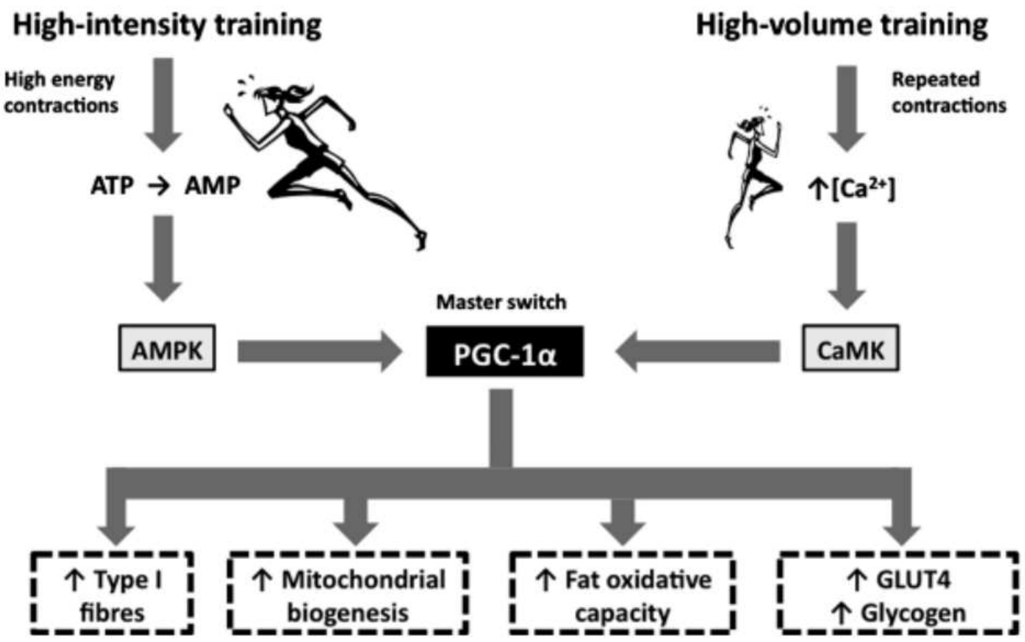


Figure 1.4.2.1 *Simplified model for the different pathways through MICT and HIIT leads to mitochondrial biogenesis (Laursen et al., 2010).*

1.4.2.2 Capillary density

Muscle capillarization plays a central role in supplying the exercising muscle with oxygen and nutrients. Improved muscle capillarization facilitates oxygen diffusion in the peripheral muscles. Skeletal muscle capillarization takes longer than mitochondria to manifest in response to exercise, weeks to months (Lundby et al. 2017), and changes in capillary density appear to be greater during low-intensity exercise (Gliemann 2016). Individuals with obesity have lower capillary density and a lower capillary to fiber area ratio in both type I and II fibers (Gavin et al. 2005). However, the acute response to exercise was higher for angiogenic factors (including VEGF) after MICT than after SIT (Hoier et al. 2013); and capillary density increased after 4 weeks of MICT, whereas it remained unchanged after 4 weeks of SIT (Hoier et al. 2013). In another study (Daussin et al. 2007), the increase in capillary density after 8 weeks of HIIT and MICT was twofold higher after MICT. Two other studies comparing SIT and MICT found similar capillary adaptations after the two modalities (Scribbans et al. 2014; Cocks et al. 2016). Given the paucity of literature, it is difficult to draw a firm conclusion, partly because most studies compare SIT and not HIIT to MICT. However, MICT appears to be superior or at least not inferior to HIIT in improving capillary density in healthy subjects. HIIT may be useful in enhancing capillarity, as Tan et al. (2018) found after 18 sessions over 6 weeks, inducing an improvement in capillary contact per fibre and increasing the capillary-to-fibre ratio in both oxidative and glycolytic fibres. Another study (Cocks et al. 2016) concludes that SIT is equally effective in improving skeletal muscle capillarisation in overweight individuals, although it requires less time than traditional MICT. A direct comparison between HIIT and MICT in terms of capillary adaptation in individuals with obesity is lacking, but the lictaeratura available showed similar adaptation to other studies in lean subjects (Daussin et al. 2007; Hoier et al. 2013; Scribbans et al. 2014; Cocks et al. 2016), we can assume that MICT may be superior, or at least not inferior, to HIIT in improving capillary density in obese subjects as well.

1.5 Polarized training

As mentioned above, physical activity guidelines for adults with obesity aged 18-60 years recommend 150-300 minutes of moderate-intensity (i.e. 3-6 metabolic equivalents, MET) or 75-150 minutes of vigorous-intensity physical activity (i.e. ≥ 6 MET) or an equivalent combination of moderate-intensity and vigorous-intensity aerobic physical activity per week (Donnelly et al. 2009; Jensen et al. 2014). However, there are no studies yet that provide recommendations for the weekly percentage of combination between moderate (i.e. MICT) and intense (i.e. HIIT) for people with obesity. The training of endurance athletes and clinical populations shares training variables such as volume, frequency, and intensity of exercise. However, in contrast to MICT and HIIT alone, a key factor in determining the training stimulus in endurance sports is the distribution of HIIT and MICT in the weekly training plan, called training intensity distribution (TID) (Stöggl and Sperlich 2014). TID can be an be quantified by defining fixed parameters or physiological thresholds (i.e., heart rate, blood levels of lactate, gas exchange, power output or velocity and perceived exertion) that delineate three (Seiler 2010) or five intensity zones (Sylta et al. 2014). The most frequently used model defines zone 1 (Z1) as intensity below the first ventilatory (VT1) or lactate threshold (LT1), zone 2 (Z2) between the two ventilatory or lactate thresholds and zone 3 (Z3) above the second ventilatory (VT2) or lactate threshold (LT2). Of the various TIDs, polarized training (POL), threshold training (THR), pyramid training (PYR) and HIIT are most frequently used in the preparation of endurance athletes (Seiler 2010; Stöggl and Sperlich 2015). POL consists of high percentage of training time in Z1 (75-80%), combined with lower proportion in Z2 (<15%), and lower to moderate percentage in Z3 (15-20%) (Treff et al. 2019). PYR consists of a high percentage of training time in Z1 and a decreasing percentage of training time in Z2 and Z3 (i.e., 70% Z1, 20% Z2, 10% Z3) (Stöggl and Sperlich 2015). THR consists of spending a high percentage of training time in Z2, never less than 35%, with the remaining time divided between Z1 and Z3 (Esteve-Lanao et al. 2007a). HIT primarily utilizes interval training and intermittent intervals with a large emphasis in Z3 training (i.e., 20% Z1, 10-20% Z2, 50-70% Z3) (Stöggl and Sperlich 2015). Of these TIDs, POL and PYR appear to be associated with the best endurance performances (Stöggl and Sperlich 2015; Casado et al. 2022).

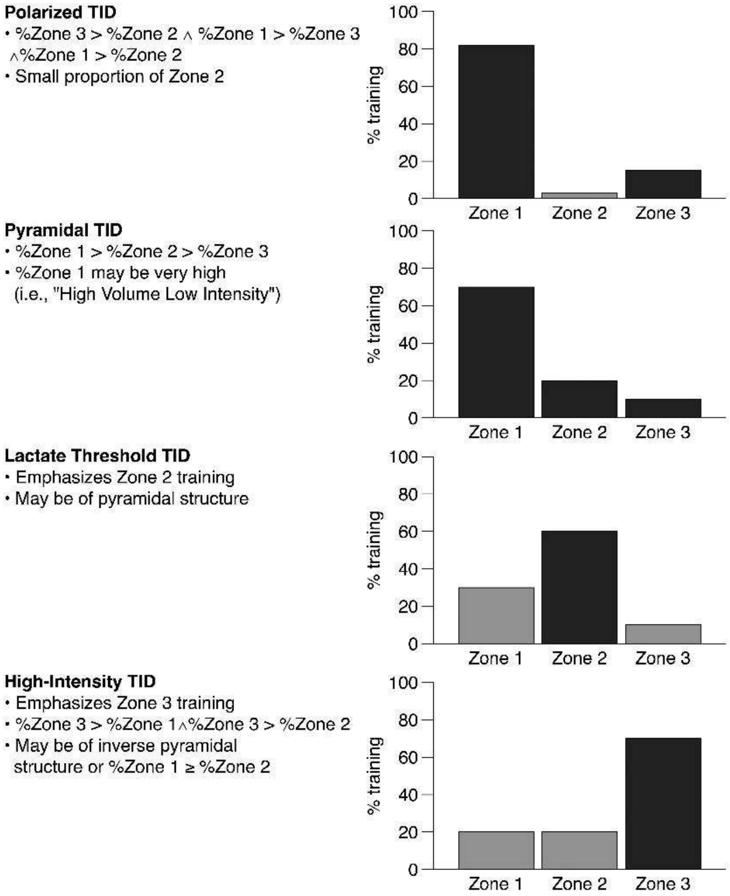


Figure 1.5 *Description of the main training intensity distribution (TID) in endurance sports (Treff et al., 2019).*

Indeed, several observational studies show that most world-class endurance athletes currently use POL and PYR TIDs (i.e., with 70-80% of weekly training with low volume at low intensity) in middle- and long-distance running (Stöggl and Sperlich 2015; Campos et al. 2022; Casado et al. 2022), swimming (González-Ravé et al. 2021) and cycling (Galán-Rioja et al. 2023). The same trend is also observed in recreational athletes (Muñoz et al. 2014; Zinner et al. 2018; Festa et al. 2020). From the analysis of those studies in which a POL TID was applied in high-level and amateur athletes, POL could be a more effective strategy to increase V'O_2max or V'O_2peak (i.e., +0.6 L min^{-1}) in a short period of time (i.e., ≤ 12 weeks). For other endurance related variables (i.e., ventilatory thresholds running economy, and race time) there was no evidence of superiority of POL compared to other TIDs. In addition, large volumes of Z2 as in THR TID in well-trained athletes may be inadequate to stimulate further cardiorespiratory adaptation but may contribute to fatigue, potentially via down-regulation of the sympathetic nervous system (Esteve-Lanao et al. 2007a; Seiler et al. 2007). In summary, it was found that in all the studies presented, regardless of the level of the athlete, 80% of weekly training was performed below VT1, with the remaining percentage in Z2 and Z3 differing depending on the competition period. The physiological rationale behind POL is based on central and peripheral adaptations. The most important central adaptation is the increase in CO, which results primarily from the end-diastolic volume of the left ventricle and myocardial contractility (Rosenblat et al. 2022). Both low-intensity aerobic exercise (Pollock 1977) and high-intensity exercise appear to increase PV and consequently SV (Milanović et al. 2015; Rosenblat et al. 2022). Therefore, combining HIIT and MICT with a POL TID in endurance athletes seems to be an even more effective strategy for this purpose. Among the peripheral adaptations, two important signaling pathways are associated with mitochondrial biogenesis (Laursen 2010). The first is related to intracellular calcium signaling and is mainly enhanced by high amounts of exercise at low intensity (Rose et al. 2007). The second is related to energy depletion of the cell, which is promoted by high-intensity exercise and activation of AMPK (Gibala et al. 2009). Furthermore, it has already been observed that the volume of low-intensity exercise favours an increase in mitochondrial content (i.e. increased CS activity) (Granata et al. 2016, 2018a; Bishop et al. 2019), whereas high-intensity exercise or SIT promotes an increase in mitochondrial respiration (Granata et al. 2018a; Rosenblat et al. 2022). The logic of POL is thus based on the principle that an appropriate combination of low (i.e., ≥ 80% of training time) and high (i.e., ≤ 20% of training time) training intensities optimize the recruitment of these pathways, thereby promoting mitochondrial adaptations and consequently the oxidative capacity of type I and IIx muscle fibres and improving the athlete's endurance performance, as summarized by Casado et al. (2023). Similar to endurance athletes, given the characteristics of untrained people with obesity, who have lower type 1 fibers content, lower mitochondrial content and functionality, and a lower capacity to use fatty acids for energy purposes at rest and during exercise (Lanzi et al. 2014; Tan et al. 2018; Georgiev et al. 2022a), it may make sense to propose POL training for obese adults. In the next sections, we will provide an overview of POL training in individuals with obesity.

1.5.1 Polarized training and individuals with obesity

In the current scientific literature, there are only few studies where it is observed the combination of HIIT and MICT (i.e., with and without POL approach) in improving body composition and physical capacities in individuals with obesity.

The study by Poon et al. (2022), was conducted on asian adults with obesity (BMI ≥ 25.0 kg m^{-2}) (i.e., considered obesity condition for asian patients) (WHO, 2016). Poon et al. (2022) alternating HIIT and MICT in each training session, without specific percentage of MICT and HIIT, three times a week for 16 weeks. Based on data provided by the authors, we tried to analyse two weeks of training. Excluding warm-up and cool-down, we observed that in the first week the participants of the HIIT-MICT group carried out two HIIT workouts and one MICT with a weekly volume of ~86 minutes (i.e., considering recovery time in HIIT session as MICT) (i.e., ~30% HIIT and ~70% MICT), while in the following week the volunteers carried out two MICT training sessions and one HIIT, for a weekly volume of ~103 minutes (i.e., ~12%HIIT and ~88% MICT). As for endurance athletes, the participants in the HIIT-MICT group performance a training with a POL approach. However, the authors did not develop a priori combination of HIIT and MICT. After the 16 weeks of training BM and %FM decreased in all the three groups (i.e., ~2kg and ~2%, respectively) compared to baseline. No significant group difference was observed for all blood markers. In addition, all the exercise groups showed a similar V'O_2max increase of ~15% (HIIT: 34.3 ± 4.4 *vs.* 18 39.1 ± 5.4; MICT: 34.9 ± 5.0 *vs.* 39.4 ± 7.2; and alternating HIIT-MICT: 34.4 ± 5.0 *vs.* 40.3 ± 4.6 mL $kg^{-1}min^{-1}$) compared to baseline and control group.

The study by Roxburgh et al. (2014) was conducted on male and female adults with obesity (BMI ≥ 30 kg m^{-2}). Authors compared two training methods, a combination of HIIT and MICT vs. MICT for five days a week for 12 weeks. The combination of HIIT and MICT provides 4 training sessions per week of MICT (i.e., 30 minutes, 15 min. of treadmill and 15 of a cycle ergometer) and one session per week of HIIT on treadmill (i.e., 8 x 60 second intervals at 100% V'O_2max, separated by 150 seconds of active recovery) for ~145 min of training a week. From the available data, we tried to analyse a week of training and we showed that in the HIIT-MICT group there was a percentage of ~94% MICT and ~6% HIIT a week. After 12 weeks, body composition remained unchanged. However, V'O_2max increased by 10.1% in in the MICT-HIIT group (i.e., 32.7 ± 9.2 *vs.* 36.0 ± 11.5 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) and 3.9% in the MICT group (33.2 ± 4.0 *vs.* 34.5 ± 6.1 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$).

The study of Zapata-Lamana et al. (2018) showed a combination of HIIT and MICT, with POL TID (i.e., ~70-80% of training volume of MICT and ~20-30% of training volume of HIIT) in young overweight and obese women. Authors compared the effects of three training protocols HIIT, MICT, and POL TID on cardiorespiratory and cardio metabolic risk factors in young overweight and obese women. The volunteers trained three times a week, for 12 weeks on a cycle ergometer. POL TID was performed in every session and consisted of three bouts of 60 s cycling exercise at 90% of $V'O_2$peak, with 2 min of active recovery between bouts and 4 min of unloaded recovery between sets followed by 30 min of cycling exercise cadence at 95% of VT1. From the available data, the weekly percentage of HIIT and MICT in the POL group was ~88% MICT and ~12% HIIT both in the training session and during a weekly training. After the training interventions BM and FM decreased significantly only in the POL group by 3.7 and 3 kg, respectively. No differences were observed between the groups at baseline in any plasma lipid parameter except for glucose levels that decreased only in the POL group. $V'O_2$peak increased significantly in all exercised groups. To study the changes in energy substrate utilization during exercise, participants performed 30 min of steady-state exercise at 60% of $V'O_2$peak before and after the intervention. Training induced a significant increase in relative fat oxidation in all groups, but relative fat oxidation increased significantly only in the POL group compared to the control group with no exercise.

In summary, the combination of MICT (30-40 min session^{-1} at 65-70% of HRmax or 90% of the VT1) and HIIT (6-12 minutes session^{-1} at 80-90% of HRmax or 90% of $V'O_2$ peak) performed in the same training session or in a weekly training program produced equal or greater effects on CRF, body composition and substrate oxidation in lean and obese sedentary adults compared to MICT or HIIT alone with equal volumes or energy expenditures per session (Roxburgh et al. 2014; Zapata-Lamana et al. 2018; Pérez et al. 2019a; Poon et al. 2022) (Figure 1.5.1). The percentage of HIIT and MICT in a weekly training in the above-mentioned studies showed high-volume at moderate-intensity (i.e., between 70-94% of weekly training) with low-volume at high-intensity (i.e., between 6-30% of weekly training).

From a physiological perspective, individuals with obesity show a reduction in type I muscle fibers, and an increase in type IIx muscle fibers, compared to lean individuals (Damer et al. 2022) with negative effects on aerobic metabolism due to a reduction in both the number and functionality of mitochondria (i.e., by 40% compared to lean counterparts) (Georgiev et al. 2022). Moreover, individuals with obesity have a reduced ability to oxidize fat during aerobic activities of both moderate and high intensity, compared to normal weight counterparts. (Lanzi et al. 2014). As previously said, exercise volume (i.e., combining low volume HIIT + high volume of MICT) is more important than exercise intensity (i.e., HIIT alone) to promote increases in mitochondrial content (Bishop et al. 2019). On the contrary, HIIT alone promote an increased in mitochondrial activity, more than MICT alone (Granata et al. 2018a). Thus, as suggested by Bishop et al. (2019) the combination of high-volume of MICT with low-volume of HIIT could be the best way to increase both mitochondrial content and functionality, as well as lipid oxidation in skeletal muscle of obese insulin-sensitive and insulin-resistant people (Georgiev et al. 2022a). In addition, the mitochondrial content is closely linked to $V'O_2$ max, both in endurance athletes and in people with cardio-metabolic disease (van der Zwaard et al. 2016). Thus, based on the above studies, high-volume at low-intensity (i.e.≥ 80% of overall training volume) combined with a low-volume at high-intensity interval training (i. e.≤ 20% of overall training volume) could be an effective strategy for improving i) body composition, ii.) endurance performance variables (e.g., $V'O_2$ max, ventilatory thresholds), while iii) reducing overreaching and injuries.

1.6 Aims of the thesis

Obesity is associated with health problems and reduced exercise tolerance (Williamson et al. 2015). Current guidelines recommend a 5% weight loss to achieve an overall improvement in cardiovascular disease (Williamson et al. 2015). However, in a short period of time (i.e. ≤ 12 weeks), as little as 2-3% weight loss is sufficient to achieve a significant overall reduction in many risk factors. In addition, the “obesity paradox" or "fit but fat" paradox” has developed in recent decades (Brown and Kuk 2015). Indeed, recent evidence suggests that weight loss does not necessarily always lead to improved health. In some cases, obese but metabolically healthy or very fit people are not at increased risk of health problems (Brown and Kuk 2015). It appears that obese but fit people have a lower mortality rate compared to lean but sedentary and untrained people (Gaesser and Angadi 2021). Sedentary behavior is just as dangerous as obesity or even more so (Same et al. 2016). The increase in sedentary behavior in turn increases body weight and impairs physical fitness. To break this vicious cycle, it is important to lose weight but also to improve physical fitness. As mentioned previsously in the first chapter of this thesis, HIIT and MICT are useful training strategies to achieve an improvement in body composition. On the contrary, over a short period of time (i.e. ≤ 12 weeks), HIIT appears to be more complete than MICT in improving CRF (MacInnis and Gibala 2017). However, studies in athletes have suggested that a combination of moderate (i.e. ~70-80% of total training volume) and high-intensity training (i.e. ~20-30% of total training volume) produces greater improvements in body composition and endurance performance-related variables (e.g. $V'O_2max$ and ventilatory thresholds) (Stöggl and Sperlich 2014a; Kim et al. 2021a; Campos et al. 2022) than HIIT or MICT alone. To our knowledge, no studies to date have used a priori proportions of MICT and HIIT (i.e. using a POL approach) in individuals with obesity during weekly training to improve body composition, CRF and fat oxidation rate during walking or running. Therefore, the aim of the thesis is to explore the effects of different aerobic training models on body composition, physiological variables and physical activity levels and physical and mental health. The specific objectives that will be analysed in the following chapters are:

1. compare the effects of 12 weeks of a combination of HIIT and MICT (combined training; COMB with a POL approach) and HIIT alone on body composition, $V'O_2$ peak and fat oxidation rate in healthy adults with obesity;

2. determine the effects of 24 weeks of POL TID or THR TID, modified from Veronique Billat (www.billatraining.com) (Molinari et al. 2020b), with reverse periodization (i.e. volume is increased over the months while intensity is maintained) (Stone et al. 2021) on body composition, V'O_2max and ventilatory thresholds in healthy men with obesity;

3. evaluate the effects of a 3-week combination of COMB using a POL approach and MICT alone on: Body composition, V′O_2peak and substrate oxidation rates in adolescents with obesity admitted for a 3-week multidisciplinary body weight reduction program (BWRP) that includes moderate energy restriction, nutritional education and psychological counselling (the same for all participants).

After the first study, for aim 2, we decided to extend the training weeks and use the TID concept, as most studies applying endurance training in adults with obesity have shown that i. exercise intensity is expressed in terms of percentage of HRmax or V’O_2max (Su et al. 2019), ii. there are few studies that have investigated the concept of TID in individuals with obesity (Streb et al. 2021), and iii. the exercise programs were conducted during a limited number of training weeks (i.e. 12 weeks on average) (Su et al. 2019). In addition, the use of COMB in adolescents with obesity was added. As obesity in childhood and adolescence is associated with an increase in comorbidities previously identified in the adult population, such as type 2 diabetes mellitus, hypertension, non-alcoholic fatty liver disease (NAFLD), obstructive sleep apnoea (OSA) and dyslipidaemia (Kansra et al. 2021), to our knowledge, no study in adolescents with obesity has a priori used a percentage of a combination of MICT and HIIT with a POL approach during weekly training to improve cardiorespiratory function and substrate oxidation rate compared to MICT or HIIT alone (Lazzer et al. 2017a).
It has further added an appendix to the thesis. The first refers to a new running test in the field called the running advisor billat training (RABIT®), which was developed to assess speed and HR at VT1, VT2 and at V’O_2max using the Borg scale of rating of perceived exertion (RPE) with 6-20 range. The RABIT® test has previously been used in recreational runners to identify submaximal and maximal training zones with a good degree of accuracy and repeatability (Giovanelli et al. 2020; Molinari et al. 2020b) Identifying the three intensity zones based on individual characteristics is necessary for prescribing interval and continuous aerobic training in athletic and non-athletic individuals, rather than using arbitrary percentages of HRmax and V’O_2max (Jamnick et al. 2020). Athletes and not-athletes’ individuals cannot always perform a GRAD test which requires expensive devices and specific knowledge. Thus, the usefulness of the RABIT test could be related

to a good estimation of speed and HR at the ventilatory thresholds based on the individual characteristics of each subject.

The second appendix is related to the relationship between adiponectin levels and aerobic physical activity in individuals with obesity (Kahn et al. 2019; Thyfault and Bergouignan 2020). As previously described, the endocrine functions of adipose tissue are dysregulated in both overweight and obesity and play a crucial role in the prevention and/or development of metabolic diseases (Nigro et al. 2014). Adiponectin levels are greatly reduced in obesity and metabolic diseases. Indeed, the biological activity of adiponectin including the regulation of glucose levels, lipid metabolism and insulin sensitivity downregulated in individuals with obesity (Nigro et al. 2014). However, aerobic physical activity can stimulate the production and secretion of adipokines and improve the endocrine functions of adipose tissue (Minniti et al. 2022). Both HIIT and MICT, showed positive changes in patient's body composition, $V'O_2max$ and adiponectin levels (De Feo 2013), while other studies showed that adiponectin concentrations do not change after long-term exercise (Mitchell et al. 2019). However, to our knowledge, there are no studies that have investigated the effects of a POL (i.e., 80% MICT-20% HIIT) compared to a threshold training programme (i.e., 65-70% MICT-30-35% HIIT) on body composition, and cardiometabolic parameters related to adiponectin expression.

Chapter 2

12-week combined training with a polarized approach and MICT in adults with obesity

Adapted from:

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Effects of 12-week combined training versus high intensity interval training on cardiorespiratory fitness, body composition and fat metabolism in obese male adults.

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2.1 Introduction

As discussed earlier, in the last two decades, the number of obese adults has tripled in developed countries (Inoue et al. 2018). This is mainly due to excess food intake, an increase in sedentary time, and a decrease in physical activity (Swinburn 2013; Guthold et al. 2018). Health consequences associated with obesity include hypertension (Henry et al. 2012), type 2 diabetes, cardiovascular disease (CVD), some types of cancer and psychosocial complications (Williams et al. 2015).

Compared to lean counterparts, individuals with have lower CRF (Lin et al. 2015) and impaired capacity to oxidize lipids at rest (Lanzi et al. 2014a) and during physical activity (Berggren et al. 2008), associated with low insulin sensitivity and a higher clustering of metabolic syndrome risk factors (Rosenkilde et al. 2010; Robinson et al. 2015). This condition has worsened in recent years due to the coronavirus disease 2019 (COVID-19) pandemic. Studies have shown that as physical activity decreases, V’O_2 max also decreases by approximately 0.3–0.4%/day (Narici et al. 2021), with negative effects on body weight (increases of ~1.5 kg per month) (Pellegrini et al. 2020). On the other hand, recent evidence suggests that obese adults with a higher level of CRF (typically expressed as V’O_2 max) or V’O_2 peak have a lower risk of morbidity and mortality than inactive lean individuals (Gaesser and Angadi 2021) and a similar fat oxidation rate compared to lean individuals matched for CRF (Croci et al. 2014). For these reasons, aerobic training is recognized as an important lifestyle intervention in weight management programs for obese individuals as it creates an energy deficit to reduce BM (Petridou et al. 2019), improves CRF (Rugbeer et al. 2021b), and optimizes fat oxidation capacity (Achten and Jeukendrup 2004b; Hetlelid et al. 2015).

In this context, MICT is the most prescribed exercise modality in weight management programs, although HIIT has emerged as an attractive, time-efficient option compared to MICT(MacInnis and Gibala 2017). HIIT typically combines high-intensity bouts (i.e., duration between 1-4 min at ≥ 85% of HRmax) separated by recovery periods of low-intensity activity or rest with an average total duration between 4-16 min (Gibala et al. 2014; MacInnis and Gibala 2017). In individuals with obesity, HIIT improves CRF (Batacan et al. 2017; Su et al. 2019), body composition (Türk et al. 2017b; Andreato et al. 2019) and fat oxidation (Vaccari et al. 2020) to a greater extent and in a shorter period (i.e., between 4 and 12 weeks) than MICT. In contrast, several systematic reviews and meta-analyses have reported similar improvements in V’O_2 peak (Rugbeer et al. 2021) and body composition (Wewege et al. 2017a; Keating et al. 2017) induced by HIIT and MICT in obese adults. However, the heterogeneity (Su et al. 2019; Rugbeer et al. 2021) and the lack of equalization (Wewege et al. 2017; Keating et al. 2017) among protocols comparing the effects of HIIT and MICT in most of the studies included in the systematic reviews and meta-analysis may have led to contrasting findings.

Recent studies have shown that the combination of MICT (30-40 min/session at 65–70% of HRmax or 90% of the first ventilatory threshold) and HIIT (6-12 min/session at 80-90% of HRmax or 90% of $V'O_2$ peak) (Roxburgh et al. 2014; Zapata-Lamana et al. 2018; Poon et al. 2022) performed in the same training session or in a weekly training program induced equal or greater effects on CRF, (Poon et al. 2022;,Roxburgh et al., 2014; Zapata-Lamana et al., 2018) body composition (Roxburgh et al. 2014; Zapata-Lamana et al. 2018; Poon et al. 2022) and substrate oxidation (Zapata-Lamana et al. 2018; Borowik et al. 2020) in lean and obese sedentary adults compared to MICT or HIIT alone with equal volumes or energy expenditures per session. Analysing data from previous studies, we observed that weekly aerobic exercise included a combination of high exercise volumes at moderate intensity (i.e., 70-94% of weekly exercise) and low exercise volumes at high intensity (i.e., 6-28% of weekly exercise)(Poon et al. 2022;,Roxburgh et al. 2014; Zapata-Lamana et al. 2018). In addition, studies on athletes have suggested that a combination of moderate- (i.e., ~70-80% of total training volume)(Campos et al. 2022) and high-intensity training (i.e., ~20-30% of total training volume)(Campos et al. 2022) provides greater improvements in body composition (Kim et al. 2021b) and endurance performance-related variables (e.g., $V'O_2$ max and lactate thresholds)(Stöggl and Sperlich 2014b; Pérez et al. 2019b) than HIIT or MICT alone. To date, to our knowledge, no studies on individuals with obesity have used a priori percentages of MICT and HIIT during weekly training to optimize cardiorespiratory function and the fat oxidation rate during walking or running.

Thus, the aims of the present chapter were to determine the effects of 12 weeks of a combination of HIIT and MICT (combined training; COMB) (Borowik et al. 2020) and HIIT alone on body composition, $V'O_2$ peak, and the fat oxidation rate in healthy adults with obesity. COMB training in our study involves a combination of high-volume low-intensity exercise (≥ 80% of overall training volume) and low-volume high-intensity training (≤ 20%).

2.2. Material and methods

Subjects

Thirty-five male adults with obesity were recruited by researchers from the School of Sport Sciences of the University of Udine. All volunteers provided a full medical history and underwent physical and nutritional examinations. Their BM was stable during the previous two months. The inclusion criteria were as follows: 1) aged between 18 and 50 years, 2) BMI ≥ 30 kg m^{-2}, and 3) physically inactive (i.e., performing less than 30 min of continuous aerobic activity on most days) based on the International Physical Activity Questionnaire Short Form (IPAQ-SF) (Craig et al. 2003). The exclusion criteria were as follows: 1) previous participation in weight management programs or 2) presence of cardiovascular, respiratory, neurologic, musculoskeletal, metabolic and/or endocrine diseases. None of the volunteers were taking medications regularly or using any medications known to influence energy metabolism. The data reported in the manuscript are not a part of a larger dataset.

Study protocol

The study was approved by the Ethics Committee of the Friuli-Venezia-Giulia Region (Italy) (protocol number 1764). Before the study began, the purpose and objective of the study were carefully explained to each participant, and written informed consent was obtained. Participants followed a 12-week weight management program involving one of two types of physical training programs (COMB *vs*. HIIT). Due to the restrictions implemented in Italy during the study period due to the third wave of the COVID-19 pandemic (Pelagatti and Maranzano 2021), participants were followed at their own homes (see below for details), ensuring both the ecological validity of the study and participant safety as well as adhering to public health recommendations at that time. Participants were randomly allocated (using sealed envelopes and a 1:1 ratio) into two groups: the COMB (n = 18) and HIIT groups (n = 16). A volunteer in the HIIT group left the study before the start of the study period due to heart disease. Full testing sessions were conducted just before the beginning (PRE) and at completion of the 3-month weight-management period (POST). The testing sessions were conducted during one visit and included assessment of anthropometric characteristics, body composition, and substrate oxidation during graded exercise; $V'O_2$ peak; and physical and dietary habits. In addition, physical capacities were monitored weekly to individualize physical training. At the beginning of the intervention period, all participants received the same general nutritional advice based on the Italian Guidelines for healthy nutrition (CREA 2019) to avoid confounding effects due to nutritional variables on the outcomes.

Measurements

Anthropometric characteristics and body composition

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. Height was measured to the nearest 0.5 cm on a standardized wall-mounted height board. BMI was calculated as BM (kg) × height^{-2} (m). Waist circumference (WC) was measured at the narrowest point between the lower costal border and the top of the iliac crest (Kagawa et al. 2008). Hip circumference (HC) was measured at the greatest posterior protuberance (Kagawa et al. 2008). Body composition was calculated by bioelectrical impedance analysis (BIA, Human IM Plus; DS Dietosystem, Milan, Italy) according to the method of Lukaski et al. (Lukaski et al. 1986). The values of fat mass (FM) and fat-free mass (FFM) were obtained with equations derived in obese people of either different ages or BMIs (fat-specific formulae) by utilizing a two-compartment model (Gray et al. 1989).

Physical capacities and maximal fat oxidation rate

The V'O_2 peak values and maximal fat oxidation rate were determined by a graded exercise test on a motorized treadmill (H/P/Cosmos Sports and Medical Gmbh, Germany) under medical supervision. Before the start of the study, individuals were familiarized with the equipment and the procedures. All the participants avoided strenuous exercise and maintained the same eating habits the day before the test and came to the laboratory after a 12-h fast.

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. When the respiratory exchange ratio (RER) value reached 1, the duration of each step was reduced from 4 min to 1 min until voluntary exhaustion. We modified the protocol proposed by Lazzer et al.(Lazzer et al. 2017b). The starting treadmill speed was set to 3 km h^{-1}. Then, it increased by 1 km h^{-1} each step, except in the transition from the first to the second stage, in which it increased by 2 km h$^{-1.}$ The incline of the treadmill was kept constant throughout the test at 1%. During the test, 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 a 3-L calibration syringe and calibration gas (16.00% O_2; 4.00% CO_2), respectively. For the duration of the entire test, an electrocardiogram was continuously recorded and displayed online for visual monitoring, and the HR was recorded with a dedicated monitor (Garmin, US). VO_2 peak was determined for each subject from the last 30 s of the graded exercise tests.

Fat oxidation rates were obtained from $V'O_2$ and $V'CO_2$ values determined during the last minute of each workload level (Achten and Jeukendrup 2004) using the following equations (Frayn 1983):

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

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

where P_{oxi} is the protein oxidation rate. P_{oxi} was estimated by assuming that protein oxidation contributed approximately 12% of resting energy expenditure (Frayn 1983):

Protein oxidation rate (g min^{-1}) = $[\text{energy expenditure kJ min}^{-1} \times 0.12] \times 16.74^{-1}\ (\mathrm{kJ\ g^{-1}})$.

The results of the graded exercise test were used to compute the relationship between the fat oxidation rate as a function of exercise intensity, expressed as $\%V'O_2$ peak. The best fit was obtained with a second-order polynomial relationship. Before and after the training program, the graded exercise test was performed following the same protocol.

Dietary and physical activity habits

Participants were invited to compile a 4-day dietary record (4-dDR), recording food and beverage consumption on 2 weekdays and 2 weekend days, at two time points: PRE and POSTWith the 4-dDR, instructions on how to record the type and portion size of the foods consumed were provided. The intakes of selected macro- and micronutrients were derived after uploading individual food information from the 4-dDRs to Microdiet V4.4.1 software (Microdiet software–Downlee Systems Ltd., High, Peak, UK), which contains the Italian "Food Composition Database for Epidemiological Studies in Italy" (Gnagnarella et al., 2015), along with information from nutritional labels, when needed. Physical activity levels were evaluated with the IPAQ-SF (Craig et al. 2003). The questionnaire records vigorous-intensity activity, moderate-intensity activity, walking and sitting duration during the previous 7 days. The IPAQ-SF scores were converted into metabolic equivalents (MET-min week^{-1}) using the Guidelines for Data Processing and Analysis of the IPAQ (Craig et al. 2003). Furthermore, health-related quality of life was investigated with the 12-item Short Form

Health Survey (SF-12). The questionnaire is composed of 12 items from which physical (SF-12_PI) and mental health (SF-12_MI) indices are obtained (Ware et al. 1996).

Training program

The training program included three training sessions per week for 12 weeks under otherwise normal living conditions. Subjects ran or walked (or a combination of the two) on flat terrain, on a track or city circuit. Each participant monitored their walking/running speed with the Polar Flow smartphone app (Polar Electro Oy, Finland) and their HR with the optical heart rate sensor Polar Verity Sense (Polar Electro Oy, Finland).

In each training session, the COMB group underwent a combination of high-volume exercise at low intensity (≥ 80% of overall training volume) with low-volume exercise at high intensity (≤ 20%) (Campos et al. 2022), as proposed by Borowik et al. (Borowik et al. 2020). Each session consisted of 5 min of warm-up (50% of $V'O_2$ peak) followed by 3 repetitions of 2-min bouts at high intensity (95% of $V'O_2$ peak), separated by 1 min of walking at low intensity (50% of $V'O_2$ peak), followed by 30 min of MICT (60% of $V'O_2$ peak) (Su et al. 2019) (Figure 2.1 panel A).

The HIIT group performed 10 min of warm-up at low intensity (50% of $V'O_2$ peak) followed by 5–7 repetitions of 2-min bouts at high intensity (95% of $V'O_2$ peak) (Su et al. 2019; Andreato et al. 2019), separated by 1 min of walking at low intensity (50% of $V'O_2$ peak), followed by 5 min of cool down (50% of $V'O_2$ peak) (Figure 2.1 panel B). Exercise intensity was manipulated by adjusting the pace (expressed in min km^{-1}) corresponding to the values of 50, 60 and 95% of $V'O_2$ peak measured during the graded test and then calculating the corresponding HR values.

Both groups repeated these training protocols in each training session. If participants improved their performance capacity, such that their HR tended to decrease, their speed was increased to ensure that the HR reached the specified values. After each training session, all participants reported their rating of perceived exertion (RPE) on the Borg 6-20 Scale (Borg 1970), their mean HR (bpm) and the distance covered (in km). Research assistants and physical trainers were responsible for verifying that each participant performed the exercises correctly and completed at least 90% of the training sessions through the online platform Polar Coach (Polar Electro Oy, Finland). 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 approximately 1.5 MJ per session measured during the graded exercise test, as shown by Vaccari et al. (Vaccari et al. 2020). All volunteers were also advised to practice leisure physical activities during the weekend and holidays.

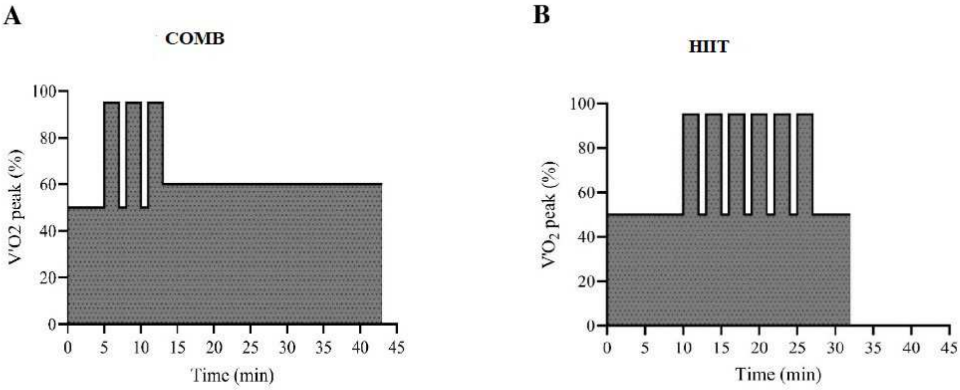

Figure 2.1 *Schematic representation of training protocols: Combined training (COMB, panel A) and High Intensity Interval Training (HIIT, panel B).*

Statistical analyses

The data were analysed using GraphPad Prism version 9.1.0 (IBM, Chicago, USA), with a significance threshold of $p < 0.05$. All the results are expressed as the means and standard deviations (SDs). The normality of data distribution was evaluated using the Shapiro–Wilk test. Sphericity was verified by Mauchly's test; if the sphericity assumption was violated, a Greenhouse–Geisser correction was used. To assess training adherence, energy consumption and the total duration of training, unpaired Student's t tests were used. Anthropometric characteristics, body composition, V'O$_2$ peak, training characteristics and data derived from questionnaires and food diaries were analysed with a 2-way ANOVA or a general linear mixed model that included the between-subjects factor of group (COMB or HIIT) and the within-subjects factor of time (PRE *vs*. POST, i.e., repeated-measures analysis). Significant main effects were further analysed by the Šídák post hoc test. The same analyses were applied for the fat oxidation rate during exercise, adding the % of V'O$_2$ peak as a fixed factor to examine differences in fat oxidation rates in response to HIIT or COMB training separately. A three-way ANOVA or a general linear mixed model (2 groups × 2 time points × 9 stage measurements) was conducted to examine differences in fat oxidation rates during the test between the COMB and HIIT groups. Finally, the corrected effect size (ES) was calculated (Lakens 2013). An ES < 0.20 was considered small, < 0.50 was considered medium, and > 0.50 was considered large, as proposed by Cohen et al.(Cohen 1988). To estimate the sample size a priori, power analysis of 12 participants per group with an F test for repeated-measures ANOVA with a statistical power of 0.80, a probability α level of 0.05, and an effect size f of 0.40 (G-Power software, v. 3.1.9.2, Universität Kiel, Kiel, Germany) revealed a predicted improvement of V'O$_2$ peak by 16% (Vaccari et al. 2020).

2.3 Results

Anthropometric characteristics and body composition

Before the intervention, no differences were observed between the groups in anthropometric characteristics or body composition except for FM (%) (+4.0 ± 5.0% COMB group, P = 0.001, Table 2.1). At POST, the mean weight loss was 2.55 ± 2.26 kg (P = 0.004; ES = 0.16, *small*) and 3.43 ± 3.97 kg (P <0 .001; ES = 0.37, *medium*) (Table 2.1) in the COMB and HIIT groups, respectively. BMI decreased by 0.83±0.75 kg m^{-2} (P=0.003; ES=0.18, *small*) in the COMB group and by 1.15 ± 1.31 kg m^{-2} (P < 0.001; ES = 0.37, *medium*) in the HIIT group. The mean FM loss was 3.84±1.72 kg (P<0.001; ES=0.34, *medium*) and 3.92±3.08 kg (P<0.001; ES=0.80, *large*) in the COMB and HIIT groups, respectively, and FM (%) decreased similarly in the COMB (2.77 ± 1.63%) and HIIT groups (2.63 ± 2.05%) (P<0.001; ES=0.73, *large*), while FFM did not change significantly in the COMB and HIIT groups (Table 1). WC decreased both in the COMB group (-2.53 ± 2.58 cm, P = 0.005; ES = 0.47, *medium*) and HIIT group (-2.30 ± 3.93 cm, P = 0.020; ES = 0.36, *medium*), and the waist-to-hip ratio decreased similarly in both groups (-0.02±0.02 cm, main effect of time, P < 0.001; ES = 0.15, *small*). The HC did not change significantly in either group (Table 2.1). There was no significant group × time interaction on any anthropometric or body composition variable (0.345< P < 0.843) (Table 2.1).

Table 2.1 *Anthropometric characteristic before (PRE), and after 12-weeks (POST) of weight management program in combined training (COMB) and high-intensity interval training (HIIT) groups.*

	COMB (n: 18)		HIIT (n: 16)		P		
	PRE	POST	PRE	POST	G	T	G x T
Age (y)	40.3 ± 6.9		38.3 ± 7.1		0.434		
Height (m)	1.76 ± 0.07		1.77 ± 0.07		0.750		
Body mass (kg)	106.6 ± 16.0	104.0 ± 16.0*	103.8 ± 9.3	100.2 ± 9.6*	0.489	0.001	0.345
BMI (kg m^{-2})	34.5 ± 5.3	33.6 ± 5.2*	33.2 ± 2.3	32.1 ± 2.6*	0.352	0.001	0.389
Waist circumference (cm)	106.6 ± 10.8	104.1 ± 11.3*	103.6 ± 6.2	101.3 ± 6.3*	0.363	0.001	0.843
Hip circumference (cm)	112.9 ± 7.9	112.3 ± 7.7	111.2 ± 4.5	110.5 ± 3.7	0.450	0.046	0.813
Waist-to-hip ratio	0.94 ± 0.06	0.93 ± 0.07*	0.93 ± 0.06	0.92 ± 0.06*	0.610	0.001	0.732
Fat-free mass (kg)	62.9 ± 6.5	63.9 ± 6.7	65.7 ± 7.8	66.0 ± 7.3	0.322	0.110	0.383
Fat Mass (kg)	43.6 ± 10.2	40.1 ± 10.2*	38.1 ± 4.7	34.2 ± 4.7*	0.052	0.001	0.623
Fat Mass (%)	40.5 ± 3.5	37.7 ± 4.1*	36.8 ± 3.7	34.2 ± 3.2*	0.006	0.001	0.838

All values are presented as mean ± standard deviation.
BMI: body mass index
G: group effect, T: time effect; G × T: groups × time effect.
**Significantly different from PRE, $P < 0.05$*

Peak oxygen uptake

At PRE, no significant differences were found between the COMB and HIIT groups in terms of V'O_2 peak, V'O_2 peak normalized by FFM or HRpeak (Table 2.2).

At POST, the absolute V'O_2 peak increased in the COMB (+16.7±9.6%, P<0.001; ES=1.11, *large)* and HIIT (+16.0 ± 15.9%, P < 0.001; ES = 0.92, *large*) groups. Additionally, V'O_2 peak normalized by FFM increased by 14.4 ± 10.3% (P<0.001; ES=1.02, *large*) in the COMB group and by 15.5 ± 17.2% (P < 0.001; ES = 1.17, *large*) in the HIIT group. However, HRpeak did not change significantly (main effect of time, P = 0.092) (Table 2.2). There was no significant group × time interaction effect on HRpeak, V'O_2 peak, V'O_2 peak normalized by FFM or HRpeak (Table 2.2).

Table 2.2 *Physical capacities and physical activity habits before (PRE) and after 3 months (POST) of weight management program in combined training (COMB) and high-intensity interval training (HIIT) groups.*

	COMB (n: 18)		HIIT (n: 16)		P		
	PRE	POST	PRE	POST	G	T	G x T
$V'O_2$peak ($L\ min^{-1}$)	2.95 ± 0.43	3.42 ± 0.40*	3.12 ± 0.45	3.58 ± 0.52*	0.254	0.001	0.962
$V'O_2$peak ($mL\ min^{-1}\ Kg^{-1}$ FFM)	47.0 ± 6.4	53.8 ± 6.7*	47.5 ± 5.1	54.5 ± 6.5*	0.758	0.001	0.942
HRpeak (bpm)	176.1 ± 15.6	174.5 ± 11.5	175.8 ± 11.2	172.1 ± 13.5	0.794	0.092	0.539
IPAQ_TOT (MET-min $week^{-1}$)	1665 ± 666	2569 ± 807*	1673 ± 975	2247 ± 735*	0.727	0.040	0.633
IPAQ_VIG (MET-min $week^{-1}$)	1005 ± 529	1163 ± 442	1136 ± 579	1401 ± 932	0.361	0.238	0.796
IPAQ_MOD (MET-min $week^{-1}$)	435 ± 250	842 ± 344	462 ± 313	712 ± 326	0.826	0.137	0.710
IPAQ_WALK (MET-min $week^{-1}$)	710 ± 448	865 ± 363	649 ± 379	635 ± 476	0.683	0.360	0.610
SF12_PI (pt)	49.6 ± 7.6	52.6 ± 3.6	50.4 ± 7.5	51.0 ± 6.5	0.796	0.237	0.440
SF12_MI (pt)	41.5 ± 9.3	42.0 ± 11.9	46.6 ± 9.9	48.2 ± 7.7	0.024	0.650	0.825

All values are presented as mean ± standard deviation

$V'O_2$peak: peak oxygen uptake, $V'O_2$peak FFM^{-1} : peak oxygen uptake normalized by fat-free mass, IPAQ_TOT: International Physical Activity Questionnaire, IPAQ_VIG: vigorous activity, IPAQ_MOD: moderate-intensity activity, IPAQWALK: 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.

*G group effect, T: time effect; G × T: groups × time effect. *Significantly different from PRE, $P < 0.05$*

Fat oxidation rate

At baseline, fat oxidation rates during the graded test were not significantly different between groups (main effect of group, $P = 0.914$). The maximal fat oxidation (MFO) rate was observed at 46 ± 6% of VO_2 peak in the COMB group (0.31 ± 0.04 g min^{-1}, Figure 2.2 A) and at 44 ± 8% of $V'O_2$ peak in the HIIT group (0.33 ± 0.07 g min^{-1}, Figure 2.2 B). On average, at exercise intensities above 60 ± 3% of $V'O_2$ peak, the fat oxidation rate decreased markedly in both groups, and the contribution of fat oxidation to the energy supply became negligible above 74 ± 6% of $V'O_2$ peak. At POST, fat oxidation rates increased in the COMB (main effect of time, $P < 0.001$) and HIIT groups (main effect of time, $P = 0.009$) without differences between groups at any exercise intensity (main effect of group, $P = 0.984$; group × time interaction, $P = 0.986$) (Figure 2.2 A, B). The exercise intensity corresponding to the MFO rate, expressed as a percentage of $V'O_2$ peak, increased in a similar manner in both groups (by 6.0 ± 7.0%; main effect of time, $P = 0.006$). No main effect of group or group × time interaction was found for the MFO rate.

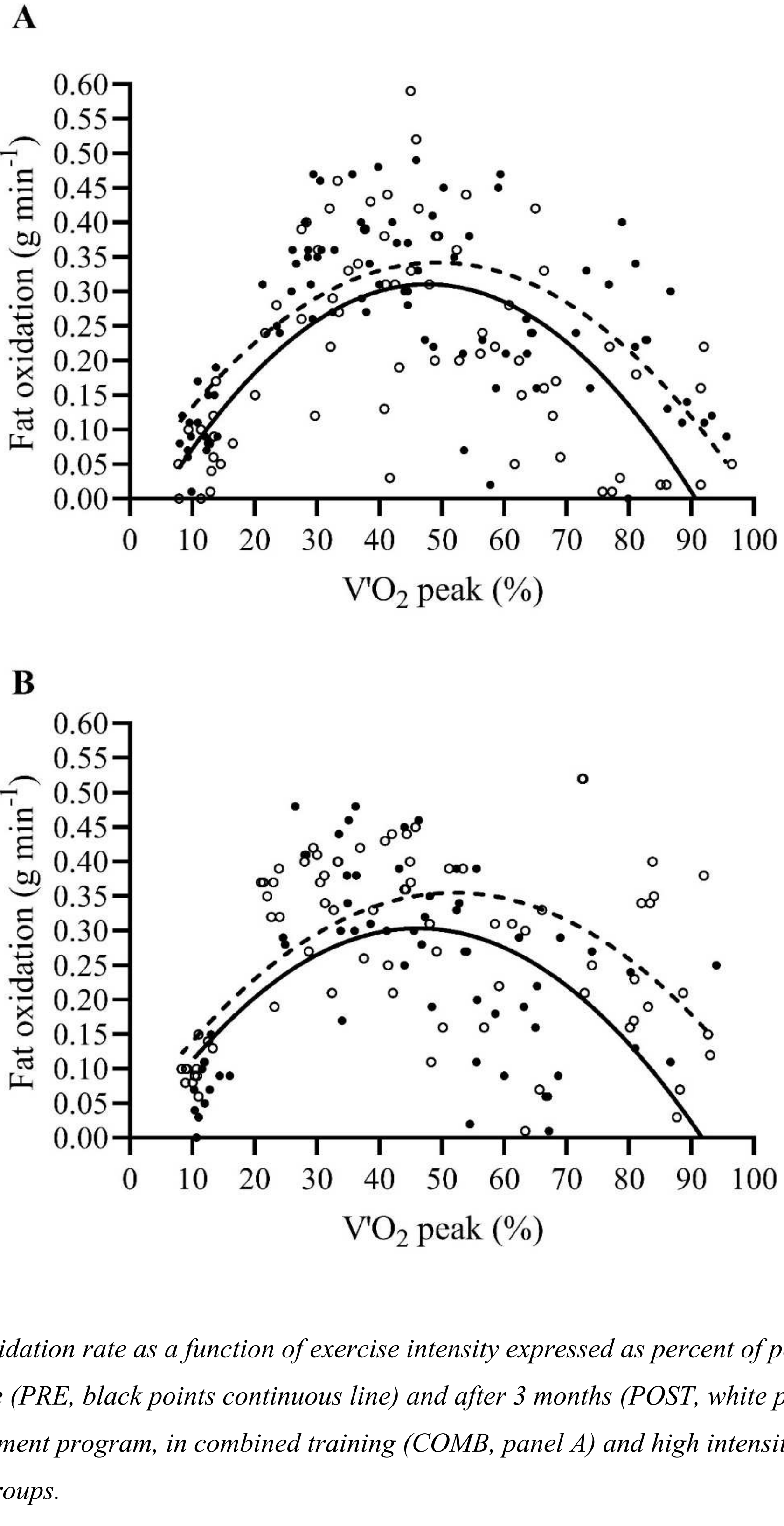

Figure 2.2 *Fat oxidation rate as a function of exercise intensity expressed as percent of peak oxygen uptake (V'O$_2$peak) before (PRE, black points continuous line) and after 3 months (POST, white points dashed line) of weight management program, in combined training (COMB, panel A) and high intensity interval training (HIIT, panel B) groups.*

Training characteristics

At the end of the training intervention, subjects had performed 34.6 ± 1.2 and 33.7 ± 1.8 training sessions in the COMB and HIIT groups, respectively ($P = 0.137$), out of 36 total sessions, without adverse events. On average, the mean HR during the training sessions was 128 ± 13 bpm and 141 ± 11 bpm in the COMB and HIIT groups, respectively ($P < 0.001$). The mean HR increased in both groups during the 12 weeks of the intervention (main effect of time, $P < 0.001$) (Figure 2.3 B), but the COMB group exhibited a lower HR (by 10.6 ± 2.2%) than the HIIT group (main effect of group, $P < 0.001$) (Figure 2.3 B) without a significant group × time interaction. On average, the energy expended during the training sessions was 22.9 ± 2.7 and 21.8 ± 2.6 kJ kg^{-1} of FFM in the COMB and HIIT groups, respectively ($P = 0.208$). However, the COMB group exhibited a greater duration of each training session (42.6 ± 3.4 min) than the HIIT group (32.6 ± 2.5 min, $P < 0.001$). The COMB group performed 86.0 ± 1.0% of the total training volume at MICT and 14.0 ± 1.0% at HIIT, while the HIIT group spent 62.0 ± 2.0% of the total training below MICT and 38.0 ± 2.0% at HIIT; thus, the groups significantly differed ($P < 0.001$).

During the 12 weeks of the training intervention, the mean RPE values were lower in the COMB group (13.0 ± 2.2) than in the HIIT group (14.3 ± 3.4) (main effect of group, $P = 0.035$) (Figure 2.3 A), with an increase in effort perceived over the 12 weeks in both groups (main effect of time, $P < 0.001$) (Figure 2.3A). There was a significant group × time interaction in the RPE (group × time interaction, $P = 0.002$). The mean HR (bpm) increased in both groups during the 12 weeks of the intervention (main effect of time, $P < 0.001$) (Figure 2.3B), but the COMB group exhibited a lower HR (by 10.6 ± 2.2%) than the HIIT group (main effect of group, $P < 0.001$) (Figure 2.3 B) without a significant group × time interaction. On average, the distance (km) covered by each participant during the 12 weeks of training was 167.9 ± 23.6 km in the COMB group and 156.8 ± 30.7 km in the HIIT group (main effect of group, $P = 0.223$) (Figure 2.3 C).

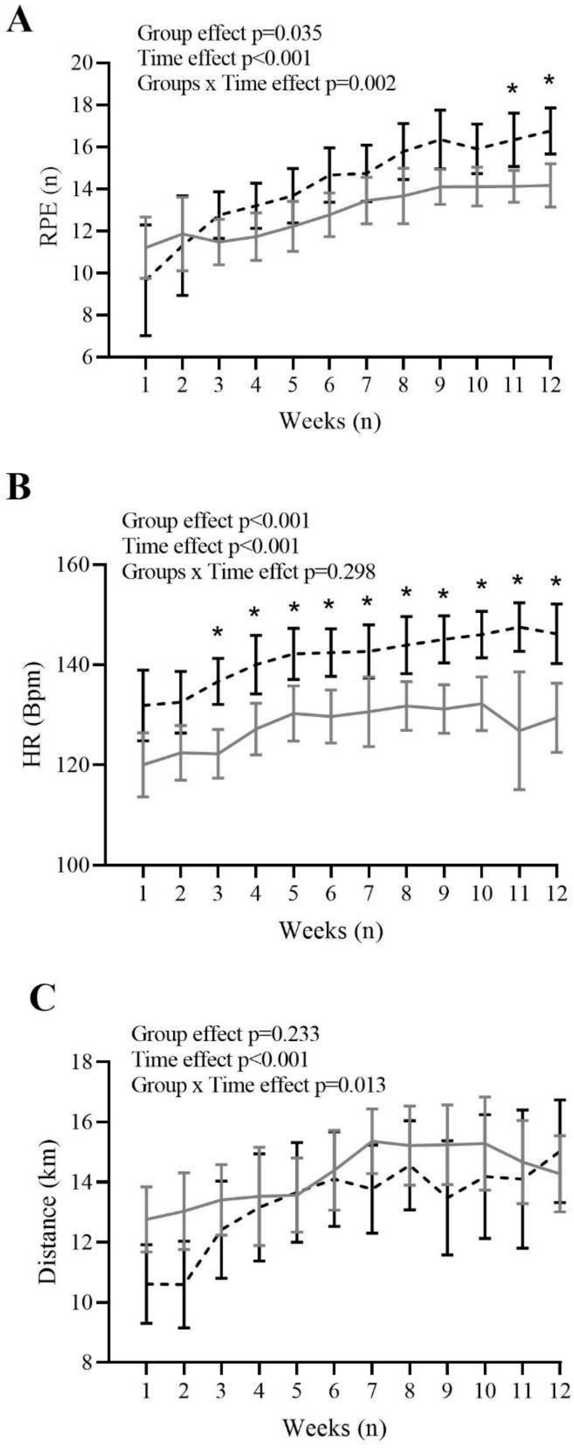

Figure 2.3 *Mean of rating of perceived exertion (RPE, panel A), Heart Rate (HR, panel B), and Distance covered (km, panel C) during the 12 weeks of training programs in combined training (COMB, grey continuous line) and high intensity interval training (HIIT, black dashed line) groups.*

All values are presented as mean ± standard deviation
**Significantly different from COMB, $P < 0.05$.*

Physical activity and nutritional habits

At baseline, physical activity habits, evaluated by the IPAQ, were similar between the two groups (Table 2.2). After the training period, total (IPAQ_TOT) physical activity increased by 98.0 ± 21.3% and 84.8 ± 15.8% ($P=0.033$; ES = 1.19, *large* and $P = 0.044$; ES = 0.65, *medium,* respectively) in the COMB and HIIT groups. Vigorous activity (IPAQ_VIG), moderate activity (IPAQ_MOD) and physical activity derived from walking (IPAQ_WALK) did not change significantly in either group (Table 2.2). The quality of life assessed by the SF-12, including physical and mental indices, showed no significant differences over time in either group (Table 2.2).

At PRE, no significant differences were found between the COMB and HIIT groups in terms of energy intake (8667 ± 2183 *vs.* 8981 ± 2620 kJ day^{-1}; main effect of group, $P= 0.721$) or macronutrient percentage contribution to total energy intake in terms of carbohydrates (42.6 ± 5.9 *vs.* 39.7 ± 8.5%; main effect of group, $P = 0.436$), fat (33.1 ± 5.6 *vs.* 37.5 ± 7.5%; main effect of group, $P = 0.232$) or protein (16.7 ± 2.9 *vs.* 18.0 ± 3.0%; main effect of group, $P = 0.807$). At POST, the mean energy intake was significantly lower than at PRE in both groups, without differences between groups (group × time interaction, $P = 0.981$); the mean energy intakes were 7380 ± 1823 kJ day^{-1} (main effect of time, $P < 0.001$; ES = 0.62, *large*) in the COMB group and 7681 ± 2290 kJ day^{-1} (main effect of time, $P < 0.001$; ES=0.51, *large*) in the HIIT group. The proportions of carbohydrate, lipid, and protein contributions to total energy intake did not change significantly in either group.

2.4 Discussion

The present chapter showed that 12 weeks of COMB training and HIIT performed by adults with obesity induced (1) significant reductions in BM and FM; (2) significant improvements in $V'O_2$ peak; and (3) similar increases in fat oxidation rates during submaximal exercise. Furthermore, (4) the COMB group exhibited lower values of RPE and HR during training than the HIIT group.

The first main finding was that both COMB training and HIIT produced similar decreases in BM and FM, by ~3 kg and ~4 kg, respectively. Although energy intake decreased similarly in both groups after the training intervention, we observed that the combination of moderate energy restriction and aerobic training was effective in improving body composition (Vaccari et al. 2020; Reljic et al. 2021). In particular, in the COMB group, the analisis of the current scientific licterature showed that a short initial bout of high-intensity training (i.e., 1 × 2 minutes of walking or running at 130% of $V'O_2$ max, or 5 × 1 min of cycling at 100% power max [P_{max}] separated by 1 min of passive recovery) before prolonged moderate-intensity exercise increased fat oxidation (%) during the moderate-intensity exercise and during the initial stage of recovery to a greater extent than MICT alone (Borowik et al. 2020; Mello-Silva et al. 2022). In contrast, HIIT increased excess postexercise oxygen consumption (EPOC) compared to MICT during the slow phase of O_2 kinetics in the recovery phase (i.e., from 30 min to 22 h after the end of the training session) (Panissa et al. 2021). This occurs due to greater postexercise fat utilization to sustain energy demands while glycogen resynthesis occurs (Moniz et al. 2020) and occurs through significant increases in circulating hormones that promote fat oxidation (i.e., catecholamines and growth hormone) (Moniz et al. 2020), although the results are conflicting (Lazzer et al. 2017b). Thus, through different physiological mechanisms, our results demonstrate that combining reduced energy intake with physical exercise (i.e., COMB training or HIIT) is a useful strategy for both weight- and FM-loss programs for obese adults. In the first chapter we showed that exercise intensity and a combination of volume and intensity may be effective in the initial stage of weight reduction programs for decreasing BM and FM (Wewege et al. 2017; Keating et al. 2017) due to similar improvements in skeletal muscle capacity for the uptake and oxidation of fatty acids (Purdom et al. 2018) and for increasing both glycogen content and utilization (Murray and Rosenbloom 2018), which are typically impaired in adults with obesity (Georgiev et al. 2022a). In addition, FFM was maintained in both exercise groups, which might be helpful for maintaining weight loss (Wewege et al. 2017b; Keating et al. 2017).

The second main finding was that both COMB training and HIIT significantly increased $V'O_2$ peak by ~16% in both groups, confirming previous results observed in people with obesity (i.e., improvements ranging between 15% and 25%) (Roxburgh et al. 2014; Zapata-Lamana et al. 2018;

Poon et al. 2022) and highly trained endurance athletes (Stöggl and Sperlich 2015; Rosenblat et al. 2019). As mentioned in the Introduction, the main features of COMB training reported in previous studies were a combination of MICT (30-40 min/session at 65–70% of HR_{max} or 90% of the first ventilatory threshold) and HIIT (6-12 min/session at 80-90% of HR_{max} or 90% of VO_2 peak) (Roxburgh et al. 2014; Zapata-Lamana et al. 2018; Poon et al. 2022) performed in the same training session and 3 days/week during the long-term training period (12-16 weeks); these features elicited equal or superior improvements in VO_2 peak compared to HIIT alone. The present study applied a combination of high-volume low-intensity exercise (≥ 80% of the overall training volume) and low-volume high-intensity exercise (≤ 20%) derived from analysis of the above studies (Roxburgh et al. 2014; Zapata-Lamana et al. 2018; Poon et al. 2022), as shown in middle- and long-distance runners(Campos et al. 2022). HIIT is superior to MICT in improving CRF when energy expenditure is held constant (Su et al. 2019). To our knowledge, this study is the first to apply an a priori combination of HIIT and MICT in obese individuals, considering primarily the weekly distribution of HIIT and MICT rather than the individual training session itself. It is possible that during the initial stage of a weight reduction program, exercise intensity (rather than volume) is critical in male adults with obesity (Gibala et al. 2014; Su et al. 2019). Nonetheless, based on the few available data, we confirmed the positive results obtained in previous studies, expanding upon these findings with an a priori manipulation of HIIT and MICT (i.e., ~ 85% MICT and ~15% HIIT). Further studies are needed to compare various combinations of COMB training over a long period of time (i.e., ≥ 6 months).

In the present study, HIIT may have enhanced $V'O_2$ peak by increasing stroke volume and maximal cardiac output (central adaptation) (Astorino et al. 2017). In contrast, COMB training may have increased $V'O_2$ peak by increasing mitochondrial content (peripheral adaptation) (Granata et al. 2018b). Exercise volume (i.e., a combination of HIIT+MICT) is more important than exercise intensity (i.e., HIIT alone) for promoting increases in mitochondrial content (Bishop et al. 2019).

Another main finding was the increase in fat oxidation over a wide range of exercise intensity (i.e., 20-80% of VO_2 peak), obtained in both the COMB and HIIT groups, without significant differences between the two groups. Previous studies have shown that HIIT increases fat oxidation in individuals with obesity to a greater extent than MICT alone (Alkahtani et al. 2013; Vaccari et al. 2020b; Atakan et al. 2022). However, our study showed that COMB training increased fat oxidation in obese individuals in a similar manner to HIIT, as demonstrated earlier in obese young women (Zapata-Lamana et al. 2018). The key mechanisms underlying these metabolic adaptations induced by HIIT and COMB training appear to be different. Contrary to the theory that above 80% of $V'O_2$ max, the contribution of fat oxidation is near negligible (Achten et al. 2003; Lazzer et al. 2017), recent studies have shown that during HIIT, fat oxidation rates increase (i.e., threefold higher in well-trained athletes than in moderately active people) (Hetlelid et al. 2015), as confirmed by metabolomics analysis (Zagatto et al. 2021). In fact, fat oxidation during HIIT, compared to continuous exercise, occurred mainly due to increased levels of fatty acid transport proteins taking up plasmatic free fatty acids (Talanian et al. 2010) in type II fibres of human skeletal muscle (Kristensen et al. 2015). On the other hand, in COMB training, increased fat oxidation during MICT (Borowik et al. 2020) primarily occurs in type I fibres (Skelly et al. 2021) that exhibit high rates of intramuscular fatty acid oxidation (Shaw et al. 2020). Thus, in line with a recent meta-analysis (Atakan et al. 2022), our study showed that combining low-volume HIIT with high-volume moderate-intensity training (i.e., COMB training) was effective for improving fat oxidation, metabolic health and body composition in individuals with obesity (Atakan et al. 2022).

In contrast to the physiological data, during the 12 weeks of the training intervention, the COMB group exhibited lower HR and RPE values than the HIIT group, in line with previous research in which both MICT and COMB training were perceived to be less strenuous (Zapata-Lamana et al. 2018; Sun et al. 2019) than HIIT, despite similar improvements in anthropometric and physiological parameters. Nevertheless, the COMB group covered more kilometres on average (i.e., ~11 km for each participant) with a greater duration of each training session (i.e., ~30%) than the HIIT group. Although HIIT is an effective, time-efficient exercise protocol for improving body composition and physical capacities in individuals with obesity (Batacan et al. 2017; Su et al. 2019), over a long period of training, high-intensity workout programs could increase lower limb injury rates (Rynecki et al. 2019), and overweight and obese patients experience fear of joint damage (Hamer et al. 2021). Thus, a weekly training distribution with a low volume of HIIT and the largest volume of MICT could be recommended in obese adults to increase adherence to international physical activity guidelines while minimizing the risk of joint injuries.

The present study has some limitations. First, although we showed that 12 weeks of COMB training or HIIT, administered with the same energy restriction, improved body composition and physical capacities, it remains unclear whether the training program was the determining factor for improving body composition, as we did not have an inactive control group to rule out dietary factors. Second, our study was carried out on healthy individuals with obesity; thus, it is not possible to extend our results to obese people with one or more comorbidities. Third, since we compared only one a priori COMB training with HIIT, it is difficult to conclude whether our selected combination ratio was indeed optimal or whether there are even better combinations.

In conclusion, COMB training and HIIT improved anthropometric and cardiovascular parameters as well as fat oxidation rates to a similar extent. However, COMB training was less intense than HIIT, confirmed by lower values of RPE and HR. Future studies should investigate various combinations of HIIT and MICT (i.e., pyramidal or threshold) over a long period of time (≥6 months) and compare them to HIIT and MICT alone to identify the optimal combination of HIIT and MICT. Such research would provide more evidence on the use of COMB training in obese adults for improving body composition and optimizing and maintaining aerobic fitness.

Chapter 3

24-week polarized training vs. threshold training in adults with obesity

Adapted from:

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Effects of 24-week polarized training vs. threshold training in obese male adults.

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3.1 Introduction

As already mentioned, people with obesity show lower CRF (Lin et al. 2015) and lower values of oxygen consumption in both gas exchange threshold (GET) (Zhou 2021) and respiratory compensation point (RCP) compared to lean individuals (Maciejczyk et al. 2014) due to lower aerobic capacity and poor oxygen transport in muscles involved in weight-bearing activities (i.e., walking or running) (Zhou 2021). However, recent evidence suggests that improvements in CRF in obese adults increase the values of oxygen consumption at ventilatory thresholds (Guio de Prada et al. 2019) while reducing morbidity and mortality levels compared to inactive lean individuals (Gaesser and Angadi 2021). Thus, endurance training are cornerstones in weight management programs for obese people, first by creating an energy deficit to reduce BM, particularly FM (Petridou et al. 2019), and second by improving CRF (Rugbeer et al. 2021).

As seen above, MICT (i.e., duration between 20-60 min at <80% maximum heart rate (HRmax) or 49-75%V'O_2max) and HIIT (i.e., duration between 1-4 min at ≥ 85% of HRmax or ≥80% of V'O_2max) (MacInnis and Gibala 2017) are the most prescribed exercise modalities in weight management programs (Su et al. 2019). In individuals with obesity, HIIT improves CRF (Su et al. 2019) and body composition (Andreato et al. 2019) more than MICT alone in a short period of time (i.e., between 4 and 12 weeks), although several systematic reviews and meta-analyses revealed similar improvements in V'O_2peak (Rugbeer et al. 2021b) and body composition (Keating et al. 2017) induced by HIIT and MICT.

In addition, recent studies suggest that the combination of high- and moderate-intensity training, with a polarized (POL) approach, characterized by covering approximately 70-90% of training volume below the GET and the remaining 10-30% of training volume near the V'O_2max (Seiler 2010a), is useful to maximize physiological parameters (e.g., V'O_2max, ventilatory thresholds) and improve body composition in athletes (Rosenblat et al. 2019; Kim et al. 2021a) and obese subjects (Poon et al. 2022; D'Alleva et al. 2023a), more than HIIT or MICT modalities alone. Nevertheless, most studies in which endurance training is applied in obese adults showed that i) the exercise intensity of training is given in relation to percentage of HRmax, or V'O_2max (Su et al. 2019), while ventilatory thresholds provide better information about endurance training using the concept of TID, typically applied to endurance athletes (Meyer et al. 2005; Stöggl and Sperlich 2015), ii) a paucity of studies have examined the concept of training periodization in adults with obesity (Streb et al. 2021), and iii) endurance training was applied during a limited number of training weeks (i.e., on average 12 weeks) (Su et al. 2019).

Finally, the study by Collins et al. (Collins et al. 2022) indicated that most people with obesity will drop out before or within 2 to 3 months of exercise training onset. Accordingly, to increase the awareness and adherence of participants to the training, adding a challenging sports performance at the end of the training programs would be useful (Keytsman et al. 2019). To date, this strategy has been used on people with multiple sclerosis, achieving excellent results in terms of adherence to training (Keytsman et al. 2019). However, to our knowledge, no study on obese adults compared two types of TIDs, with linear or reverse periodization, for long periods of time (i.e., ≥ 6 months). Thus, the aims of the present chapter were i) to determine the effects of 24 weeks of either POL TID or threshold (THR) TID (i.e., ≥20% of overall volume conducted at intensity between the ventilatory thresholds) (Campos et al. 2022) , modified by Veronique Billat (www.billatraining.com) (Molinari et al. 2020b), with reverse periodization (i.e., volume increased over the months, while intensity is maintained) (Stone et al. 2021) on body composition, $V'O_2max$, and ventilatory thresholds in healthy adults with obesity. ii) At the end of the 24 weeks, a running competition depending on the level reached by each participant, such as a half marathon, 30-km race, or marathon, was employed to encourage adherence to the project.

3.2 Material and Methods

Subjects

Twenty-eight male adults with obesity were enrolled in the study. These subjects all took part in a previous study that ended two months before the start of this study. All subjects had a full medical history and underwent physical and nutritional examinations. BM was stable during the previous two months. None of the subjects showed cardiovascular, respiratory, neurological, skeletal, metabolic and/or endocrine diseases, and none of the subjects took medications regularly or used drugs known to affect energy metabolism. Of these subjects, twenty participants completed the 24 weeks of training (Table 3.1 and 3.2).

Experimental Design

The study was approved by the Ethics Committee of the Friuli-Venezia-Giulia Region (Italy) (protocol number 1764). Before the study began, the purpose and objective were carefully explained to each subject, and written informed consent was obtained.

The training intervention was performed from November 2021 to May 2022 (24 weeks). Between the previous study that had finished in September 2021 and the beginning of this study, an 8-week preintervention period was employed to standardize the training load for all subjects. During the preintervention period, participants were instructed to perform 3 sessions/week, with a progressive working volume from 100 to 200 min week^{-1} performed at low intensity (speed corresponding to 60% of V'O_2peak) derived from the previous graded exercise test.

After the 8-week preintervention period, participants followed a 24-week weight management program and were randomly divided into two groups, POL (n = 10) and THR (n = 10), with reverse periodization, using the three zones model to calculate the TID for both groups (Stöggl and Sperlich 2014a) (see below for details). The participants were trained in their normal living conditions, ensuring the ecological validity of the study. Full testing sessions were conducted before the beginning (PRE) and at completion of the 24-week weight management period (POST). The testing sessions were conducted during one visit, including the assessment of anthropometric characteristics, body composition and graded exercise test on a 400 m track (Gemona del Friuli, Udine, Italy) to measure the ventilatory thresholds and V'O_2max. All tests were performed under medical supervision. The physical capacities were monitored weekly to individually adjust physical training. At PRE and throughout the weight management period, each participant received the same nutritional advice to avoid confounding nutritional variables on the outcomes (CREA 2019). At the end of the 24-week training period, a running challenge was proposed consisting of a half marathon, 30-km race or a marathon, depending on the level reached by the participants.

Training program

The training intervention was performed for 24 consecutive weeks, with 3 sessions per week. The sessions consisted of walking or running (or a combination of the two methods) on flat terrain, a track or city circuits. The training was divided into three 8-week macrocycles structured as 3+1 mesocycles, with linear periodization (see Supplementary file for detailed program). In both groups, on average, training load (TL) increased by ~30% between the first and the second 8-week macrocycles. Then, between the second and the third 8-week macrocycles, TL increased by ~10% (see supplementary data for extended program). In all 3+1 mesocycles, the last week was a recovery week, and TL was reduced by 30%. TID was calculated every 8 weeks with the three-zone model using the speed reached at GET, RCP and V'O_2max (Bellinger et al. 2020): zone 1 (Z1), for intensities below GET; zone 2 (Z2), for intensities between GET and RCP; and zone 3 (Z3), for intensities above RCP. POL TID was verified with the polarization index (i.e., polarized: Z1 > Z3 > Z2) (Stöggl and Sperlich 2015; Treff et al. 2019), POL and THR TID were matched for the same TL. TL was quantified with the training impulse (TRIMP), where each zone has a weighting factor that is multiplied by the duration in this zone (Foster et al. 2001). All participants uploaded their own workouts to an online training diary, Polar Flow (Polar Electro Oy, Finland) or Gamin Connect (Garmin, Olathe, USA). Research assistants and physical trainers verified that each subject performed the exercises correctly and registered for each training session: training duration, time spent in each endurance training zone, and rate of perceived exertion (RPE) using the Borg 6-20 Scale (Borg 1970), as recorded by the participants. When the mean HR decreased by 5 bpm for two consecutive training sessions in one of the three training zones, the speed was increased to maintain the prescribed intensity. At the end of the 24-week training period, seventeen of the twenty participants performed a challenge, running a half marathon, a 30-km race or a marathon, depending on the level reached by the participants at the end of the study. The challenge included a lap of 10.2 km to be repeated according to the distance. For safety reasons, we placed refreshment points with water, mineral salts, and fruits along the track. The challenge was performed under medical supervision. The performance of the subjects was monitored using their Garmin (Garmin, Olathe, USA) or Polar (Polar Electro Oy, Finland) watches.

Measurements

Anthropometric characteristics and body composition

BM was measured with a manual weighing scale (approximation 0.1 kg) (Seca 709, Hamburg, 165 Germany) with the subject dressed only in light underwear and no shoes. A wall-mounted height board was used to measure the stature. BMI was calculated as BM (kg) × stature^{-2} (m). The waist circumference (WC) and hip circumference (HC) were measured with the method of Kagawa et al. (Kagawa et al. 2008). Body composition was measured by bioelectrical impedance (BIA, Human IM Plus; DS 171 Dietosystem, Milan, Italy) (Lukaski 1987). The fat mass (FM) and fat free mass (FFM) values were obtained with the equations described by Gray et al. (Gray et al. 1989) derived in obese people of either age (fat-specific formulae).

Graded exercise test (GRAD)

To determine V'O_2max, HRmax, and ventilatory thresholds, participants carried out a graded exercise test on a 400 m track (Gemona del Friuli, Udine, Italy) under medical supervision. A collaborator with a bike paced the runners, and the participants were instructed to follow the bike. The duration of each step was one minute, and the speed increased by 0.5 km/h every minute until volitional exhaustion. V'O_2, V'CO_2 and HR were measured during this test using a wearable metabolic unit (K5; Cosmed, Roma, Italy) and a chest strap (Garmin HRMrun, Olathe, USA), respectively. We calibrated the volume and gas analysers before every test using a 3-L calibration syringe and calibration gas (16.00% O2 and 5.00% C'O2), respectively. We determined the GET and RCP with the V-slope method (Beaver et al. 1986). V'O_2max was calculated as the average 30-s V'O_2 according to previously established criteria (Howley et al. 1995): (i) plateau in V'O_2 (i.e., increase <150 ml min^{-1}), (ii) RER > 1.1, and (iii) ≥90% of theoretical HRmax.

Dietary habits

Participants were invited to collect a 4-day dietary record collecting the food and beverage consumption of 2 weekdays and 2 weekend days, instructions on how to record the type and portion size of the foods consumed, at PRE and POST as previously described (Vaccari et al. 2020).

Statistical analysis

The data were analysed using GraphPad Prism (version 9.4.0), with significance set at $p < 0.05$. The results obtained are expressed as the means and standard deviations (SDs). The Shapiro–Wilk test was used to evaluate the normality of the data. Sphericity was verified by Mauchly's test. A Greenhouse–Geisser correction was used in cases of sphericity assumption violations. For baseline characteristics and training adherence comparison, Student's t test was used for unpaired data. Anthropometric characteristics, body composition, V′O_2max, ventilatory thresholds and training characteristics were analysed with a 2-way ANOVA that included the between-subjects factor of the training model (POL and THR groups) and the within-subjects factor of time (PRE vs. POST, i.e., repeated measured analysis). Significant main effects were further analysed by the Bonferroni post hoc test. Finally, effect sizes comparing pre-post changes within each group were calculated as the corrected effect size (ES) (Lakens 2013). ES < 0.20 was considered small, < 0.50 medium and > 0.50 large (Cohen 1988). To determine the sample size a priori, power analysis showed 7 participants per group selected an F test for ANOVA-repeated measures-within factors analysis with a statistical power of 0.80, a probability α level of 0.05, and an effect size f of 0.35 (G-Power software, v. 3.1.9.2, Universität Kiel, Kiel, Germany).

3.3 Results

Anthropometric characteristics and body composition

At baseline, no differences in the anthropometric characteristics and body composition were observed between groups (Table 3.1). At POST, BM decreased in both groups by 3.66 ± 3.11 kg (ES 0.31 *medium*) and 2.73 ± 3.17 kg (ES = 0.24 *medium*) for the POL and THR groups, respectively (time effect $P < 0.001$). BMI was reduced by 1.22 ± 1.04 kg m^{-2} (ES 0.37 *medium*) for the POL group and 0.75 ± 0.96 kg m^{-2} (ES 0.37 *medium*) for the THR group (time effect $P<0.001$). FM (kg) decreased by 4.21 ± 3.10 kg (ES 0.57 *large*) in the POL group and 3.34 ± 2.45 kg (ES 0.47 *medium*) in the THR group (time effect $P<0.001$). FM (%) decreased in both groups (3.10 ± 2.34 and 2.73 ± 2.00%, time effect Pc<c0.001) for POL (ES 0.74 *large*) and THR (ES 0.57 *large*). FFM (%) increased on average by 2.93 ± 2.13 (%) (time effect $P<0.001$) in the POL (ES 0.74 *large*) and THR (ES 0.57 *large*) groups. FFM (kg), WC, HC and waist-to-hip ratio did not change significantly in either group (Table 3.1).

***Table 3.1**. Anthropometric characteristics before (PRE), and after 24 weeks (POST) of weight management program in polarized training (POL) and threshold training (THR) groups.*

	POL (n: 10)		THR (n: 10)		P		
	PRE	POST	PRE	POST	G	T	G x T
Age (y)	42.1 ± 6.1		40.0 ± 6.5		0.724		
Stature (m)	1.75 ± 0.08		1.78 ± 0.05		0.401		
Body mass (kg)	98.2 ± 11.6	94.6 ± 11.5*	100.0 ± 9.6	97.3 ± 11.7*	0.670	0.001	0.443
BMI (kg m^{-2})	32.0 ± 3.3	30.8 ± 3.1*	31.4 ± 1.8	30.6 ± 2.4*	0.743	0.001	0.450
Waist (cm)	103.0 ± 7.5	102.3 ± 7.8	102.0 ± 4.5	101.3 ± 5.6	0.695	0.577	0.917
Hip (cm)	110.1 ± 5.1	109.5 ± 5.9	109.0 ± 3.0	108.6 ± 3.4	0.471	0.988	0.478
Waist-to-hip ratio	0.94 ± 0.05	0.93 ± 0.05	0.94 ± 0.05	0.93 ± 0.04	0.981	0.482	0.247
Fat-free mass (kg)	62.3 ± 5.9	62.8 ± 5.9	64.6 ± 5.1	65.6 ± 6.3	0.348	0.050	0.495
Fat Mass (kg)	35.9 ± 7.2	31.7 ± 7.3*	35.0 ± 6.0	31.7 ± 7.3*	0.885	0.001	0.514
Fat-free mass (%)	63.6 ± 3.7	66.7 ± 4.3*	65.0 ± 3.7	67.7 ± 4.6*	0.532	0.001	0.695
Fat Mass (%)	36.4 ± 3.7	33.3 ± 4.3*	35.0 ± 3.7	32.3 ± 4.6*	0.533	0.001	0.700

All values are presented as mean ± standard deviation

BMI: body mass index

G: group effect, T: time effect; G × T: groups × time effect.

**Significantly different from PRE, $P < 0.05$.*

Aerobic physical capacities

At PRE, the percentage (%) of $V'O_2max$ at RCP was greater in the THR group than in the POL group by +3.83 ± 6.17% ($P = 0.004$), and $V'O_2$ at GET ($P = 0.004$) was greater in the THR group than in the POL group by +12.0 ± 14.3% ($P = 0.047$) (Table 3.2). However, the other physiological parameters were not significantly different between the groups (Table 3.2).

At POST, $V'O_2max$ (L min^{-1}) increased by 0.3 0± 0.41 and 0.18 ± 0.28 L min^{-1} in the POL (ES 0.61 *large*) and THR (ES 0.37 *medium*) groups, respectively (time effect $P < 0.001$). $V'O_2max$ (L kg^{-1} min^{-1}) increased in the POL (+19.1 ± 13.0%, ES 0.87 *large*) and THR (+7.81 ± 8.10%, ES 0.50 *large*) groups (time effect $P < 0.001$). The speed at $V'O_2max$ ($vV'O_2max$) increased in the POL group by 0.95 ± 0.72 km h^{-1} (ES 0.53 *large*) and by 0.35 ± 0.70 km h^{-1} (ES 0.21 *medium*) (time effect $P < 0.001$) in the THR group. No difference was found for HRmax and RER at $V'O_2max$ in either group (Table 3.2). $V'O_2$ (L min_{-1}) under RCP increased by 0.25 ± 0.47 L min^{-1} in the POL group (ES 0.50 *large*) and 0.10 ± 0.21 L min^{-1} in the THR group (ES 0.27 *medium*) (time effect $P = 0.034$). $V'O_2$ (L kg^{-1} min^{-1}) under RCP increased in the POL (+13.0 ± 15.8%, $P = 0.004$, ES 0.70 *large*) and THR (+6.0 ± 6.2%, $P = 0.200$, ES 0.48 *medium*) groups (time effect $P<0.001$). The speed at RCP increased in both groups (1.10 ± 0.53 and 0.55 ± 0.73 km h^{-1}, time effect $P<0.001$) in the POL (ES 0.60 *large*) and THR (ES 0.32 *medium*) groups. No difference was found for HR, RER, % of $V'O_2max$ and HRmax under RCP in either group (Table 3.2).

$V'O_2$ (L min^{-1}) at GET increased in both groups (0.35 ± 0.34 and 0.30 ± 0.26 L min^{-1}, time effect $P < 0.001$) in the POL (ES 0.83 *large*) and THR (ES 0.88 *large*) groups, respectively. $V'O_2$ (L kg^{-1} min^{-1}) at GET increased in the POL (4.59 ± 3.20 ml kg^{-1} min^{-1}, ES 0.71 *large*) and THR (3.77 ± 2.31 ml kg^{-1} min^{-1}, ES 0.87 *large*) groups (time effect $P = 0.001$). The speed at GET increased in both groups (1.00 ± 0.55 and 0.95 ± 0.93 km h^{-1}, time effect $P < 0.001$) in the POL (ES 0.60 *large*) and THR (ES 0.61 *large*) groups. No difference was found for HR, RER, % of $V'O_2max$ or HRmax at GET in either group (Table 3.2).

Table 3.2 *Physiological Parameters before (PRE) and after 24 weeks (POST) of weight management program in polarized training (POL) and threshold training (THR) groups.*

	POL (n:10)		THR (n: 10)		P		
	PRE	POST	PRE	POST	G	T	G x T
Maximal oxygen uptake							
V'O$_2$(L min^{-1})	3.65 ± 0.41	3.95 ± 0.52*	3.93 ± 0.48	4.11 ± 0.44*	0.265	0.001	0.464
V'O$_2$(ml kg $^{-1}$min^{-1})	37.4 ± 4.0	42.1 ± 6.4*	39.4 ± 3.8	42.4 ± 5.4	0.570	0.001	0.283
HRmax (bpm)	177.3 ± 12.7	174.1 ± 8.4	179.5 ± 8.8	175.5 ± 10.8	0.670	0.050	0.796
RER max	1.12 ± 0.05	1.08 ± 0.03	1.09 ± 0.05	1.10 ± 0.04	0.683	0.190	0.110
Speed (km h^{-1})	11.9 ± 1.7	12.9 ± 1.9*	12.8 ± 1.5	13.2 ± 1.8	0.440	0.001	0.070
Respiratory compensation point							
V'O$_2$(L min^{-1})	3.20 ± 0.45	3.45 ± 0.50*	3.55 ± 0.37	3.65 ± 0.37*	0.119	0.036	0.371
V'O$_2$(ml/kg/min)	32.7 ± 4.5	36.8 ± 6.2*	35.8 ± 3.8	37.7 ± 4.8	0.321	0.001	0.181
V'O$_2$, %max	87.5 ± 5.5	87.3 ± 4.8	90.4 ± 1.8	90.2 ± 5.2	0.040	0.893	0.998
HR (bpm)	164.0 ± 10.6	163.3 ± 9.8	169.2 ± 8.8	165.5 ± 10.7	0.360	0.230	0.385
HR, %max	92.6 ± 3.9	93.8 ± 3.1	94.0 ± 2.0	94.3 ± 2.1	0.312	0.329	0.583
RER	1.02 ± 0.05	0.99 ± 0.03	1.00 ± 0.05	1.01 ± 0.03	0.848	0.468	0.073
Speed (km/h)	10.2 ± 1.6	11.3 ± 1.9*	11.3 ± 1.4	11.8 ± 1.6*	0.230	0.001	0.060
Gas exchange threshold							
V'O$_2$(L min^{-1})	2.52 ± 0.41	2.87 ± 0.40*°	2.90 ± 0.33	3.21 ± 0.35*	0.030	0.001	0.746
V'O$_2$(ml/kg/min)	26.2 ± 6.5	30.8 ± 6.0*	29.4 ± 3.8	33.1 ± 4.3*	0.225	0.001	0.482
V'O$_2$, %max	70.0 ± 12.5	73.2 ± 8.9	75.0 ± 5.4	78.2 ± 7.6	0.129	0.135	0.960
HR (bpm)	143.4 ± 14.1	145 ± 11.6	149.5 ± 10.9	151.7 ± 11.3	0.154	0.528	0.897
HR, %max	81.0 ± 7.1	83.4 ± 7.1	83.0 ± 2.9	86.5 ± 4.0	0.192	0.065	0.692
RER	0.91 ± 0.06	0.90 ± 0.04	0.90 ± 0.06	0.93 ± 0.04	0.496	0.572	0.175
Speed (km/h)	8.60 ± 1.60	9.60 ± 1.72*	9.23 ± 1.37	10.20 ± 1.50*	0.327	0.001	0.894

All values are presented as mean ± standard deviation$V'O_2$: oxygen consumption, HR: heart rate, RER: respiratory exchange ratio, $V'O_2$ %max: percentage of maxima oxygen uptake, HR %max: percentage of heart rate max.

G: group effect, T: time effect; G × T: groups × time effect.

**Significantly different from PRE, $P < 0.05$.*

° Significantly different POL vs. THR at baseline, $P < 0.05$.

Training characteristics

Twenty-eight obese adult males were recruited for the study. Of these, twenty subjects completed the 24-week training. Of the eight who dropped out, five had family or work reasons, while three were injured. One of three injured participants showed ankle problems outside the training program; the other two injuries were caused by algo dystrophy to the knee in one subject and medial meniscus inflammation of the left knee in the other subject. At the end of the training intervention, subjects performed 92.3 ± 10.1 and 87.7 ± 10.8% of training sessions for the POL and THR groups, respectively ($P = 0.253$). Average weekly TL (a.u. week^{-1}), time spent in Z1 (min week^{-1}) and time spent in Z3 (min week^{-1}) increased similarly in both groups by 16.9 ± 21.2, 18.0 ± 26.5 and 30.7 ± 40.6% over the three 8-week macrocycles (time effect $P < 0.001$), without differences between the values of weekly TL (a.u. week^{-1}) and time spent in Z3 (min week-1) (Table 3.3). Moreover, the POL group spent more time in Z1 (+61.0±20.4 min week^{-1}, $P<0.001$) than the THR group over the three 8-week macrocycles. The average weekly time in Z2 (min week^{-1}) was greater in the THR group by +31.0 ± 20.2 min week^{-1} than in the POL group ($P < 0.001$), without an increase over the 8-week macrocycles (Table 3.3). The total volume (min week^{-1}) was greater in the POL group than in the THR group (+31.3 ± 23.0 min week^{-1}, $P < 0.001$) over the three 8-week macrocycles in both groups (Table 3.3).

Table 3.3 *Average training intensity distribution and training load for week 1–8, week 9–16 and week 17-24 before of weight management program in polarized training (POL) and theshold training (THR) groups.*

	POL (n: 10)			THR (n: 10)			P		
	Weel 1-8	Week 9-16	Week 17-24	Week 1-8	Week 9-16	Week 17-24	G	T	G x T
Z1 (min week^{-1})	154 ± 20	183 ± 31	204 ± 60	95 ± 17	129 ± 26	135 ± 51	0.001	0.008	0.715
Z2 (min week^{-1})	5 ± 2	8 ± 3	10 ± 0	39 ± 21	29 ± 15	37 ± 20	0.001	0.601	0.890
Z3 (min week^{-1})	10 ± 2	14 ± 3	14 ± 2	8 ± 5	10 ± 9	13 ± 8	0.340	0.040	0.731
∑ volume (min week^{-1})	167 ± 22	205 ± 30	223 ± 66	141 ± 20	176 ± 31	185 ± 42	0.001	0.030	0.520
TL (a.u. week^{-1})	191 ± 28	240 ± 32	260 ± 55	188 ± 33	239 ± 34	249 ± 36	0.793	0.001	0.958

All values are presented as mean ± standard deviation.
Z1: time spent at intensity below gas exchange threshold, Z2: time spent at intensity between gas exchange threshold and respiratory compensation point, Z3: time spent at intensity between respiratory compensation point and maximal oxygen uptake, ∑ volume: average total weekly volume, TL: training load. G: group effect, T: time effect; G × T: groups × time effect.

RPE did not differ between the two groups during the 24 weeks of the training intervention (Figure 3.1).

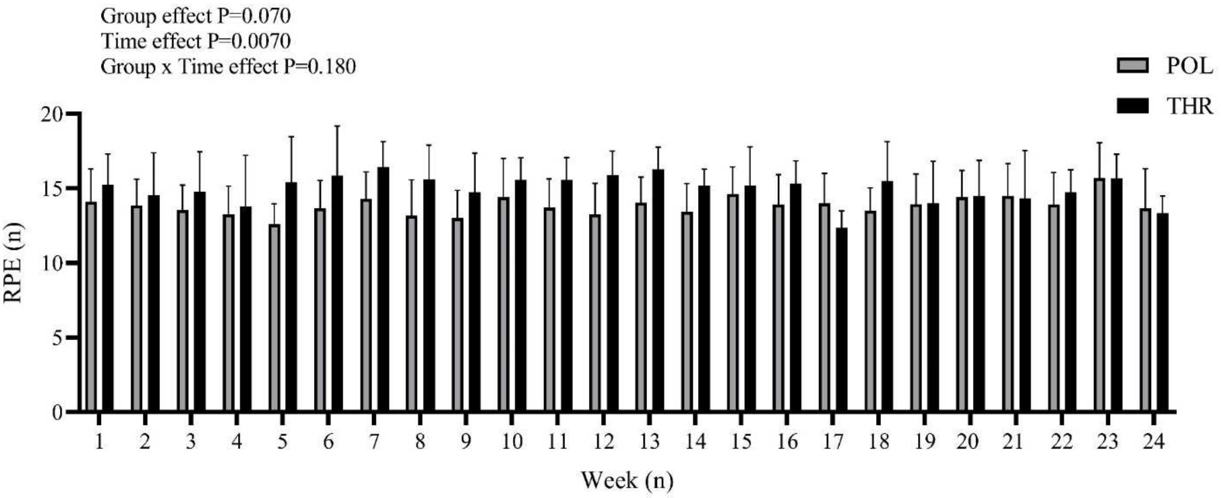

Figure 3.1 *Average values of rating of perceived exertion (RPE) during the 24 weeks of the training intervention between POL (grey columns) and THR (black columns) groups*

The POL group spent more time (%) in Z1 than the THR group (91.0 ± 2.4 vs. 71.3 ± 9.6%, P < 0.001), while the POL group spent less time (%) in Z2 than the THR group (4.0 ± 4.0 vs. 23.0 ± 11.2%, P < 0.001) over the 8-week macrocycles (Figure 3.2). The time (%) spent in Z3 did not differ between the two groups (Figure 3.2). At the end of the 24 weeks of training, eight participants completed the half marathon distance in 2:47:50 ± 0:7:44 h:m:s, range [2:38:57-3:01:06 h:m:s], three completed the 30-km race in 4:03:04 ± 0:42:03 h:m:s, range [3:35:59-4:51:30 h:m:s], and six participants completed a marathon distance in 4:19:46 ± 0:40:50 h:m:s, range [3:28:14-5:18:19 h:m:s].

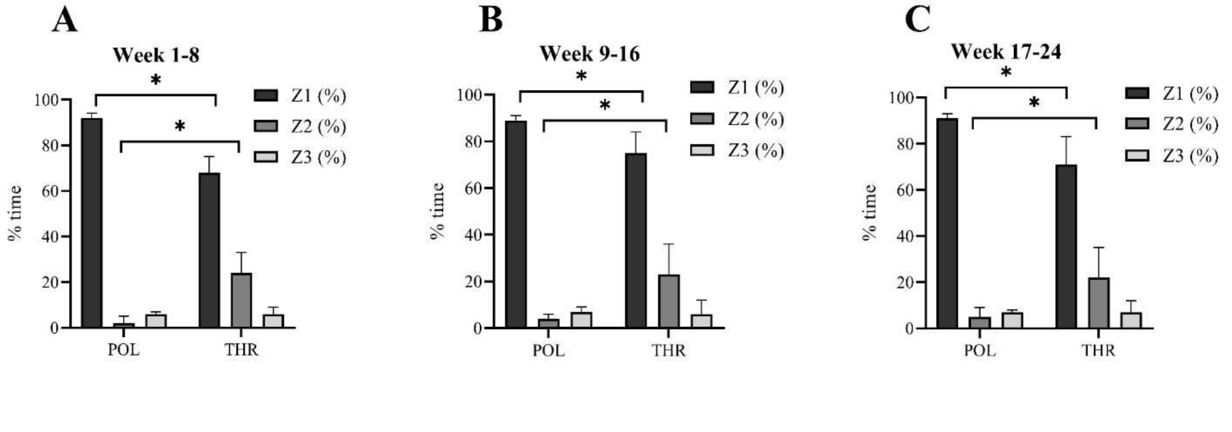

Figure 3.2 *Total running time in each zone (% time) for week 1–8 (panel A), week 9–16 (panel B) and week 17-24 (panel C) during the weight management program in polarized training (POL) and threshold training (THR). *Significantly different from THR, P < 0.05.*

Nutritional habits

At PRE, no significant differences were found between the POL and THR groups in terms of energy intake (6405 ± 611 vs. 8185 ± 2306 kJ day^{-1}, group effect P = 0.060) and macronutrient percentage contribution to total energy intake: carbohydrates (39.5 ± 7.9 vs. 42.4 ± 12.1%E, group effect P = 0.628), fats (35.6 ± 5.3 vs. 31.3 ± 6.1%E, group effect P = 0.091) and proteins (19.3 ± 5.2 vs. 19.2 ± 6.2%E, group effect P = 0.920). At POST, mean energy intake was unchanged in both groups (time effect P=0.701), without differences between groups (interaction G × T, P = 0.701). The carbohydrate, lipid, and protein percentage contributions to total energy intake did not change significantly in either group.

3.4 Discussion

The present chapter shows that a 24-week of POL or THR TID, with reverse periodization, applied in male adults with obesity induced the following in both groups: (1) significant reductions in BM and FM; (2) significant improvements in $V'O_2max$ and ventilatory thresholds; and (3) high adherence to training during the training period and a lower dropout rate.

The first outcome described in this chapter was that POL and THR TID combined with a no reduction in caloric intake resulted in similar reductions in BM and FM of ~4 and ~12%, respectively. Although weight loss of at least 5% of BM should be achieved for "successful" obesity treatment (Williamson et al. 2015), we observed that reduced caloric intake combined with physical exercise reduced BM (i.e., ~4 kg) more than physical exercise alone (i.e., ~1.7 kg) (Bellicha et al. 2021). The positive effects of POL and THR TID on reducing BM and FM have previously been noted in endurance athletes (Pérez et al. 2019a) and confirmed in the present study in obese volunteers. These results could be due to long-term adaptations to endurance training (i.e.,> 12 weeks), such as increasing skeletal muscle capacity for fatty acid uptake and oxidation and concomitant increases in glycogen content and glycogen utilization with higher levels of glycolytic enzymes (Murray and Rosenbloom 2018), which are typically impaired in obese individuals (Georgiev et al. 2022b). Thus, to our knowledge, this study is the first to apply a TID -approach using POL or THR training with reverse periodization in obese adult men and confirms that this approach may be equivalent or superior to classic HIIT or MICT in reducing BM and FM loss. In addition, as in the previous chapter, we outlined the role of physical exercise in maintaining FFM during weight management program (Reljic et al. 2021).

The second outcome achieved in the present chapter was that the POL and THR groups significantly improved their physical capacities (i.e., $V'O_2$ at ventilatory thresholds and $V'O_2max$), confirming previous research conducted in recreational and highly trained endurance athletes (Stöggl and Sperlich 2015; Rosenblat et al. 2019). V'O_2 at GET, expressed both in absolute values or relative to BM, increased by ~15 and 20% for the POL group and ~11 and 13% in the THR group, without differences between the two groups. This amount is greater than the 9% increase in V'O_2 at GET reported in a previous study conducted in individuals with obesity after 16 weeks of HIIT (Guio de Prada et al. 2019). Previous studies on endurance athletes have shown that V'O_2 at GET increased only with POL training (Stöggl and Sperlich 2014a), while THR training increased V'O_2 at GET in untrained subjects (Gaskill et al. 2001). The high volume in Z1 performed in both groups may have led to favorable adaptations, such as enhanced mitochondrial content and respiratory function (Granata et al. 2018a), as well as improved fat and glucose utilization during endurance exercise (Murray and Rosenbloom 2018; Purdom et al. 2018). V'O_2 at RCP, expressed both in absolute and relative values, increased by ~9 and 13% for the POL group and ~ 5 and 3% in the THR group. This increase confirms that in people with obesity, intensity training above or below the RCP increases the values of V'O_2 associated with the RCP (Guio de Prada et al. 2019) However, in our study, we observed that the effect of POL training in improving V'O_2 values at RCP was greater than the effect of THR training, as shown by comparing pre-post changes within each group (i.e., ES *large* vs. *medium* for POL and THR, respectively). This finding agrees with previous studies in which POL training improved V'O_2 and speed at RCP more than THR training when applied to endurance athletes (Stöggl and Sperlich 2014a; Rosenblat et al. 2019). However, to our knowledge, this study is the first to compare two different TIDs with POL and THR approaches in obese subjects, precluding direct comparisons with a population sample similar to ours. From a physiological perspective, the high training volume spent in Z1 by the POL group may have increased type I muscle fibre density (Bathgate et al. 2018) while simultaneously increasing capillary density (van der Zwaard et al. 2021). Indeed, the proportion of type I skeletal muscle fibres and capillary in contacts with type I fibres were positively correlates with RCP (Mitchell et al. 2018). Alternatively, a weekly training routine (i.e., ≥30% of the overall training volume) conducted below and above the RCP might be very demanding for the THR group and therefore not produce greater adaptations compared to the use of greater volumes in Z1 (Esteve-Lanao et al. 2007). V'O_2max, expressed both in absolute or relative values, increased by ~8 and 12% for the POL group and ~ 4 and 6% in the THR group, but these improvements were smaller than the improvements reported in previous studies (i.e., ranged between 9 and 25%) (Zapata-Lamana et al. 2018; Guio de Prada et al. 2019; D'Alleva et al. 2023), probably because the participants of our study were not totally deconditioned. Additionally, comparing pre-post changes within each group showed that POL training improved V'O_2max to a greater extent than

THR training (i.e., ES large vs. medium for POL and THR, respectively) in agreement with previous studies in which POL training improved V’O$_2$max more than THR when applied to endurance athletes (Stöggl and Sperlich 2014a; Rosenblat et al. 2019). Since peripheral factors limit V’$\dot{O}_2$max in untrained subjects (Wagner 2008), exercise volume is more effective than exercise intensity in increasing O$_2$ extraction capacity and mitochondrial content during whole-body maximal exercise in overweight and diabetic male adults (Van Ryckeghem et al. 2022).

The third outcome of this chapter was that the participants in both groups had high adherence to training and a lower dropout rate during the 24 weeks of the training intervention, and this adherence did not differ between groups. Recent studies in which endurance training was applied in obese subjects over a long period of time (i.e., ≥ 6 months) showed a high dropout rate (i.e., ranged between 31 and 44%) (Collins et al. 2022; Perna et al. 2022), while this rate was ~ 26% in our study. Specifically, we observed that using the concept of TID with POL or THR training with reverse periodization together with a running competition at the end of the study may helped to maintained high adherence to training in our study (i.e., ~90%), in line with previous studies on nonobese subjects (Keytsman et al. 2019). In addition, ~7% of our participants left the study due to an injury. Typically, the rate of running-related injuries (RRIs) among novice runners is ~30% (Peterson et al. 2022), and obesity is a major risk factor for RRIs (Bertelsen et al. 2018). Since the rate of progression in training volume and intensity may contribute to the risk of RRI (Damsted et al. 2019), an approach to running through periodizing POL or THR may represent an efficacious strategy to increase adherence to training while reducing both RRI and dropout rate over a long period of training in obese subjects.

Limitations

Our study presents some limitations. First, although in our study we showed that 24 weeks of POL or THR TID improved body composition and physical capacities in healthy obese male, with low injury rates at POST, the low number of participants, already trained, and the absence of a control group, do not allow to draw definitive conclusions. Second, our study was carried out on obese subjects not totally deconditioned, thus it is not possible to extend our results to obese people completely deconditioned. Third, since our study was conducted only in obese male adults, further studies will be needed to confirm our results in obese female adults. Finally, although our results are promising, further studies will be needed to confirm what has been found with the addition of a control group.

Practical Applications

Male adults with obesity who want to approach running and simultaneously improve their body composition and physical capacities while minimizing the risk of injury should choose TIDs with POL or THR approaches, both with a high volume of training below the GET.

Conclusions

In conclusion, 24-week training with POL or THR TID with reverse periodization improves body composition and physical capacities in adults with obesity. Moreover, adding a running competition at the end of the training programs may be helpful to increase adherence to training while reducing the dropout rate. Future studies will be needed to determine if exercise adherence can be improved by adding competition compared with a group without competition at the end of the exercise program.

Chapter 4

3-week combined training with a polarized approach and MICT in adolescents with obesity

Adapted from:

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Effects of combined training or moderate intensity continuous training during a 3-week multidisciplinary body weight reduction program on cardiorespiratory fitness, body composition, and substrate oxidation rate in adolescents with obesity.

Sci Rep. 2023 Oct 17;13(1):17609. doi: 10.1038/s41598-023-44953-3.

4.1 Introduction

Th World Health Organization (WHO) showed that the prevalence of adolescents with obesity aged 10 to 19 years have increased from 4% in 1975 to 18% in 2016 (Bibiloni et al. 2014). The main causes of obesity are a sedentary lifestyle, unhealthy diet, and reduced physical activity level with negative consequences on physical and mental health, such as the increased risk of cardiovascular disease, diabetes, hypertension, dyslipidaemia, inflammation, and psychosocial complications (Kodama et al.2020). Compared with lean counterparts, adolescent with obesity exhibit exercise intolerance, lower CRF (i.e., when expressed in ml kg FFM^{-1}min^{-1}) (Dupuis et al. 2000), and impaired capacity to oxidize lipids at rest (Lanzi et al. 2014) and during physical activity (Berggren et al. 2008) which may compromise their current and future health (Cole and Lobstein 2012). Indeed, as for adults even for adolescents CRF (i.e., expressed as V'O$_2$ max or V'O$_2$ peak) is one of the most important indicators of health and fitness (Gaesser &Ansaldi, 2021). CRF is inversely related to mortality and morbidity in various chronic diseases (Gaesser and Angadi 2021). However, adolescent with obesity presented FM infiltration within the skeletal muscle, which may compromise the contractile component of the muscle, impairing the ability of the muscular tissue to uptake oxygen (Georgiev et al., 2022). For this purpose, aerobic training become critical in weight management programs for adolescents with obesity to create an energy deficit that reduces BM and FM (Petridou et al. 2019), improves CRF (Rugbeer et al. 2021) and optimizes substrate oxidation capacity.

Two types of exercise training have received much attention in recent years: MICT and HIIT. MICT consists of performing aerobic exercise at a constant and moderate intensity for a prolonged duration (i.e., 30-50 minutes at ≤80% of maximal heart rate, HRmax) (Guo et al. 2023), whereas HIIT consists of alternating short periods of intense exercise (i.e., duration between 1 and 4 min at ≥85% of HRmax) (Gibala et al. 2014; MacInnis and Gibala 2017) with low-intensity exercise recovery periods with a total duration between 4 and 16 minutes. Both MICT and HIIT have been shown to improve body composition (Lazzer et al. 2017; Cao et al. 2021), CRF (Cao et al. 2021; Guo et al. 2023), and metabolic health (Cao et al. 2021; Guo et al. 2023) in adolescents with obesity, although HIIT is a time-efficient form of exercise (Cao et al. 2021). However, a previous meta-analysis reported the occurrence of adverse events such as leg discomfort, joint sprains, and asthma during and after HIIT (Martland et al. 2020). Furthermore, given the heterogeneity and low number of studies comparing the HIIT and MICT protocols, there is limited evidence on the comparative effects of MICT and HIIT in adolescents with obesity (Thivel et al. 2019).

Analysis data from previous studies found that the combination of MICT (i.e., 70-80% of total training volume, 30-40 min/session at 65-70% of HRmax or 90% of the first ventilatory threshold) and HIIT (i.e., ~20-30% of total training volume, 6-12 minutes/session at 80-90% of HRmax or 90% of $V'O_2$peak) (Zapata-Lamana et al. 2018; Poon et al. 2022; D'Alleva et al. 2023) with a polarized approach (Seiler 2010) performed in the same training session or a weekly training program resulted in equal or greater effects on CRF, body composition (Zapata-Lamana et al. 2018; Poon et al. 2022; D'Alleva et al. 2023b) and substrate oxidation (Zapata-Lamana et al. 2018; Borowik et al. 2020; D'Alleva et al. 2023b)in lean and obese sedentary adults compared with MICT or HIIT alone with equal volume or energy expenditure per session. Since people with obesity have reduced CRF (Gaesser and Angadi 2021), reduced mitochondrial function and poor metabolic flexibility (Georgiev et al. 2022), combining HIIT and MICT with a polarized approach would be an effective strategy to improve both mitochondrial content and mitochondrial functionality compared to MICT alone (Bishop et al. 2019). Furthermore, to the best of our knowledge, no study in adolescents with obesity has used a priori percentage of a combination of MICT and HIIT with a polarized approach during weekly training to improve cardiorespiratory function and substrate oxidation rate compared with MICT alone. To optimize cardiorespiratory function and substrate oxidation during moderate or interval training, several authors recommended walking or running instead of cycling (Lafortuna et al. 2010; Bogdanis et al. 2021).

Therefore, the present chapter aimed to evaluate the effects of a 3-week combination of HIIT and MICT (combined training; COMB) using a polarized approach (D'Alleva et al. 2023) and MICT alone on body composition, $V'O_2$ peak, and substrate oxidation rates in adolescents with obesity hospitalized for a 3-week multidisciplinary body weight reduction program (BWRP), entailing moderate energy restriction, nutritional education, psychological counseling (the same for all participants).

4.2 Material and methods

Participants

Twenty-one boys with severe obesity (body mass index standard deviation score, BMI SDS > 2, 16.0 ± 1.4 years) (Cacciari et al. 2006) with a pubertal stage >3 (Tanner and Whitehouse 1962) were recruited as inpatients from the Division of Auxology, Italian Institute for Auxology, IRCCS, Piancavallo (VB), Italy. Their BM was stable (changes less than ± 1 kg) during the previous 2 months. Inclusion criteria were i. absence of cardiovascular, respiratory, neurological, musculoskeletal, metabolic, and/or endocrine disease, ii. no regular use of medications known to influence energy metabolism. The 3-week multidisciplinary BWRP included moderate energy restriction, nutritional education, psychological counseling, and two different training programs (COMB or MICT, described in detail in the following paragraph).

Study protocol

The study was approved by the Ethics Committee of the Istituto Auxologico Italiano, IRCCS, Milan, Italy (ethical committee code of approval: 2022_03_15_03; research project code: 01C212; acronym: ALPOLAROB). The study was conducted in accordance with the Declaration of Helsinki and with the 2005 Additional Protocol to the European Convention of Human Rights and Medicine concerning Biomedical Research. Before the study began, all the volunteers and their parents were fully informed of the purpose of the study and provided written informed consent. The adolescents were hospitalized for a multidisciplinary BWRP. During the first few days, all the participants underwent a physical examination including hematology, biochemistry, and urine analysis._Physical examination included assessment of anthropometric characteristics, body composition, basal metabolic rate (BMR), energy expenditure (EE), and substrate oxidation rate during submaximal exercise. Thereafter, all volunteers followed a 3-week personalized weight-management program entailing moderate energy restriction, nutritional education, and psychological counseling. Participants were randomly allocated (using sealed envelopes and a 1:1 ratio) into two groups: the COMB (n:10) and MICT groups (n:11). All the testing sessions were conducted just before the beginning (week 0, W0) and at the end of the 3-week body weight reduction program (week 3, W3).

Diet and nutritional education

Based on the initial BMR and physical activity level, all adolescents received a personalized diet during the 3-week BWRP. Energy intake was adjusted to be close to 1.2 times the initial BMR, which is approximately 15–20 % lower than the estimated daily EE. Diet composition was formulated according to the Italian recommended daily allowances (Nutrition ISo (1996) Recommended levels of energy and nutri-ents intake for the Italian population (LARN). Edra Medical Publishing and New Media). During the 3-week weight reduction program, the consumption of meals was always under the supervision of a dietician.

Physical activity

The training program included two training sessions per day (i.e., from Monday to Friday) for a period of 3 weeks under medical supervision. Volunteers walked on a treadmill. Each participant monitored his intensity with a heart rate (HR) chest strap (Polar H10, Polar Electro Oy, Finland). All subjects completed 28 ± 2 sessions of physical training. The COMB group completed a combination of high-volume, low-intensity exercise (~80% of overall training volume) and low-volume, high-intensity exercise (~20%). Each session consisted of a 5-min warm-up (50% of $V'O_2$ peak) followed by three 2-min at high intensity (95% of $V'O_2$ peak), separated by 1 min of walking at low intensity (50% of $V'O_2$ peak), followed by ~30 min of MICT (60% of $V'O_2$ peak) (D'Alleva et al. 2023b) (Figure 4.1 A). Each training session lasted 36 ± 4 minutes for the COMB program. The MICT group performed 45 ± 6 min at HR corresponding to 40% of $V'O_2$peak (Lazzer et al. 2017) (Figure 4.1 B). Exercise intensity was manipulated by adjusting treadmill speed and incline according to the values of 40, 50, 60, and 95% of $V'O_2$ peak measured during the graded test (GRAD) and then calculating the corresponding HR values. Both training programs were equated to the same energy expenditure during a training session (e.g., 20 kJ per kg of fat-free mass, FFM, about 1.4 MJ per session) (Lazzer et al. 2017). In addition, volunteers had one $\text{hour}\cdot\text{day}^{-1}$ of aerobic leisure activities at the institution on Saturday and Sunday. The research assistant and physical trainers monitored all of the adolescents' training sessions to increase participant motivation (Kelley et al. 2019) and to verify that each subject participated in each training session, performed the exercises correctly, and completed at least 95% of the training program.

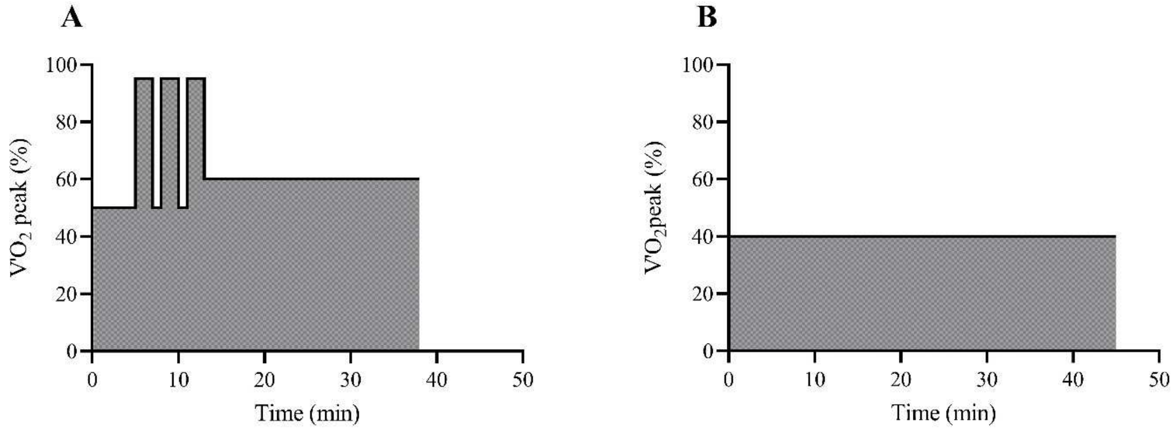

Figure 4.1 *Schematic representation of training protocols: Combined training (COMB, panel A) and Moderate-intensity continuous training (MICT, panel B).*

Measurements

Physical characteristics and body composition

BM was measured to the nearest 0.1 kg using an electronic scale (Selus, Italy) with the volunteers dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized Harpenden stadiometer (Holtain Ltd, UK). The body mass index (BMI) was calculated as BM (kg) x stature (m)$^{-2}$. For body composition analysis, a tetrapolar multifrequency impedance mete impedancemeter (BIA, Human-IM Scan, DS-Medigroup, Milan, Italy) was used after subjects had rested supine for 20 min with arms and legs relaxed and not in contact with other body parts (Lukaski 1987). A current of 800 μA was delivered at a frequency of 50 kHz for the BIA measurements. Great care was taken to standardize the variables affecting the validity, reproducibility, and precision of the measurement. FFM was calculated using the equation developed by Lazzer et al. (Lazzer et al. 2008), and fat mass (FM) was determined as the difference between BM and FFM.

Basal metabolic rate

After overnight fasting, BMR was measured in the morning (measurements between 0800 and 1000 a.m.), using an indirect open-circuit computerized calorimetry (Vmax 29, Sensor Medics, Yorba Linda, Ca, USA) and a rigid, transparent, and ventilated canopy. Before each test, calibration was performed with a reference gas mixture (95.00 % O_2 and 5.00 % CO_2). The duration of the BMR was 45 minutes. Values of oxygen consumption (V'O_2) and carbon dioxide production (V'CO_2), standardized for temperature, barometric pressure, and humidity, were recorded at 1-minute intervals. Values of the first 5-10 minutes were excluded from the analysis because they correspond to the adaptation to the procedure environment. Energy expenditure (EE) was calculated from the O_2 and CO_2 values (Weir 1949) and averaged over the entire measurement period.

Physical capacities and maximal fat oxidation rate

A GRAD test on a motorized treadmill determined the V'O_2 peak values and substrate oxidation rates (TechnoGym, Gambettola, Italy). All participants performed the GRAD test in the morning (exercise starting between 0800 and 1000 a.m.), under medical supervision.

Before the start of the study, individuals were familiarized with the equipment and the procedures. All participants avoided strenuous exercise and maintained the same eating habits the day before the test and came to the laboratory after a 12-h fast.

The GRAD test began with a 10-min rest period followed by walking in stages of a 5-minute duration. According to (Lazzer et al. 2017) treadmill speed (m s^{-1}) and incline (%) followed a sequence: 0.6 (0 %), 1.0 (0 %), 1.0 (3 %), 1.3 (3 %), 1.4 (6 %), 1.4 (9 %) and 1.4 (12 %). The workload was gradually increased until an HR of approximately 180 $beats^{-1}$ was reached. At this point, exercise was stopped to avoid cardiovascular complications associated with maximal effort, which would be particularly risky in this type of population. During the whole GRAD, ventilatory and gas exchange responses were measured continuously by indirect calorimetry (CPX Express, Medical Graphics Corp, MN, USA). During the exercise test, an electrocardiogram was continuously recorded and displayed online for visual monitoring whereas HR was measured with a dedicated monitor device (Polar Electro Oy, Finland). The flowmeter and gas analyzers of the system were each calibrated with a 3-L calibration syringe and calibration gas (16.00% O_2; 4.00% CO_2). The V'O_2peak was estimated for each subject considering the last 20 seconds of the graded exercise test. The substrate oxidation rate was determined from V'O_2 and V'CO_2 values determined during the last minute of each workload level (ACHTEN et al. 2002)using the following equations (Frayn 1983):

Fat oxidation rate (g min^{-1}) = 1.67 × V′O_2 (l mim^{-1}) - 1.67 × V′CO_2 (l mim-1) - 0.307 × Pox

Carbohydrate oxidation rate (g min^{-1}) = 4.55 × V′CO_2 (l mim^{-1}) - 3.21 V′O2 (l mim-1) - 0.459 × Pox

where Pox is the protein oxidation rate. The protein oxidation rate was estimated by assuming that protein oxidation contributed approximately 12 % of resting energy expenditure (Frayn 1983):

Protein oxidation rate (g min^{-1}) = energy expenditure (kJ min^{-1}) × 0.12-16.74 (kJ g^{-1})

The results of the graded exercise test were used to compute the relationship between substrate oxidation and exercise intensity, expressed as %V'O_2 peak, according to (Lazzer et al. 2017b). Before and after the training program, the graded exercise test was performed following the same protocol.

Energy expenditure and substrate oxidation rate during submaximal exercise

As described previously, all the volunteers were randomly split into two groups: 10 adolescents participated in COMB training and 11 adolescents participated in a MICT program. To ensure full recovery after the GRAD test, submaximal testing took place two days after the GRAD test. All the participants arrived at the laboratory after a 12-hour overnight fast. COMB and MICT were designed to have equal amounts of energy expended. Both COMB and MICT exercises were performed on a motorized treadmill (TechnoGym, Gambettola, Italy). The COMB training test included a 10-minute rest period in a standing position on a treadmill, followed by ~36 minutes of walking with 3 repetitions of 2 minutes at a high intensity (95% of V'O_2 peak) followed by ~30 minutes of MICT (60% of V'O_2 peak). The MICT exercise test comprised a 10-minute rest period in a standing position on a treadmill, followed by about 45 minutes of walking at maximal fat oxidation rate intensity previously determined individually during a GRAD test.

During the submaximal tests, V′O_2 and V′CO_2 were measured continuously (CPX Express, Medical Graphics Corp, MN, USA) during the rest and exercise periods. According to Lazzer et al. (Lazzer et al. 2017b), the substrate oxidation rate was calculated over consecutive 5-minute periods using the equations of (Frayn 1983). Energy supply (kJ min^{-1}) during exercise was calculated as the sum of each substrate oxidation rate (g min^{-1}) multiplied by the appropriate conversion factor (carbohydrate and protein = 16.7 kJ g^{-1}; fat = 37.7 kJ g-1). During the exercise tests an electrocardiographic record was performed continuously and displayed online for visual monitoring, and HR was measured with a dedicated monitor device (Polar Electro Oy, Finland).

Statistical analyses

Data were analyzed using GraphPad Prism version 9.5.1 (IBM, Chicago, USA), with significance set at $P < 0.05$. All results are expressed as mean and standard deviation (SD). Shapiro-Wilk was used to verify the normal distribution of the data. Sphericity was verified by Mauchly's test.

If the sphericity assumption was violated, the Greenhouse-Geisser correction was applied. To assess training adherence and the total duration of the training, unpaired Student's t-tests were used. Anthropometric characteristics, body composition, $V'O_2$ peak, training characteristics, BMR, energy expenditure, and substrate oxidation during submaximal tests were analyzed with a 2-way ANOVA or a general linear mixed model that included the between-subjects group factor (COMB or MICT) and the within-subjects time factor (week 0 *vs.* week 3, i.e., repeated-measures analysis). Post-hoc comparisons were performed using the Bonferroni procedure for significant differences. The same analyses were used for the substrate oxidation rate during the GRAD test, adding the % of $V'O_2$ peak as a fixed factor to examine differences in substrate oxidation (i.e., carbohydrate and fat) rates in response to COMB or MICT training separately. A three-way ANOVA or a general linear mixed model (2 groups x 2-time points x 8 stage measurements) was conducted to examine differences in substrate oxidation rates during the test between the COMB and MICT groups. Finally, the corrected effect size (ES) was calculated for pre-post differences between COMB and MICT (Faulkner and Belin de Chantemèle 2018). An ES < 0.20 was considered small, and 0.50 was considered large (Cohen 1988). To estimate the sample size a priori, power analysis of 10 participants per group with an F test for repeated-measures ANOVA with a statistical power of 0.80, a probability a level of 0.05, and an effect size f of 0.45 (G-Power software, v. 3.1.9.2, Universitat Kiel, Kiel, Germany) resulted in a predicted 12% improvement in the $V'O_2$ (Lazzer et al. 2017b)

4.3 Results

Anthropometric characteristics and body composition

At W0, no significant differences were found between the two groups for age, BM, BMI, FFM, and FM (kg). FM (%) was higher in COMB than in the MICT group (44.0 ± 4.6 *vs*. 37.8 ± 2.1%, group effect, $P = 0.003$, Table 4.1). BMR (MJ die^{-1}) and BMR (MJ kg FFM^{-1} die^{-1}) were lower in COMB than in the MICT group (7.44 ±1.05 *vs*. 8.66 ± 0.78 MJ die^{-1}, group effect $P = 0.023$, and 0.11 ± 0.01 *vs*. 0.13 ± 0.02 MJ kg FFM^{-1} die^{-1}, group effect $P = 0.019$, Table 1), respectively.

Between W0 and W3, V′O_2peak (L min $^{-1}$) and O_2pulse increased significantly only in the COMB group by + 0.28 ± 0.22 L min^{-1} (ES: 0.61, *large*, interaction G x T $P = 0.035$) and +1.62 ± 1.53 ml bpm^{-1} (ES: 0.62, *large*, interaction G x T $P = 0.038$). V'O_2peak expressed in relative values significant increase in the COMB (by 4.76 ± 4.28 ml kg FFM^{-1} min^{-1}, ES: 0.90, *large*, time effect $P < 0.001$) and MICT (by 4.15 ± 4.87 ml kg FFM^{-1} min $^{-1}$, ES: 0.61, *large*, time effect $P < 0.001$), groups. However, HRpeak did not change significantly in both groups (main effect of time, $P = 0.609$) (Table 4.1).

Table 4.1 *Anthropometric characteristics and physical capacities of adolescents before (Week 0) and at the end (Week 3) of the multidisciplinary weight-management program in combined training (COMB) and moderate-intensity continuous training (MICT) groups.*

	COMB (n: 10)		MICT (n: 11)		P		
	Week 0	Week 3	Week 0	Week 3	G	T	G x T
Age (y)	15.7 ± 1.7	15.8 ± 1.7	16.2 ± 1.1	16.3 ± 1.1	0.309	0.001	0.835
Stature (m)	1.75 ± 0.07	1.75 ± 0.07	1.72 ± 0.07	1.72 ± 0.07	0.667	0.165	0.230
Body mass (kg)	117.7 ± 19.8	112.6 ± 19.2*	111.5 ± 14.8	103.2 ± 13.9*	0.340	0.001	0.001
BMI (kg m^{-2})	38.5 ± 5.3	36.9 ± 5.2*	37.1 ± 3.1	34.3 ± 3.0*	0.335	0.001	0.001
Fat-free mass (kg)	65.4 ± 7.9	64.7 ± 7.5	69.2 ± 7.5	65.0 ± 8.4*	0.567	0.001	0.001
Fat Mass (kg)	52.3 ± 13.2	48.0 ± 12.8*	42.4 ± 7.6	38.2 ± 6.0*	0.060	0.001	0.889
Fat Mass (%)	44.0 ± 4.6	42.0 ± 4.8*	37.8 ± 2.1	37.0 ± 2.0	0.003	0.009	0.268
BMR (MJ die^{-1})	7.44 ± 1.05	7.20 ± 1.02	8.66 ± 0.78	8.13 ± 1.02*	0.022	0.012	0.286
BMR (MJ kg FFM^{-1} die $^{-1}$)	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.02	0.13 ± 0.01	0.018	0.564	0.868
V'O_2peak (L min^{-1})	2.52 ± 0.46*	2.81 ± 0.45*	3.60 ± 0.42	3.62 ± 0.36	0.001	0.014	0.035
V'O_2peak (mL min^{-1} kg FFM^{-1})	38.6 ± 5.3	43.3 ± 4.6*	52.2 ± 5.3	56.3 ± 7.5	0.003	0.001	0.773
HRpeak (bpm)	176.0 ± 4.7	176.0 ± 9.1	176.1 ± 6.8	177.8 ± 5.6	0.668	0.609	0.572
O_2pulse (ml bpm $^{-1}$)	14.3 ± 2.5	15.9 ± 2.5*	20.4 ± 2.3	20.4 ± 2.3	0.001	0.040	0.038

All values are presented as mean ± standard deviation.
BMI: body mass index, BMR: basal metabolic rate, V'O_2max: maximal oxygen uptake, HRmax: heart rate max, O_2: oxygen.
G: group effect, T: time effect; GxT: groups x time effect.
**Significantly different from PRE, $P < 0.05$.*

Substrate oxidation rate during the graded test

At baseline, CHO oxidation rates were not significantly different between the COMB and MICT (main effect of group, $P = 0.060$) during the graded test at all exercise intensities (Figure 4.2, panels A and B). Moreover, the CHO oxidation rate, expressed in absolute values, increased with exercise intensity in both groups (Figure 4.2, panels A and B). Fat oxidation rates were lower in the COMB group than in the MICT group at exercise intensities above 35 ± 6% of $V'O_2$peak (main effect of group $P = 0.009$). The maximal fat oxidation rate (MFO) rate was observed at 50 ± 9% of the $V'O_2$ peak in the COMB group (0.32 ± 0.07 g min^{-1}, Figure 4.2 panel C) and at 46 ± 8% of the $V'O_2$ peak in the MICT group (0.41 ± 0.09 g min^{-1}, Figure 4.2 panel D). The MFO, expressed in absolute values, was lower in the COMB group than in the MICT group (0.32 ± 0.06 vs. 0.41± 0.09 g min^{-1}, respectively, $P < 0.001$). At exercise intensities above 66 ± 12% of the $V'O_2$ peak, the fat oxidation rate decreased markedly in both groups, and the contribution of fat oxidation to the energy supply became negligible above 74 ± 6% of the $V'O_2$ peak.

At POST, CHO and fat oxidation rates at all exercise intensities in the COMB and MICT groups were not significantly different from those at W0 (Figure 4.2 panels A, B, C, and D). Exercise intensity corresponding to the MFO rate, expressed in absolute value increased only in the COMB group by 0.04 ± 0.03 g min^{-1} (ES. 0.38, *medium*, $P < 0.001$) (Figure 4.2 panel C and D).

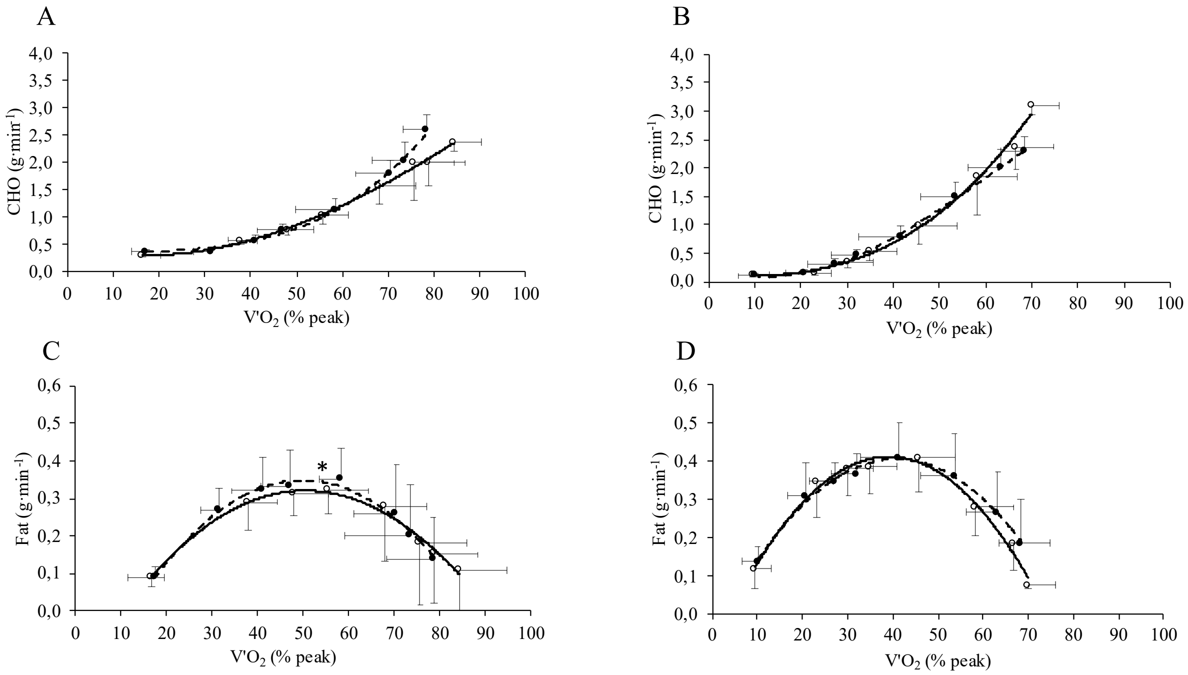

Figure 4.2 *Carbohydrate (CHO, $g\cdot min^{-1}$, panels A and B) and fat ($g\cdot min^{-1}$, panels C and D) oxidation rates as a function of exercise intensity expressed as percent of peak oxygen uptake ($V'O_2peak$) in COMB (panels A and C) and MICT (panels B and D) groups, before (Week 0, opened circle and continuous line) and at the end (Week 3, filled circle and dashed line) of the multidisciplinary weight-management program.*

**Significantly different Week 0 vs. Week 3, $P<0.05$*

Training characteristics

Participants in both groups completed 28 ± 2 sessions of physical exercise. On average, each exercise session lasted less time in the COMB group (35.9 ± 4.6 min) than in the MICT group (45 ± 6 min, $P < 0.001$). At W0, the average HR during the training sessions was 134 ± 7 bpm and 124 ± 6 bpm in the COMB and MICT groups, respectively ($P < 0.010$). HR expressed in relative values, was higher in the COMB group (76 ± 3 %HRmax), than in the MICT group (70 ± 3 %HRmax, group effect $P < 0.001$).

At W3, HR expressed in absolute values did not change in both groups (time effect $P = 0.147$ and $P = 0.170$, respectively). On average, the COMB group performed 17.0 ± 1.9% of the weekly training volume at HIIT and 83.1 ± 1.9% at MICT, whereas the MICT group spent 100% of the weekly training volume at MICT.

Energy expenditure and substrate oxidation rate during the submaximal exercise

At W0, energy expenditures (EEs) were not significantly different during the COMB and MICT exercise (1362 ± 166 *vs*. 1521 ± 256 kJ, respectively, P= 0.066). During the exercise, both groups had similar energy from CHO and protein (Table 4.2). The amount of fat oxidized during exercise was significantly lower in the COMB group than in the MICT group (552 ± 137 *vs*. 831 ± 177 kJ, group effect P =0.002) (Table 4.2).

At W3, EEs, CHO, fat, and protein oxidation rates did not change significantly in the COMB and MICT groups (Table 4.2).

Table 4.2 *Substrate oxidized during Combined training (COMB) and Moderate-intensity continuos training (MICT) exercises before (Week 0) and at the end (Week 3) of the multidisciplinary body weight-management program.*

	COMB (n:10)		MICT (n: 11)		P		
	Week 0	Week 3	Week 0	Week 3	G	T	G x T
Total EE (kJ)	1362 ± 166	1312 ± 184	1521 ± 256	1549 ± 201	0.070	0.912	0.410
EE from CHO (kJ)	644 ± 189	737 ± 211	518 ± 107	563 ± 142	0.117	0.159	0.592
EE from Fat (kJ)	552 ± 137	433 ± 157	831 ± 178	812 ± 221	0.002	0.091	0.168
EE from Protein (kJ)	174 ± 20	165 ± 22	172 ± 30	173 ± 22	0.686	0.541	0.370
CHO (g)	39 ± 11	44 ± 13	31 ± 6	34 ± 9	0.114	0.162	0.592
Fat (g)	15 ± 4	11 ± 5	22 ± 5	22 ± 6	0.002	0.092	0.169
Protein (g)	10 ± 1	10 ± 1	10 ± 2	10 ± 1	0.710	0.532	0.364

All values are presented as mean ± standard deviation.
EE: energy expenditure and CHO: carbohydrate.
G: group effect, T: time effect; GxT: groups x time effect.
**Significantly different from PRE, $P < 0.05$.*

4.4 Discussion

The present chapter showed that 3-week in-hospital multidisciplinary BWRP with COMB or a MICT training program in adolescents with obesity (1) significantly reduced BM and FM in both groups, although more pronounced in the MICT group; (2) maintained FFM only in the COMB group; (3) significantly improved V’O_2peak and O_2 pulse in the COMB group; and (4) determined an increase in MFO during the GRAD test only in the COMB group.

The first important finding was that both COMB and the MICT program helped to reduce BM and FM, by ~5 and 8 kg for BM and ~4 kg in both groups for FM. Although MICT was found to be more effective in reducing BM, both types of training contributed equally to reducing FM. A recent meta-analysis showed that both MICT and HIIT produced similar reductions in BM (i.e., between 2 and 5 kg) kg in adolescents with obesity over an average period of 12 weeks (Liu et al. 2020; Guo et al. 2023). In our study, we observed that a 3-week multidisciplinary BWRP entailing moderate energy restriction, nutritional education, psychological counseling (for all participants), and two different training methods (i.e., COMB and MICT) could determine a similar weight loss in one-third of the time compared with previous data (Liu et al. 2020; Guo et al. 2023). Nevertheless, participants allocated to the COMB or MICT group received the same balanced diets formulated according to the Italian recommended dietary allowances (Nutrition Iso, 1996), it is very difficult to determine the extent to which the different components of the training study such as moderate energy restriction, physical exercise, nutritional education, psychological counseling and the growth process of adolescence explain the reduction in BM and FM. Since most cases of obesity in childhood and adolescence are caused by insufficient physical activity (Men et al. 2023), it is important to reduce BM and FM at a young age with a specific program that includes physical training combined with energy restriction in a specialized institution to (1) reduce the risk of developing obesity in adulthood (Weihrauch-Blüher et al. 2019) and (2) reduce the prevalence of obesity-related diseases such as type 2 diabetes, stroke, coronary heart disease, and cancer (Juonala et al., 2020). From a physiological perspective, COMB and MICT helped in reducing BM and FM may be due to similar improvements in skeletal muscle capacity to increase the glycogen and fatty acid content and utilization during aerobic exercise (Murray and Rosenbloom 2018; Purdom et al. 2018). In the current study, we observed higher fat oxidation (i.e., 41-54%) and lower carbohydrate oxidation (i.e., 35-47%) during submaximal exercise in both COMB and MICT groups. Thus, to the best of our knowledge, this study is the first to use COMB training with a polarized approach in a cohort of adolescents with obesity to reduce BM and FM. In addition, FFM was maintained only in the COMB group, whereas a large BM loss in the MICT group consisted mainly of FFM. The within-group comparison showed that BMR decreased significantly in the MICT group (-6%, ES: 056, *large*) compared with the COMB group (-

2%, ES: 0.21, *medium*). Recent evidence has shown that HIIT can promote the anabolic pathway leading to increased muscle protein synthesis and muscle satellite cell activation in athletes and clinical patients (Callahan et al. 2021), and the COMB group performed a small amount of HIIT (i.e., ~16% of total weekly volume), compared to the MICT group.

The second important finding was that COMB training significantly increased V’O$_2$ peak in absolute and relative values by ~12%, confirming previous results observed in adults with obesity. Indeed, previous studies have shown that a combination of high-volume, low-intensity exercise (i.e., 80% of the overall training volume) and low-volume, high-intensity training (i.e., <20%) (Zapata-Lamana et al. 2018; Poon et al. 2018; D’Alleva et al. 2023b) resulted in equal or better improvements in V'O$_2$ peak compared to HIIT or MICT alone (i.e., improvements ranging between 15% and 25%) in adults with obesity and in highly trained endurance athletes (Stöggl and Sperlich 2014a; Poon et al. 2018; D’Alleva et al. 2023b). Compared to the above-mentioned studies, our study presents two important differences (i) it is the first study using COMB training with a polarized approach in a group of adolescents with obesity, (ii)~~,~~ it was conducted in only 3 weeks, compared to the average of 12-16 weeks of previous studies. Moreover, the results obtained in our study confirmed that the improvements obtained by COMB were similar to those obtained by HIIT (i.e., +12% V’O$_2$peak) in a group of adolescents with obesity (Lazzer et al. 2017b). However, no study to date has compared the effects of COMB and HIIT on V'O$_2$peak in a group of adolescents with obesity. The effect of COMB on improving V’O$_2$peak may be better than that of MICT, due to an increase in factors affecting oxygen delivery and extraction, including stroke volume (i.e., confirmed by an increase in O$_2$ pulse as an indirect marker of central adaptations)(Mezzani 2017), peripheral perfusion and diffusing capacity and skeletal muscle oxidative capacity (Bishop et al. 2019). Nonetheless, we observed positive results for the first time with an a priori manipulation of HIIT and MICT (i.e., ~85% MICT and ~15% HIIT) in the COMB group when applied to adolescents with obesity.

The third finding was that the increase in MFO occurred only in the COMB group (i.e., + 0.04 g min^{-1}, +6%), with no significant differences for CHO and fat oxidation at all exercise intensities in the COMB and MICT groups. Our study is consistent with current scientific literature, as HIIT and SIT, the most effective types of exercise for improving fat oxidation, produce a mean increase in fat oxidation of ~ 0.03 g min^{-1} when applied over a short period of time (i.e., < 4 weeks) (Atakan et al. 2022). Previous studies conducted in adults with obesity showed that MFO increased after a period of MICT (i.e., intensity >70% of V’O$_2$peak) with or without significant weight loss (Nordby et al. 2006; Rosenkilde et al. 2015). However, in our study the MICT group showed a large BM decrease compared to the COMB group, even MFO remained unchanged. There may be a minimum intensity to observe an increase in the absolute value of MFO. In our study, the MICT group trained at an intensity of ~40% of V'O$_2$peak, compared with 60% of V'O$_2$peak in the MICT portion of the COMB group, despite different training volumes. A previous study by our research group showed that HIIT increased fat oxidation more than MICT alone in adolescents with obesity (Lazzer et al. 2017b). Consistent with a recent meta-analysis (Atakan et al. 2022), our study showed that the combination of low-volume HIIT and moderate-volume exercise (i.e., COMB) is effective in improving fat oxidation, metabolic health, and body composition in individuals with obesity (Atakan et al. 2022). The key mechanisms underlying these metabolic adaptations induced by COMB training appear to be mediated primarily in type I fibers (Skelly et al. 2021), which have high intramuscular fatty acid oxidation rates and high mitochondrial content (Bishop et al. 2019; Shaw et al. 2020).

The present study has several limitations. First, although we have reported that 3 weeks of COMB or MICT training combined with moderate energy restriction and nutrition education improves body composition and physical performance, it is difficult to determine the determining factor for body composition improvement because we did not have an inactive control group to rule out the dietary factors. Second, in the present study we did not measure the amount of energy spent in aerobic leisure activity. Therefore, we are unable to know what extent this might have had an impact on improving body composition and physical capacities in our study population. Third, our study was conducted on adolescents with obesity in a specialized institution; therefore, it is not possible to generalize our results to home-based training. Finally, because we compared a priori only one COMB training with MICT, it is difficult to assess whether the combination ratio we chose was indeed optimal or whether there are even better combinations.

In summary, COMB and MICT training helped in improving body composition. In contrast, only the COMB group improved cardiovascular parameters and MFO. Thus, COMB training could be a reasonable alternative to MICT or HIIT to improve body composition and physical capacities in adolescents with obesity. Future studies should examine different combinations of HIIT and MICT (i.e., polarized, pyramidal, or threshold training) over a longer period (12 weeks) and compare them with HIIT and MICT alone to determine the optimal combination of HIIT and MICT.

Chapter 5

Conclusions and practical applications

The main aim of this thesis was to find the "optimal" combination of HIIT and MICT useful to improve the body composition and physical capacities of people with obesity.

Typically, HIIT and MICT were applied in the training routine of individuals with obesity (Andreato et al., 2017; Su et al., 2019; Turk et al., 2017). However, since the lack of time is the principal barrier to physical activity, HIIT could be a time efficient strategy to increased physical activity level and at the same time improve cardio metabolic health of people with obesity (Andreato et al., 2018; Su et al, 2019). Indeed, previous studies have shown that HIIT is at least as effective as MICT in improving body composition and CRF parameters (Andreato et al., 2018; Turk et al., 2017; Vaccari et al., 2020). However, as previously said, our analysis from previous studies shown that performed a combination of MICT (30-40 min/session at 65–70% of HRmax or 90% of the VT1) and HIIT (6-12 min/session at 80-90% of HRmax or 90% of $V'O_2$ peak) (Roxburgh et al. 2014; Zapata-Lamana et al. 2018; Poon et al. 2022) during the same training session or in a weekly training program induced equal or greater effects on body composition, CRF, (Poon et al. 2022;·Roxburgh et al., 2014; Zapata-Lamana et al., 2018) (Roxburgh et al. 2014; Zapata-Lamana et al. 2018; Poon et al. 2022) and substrate oxidation (Zapata-Lamana et al. 2018; Borowik et al. 2020) in lean and obese sedentary adults compared to MICT or HIIT alone with equal volumes or energy expenditures per session. Thus, in the first chapter we observed that COMB training, with a priori percentage og MICT (i.e., 80% of training) and HIIT (i.e., 20% of training), with a POL approach, may raprsent an alternative to HIIT for improving body composition, aerobic physical capacity and MFO in male adults with obesity. Improving $V'O_2$max, increased fat oxidation, while maintaining FFM, as shown in the results of the second chapter, may help patients with obesity in improving their situation to i. increase exercise performance and endurance capacity (Gaesser e Ansaldi, 2021), ii break the vicious circle of sedentary and improving their quality of life (Same et. al., 2016), iii. preventing cardiometabolic obesity disorders (Williams et al., 2015) and long-term weight regain (Vaccari et al., 2020).

Nevertheless, most studies in which endurance training is applied in obese adults showed that i) the exercise intensity of training is given in relation to percentage of HRmax, or $V'O_2$max (Su et al. 2019), while ventilatory thresholds provide better information about endurance training using the concept of TID, typically applied to endurance athletes (Anselmi et al., 2021; Meyer et al. 2005; Stöggl and Sperlich 2015), ii) a paucity of studies have examined the concept of training periodization in adults with obesity (Streb et al. 2021), and iii) endurance training was applied during a limited number of training weeks (i.e., on average 12 weeks) (Su et al. 2019). In this regard, in the second chapter we try to determine the effects of 24 weeks of either POL TID or threshold (THR) TID (i.e., ≥ 30% of overall volume conducted at intensity between or above the GET and RCP) (Campos et al. 2022), modified by Veronique Billat (www.billatraining.com) (Molinari et al. 2020b), with reverse periodization (i.e., volume increased over the months, while intensity is maintained) (Stone et al. 2021) and a personalized approach (i.e., training prescription with HR and speed at individuals ventilatory thresholds) on body composition, V'O_2max, and ventilatory thresholds in healthy adults with obesity. We showed that after the 24 weeks of training POL and THR groups significantly improved their body composition (i.e., BM and FM of ~4 and ~12%, respectively) and aerobic physical capacities (i.e., V’O_2 at ventilatory thresholds and V’O_2max), confirming previous research conducted in recreational and highly trained endurance athletes (Stöggl and Sperlich 2015; Rosenblat et al. 2019). Although, V’O_2 at GET, RCP and V’O_2max expressed both in absolute values or relative to BM, increased similarly in both groups, the effects of POL training in improving V’O_2 values at GET, RCP and V’O_2max was greater than the effect of THR training, as shown by comparing pre-post changes within each group (i.e., ES *large* vs. *medium* for POL and THR, respectively). The high volume in Z1 performed in both groups, exspecially in POL group may have increased type I muscle fibre density (Bathgate et al. 2018) while simultaneously increasing capillary density (van der Zwaard et al. 2021). Indeed, the proportion of type I skeletal muscle fibres and capillary in contacts with type I fibres were positively correlates with RCP (Mitchell et al. 2018). Alternatively, a weekly training routine (i.e., $\geq$ 30% of the overall training volume) conducted below and above the RCP might be very demanding for the THR group and therefore not produce greater adaptations compared to the use of greater volumes in Z1 (Esteve-Lanao et al. 2007). Since peripheral factors limit V’$\dot{O}_2$max in untrained subjects (Wagner 2008), exercise volume may be more effective than exercise intensity in increasing O_2 extraction capacity and mitochondrial content during whole-body maximal exercise in overweight and diabetic male adults (Van Ryckeghem et al. 2022).

In the third chapter, we aimed to evaluate the effects of a 3-week of COMB with a polarized approach (D’Alleva et al. 2023) and MICT alone on body composition, V’O_2 peak, and substrate oxidation rates in adolescents with obesity hospitalized for a 3-week BWRP. As in adults, adolescents with obesity had low levels of physical activity and low CRF (Dupuis et al., 2000). As in the first chapter, we observed that COMB training, with a prioritised proportion of MICT (i.e., 80% of training) and HIIT (i.e., 20% of training) with a POL approach could be an alternative to MICT for improving body composition, aerobic physical capacity and MFO in adolescents with obesity. At the same time, COMB could be a viable alternative to HIIT, which often causes anxiety, fatigue, total mood disturbance (Selmi et al., 2018) in young people. Future studies will be needed to compare COMB (i.e. with different combinations of HIIT and MICT) and HIIT alone in adolescents with obesity.

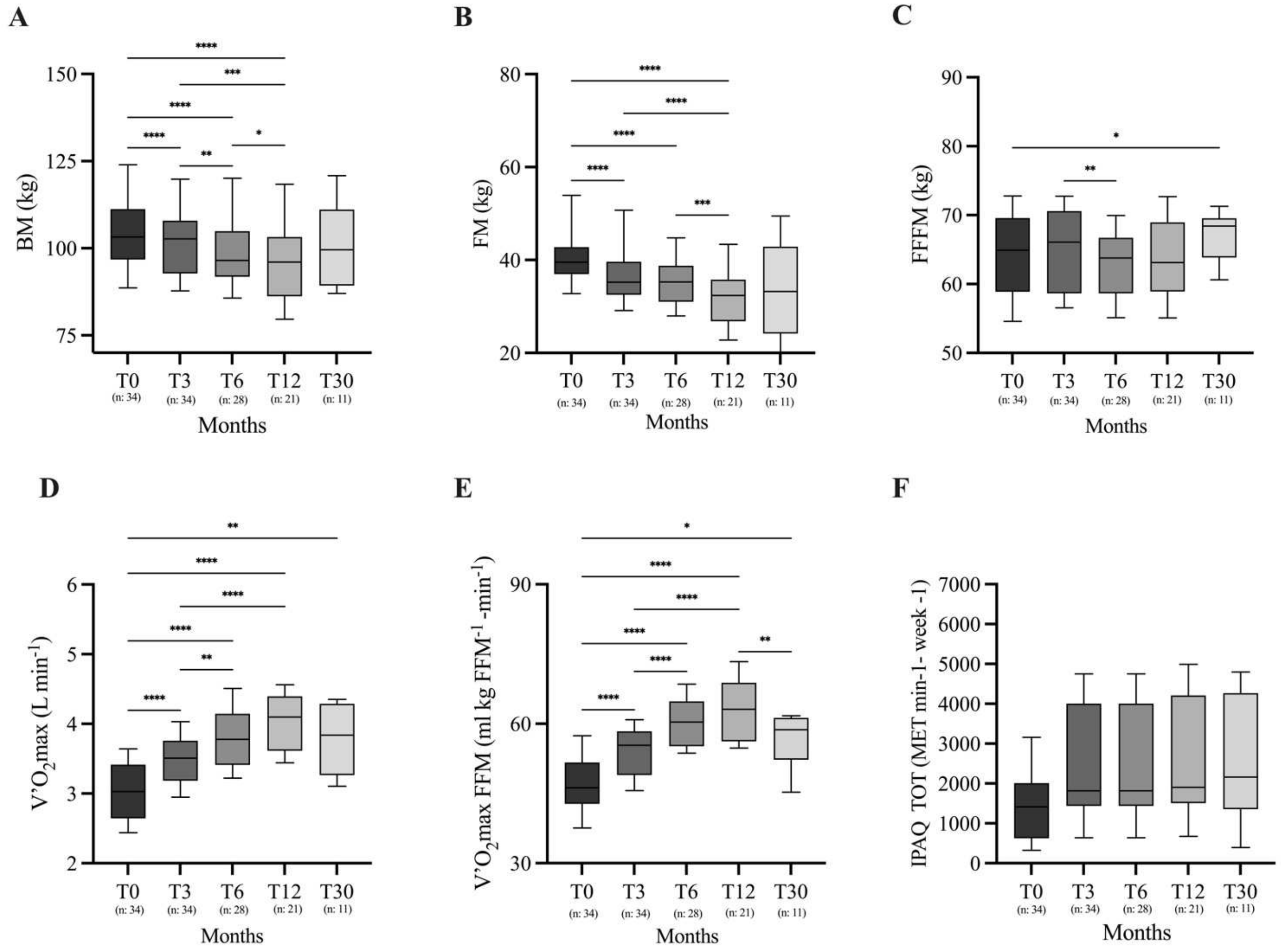

Figure 6.1 Overview of the body mass (BM, panel A), fat mass (FM, panel B), fat-free mass (FFM, panel C), absolute maximal oxygen consumption (V’O$_2$max, panel D), maximal oxygen consumption relative to FFM (V’O2max FFM, panel E) and total weekly physical activity (IPAQ TOT, panel F) of all participants during the twelve months of training and after one sixteen months of follow-up.

*P<0.05
**P<0.01
***P<0.001
****P<0.0001

Follow-up tests took place eighteen months after the end of the second study (T30), which is described in the second chapter. Previous studies have shown that the participation rate in follow-up tests is between 30 and 62% (Collins et al. 2022; Quist et al. 2022). In our case, considering the number of participants as a whole group remaining at the end of the second study (i.e. 21 participants), we have a compliance of 52% at follow-up, without distinction between the experimental groups. Compared to the end of the 12-month training intervention (T12), BM and FM values remained unchanged, although we observed a tendency to increase absolute values by +4 kg and + 3 kg, respectively, without significant statistical differences (Figure 6.1). FFM showed similar values compared to T12 and remained significantly higher compared to baseline (T0) (Figure 6.1). Aerobic physical performance was assessed by V'O_2max and V'O_2max FFM. At T30, V'O_2max remained unchanged in absolute values (L min^{-1}) compared to T12, although we observed a tendency to decrease. In contrast, V'O_2max FFM decreased significantly compared to T12 (-10%, $P < 0.05$), although FFM was maintained. The values of total weekly physical activity assessed with the IPAQ SF remained unchanged at T30 compared to T12 (Figure 6.1). In agreement with previous studies, we have shown that BM and FM were maintained in participants who maintained their V'O_2max (Quist et al., 2022). Indeed, maintaining physical activity levels, without FFM loss, confirming the pivotal role of physical activity in long-term weight regain (Vaccari et al., 2020). In our case, the volunteers who participated in the follow-up exercised 3-4 times per week, combining aerobic activities (e.g. cycling, running and swimming), individual or team sports (e.g. football and padel) and strength training. As both the POL and THR groups performed a higher volume at low intensity (70-90% of weekly training) and all participants in the follow-up test completed low-intensity sessions 2-3 times per week, this may indicate greater long-term efficacy of high volume at low intensity in maintaining cardiovascular fitness and metabolic health, in line with studies on endurance athletes (Campos et al., 2021; Casado et al., 2022).

From a physiological point of view, it appears that training volume, rather than training intensity, may be the key-factor for improvements in mitochondrial content (i.e., peripheral factor of V'O_2max) (Bishop et al. 2014). On the contrary, exercise intensity is a key- factor for enhances mitochondrial respiration (Gibala et al., 2017; Granata et al., 2018). Despite there are a paucity of studies that investigated central of peripheral factor for aerobic capacities improvements in individuals with obesity and considering the pivotal role of mitochondria for carbohydrate and fat oxidation, starting exercise with increasing training volume in individuals with obesity (i.e, below the GET) increases mitochondrial content as well, as seen by previous works (BÆkkerud et al. 2016; Tan et al. 2018). Then after the first months, once individuals have significantly improved their aerobic capacity and skills, it is useful to variate the stimulus inserting HIIT and high intensity exercises during the weekly training (De Feo 2013), with a POL TID. Thus, considering the current guidelines on physical activity in people with obesity, to our knowledge, this thesis could lay the foundation for identifying the optimal dose of MICT and HIIT during the training week

Appendix I

A new field test to estimate the three physiological training zones in adults with obesity.

Adapted from:

D'Alleva, M., Giovanelli, N., Graniero, F., Billat, V., Lazzer, S. A new field test to estimate the three physiological training zones in adults with obesity. Submitted to: Science and sports.

5.1 Introduction

Over the last two decades, the number of people with obesity has tripled worldwide (Inoue et al. 2018); likewise, the number of people with obesity participating in running competitions is increasing (Vincent et al. 2020). Since running has been used as an exercise strategy to manage obesity and improve cardiovascular and metabolic health (Lee et al. 2017) identifying intensity boundaries is essential for prescribing interval and endurance training in athletic and non-athletic individuals.

V'O_2max and ventilatory thresholds have been associated with best endurance performance in competitions from 3000 m to marathon (Thompson 2017). In addition, submaximal and maximal measurements of gas exchange obtained during a graded exercise test (GRAD), have been used to identify the three physiological training zones (i.e., three exercise intensity domains), for prescribing exercise intensity in athletes (Jamnick et al. 2020): zone 1 (z1) represents speed or HR below aerobic threshold (AerT); zone 2 (z2) represents speed or HR between the two ventilatory thresholds; and zone 3 (z3) represents speed or HR above the anaerobic threshold (AnT) (Seiler 2010; Stöggl and Sperlich 2015). Determination of ventilatory thresholds is based on the individual characteristics of each subject based on data from GRAD. Nevertheless, scientists or trainers perform tests with expensive devices, that is not always available in field conditions and requires specific knowledge.

In the last decades, low-cost field tests were developed for evaluation or training prescription. The Cooper test and the Billat test were developed to estimate V'O_2max (Cooper 1968; Billat et al. 1994a), field test protocols were developed to calculate the critical speed (CS) (Galbraith et al. 2014) and submaximal protocols were used to estimate AerT (Forte et al. 2018). However, the above tests i) estimate only one variable at a time, and ii) do not provide an overview of the different intensity domains.

Recently, a new field running test called RABIT® (Running Advisor Billat Training) was developed to assess speed and HR at AerT, AnT, and at V'O_2max using the rate of perceived exertion (RPE) Borg scale with 6-20 range (Giovanelli et al. 2020; Molinari et al. 2020b). The RABIT® test provides a protocol that requires athletes to run a 10-minute step at an easy pace (equivalent to an RPE 11), a 5-minute step at a moderate pace (equivalent to an RPE 14) and 3-minute step at hard pace (equivalent to an RPE 17) divided by 1 minute of passive recovery (Molinari et al. 2020b). The RABIT® test was adapted from (CECI and HASSMÉN 1991) who observed that runners were able to self-regulate their running intensity at three different RPE values (i.e., RPE: 11, 13, and 15). RPE value is closely related to the concept of exercise intensity and how "hard" or "easy" people perceive a physical task (Borg 1970). RPE considers workload including many factors that affect the performance: temperature, humidity, energy supply. Moreover, RPE values are used to define the boundary between moderate, heavy and severe intensity domains (Bok et al. 2022)

Although, the RABIT® test is a simple method to detect submaximal and maximal training zones in recreational runners (Giovanelli et al. 2020; Molinari et al. 2020b), several authors have proposed different RPE values corresponding to the two ventilatory thresholds and $V'O_2max$ in different populations (e.g., obese, and athletic populations) (Seiler 2010c Coquart et al. 2012; Bok et al. 2022). AerT has been observed to be located around RPE ≤13 (Coquart et al. 2012; Hydren and Cohen 2015; Bok et al. 2022), AnT corresponds to RPE values between 14-16 (Coquart et al. 2012; Hydren and Cohen 2015; Bok et al. 2022) and RPE ≥17 is employed for $V'O_2max$ (Seiler 2010; WOOD et al. 2010; Hydren and Cohen 2015).

Therefore, the aim of this chapter was to verify whether the RABIT ® test is a valid field test that can be easily performed by obese participants to identify the three physiological training zones without expensive equipment. In our study, we proposed a modified version of the RABIT ® test, in which the RPE was 13, 16, and 18, while maintaining the original duration of each step.

5.2 Materials and methods

Participants

Thirteen obese male adults (mean age 40.2 ± 4.1 years; mean BM 94.2 ± 10.2 kg; mean BMI 30.9 ± 4.4 $kg \cdot m^{-2}$, mean FM 34.3 ± 6.4 %) were enrolled in the study. All subjects had a complete medical history and underwent a physical examination. BM was stable during the previous two months. All the volunteers were moderately physically active (i.e., performed continuous aerobic activity longer 20 minutes two or three times/week) based on the International Physical Activity Questionnaire Short Form (IPAQ-SF) (Craig et al. 2003). All participants were healthy, had no current injuries and were not taking medications regularly or used drugs known to affect energy metabolism. Participants were informed of the protocol, and written informed consent was obtained.

Experimental Design

Participants performed GRAD and the RABIT test in a randomised order, with 5-7 days of recovery in between. Participants avoid strenuous exercise the day before the test. All tests were performed at the same time of day under similar weather conditions (12.4°C [3.1°C], 51.3% [9.8%] relative humidity) on a 400-m track under medical supervision. The warm-up was the same for all volunteers and consisted of fifteen minutes of easy running followed by three ten-second sprints with one minute of rest in between. Subsequently, participants were equipped with a portable metabolimeter (K5, Cosmed, Italy) to record cardiorespiratory parameters. Since all these participants had already participated in a previous study (D'Alleva et al. 2023a), volunteers were instructed to indicate an RPE value in the last minute of the previously performed GRAD by marking a point on the RPE scale. In addition, after each training session in the previous study, all participants reported their RPE score on the Borg 6-20 scale (D'Alleva et al., 2023).

Measurements

Graded exercise test (GRAD)

During the GRAD test, all the participants were instructed to follow a collaborator with the bike for pacing. The starting speed was ~70% of the speed of their 10.000 m previously run on a 400-m track. The duration of each step was 1 min, and the speed increased by 0.5 km/h every minute until volitional exhaustion. The number of steps completed was between 10 and 15Oxygen uptake (V'O_2), carbon dioxide production (V'CO_2), minute ventilation ($V_{E)}$, and heart rate (HR) were measured during this test using a wearable metabolic unit (K5; Cosmed, Roma, Italy) and a chest strap (Garmin HRMrun, Olathe, USA), respectively. We calibrated the volume and gas analysers before each test using a 3-L calibration syringe and calibration gas (16.00% O_2 and 5.00% C'O_2), respectively. We determined the AerT and AnT with the V-slope method (Beaver et al. 1986). V'O_2max was calculated as the average 30-s V'O_2 according to previously established criteria (Howley et al. 1995): (i) plateau in V'O_2 (i.e., increase < 150 ml/min), (ii) respiratory exchange ratio (RER) > 1.1, and (iii) $\geq 90\%$ of theoretical maximal heart rate.

RABIT test

The test included three incremental exercise stages: 10 min at RPE 13, 5 min at RPE 16, and 3 min at RPE 18 (Figure 5.1). Each step was followed by a 1-min standing rest period. Participants were instructed to 'hold' the target RPE and change their running speed according to the given RPE. The RPE scale could be viewed by participants at regular intervals (i.e., every 200 m). We asked participants to run without the watch to avoid external influences. Throughout the RABIT test, the volunteers wore the K5 and a chest strap (Garmin HRMrun, Olathe, USA) to collect cardiorespiratory and ventilatory parameters, pacing response and HR. Then, we averaged the data from the last minute of each step and compared the first step (RPE 13) with AerT, the second step (RPE 16) with AnT and the third (RPE 18) with maximum values.

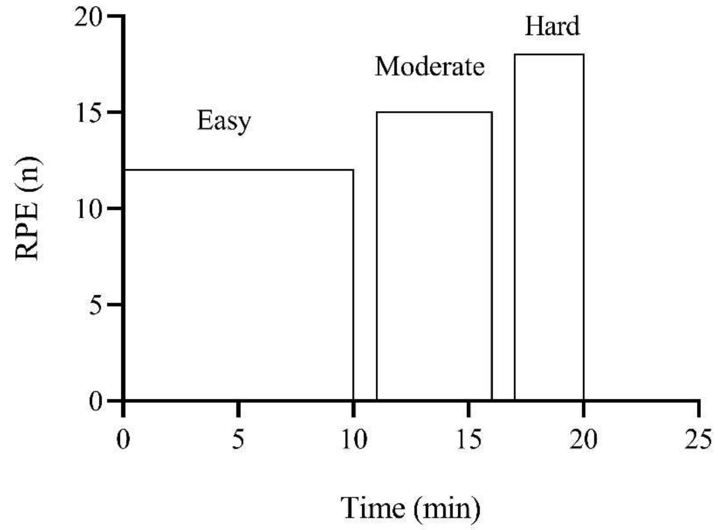

Figure 5.1 *Schematic representations of RABIT ® test. Easy: run ten minutes at 13 of the Borg scale. Moderate: run five minutes at 16 of the Borg scale. Hard: run three minutes at 18 of the Borg scale. Between each step subjects rested for one minute.*

Statistical analysis

The data were analysed using GraphPad Prism (version 9.4.0), with significance set at $p<0.05$. All the parameters (V'O$_2$, RER, V$_E$, HR, running speed) are expressed as means and standard deviations (SDs) for GRAD and RABIT test. The Shapiro–Wilk test was used to evaluate the normality of the data. A Greenhouse–Geisser correction was used in cases of sphericity assumption violations. Only for HR analyses we used data from 9 participants due to technical problem with the chest strap (Garmin HRMrun, Olathe, USA). V'O$_2$, RER, VE, HR and speed measured during the RABIT test at RPE 18, 16 and 13 were compared with AerT, AnT and V'O$_2$max, respectively, obtained during GRAD. A paired T- test was used to compare the results of GRAD and the RABIT test. Identification of the slow component of HR during the RABIT test at RPE 16 was done using a linear regression line and correlation analysis by the method of last squared residuals (Zucccarelli et al., 2018). The Bland-Altman test was used to verify the parameters obtained during the RABIT (Martin Bland and Altman 1986). Statistical significance was set at $P<0.05$. Pearson coefficient was used for the correlation between GRAD and RABIT. The correlation was classified as low ($r= 0.30 – 0.50$), moderate ($r = 0.50 – 0.70$) high and very high ($r = 0.70 – 1.00$) (Atkinson and Nevill 1998). The predictive accuracy of the RABIT parameters was set within ±5% of GRAD parameters and are reported as percentages values (Phang et al. 1990). Effect sizes (ES) were calculated between the parameters of the RABIT and GRAD using the Cohen's d ($0<d<0.20$ small; $0.20<d<0.50$, medium; $0.50<d$, large).

5.3 Results

Maximum parameters

At RPE 18, the values of V’O_2, V_E, HRmax, and maximal running speed were similar to the parameters obtained during the GRAD test (Table 5.1; Figure 5.2A, 2B and 2C; Figure 5.3A). RER max at RPE 18 of the RABIT test was lower by -3.78 ± 3.19% ($P<0.001$) compared to GRAD test. Linear regression between GRAD and RABIT for V’O_2max, HRmax, and maximal running speed were $R^2 = 0.94$ ($P < 0.001$), $R^2 = 0.52$ ($P < 0.030$), and $R^2 = 0.97$ ($P < 0.001$) respectively. The accurate prediction was 85% for V’O_2max, 82% for RERmax, 54% for minute ventilation, 93% for HRmax and 92% for running speed. The ES and 95% confidence intervals (95%CI) were for V’O_2max (ES: 0.09 *small*; 95%CI: -0.50 to 1.75), for RERmax (ES: 1.56 *large*; 95%CI: -0.06 to -0.02), for V_E (ES: 0.20 *medium*; 95%CI: -8.50 to 2.43), for HRmax (ES: 0.09 *small*; 95%CI: -5.71 to 5.30) and for maximal running speed (ES: 0.10 *small*; 95%CI: -0.42 to 0.01).

Anaerobic threshold

At RPE 16, V’O_2, RER, V_E and HR measured during the RABIT test were not significantly different from the corresponding parameters obtained at AnT during the GRAD test (Table 5.1 and Figure 5.2D, 2E, 2F). The running speed was significantly lower in the RABIT test in comparison to GRAD test (-5.03 ± 8.91%, $P = 0.041$) (Figure 3B). Linear regression between GRAD and RABIT tests for V’O_2, HR, and running speed were $R^2= 0.89$ ($P<0.001$), $R^2=0.47$ ($P <0.004$), and $R^2 = 0.82$ ($P < 0.001$), respectively. The accurate prediction was 62% for V’O_2, 67% for RER, 46% for V_E, 78% for HR and 77% for running speed. The ES and 95%CI were for V’O_2 (ES: 0.07 *small*; 95%CI: –1.21 to 2.40), for RER (ES: 0.33 *medium*; 95%CI: -0.05 to 0.01), for V_E (ES: 0.48 *medium*; 95%CI: -15.2 to 2.40), for HR (ES: 0.11 *small*; 95%CI: -7.65 to 6.31) and for running speed (ES: 0.26 *medium*; 95%CI: 1.02 to -0.02). During RPE 16, the slope of the HR versus time regression lines were not significantly different between minutes four and five ($P=0.44$) (Figure 5.4).

Aerobic threshold

At RPE 13, the values of V’O_2, RER, V_E and HR measured during the RABIT test were similar to the corresponding parameters measured at AerT during the GRAD test (Table 1 and Figure 5.2G, 2H, 2I). The running speed during RABIT test was significantly lower than the speed at AerT measured during GRAD test (-7.00 ± 9.64%, $p<0.001$). Linear regression between GRAD and RABIT tests for V’O_2, HR, and running speed were $R^2 = 0.78$ ($P < 0.001$), $R^2 = 0.76$ ($P < 0.001$), and $R^2 = 0.78$ ($P < 0.001$), respectively. The accurate prediction was 46% for V’O_2, 91% for RER, 46% for V_E, 67% for

HR and 62% for running speed. The ES and 95%CI were for V'O$_2$ (ES: 0.12 *small*; 95%CI: –2.87 to 1.43), for RER (ES: 0.01 *small*; 95%CI: -0.03 to 0.01), for V$_E$ (ES: 0.32 *medium*; 95%CI: -8.53 to 3.54) , for HR (ES: 0.01 *small*; 95%CI: -12.54 to 1.43) and for running speed (ES: 0.28 *medium*; 95%CI: -1.04 to -0.10).

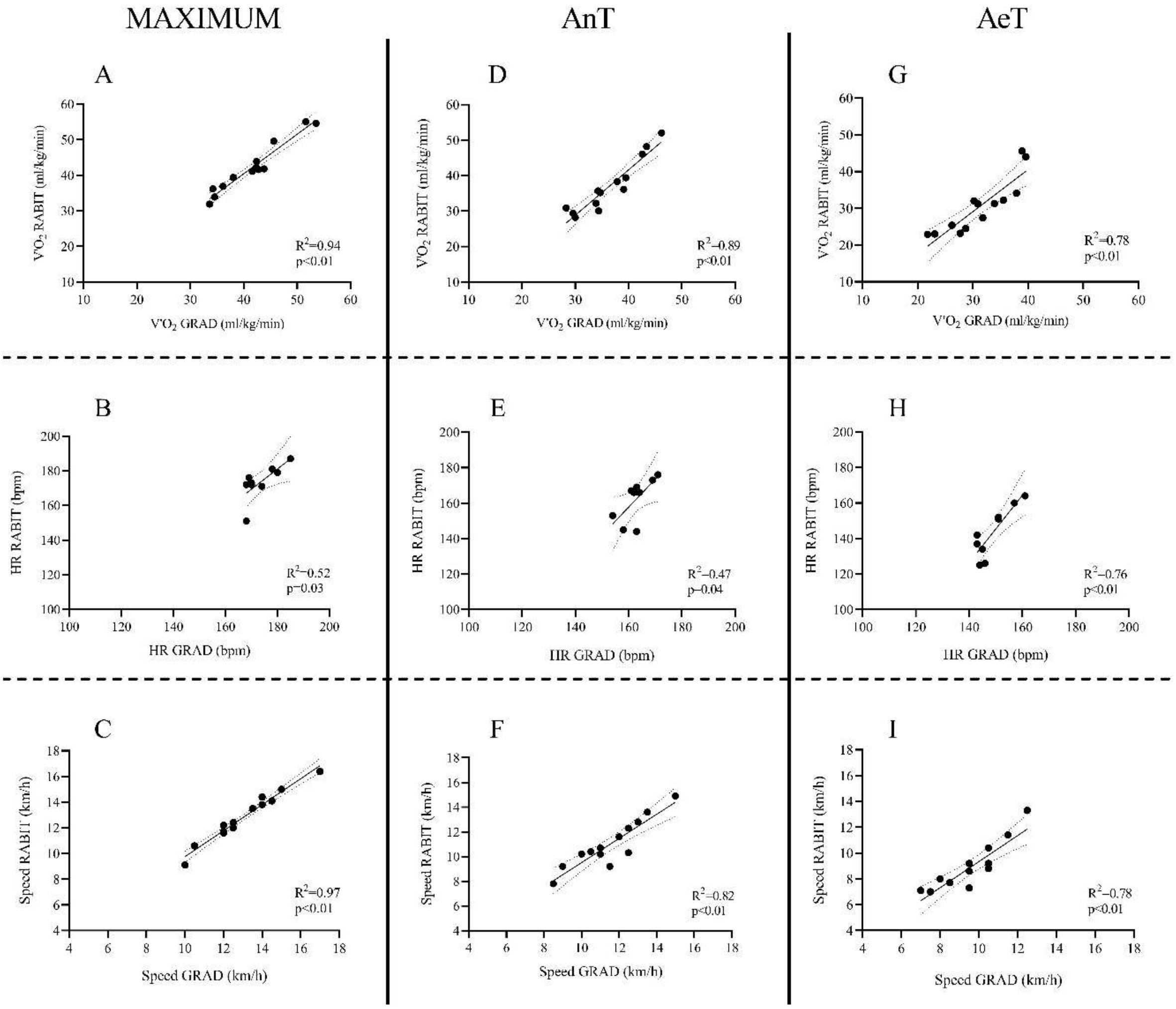

Figure 5.2 *Correlation and confidence intervals (95%) between the results of RABIT vs. GRAD test in the cardiorespiratory parameters at three intensities (Maximum: A, B, C; AnT: D, E, F; AerT: G, H, I). V'O$_2$: oxygen uptake; HR: heart rate; AerT: aerobic threshold; AnT: anaerobic threshold.*

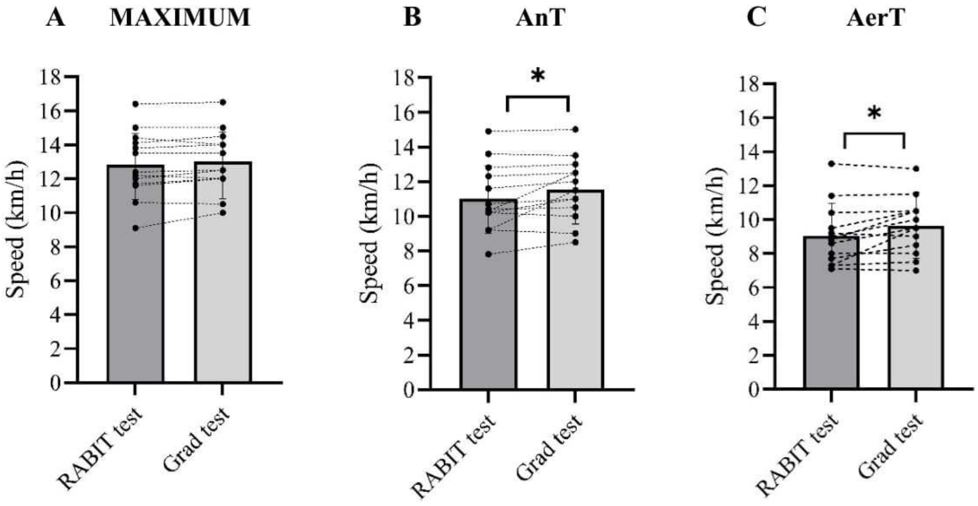

__Figure 5.3__ Individual differences between the speed reached during RABIT vs. GRAD test at three intensities: RPE 18 and Maximum: (panel A), RPE 16 and AnT (panel B), RPE 13 and AerT (panel C). RPE: rating of perceived exertion; AerT: aerobic threshold; AnT: anaerobic threshold.
**P<0.05*

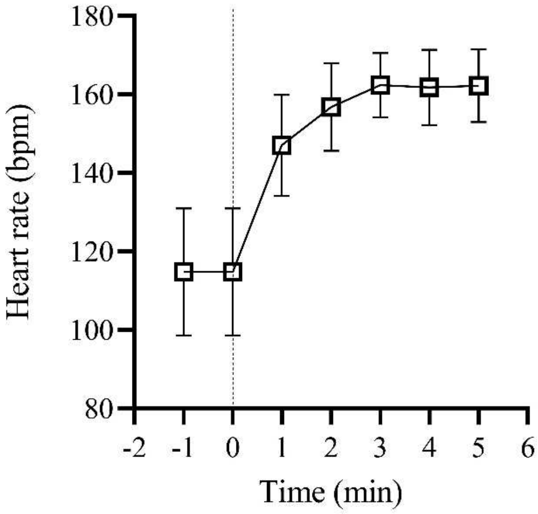

__Figure 5.4__ Mean (±SD) values of heart rate (HR) during RABIT test at RPE 16. Vertical lines indicate that exercise started at time 0. See results for further details.

__Table 5.1__ Parameters obtained during the RABIT test and GRAD test.

	RABIT test	Graded test	Differences (%)	95% Limits of agreement		Accurate prediction	p
Maximum – RPE 18							
Oxygen uptake (ml/kg/min)	42.1 ± 7.2	41.5 ± 6.3	1.2 ± 4.3	-7.2	9.6	85	0.258
Respiratory exchange ratio	1.05 ± 0.02	1.09 ± 0.03	-3,6 ± 3.1	-9.6	2.3	82	0.001
Minute ventilation (L/min)	126.7 ± 16.3	123.6 ± 13.5	2.2 ± 7.6	-12.5	17.1	54	0.250
Heart rate (bpm)	173 ± 10	172 ± 7	-0,2 ± 4.3	-8.5	8.3	93	0.964
Speed (km/h)	12.8 ± 2.0	13.0 ± 1.8	-1,7 ± 3.1	-7.9	4.3	92	0.060
Anaerobic threshold – RPE 16							
Oxygen uptake (ml/kg/min)	37.0 ± 7.6	36.5 ± 5.6	-1 ± 7.7	-5,3	6.5	62	0.489
Respiratory exchange ratio	0.98 ± 0.03	0.99 ± 0.03	-2,1 ± 5.2	-0,1	0.08	67	0.194
Minute ventilation (L/min)	103.8 ± 15.3	97.4 ± 11.0	5.9 ± 15.0	-23.4	35.3	46	0.140
Heart rate (bpm)	162 ± 12	161 ± 6	-0,6 ± 5.8	-18,5	17.1	78	0.831
Speed (km/h)	11.0 ± 2.0	11.5 ± 1.8	-4.9 ± 7.7	-2,1	1.1	77	0.041
Aerobic threshold – RPE 13							
Oxygen uptake (ml/kg/min)	30.5 ± 7.5	31.3 ± 5.8	-3,2 ± 10.6	-7,7	6.3	46	0.478
Respiratory exchange ratio	0.91 ± 0.03	0.91 ± 0.04	-0,1 ± 3.7	-0,07	0.05	91	0.337
Minute ventilation (L/min)	75.2 ± 8.0	72.8 ± 6.9	4.3 ± 13.5	-15.6	22.1	46	0.386
Heart rate (bpm)	143 ± 14	143 ± 7	-4,1 ± 6.6	-23,4	12.3	67	0.104
Speed (km/h)	9.00 ± 1.82	9.60 ± 1.70	-7,6 ± 9.2	-2,4	1.0	62	0.020

All values are expressed as mean ± standard deviation.

[1] *Accurate prediction: percentage of all subjects whose RABIT parameters were within 95% to 105% of GRAD parameters*

5.4 Discussion

The aim of this study was to validate a single-field test to determine the parameters related to $V'O_2max$, AnT, and AerT in subjects with obesity. The main results show that 1) for parameters related to maximal intensity, no significant differences were shown between the two methods, 2) for AnT-related parameters and 3) for AerT-related parameters, the RABIT test showed significantly lower values only for the running speed.

At first, for maximal intensity, we showed similar values of the analysed parameters when the two tests are compared, with an elevated accurate prediction (i.e., ~70% or more). Our results confirm that a 3-min stage duration is sufficient to measure $V'O_2max$ and related parameters in people with obesity, in line with previous studies carried out on athletic and non-athletic populations (Hansen et al. 2016; Giovanelli et al. 2020; Molinari et al. 2020b). Although our participants had previously been familiarised with the RPE Borg scale and were expected to maintain the desired RPE value from the beginning of each phase of the RABIT test, it appears that focusing on the goal of running at a "hard" intensity is more important than focusing on the RPE value itself, as previously observed in studies with different RPE values during the 3-min "hard" step (i.e., the RPE ranged from 15-17) (Giovanelli et al. 2020; Molinari et al. 2020b). Therefore, HR and speed derived from the RABIT test at maximal intensity are useful for training prescriptions. Indeed, at this intensity the speed and HR obtained during the RABIT test were only ~2% and ~0.5% lower than the speed and HR measured during the GRAD test, although for the HR values the 95% limits of agreement were ± ~8% in line with the study of Giovanelli et al., (Giovanelli et al. 2020). Thus, the speed and HR obtained during the RABIT test can be used to provide information on the data related to the maximum intensity and to plan interval training for improving $V'O_2max$ in adults with obesity.

At RPE 16, the RABIT test may also be useful to identify HR and speed corresponding to AnT, albeit with lower reliability compared to maximal intensity. HR obtained by the RABIT and GRAD tests was not significantly different, suggesting that it can be used to monitor and plan training sessions at steady state condition between 30-60 minutes or long interval training sessions (L. V. Billat, 2001). However, Zuccarelli et al. (ZUCCARELLI et al. 2018) reported the presence of a slow component in HR above AerT in patients with severe obesity (i.e., $BMI \geq 40\ kg\ m^{-2}$). However, when comparing HR at the end of four- and five-minutes during RABIT at RPE 16, we did not detect the HR slow component ($p=0.44$). It is possible that the lower degree of obesity and better cardiorespiratory fitness of our participants allowed them to reach the steady state at an intensity close to AnT. In addition, speed corresponding to AnT was significantly lower in the RABIT test by ~5%, despite a predictive accuracy of ~80% and a higher correlation. The difference between the two tests shown in our study was greater than that observed in endurance athletes (i.e., ~3%) (Giovanelli et al. 2020). Previous studies have shown that for 30-60 minutes running or cycling at fixed RPE equivalent to AnT (i.e., RPE 15-17), exercise intensity (i.e., expressed as power output or speed) was ~8% lower during cycling (i.e., not reported during running) in the first 5-7 min of exercise than the power or speed reached during the GRAD test at the same relative intensity (Cochrane-Snyman et al. 2019; O'Grady et al. 2021; O'Malley et al. 2022) . The authors hypothesised that participants changed their velocity or power output to maintain the same perception of effort as the duration of the bout increased. Because RPE values are an integration of several factors including cardiovascular, respiratory, and metabolic variables (Borg 1982), it is possible that the speed or power output at fixed RPE corresponding to AnT decreased, due to growing levels of metabolites accumulation (e.g., H^+ ions), nociceptive stimulation (Mauger 2014) and the work performed by the respiratory muscles compared with the GRAD test. Thus, all the above-mentioned mechanisms were directly involved in the changes in workload (speed) required to regulate load at a constant RPE. From a practical perspective, this difference may be irrelevant, and speed can be used to plan training sessions when HR is not available. At RPE 13, values obtained with the RABIT test were less accurate than at higher intensities. HR showed similar values between the two tests with a higher accurate prediction (i.e., ~ 70%) and higher correlation, suggesting that determination of HR for AerT with the RABIT test at this intensity, which is typically maintained for 150-180 min (Billat, 2001) may be useful for exercise prescription, although the durability of AerT in adults with obesity has never been assessed. Because we only had the HR values of 9 of the 13 participants, these data are promising but should be taken with caution. A larger number of participants and a higher predictive accuracy value could provide further confirmation of our data. Moreover, our results confirm the data observed in trained runners (Giovanelli et al. 2020). The speed corresponding to AerT was statistically different between the two tests. The difference was ~7% between the RABIT and GRAD tests, despite an accurate prediction of ~60% and a higher correlation. However, exercise at RPE 13 is in the moderate intensity domain, where metabolite production equals metabolite clearance and athletes experience fewer sensations such as discomfort and pain (GAESSER and POOLE 1996). Therefore, it is not clear why higher intensity fixed effort exercise appears to be more reproducible than lower intensity fixed effort exercise, in athletic and non-athletic populations (Cochrane-Snyman et al. 2019; Giovanelli et al. 2020; O'Malley et al. 2022). From a practical perspective, the use of HR or speed derived from the RABIT test should be taken with caution. We suggest combining the use of HR and velocity to prescribe moderate-intensity exercise training in adults with obesity.

Limitations

Our study has some limitations. First, although the RABIT test was based on solid premises, the values of speed at RPE 13 and RPE 16 should be used with caution, nonetheless our data showed higher accurate prediction. It could be useful to combine the data of HR and the speed to optimize training intensity prescription. Second, despite the validity of our data, the inclusion of several familiarisation trials could further improve the validity and reproducibility of performance indices during the self-regulated RPE test. Third, since our study was conducted only in male adults with obesity, further studies will be needed to confirm our results in lean and overweight recreational runners.

Practical applications

The results of the present study suggest RABIT test could be useful for detecting the parameters (speed and HR) associated with the exercise training zones outside laboratory environments without the need for expensive $V'O_2$ measurement equipment by simply using the RPE-Borg scale. However, the RABIT test should be used with caution for determining AerT because of the low predictive results. Therefore, for training optimization, we recommend a combination of HR and the speed to obtain more accurate information.

Conclusions

In conclusion, the findings of the present study showed that RABIT is a simply low-cost field test useful for trained adults with obesity to immediately identify the three physical training zones and plan their training sessions. Athletes and coaches must be aware of these results to use this test when it is appropriate.

Appendix II

Improvement of adiponectin in relation to physical performance and body composition in young obese males subjected to twenty-four weeks of training programs.

Adapted from:

Mallardo M, **D'Alleva M**, Lazzer S, Giovanelli N, Graniero F, Billat V, Fiori F, Marinoni M, Parpinel M, Daniele A, Nigro E.

Improvement of adiponectin in relation to physical performance and body composition in young obese males subjected to twenty-four weeks of training programs.

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6.1 Introduction

The global prevalence of obesity is alarmingly increasing worldwide independently of gender, age and race (Boutari and Mantzoros 2022). The development of a different but associated variety of adverse sequelae, including metabolic syndrome, type II diabetes (T2D), dyslipidemia, cardiovascular disease, and different cancers, is making obesity more impactful in terms of morbidity, and mortality worldwide (Strissel et al. 2007). Among the main factors predisposing to obesity there are unhealthy lifestyle, including the lack of physical activity and a sedentary lifestyle (Guzel et al. 2022). On the other hand, there is increasing evidence that physical activity and exercise are broadly as effective as pharmacological interventions in preventing the adverse sequelae (including metabolic syndrome, T2D, cardiovascular diseases, and different cancers) associated with obesity (Su et al. 2019; Poon et al. 2022). A recent meta-analysis evidenced that lifestyle modification, including physical activity, can lead to significant improvement in obesity and inflammation status among overweight/obese individuals (Rahimi et al. 2022). Indeed, both endurance and resistance training programs result a common treatment method for improving the health status of individual that are obese or overweight, and/or with metabolic issues (Van Der Klaauw and Farooqi 2015).

The molecular basis underlying physical activity in the control of weight gain are still not completely known and represent an open field of research. Previous studies showed a direct effect of physical activity on adipose tissue metabolism (Jiang et al. 2022). Adipose tissue is an active endocrine tissue secreting adipokines involved in regulation of different processes such as energy metabolism, and inflammation (Nigro et al. 2014). The endocrine functions of adipose tissue are dysregulated in both overweight and obesity and are crucial in the prevention and/or in pathogenesis of the metabolic diseases (Nigro et al. 2014). Physical activity is able to stimulate the production and secretion of adipokines improving the endocrine functions of adipose tissue ((Minniti et al. 2022). One of the obesity-associated hormones is adiponectin, whose serum levels are inversely related to BMI and insulin resistance. Adiponectin accounts for up to 0.05% of the total serum proteins in circulation, where it can be found in three oligomeric forms, with different molecular weight: trimers of Low Molecular Weight (LMW), hexamers of Medium Molecular Weight (MMW), and oligomers of High Molecular Weight (HMW). These latter elicit the most relevant biological activity of adiponectin, consisting in the homeostatic regulation of glucose levels, lipid metabolism, and insulin sensitivity. Adiponectin levels are highly decreased in obesity and metabolic-related disorders while weight loss leads to increasing adiponectin levels (Nigro et al. 2014).

Whether physical exercise exerts its beneficial effects also through the regulation of adiponectin, regardless of modifications in body composition is still a matter of debate. In addition, it was well known that the type of physical exercise (aerobic/anaerobic) differently influences body composition, cardiometabolic health and hormone balance (Lu et al. 2022). Some studies, performed on patients with obesity and involving high intensity interval training (HIIT) and moderate intensity continuous training (MICT), showed positive changes in patient's body composition, V'O_2max and adiponectin levels ((De Feo 2013; Moghadam et al. 2020), while others demonstrated that adiponectin concentrations do not change after long-term exercise (Mitchell et al. 2019). However, to our knowledge, no studies examined the effects of a Polarized (POL) *vs* a Moderate (threshold training (THR) modified by Veronique Billat) (Molinari et al. 2020a), volume/intensity program on body composition, and cardiometabolic parameters in relation to adiponectin expression. The POL training program consists of high volume of training at moderate intensity (i.e., approximately 80% of the volume) and the remaining 20% conducted at severe intensity domain (Seiler 2010) while the THR training consisted by a higher prevalence of training between heavy and severe intensity domain (i.e., THR with ≥ 20% of overall volume conducted at intensity between the ventilatory thresholds) (Campos et al. 2022).

Thus, the aim of the present study was to determine the effects of 24 weeks of POL and THR training programs on body composition, physical capacities and adiponectin values in saliva and in serum from male adult obese subjects at T0 (before starting the program) and at T1 (at the end of the program). In addition, the adiponectin oligomeric distribution was related to metabolic and fitness features. The two programs were selected in the light of some studies that emphasized how trainings combining the low volume activity of HIIT, and the high volume of MICT (POL) represent a useful strategy to improve body composition, more effective that HIIT or MICT modalities alone (Ryan and Li 2022; Zouhal et al. 2020) We have analyzed saliva since it constitutes a clinically informative, biological fluid containing specific soluble biomarkers. As a biological fluid, saliva offers several advantages over blood: it is easily collected and stored and therefore stress-free for patients; we therefore analysed and compared both biological fluids.

6.2 Materials and methods

Participants and sample collection

Thirteen male subjects with obesity, part of a previous larger study including twenty subjects (under review) were recruited for the present study from the School of Sport Sciences of the University of Udine (mean age 40.8 ± 6.2 years; mean BMI: 32.0 ± 3.0 kg m^{-2}). All subjects had a full medical history, physical and nutritional examination. Body mass (BM) was stable during the previous two months. The inclusion criteria were: 1) age between 18 and 50 years, 2) BMI ≥ 30 kg m^{-2}, and 3) to be moderately physically active (i.e., perform continuous aerobic activity longer than 20 min at least twice a week) based on International Physical Activity Questionnaire Short Form (IPAQ-SF) (Craig et al. 2003). Conversely, exclusion criteria were the presence of cardiovascular, respiratory, neurologic, muscular-skeletal diseases. All participants were male and Caucasians. The study was approved by the Ethics Committee of the Friuli-Venezia-Giulia Region (Italy) (protocol number 1764) and conducted according to the ethical principles of the Declaration of Helsinki. Informed consent was obtained from all participants before the study began.

Before (T0) and after the end (T1) the training period, blood samples were collected after a 12-hours overnight fasting period and centrifuged to collect serum. Serum aliquots were immediately frozen in liquid nitrogen and stored at -80°C. For saliva samples, participants were asked not to consume food and drinks for at least 2.5 hours before 10:30 am. Drinking water was allowed before saliva collection began. Subsequently, participants were asked to brush their teeth (new toothbrushes and toothpaste were provided and they were the same for all donors) and finally to rinse their mouth several times with tap water to avoid any contamination from food residues or toothpaste flavourings. After 1 h from tooth brushing, saliva samples were collected, centrifuged at 13.200 rpm and stored at -80°C for the subsequent analysis. Assessment of anthropometric characteristics together with blood and saliva sample’s collection were conducted at T0 and T1 under medical supervision.

During the 24 weeks of the weight management program, each participant received the same nutritional advice to avoid confounding nutritional variables on the outcomes. All participants completed a 4-days dietary record (4-dDR), collecting the food and beverage consumption of 2-week days and 2 weekend days, at T0 and T1.

Training program

Participants involved in the present study (n: 7 POL and n: 6 THR) performed 3 sessions per week, for 24 consecutive weeks, by walking or running (or a combination of the two methods) in their normal living conditions. The 2 training programs were divided in three 8-week macrocycles, structured as 3+1 mesocycles. In both groups, during the first two 8-week macrocycles, training load (TL) increased by ~30% in the first three weeks of each mesocycles. Between the second and the third 8-week macrocycle, TL increased by ~10%. The last week of each mesocycle was a recovery week. Every 8-weeks, the three zones model was used for the calculation of the training intensity distribution (TID) using the speed reached at gas exchange threshold (GET), respiratory compensation point (RCP) and V'O_2max (Bellinger et al., 2019): zone 1 (Z1), for intensities below GET; zone 2 (Z2), for intensities between GET and RCP; and zone 3 (Z3), for intensities above RCP. To check the POL TID, we used the polarization index (i.e. polarized: Z1 > Z3 > Z2) ((Poole and Jones 2012). POL and THR TID programs were matched for the same TL obtained by the training impulse (TRIMP), where each zone has a weighting factor that is multiplied by the duration in this zone (Lucía et al. 2003). During the 24 weeks of training, all participants recorded their workouts using an online training diary Polar Flow (Polar Electro Oy, Finland) or Gamin Connect (Garmin, Olathe, USA). The training was checked online registering the training sessions: duration, time spent in each endurance training zone, and rate of perceived exertion (RPE) using the Borg 6-20 Scale (Borg 1970). For maintaining the intensity prescribed, the speed in each zone was increased when the mean HR decreased by 5 bpm for two consecutive training sessions.

Anthropometric and adiponectin measurements

The anthropometric and biochemical parameters of the study participants are shown in Table 1. Body mass (BM) was measured with a manual weighing scale (approximation 0.1 kg) (Seca 709, Hamburg, 165 Germany). Measurement of stature was performed using a wall-mounted height board. BMI was calculated as BM (kg)×stature (m^{-2}). Measurement of waist circumference (WC) and Hip circumference (HC) was performed using the method of Kagawa (Kagawa et al. 2008). Body composition assessment was performed using the bioelectrical impedance (BIA, Human IM Plus; DS 171 Dietosystem, Milan, Italy). The values of fat mass (FM) and fat free mass (FFM) were obtained with equations of (Gray et al. 1989). The concentration of total adiponectin in serum and in saliva was measured two times in each individual in triplicate by an enzyme-linked immunosorbent assay

(ELISA) using a polyclonal antibody produced in-house versus a human adiponectin amino acid fragment (H2N-ETTTQGPGVLLPLPKG-COOH) as previously described (Nigro et al., 2015).

Graded exercise test (GRAD)

A graded exercise test on a 400m track was carried out to determine physical capacities of each subject: V'O_2max, heart rate max (HRmax), and ventilatory thresholds. During the test, participants followed a researcher on a bike that gave the pace. The duration of each step was one minute, and the speed increased by 0.5 km/h every minute until the volitional exhaustion. Wearable metabolic unit (K5; Cosmed, Roma, Italy) and a chest strap (Garmin HRMrun, Olathe, USA) were used to measure oxygen uptake (V'O_2), carbon dioxide production (V'CO_2) and heart rate (HR). We calibrated the volume and gas analyzers before every test using a 3-L calibration syringe and calibration gas (16.00% O_2 and 5.00% C'O_2), respectively. GET and RCP were determined with the V-slope method. V'O_2max was calculated as the average 30-s V'O_2 according to the following criteria (Howley et al. 1995): (i) plateau in V'O_2 (i.e., increase <150 ml min−1), (ii) Respiratory Exchange Ratio (RER) > 1.1, and (iii) ≥ 90% of theoretical maximal heart rate.

Western blotting analysis

Serum and saliva samples from all participants were quantified for total proteins by Bradford's method (Bio-Rad, Hercules, CA, USA) and 10 µg of total proteins were treated with 1X Laemmli buffer, heated at 95°C for 10 min and loaded on 10% SDS-PAGE gel as previously described (31). The blots were developed by ECL (Amersham Biosciences, Piscataway, NJ, USA) using Kodak BioMax Light film, digitalized with a scanner (1.200 dpi) and analyzed by densitometry with the ImageJ software (http://rsbweb.nih.gov.ij/). All experiments were performed in triplicate.

Statistical analysis

The data were analysed using GraphPad Prism (version 9.4.0), with significance set at $p < 0.05$. The results obtained were expressed as means and standard deviations (SDs). Shapiro–Wilk test was used for evaluation of the normal distribution of the data. Sphericity was verified by Mauchly's test. Greenhouse–Geisser correction was used in cases of sphericity assumption violations. Anthropometric characteristics, body composition, V′O_2max, ventilatory thresholds, training characteristics and blood analysis parameters were analysed with a student's test for paired data. Bivariate associations were determined by Pearson's or Spearman's correlation coefficients (non-normally distributed data).

6.3 Results

Anthropometric characteristics and cardiometabolic parameters of obese subjects

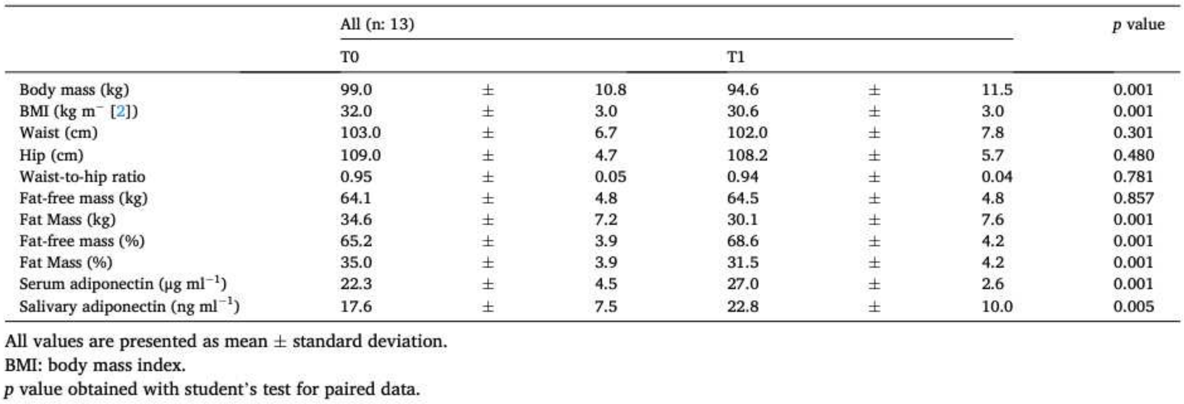
The anthropometric and biochemical parameters of the study participants are shown in the Table 1. All participants were considered together in the pre-post analysis independently from the training because the 2-way ANOVA analysis between subjects performing the two programs (POL and THR groups) showed no significant differences in all considered variables.

At T1, BM and BMI statistically decreased by 4.46 ± 2.90 kg ($P < 0.001$) and 1.43 ± 0.92 kg m^{-2} ($P < 0.001$) (Table 6.1). As well, FFM (%) statistically increased by 3.32 ± 2.12% ($P <0.001$) (Table 6.1). Then, FM (kg) and FM (%) statistically decreased by 4.47 ± 2.78 kg ($P < 0.001$) and by 3.32 ± 2.12 % ($P <0.001$) (Table 6.1). FFM (kg), WC, HC and waist-to-hip ratio did not change significantly (Table 6.1).

Regarding cardiometabolic parameters, at T1, $V'O_2max$ (L min^{-1}) and $V'O_2max$ (L kg^{-1} min^{-1}) statistically increased by 0.20 ± 0.26 L min^{-1} ($P = 0.025$) and 3.96 ± 3.48 ml kg^{-1} min^{-1} ($P = 0.015$) (Table 6.2). HRmax decreased by 4.80 ± 7.37 bpm ($P = 0.040$). Maximal respiratory exchange ratio (RERmax) did not change significantly (Table 6.2). $V'O_2$ (ml kg^{-1} min^{-1}) at RCP statistically increased by mean 3.10 ± 3.54 ml kg^{-1} min^{-1} ($P = 0.008$) (Table 6.2).

No statistical difference was found for $V'O_2$ (L min^{-1}), HR, Respiratory exchange ratio (RER), % of $V'O_2max$ and HRmax under RCP (Table 6.2). $V'O_2$ (L min^{-1}) and $V'O_2$ (ml kg^{-1} min^{-1}) at GET statistically increased by 0.28 ± 0.32 L min^{-1} ($P = 0.008$) and by 4.22 ± 2.96 ml $kg^{-1}min^{-1}$ ($P < 0.001$) (Table 6.2). In addition, no difference was found for HR, RER, % of $V'O_2max$ or HRmax at GET in (Table 6.2).

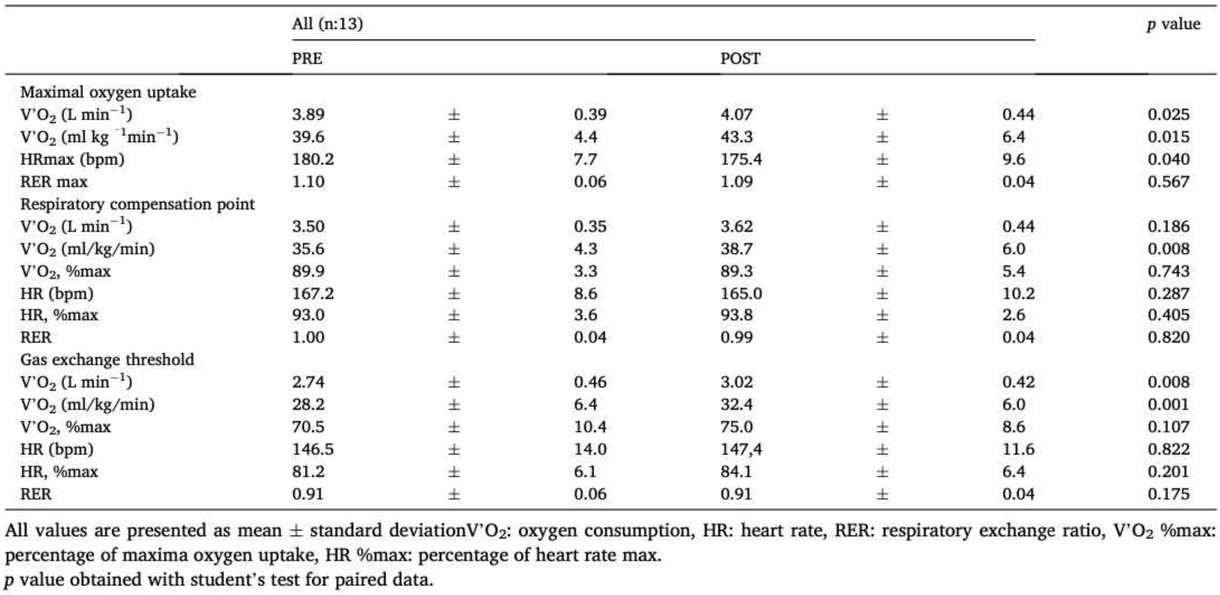
Table 6.1 *Anthropometric characteristics and adiponectin values before (T0), and after 24 weeks (T1) of a training program in both groups.*



	All (n: 13)						p value
	T0			T1			
Body mass (kg)	99.0	±	10.8	94.6	±	11.5	0.001
BMI (kg m^{-} [2])	32.0	±	3.0	30.6	±	3.0	0.001
Waist (cm)	103.0	±	6.7	102.0	±	7.8	0.301
Hip (cm)	109.0	±	4.7	108.2	±	5.7	0.480
Waist-to-hip ratio	0.95	±	0.05	0.94	±	0.04	0.781
Fat-free mass (kg)	64.1	±	4.8	64.5	±	4.8	0.857
Fat Mass (kg)	34.6	±	7.2	30.1	±	7.6	0.001
Fat-free mass (%)	65.2	±	3.9	68.6	±	4.2	0.001
Fat Mass (%)	35.0	±	3.9	31.5	±	4.2	0.001
Serum adiponectin (μg ml^{-1})	22.3	±	4.5	27.0	±	2.6	0.001
Salivary adiponectin (ng ml^{-1})	17.6	±	7.5	22.8	±	10.0	0.005

All values are presented as mean ± standard deviation.
BMI: body mass index.
p value obtained with student's test for paired data.

Table 6.2 *Cardiometabolic Parameters before (T0) and after 24 weeks (T1) of a training program in both groups.*



	All (n:13)						p value
	PRE			POST			
Maximal oxygen uptake							
$V'O_2$ (L min^{-1})	3.89	±	0.39	4.07	±	0.44	0.025
$V'O_2$ (ml kg $^{-1}$min^{-1})	39.6	±	4.4	43.3	±	6.4	0.015
HRmax (bpm)	180.2	±	7.7	175.4	±	9.6	0.040
RER max	1.10	±	0.06	1.09	±	0.04	0.567
Respiratory compensation point							
$V'O_2$ (L min^{-1})	3.50	±	0.35	3.62	±	0.44	0.186
$V'O_2$ (ml/kg/min)	35.6	±	4.3	38.7	±	6.0	0.008
$V'O_2$, %max	89.9	±	3.3	89.3	±	5.4	0.743
HR (bpm)	167.2	±	8.6	165.0	±	10.2	0.287
HR, %max	93.0	±	3.6	93.8	±	2.6	0.405
RER	1.00	±	0.04	0.99	±	0.04	0.820
Gas exchange threshold							
$V'O_2$ (L min^{-1})	2.74	±	0.46	3.02	±	0.42	0.008
$V'O_2$ (ml/kg/min)	28.2	±	6.4	32.4	±	6.0	0.001
$V'O_2$, %max	70.5	±	10.4	75.0	±	8.6	0.107
HR (bpm)	146.5	±	14.0	147,4	±	11.6	0.822
HR, %max	81.2	±	6.1	84.1	±	6.4	0.201
RER	0.91	±	0.06	0.91	±	0.04	0.175

All values are presented as mean ± standard deviation$V'O_2$: oxygen consumption, HR: heart rate, RER: respiratory exchange ratio, $V'O_2$ %max: percentage of maxima oxygen uptake, HR %max: percentage of heart rate max.
p value obtained with student's test for paired data.

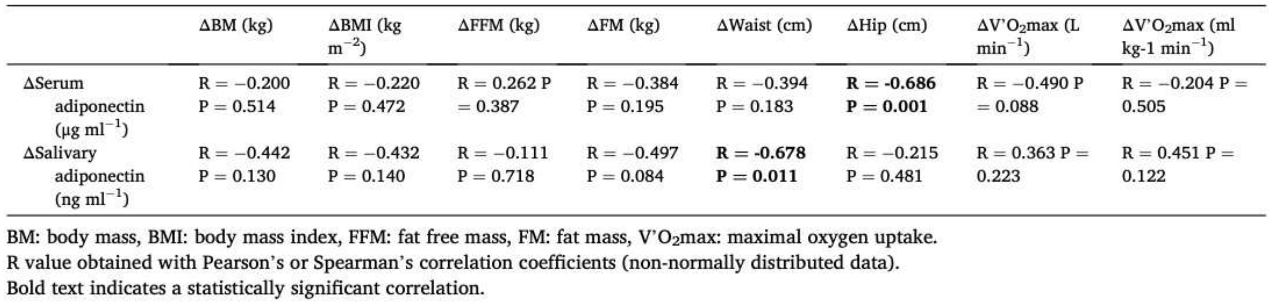
Total Adiponectin levels increase in saliva and in serum of obese subjects undergone training programs.

To investigate whether the circulating adiponectin levels are related to the lifestyle modifications, total salivary and serum adiponectin levels were analyzed adiponectin at T0 and at the end of the training period (T1) (Table 6.1); interestingly, salivary adiponectin were significantly higher in obese subjects at T1 compared to the baseline by +5.22 ± 4.74 ng mL^{-1} ($P < 0.001$).

In line with salivary findings, total serum adiponectin levels, as shown in Table 6.1, are significantly increased by +4.72 ± 3.52 µg mL^{-1} ($P < 0.001$) in obese subjects at T1 compared to T0.

Furthermore, we investigated the potential relationships between the changes in salivary and serum adiponectin and the observed modifications in BM, BMI, FFM, FM, waist, hip, V'O$_2$max (L min^{-1}), and V'O$_2$max (ml kg^{-1} min^{-1}) (see Table 6.3). There were significant correlations between Δ serum adiponectin and Δ Hip (Figure 6.1, panel A) and between Δ salivary adiponectin and ΔWaist (Figure 6.1, panel B).

Table 6.3 *Correlations between the change (Δ) in serum adiponectin and salivary adiponectin with the change in the measured variables.*



	ΔBM (kg)	ΔBMI (kg m^{-2})	ΔFFM (kg)	ΔFM (kg)	ΔWaist (cm)	ΔHip (cm)	ΔV'O$_2$max (L min^{-1})	ΔV'O$_2$max (ml kg-1 min^{-1})
ΔSerum adiponectin (µg ml^{-1})	$R = -0.200$ $P = 0.514$	$R = -0.220$ $P = 0.472$	$R = 0.262$ $P = 0.387$	$R = -0.384$ $P = 0.195$	$R = -0.394$ $P = 0.183$	**$R = -0.686$ $P = 0.001$**	$R = -0.490$ $P = 0.088$	$R = -0.204$ $P = 0.505$
ΔSalivary adiponectin (ng ml^{-1})	$R = -0.442$ $P = 0.130$	$R = -0.432$ $P = 0.140$	$R = -0.111$ $P = 0.718$	$R = -0.497$ $P = 0.084$	**$R = -0.678$ $P = 0.011$**	$R = -0.215$ $P = 0.481$	$R = 0.363$ $P = 0.223$	$R = 0.451$ $P = 0.122$

BM: body mass, BMI: body mass index, FFM: fat free mass, FM: fat mass, V'O$_2$max: maximal oxygen uptake.
R value obtained with Pearson's or Spearman's correlation coefficients (non-normally distributed data).
Bold text indicates a statistically significant correlation.

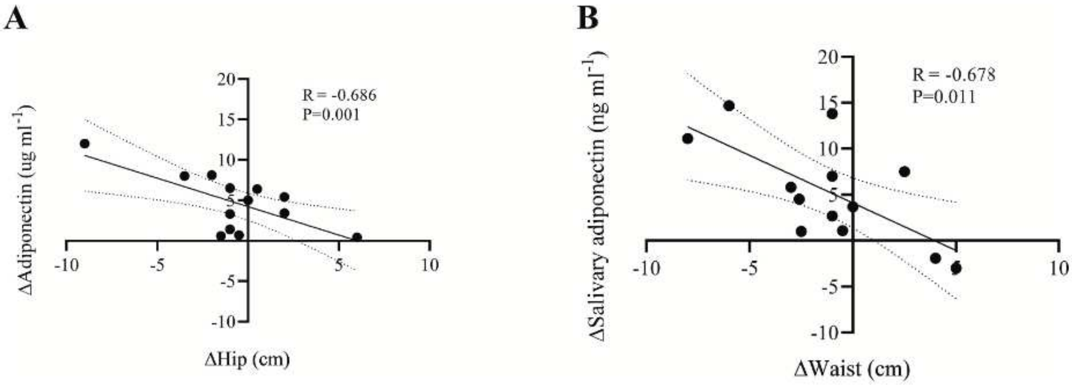


Figure 6.1 *Correlation between changes in Adiponectin (ΔSerum adiponectin) and Hip (ΔHip) (panel A), and correlation between changes in salivary adiponectin (ΔSalivary adiponectin) and Waist (ΔWaist) (panel B).*

HMW Adiponectin oligomers increase in salivary and serum rom obese subjects

Western blotting analysis performed on saliva samples from all subjects involved in the study confirmed that the adiponectin levels are statistically increased in obese subjects (Figure 6.2, panel B). The analysis performed on serum shows three bands corresponding to HMW (≥ 250 kDa), MMW (≥ 180 kDa), and LMW (≥ 70 kDa) oligomers (Figure 6.3, A). Also in serum, data confirmed that the adiponectin levels are statistically increased in obese subjects with a specif regard to HMW oligomers (Figure 6.3, A) as indicated by the densitometric analysis (Figure 6.3, B, $P < 0.01$).

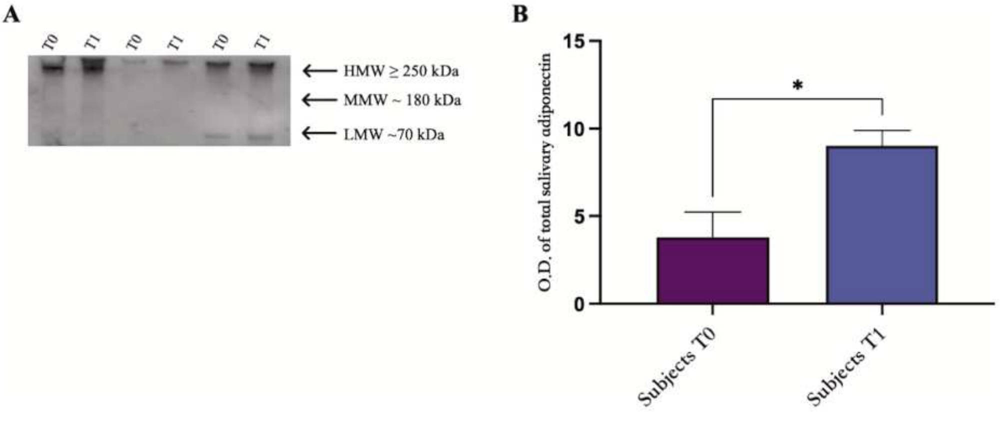


Figure 6.2 *Western blotting analysis of adiponectin oligomers in saliva samples from obese subjects at baseline (T0) and post physical intervention (T1). (A) One representative image of western blot oligomeric distribution [HMW (≥250 kDa), MMW (180 kDa), and LMW (70 kDa)] of three subjects at baseline (T0) and post physical intervention (T1). (B) Graphical representation of pixel quantization of all analyzed subjects included in the study. Pixel quantization was performed by densitometric analysis with the ImageLab software; p.< 0.01*

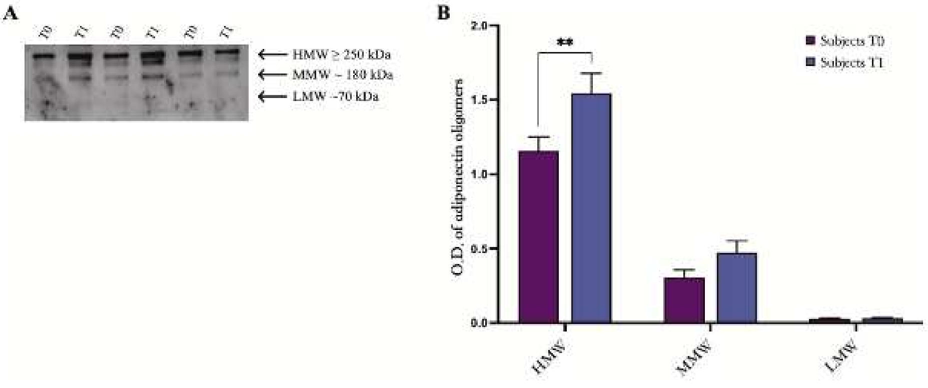


Figure 6.3 *Western blotting analysis of adiponectin oligomers in sera from obese subjects at baseline (T0) and post physical intervention (T1). (A) One representative image of western blot oligomeric distribution [HMW (≥250 kDa), MMW (180 kDa), and LMW (70 kDa)] of three subjects at baseline (T0) and post physical intervention (T1). (B) Graphical representation of pixel quantization of all analyzed subjects included in the study. Pixel quantization was performed by densitometric analysis with the ImageLab software;* $p.< 0.01$.

6.4 Discussion

In the present paper we have determined the effects of two different 24-weeks training programs, a high (POL) *vs* an intermediate (THR) volume/intensity program to verify the potential beneficial effects on salivary adiponectin expression, cardiometabolic capacities and body composition in male adults with obesity. Howevere, given the small number of participants both groups were analysed as a single. Our data shows that a 24-week of POL and THR training program determines in adults with obesity: i. an increase in serum and salivary adiponectin concentrations at T1 compared to T0; ii. an improvement in anthropometric (Fat Free Mass, Fat Mass, BMI, BM) and cardiometabolic parameters (V’O_2max, HRmax). However, the two different training programs did not determine any difference in terms of improvement of body composition and cardiovascular parameters suggesting that even at lower volume, 24 weeks of training is effective in individuals with obesity. Indeed, at the end of the six months training programs, the anthropometric parameters (BMI, FFM, FM, FM) and the cardiometabolic values (V’O_2max both in absolute values or relative to BM, V’O_2 at RCP and GET) improved in both groups. Previously, contrary to our findings, it was found that a POL training induced equal or superior effects on improving V’O_2max and body composition in endurance athletes (Seiler 2010; Stöggl and Sperlich 2014), as well in obese sedentary subjects (Poon et al. 2022; D’Alleva et al. 2023), compared to MICT and HIIT modalities alone. Although a more recent study confirmed that a high-intensity physical exercise exerts more vigorous effects in the regulation of body composition compared to a moderate exercise (Poon et al., 2022), the high volume in Z1 performed in both groups (i.e., ~90% for POL *vs*.~70% for THR), despite the difference between the two groups, may have led to positive adaptations, such as increased of mitochondrial content and respiratory function, as a marker of an enhanced oxidative metabolism (Bishop et al. 2019), closely linked to the values of V'O_2max (van der Zwaard et al. 2016) and V'O_2 at the two ventilatory thresholds (Mitchell et al. 2019). Thus, over a long period of time (i.e., ≥ 24 weeks), may be required a minimum dose of training performing at low intensity for improving body composition and physical capacities in subjects with obesity, in line with studies on endurance athletes (Casado et al. 2022). However, it is also to notice that the lack of different effects between the two training programs might also be traced to: a) the limited number of participants; b) difference in the volume between the two programs was too low; c) timing of the two programs needs to be longer. In addition, as adiponectin concentrations are related to the adiposity changes, being lower in obese subjects and increasing after weight loss, the extent of weight loss following a training program might represent a confounding factor for the direct effects of physical exercise on adiponectin.

A growing body of research indicates that physical activity exerts beneficial effects also through the regulation of the endocrine functions of the adipose tissue. Indeed, it seems that adiponectin secretion is regulated by physical activity and that such event gives a contribution toward a health status of overfat individuals (Da Silveira Campos et al. 2017). However, whether the involvement of adiponectin is dependent on the type and intensity of exercise is still a matter of debate.

In our population, salivary and serum adiponectin levels are significantly increased in obese subjects at the end of both training programs (POL and THR) without significant differences. Contrary to our data, a study on 148 obese adolescents, reported that only an aerobic plus resistance training is effective in increasing adiponectin concentration compared to a resistance training only (Da Silveira Campos et al. 2017). More recently, Moghadam et al., in accordance with our data, reported that either a 12-week high-intensity interval training (HIIT) or a moderate-intensity continuous training (MICT) increased adiponectin (Moghadam et al. 2020).

The duration of the training program might also affect adiponectin regulation; Swisher et al. reported an insignificant increase in serum adiponectin following 12 weeks of moderate-intensity aerobic exercise in women (Swisher et al. 2015). On the contrary, 24-months Mediterranean Diet (MedDiet) and physical activity training in a pre-pubertal population with obesity did not change adiponectin levels (Cobos-Palacios et al. 2022)). On the other hand, in accordance with our data, previously it has been reported that a combined exercise program (resistance and aerobic physical exercise training) of 12 weeks determines an increase in adiponectin levels, with positive correlations with the percent of lean body and negative correlations with percent body fat (Jeon et al. 2013)

Our data outline a correlation between adiponectin expression and both body composition and cardio-metabolic parameters suggesting that the effectiveness of physical exercise in regulating the endocrine functions of adipose tissue are related to these accessory parameters.

Finally, we have evaluated serum and salivary adiponectin in relation to metabolic and fitness parameters. We showed that serum adiponectin did not correlates with $V'O_2\%max$ and inversely correlate with hips circumference; similarly, salivary adiponectin did not directly correlate with $V'O_2\%max$ and inversely correlate with waist circumference. Both models suggest that adiponectin regulation is related to the improvement of body composition in terms of adipose tissue reduction and to the beneficial effects of physical exercise on fitness variables. In line to our data, a very recent paper analysed adiponectin mRNA expression in gluteal and abdominal adipose tissue of overweight and obese subjects in relation to a 6-months physical exercise program; the authors did not find significantly changes in adiponectin or any correlation to the maximal oxygen consumption (Ryan and Li 2022). It is to notice that there are only few papers analysing specifically the effects of a physical exercise program in obese patients, while most of the studies considered a combination of physical activity program together with a diet protocol (Venojärvi et al. 2022). On the contrary, Miyatake et al. (2014) found that circulating adiponectin levels were associated with peak oxygen uptake although the authors examined normal weight subjects (Miyatake et al. 2014). The applicability of this study is also limited due to the lack of a detailed nutritional protocol and by unchecked compliance of the patients. However, our data suggest the usefulness of saliva as a non-invasive and easy to collect biological sample to evaluate cardiometabolic status and body composition status of obese subjects undergone a training program.

In conclusion, our results suggest that a 24-weeks physical training is not only an effective tool in the management of weight but also in the physical fitness in relation to the endocrine activity of adipose tissue as suggested by the enhanced adiponectin levels at the end of the training period. In addition, saliva resulted a valid alternative tool to sera for the evaluation of adiponectin secretion by adipose tissue, allowing a less invasive sample collection. Further studies are needed on a larger cohort of patients and for a longer period.

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