



Effects of 3-month high-intensity interval training vs. moderate endurance training and 4-month follow-up on fat metabolism, cardiorespiratory function and mitochondrial respiration in obese adults

Filippo Vaccari^{1,2} · Angelina Passaro^{3,4} · Andrea D'Amuri³ · Juana Maria Sanz³ · Francesca Di Vece⁴ · Eleonora Capatti⁴ · Benedetta Magnesa¹ · Marina Comelli¹ · Irene Mavelli¹ · Bruno Grassi¹ · Federica Fiori¹ · Giulia Bravo¹ · Alice Avancini⁵ · Maria Parpinel¹ · Stefano Lazzer^{1,2}

Received: 23 December 2019 / Accepted: 25 May 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Purpose The purpose of this study was to investigate, in obese adults, changes in body composition, physical capacities, fat oxidation and ex vivo mitochondrial respiration induced by a 3-month either moderate-intensity continuous training (MICT) or high-intensity interval training (HIIT); afterwards, the patients were followed for four months.

Methods Thirty-two patients (mean age 39 years; mean body mass index [BMI] $36 \text{ kg} \cdot \text{m}^{-2}$) participated in this study attending ~34 sessions of training. At baseline (PRE), at the end of the program (POST) and after follow-up, body composition, peak O_2 uptake ($\text{V}'\text{O}_{2\text{peak}}$) and fat oxidation rate were measured. *Vastus lateralis* biopsies for the evaluation of mitochondrial respiration were performed only at PRE and POST.

Results At POST, body mass (BM) and fat mass (FM) decreased (-6 and -14% , respectively, $P < 0.05$) in MICT and HIIT; $\text{V}'\text{O}_{2\text{peak}}$ increased in both groups ($+6$ and $+16\%$, respectively, $P < 0.05$). Maximal fat oxidation rate increased only after HIIT ($P < 0.001$). Maximal ADP-stimulated mitochondrial respiration normalized by citrate synthase increased ($P < 0.05$) by 67% and 36% in MICT and HIIT, respectively, without significant difference. After follow-up, BM and FM were still lower (-4 and -20% , respectively, $P < 0.050$) compared with baseline in both groups. Only after HIIT, $\text{V}'\text{O}_{2\text{peak}}$ ($+8\%$) and maximal fat oxidation rate were still higher ($P < 0.05$).

Conclusions HIIT was more effective in improving and maintaining $\text{V}'\text{O}_{2\text{peak}}$ and fat oxidation. These results may be relevant for an appropriate prescription of training programs designed to optimize aerobic fitness in obese subjects.

Keywords Obesity · HIIT · Lipid oxidation · Mitochondrial respiration · Aerobic function

Communicated by Philip D. Chilibeck.

✉ Filippo Vaccari
vaccari.filippo@spes.uniud.it

¹ Department of Medicine, University of Udine, P.le Kolbe 4, 33100 Udine, Italy

² School of Sport Sciences, University of Udine, Udine, Italy

³ Department of Medical Science, University of Ferrara, Ferrara, Italy

⁴ Department of Medicine, Azienda Ospedaliera Universitaria di Ferrara, Ferrara, Italy

⁵ Biomedical, Clinical and Experimental Sciences, Department of Medicine, University of Verona, Verona, Italy

Abbreviations

a- $\bar{\text{rO}}_2$ diff	Arteriovenous oxygen difference
BM	Body mass
BMI	Body mass index
CO	Cardiac output
CI	Oxidative phosphorylation complex I
CII	Oxidative phosphorylation complex II
DAP	Diastolic arterial pressure
ETS	Mitochondrial electron transport system
FAT	Lipid oxidation rate during the incremental test
FM	Fat mass
FFM	Fat-free mass
HIIT	High-intensity interval training
HOMA-IR	Homeostasis Model Assessment

HR	Heart rate
IPAQ-SF	International Physical Activity Questionnaire Short Form
MICT	Moderate-intensity continuous training
SAP	Systolic arterial pressure
SF12	Short-Form 12, questionnaire about health-related quality of life
SF12_MI	Short-Form 12, mental index
SF12_PI	Short-Form 12, physical index
SVC	Systemic vascular conductance
V'CO ₂	Pulmonary CO ₂ output
V'O ₂	Pulmonary O ₂ uptake

Introduction

Obesity is a common health problem that is widely spread worldwide and is a major risk factor for type 2 diabetes mellitus, cardiovascular and respiratory diseases, musculoskeletal disorders and some types of cancer (Williams et al. 2015). It is generally accepted that the primary cause of obesity is energy imbalance, i.e. the amount of calories which are introduced is greater than the amount of calories which are utilized. In this context, molecular processes and pathways that directly regulate energy metabolism or caloric intake appear to be feasible targets for therapy. Compared to lean subjects, obese people have an impaired capacity to oxidize lipids (Lanzi et al. 2014), associated with low insulin sensitivity (Kelley and Simoneau 1994) and ease in gaining weight (Zurlo et al. 1990). Further, obesity is a risk factor for cardiovascular and respiratory diseases, musculoskeletal disorders and some types of cancer (Thrush et al. 2013; Devarshi et al. 2017; de Mello et al. 2018).

Optimizing fat oxidation capacity is an important objective both for performance (Hetlelid et al. 2015) and health (Achten and Jeukendrup 2004). Whole-body fat oxidation does not seem to be related with intrinsic mitochondrial oxidative capacity (Nordby et al. 2006; Dandanell et al. 2018), but rather to the amount of lean body mass (Nordby et al. 2006), mitochondrial content (Dandanell et al. 2018) and maximal oxygen uptake (V'O₂max) (Nordby et al. 2006; Hetlelid et al. 2015; Dandanell et al. 2018).

Numerous studies have demonstrated that skeletal muscle metabolism and mitochondrial content/function are impaired in obesity, but there is no general agreement about the issue (Ara et al. 2011; Nair et al. 2011; Fisher-Wellman et al. 2014). Mitochondrial content (Ritov et al. 2005; Larsen et al. 2012), the levels of mitochondrial proteins (Wijngaarden et al. 2013) and their (predominantly nuclear) genes (Mootha et al. 2003; Patti et al. 2003; Ritov et al. 2009; Hwang et al. 2010) have been shown to be reduced in the skeletal muscle of obese individuals, as well as in type 2 diabetes individuals, compared to lean controls. Mitochondrial dysfunction

and impaired enzymatic activity of oxidative phosphorylation complexes have been confirmed more recently in obese skeletal muscle (Formentini et al. 2017; Devarshi et al. 2017; de Mello et al. 2018).

Compared to other methods, high-resolution respirometry in permeabilized muscle fibres, the approach which was utilized in the present study allows a more 'physiological' evaluation of mitochondrial function *ex vivo*. In studies carried out by this method in obese subjects, an impaired muscle mitochondrial metabolism was reported, in terms of mitochondrial respiratory capacity (state 3 respiration) (Vijgen et al. 2013). In obese sedentary adults, moderate-intensity physical activity combined with weight loss was reported to increase the enzymatic activities of the electron transport chain, which occurred without a significant increase in mitochondrial DNA, and was ascribed to an increase in mitochondrial cristae (Menshikova et al. 2007). However, mitochondrial adaptations resulting from different training modalities have been slightly investigated in obese subjects.

Thus, the aim of the present study was to determine the effects of 3 months of either high-intensity interval training (HIIT) or moderate-intensity continuous training (MICT) on body composition, V'O₂peak, fat oxidation, and cardiovascular and mitochondrial functions in healthy obese volunteers.

Greater responses were hypothesized for all variables following HIIT vs. MICT. An evaluation was also performed 4 months after the termination of the interventions, in order to evaluate the maintenance of the observed changes, considering that the weight maintenance after its reduction is a major issue (Donnelly et al. 2009). During the experimental period and follow-up, identical nutritional advices have been provided for both groups in order to avoid confounding nutritional variables on the outcomes.

Subjects and methods

Subjects

Thirty-two obese volunteers (17 males and 15 females) were recruited from the Exercise Physiology Laboratory of the University of Udine, where they underwent a medical and dietetic evaluation. The inclusion criteria were age between 18 and 50 years and body mass index (BMI) ≥ 30 kg m⁻². Subjects who had previously participated in weight management programs had cardiovascular, respiratory, neurologic, muscular-skeletal, metabolic and/or endocrine diseases or those who were taking any drugs known to influence energy metabolism and cardiorespiratory adjustments to exercise were excluded. No subject was taking beta-blockers. The Ethics Committee of the Friuli-Venezia-Giulia Region approved this study (protocol number 1764). Before the study began, the purpose and objectives had been carefully

explained to each subject and written informed consent had been obtained. A physical activity questionnaire was administered to exclude potential volunteers who engaged in any continuous activity longer than 20 min more than once a week, indicative of a moderate physical activity level (IPAQ-SF) (Craig et al. 2003).

Study protocol

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

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

Physical activity

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

All subjects completed 34 ± 0.14 sessions of physical training. The intensity of MICT on the treadmill was set at a heart rate (HR) corresponding to 60% of the initial $\dot{V}O_{2peak}$, the duration of the training session was 44 ± 8 min. HIIT consisted of 10 min of warm up (50% of $\dot{V}O_{2peak}$) followed by 3–7 repetitions of 3 min bouts of high-intensity walking (100% of $\dot{V}O_{2peak}$), interspersed by 1.5 min walking at low intensity (50% of $\dot{V}O_{2peak}$) and followed by 5 min of cool down (50% of $\dot{V}O_{2peak}$); the duration of the training session was 33 ± 4 min. Exercise intensity was set up by adjusting the slope of the treadmill and verifying that the HR values corresponded to the values of 50 or 100% of $\dot{V}O_{2peak}$. When subjects improved their performance capacity and their HR tended to decrease, the slope was increased to ensure that HR reached values previously selected. The amounts of energy expended during the

training sessions were similar for both groups: 20 kJ per kg of fat-free mass (FFM), which corresponds to about 1.5 MJ per session.

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

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

Diet and nutritional education

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

Measurements

Anthropometric characteristics and body composition

The medical history and a physical examination of subjects were taken at the time of admission to the weight management program. Body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm

on a standardized wall-mounted height board. BMI was calculated as $\text{BM (kg)} \times \text{stature}^{-2} \text{ (m)}$. Body composition was measured by bioelectrical impedance (BIA, Human IM Plus; DS Dietosystem, Milan, Italy) according to the method of Lukaski et al. (Lukaski et al. 1986). Fat mass (FM) and fat-free mass (FFM) were calculated with equations derived in obese people either of different ages or BMI (fat-specific formulae) by utilizing a two-compartment model (Gray et al. 1989).

Dietary and physical activity habits

Dietary data were collected using a 4-day dietary (4-DD) record (food diary) given to the subjects in 3 different occasions in order to analyse their eating behaviour before the beginning of the intervention, at the end of the intervention and after the follow-up period. The diaries were given together with instructions on how to record type, quantity and mode of consumption of foods over a 24-h period on four separate days, including one during the weekend. Data extracted from food diaries were analysed using the Microdiet software (V2.8.6, Downlee Systems Ltd., High, Peak, UK) containing the Italian food composition database for epidemiological studies (Gnagnarella et al. 2015), integrated with information from nutritional labels when data were missing, data and the brand were specified in the diary.

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

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

Physical capacities and maximal fat oxidation rate

Peak oxygen uptake ($\dot{V}\text{O}_{2\text{peak}}$) 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. Subjects were asked to avoid strenuous exercise the day before the test and came to the laboratory after a 12-h fasting. Before the beginning of the study, subjects were familiarized with the equipment and the procedures. Each test was undertaken at the same time of the day in the different periods of the study and comprised

a 5-min rest period followed by walking in stages of 4-min duration until voluntary exhaustion. The rates in m s^{-1} and incline in % followed a sequence: 1.11 (0%), 1.11 (3%), 1.39 (3%), 1.39 (6%), 1.53 (6%), 1.53 (9%), 1.53 (12%), 1.53 (13%), 1.53 (15%), 1.53 (18%), 1.53 (21%) and 1.53 (24%) (Lazzer et al. 2017). During the experiment, ventilatory and gas exchange responses were measured continuously by indirect calorimetry (CPET, Cosmed, Italy). The flowmeter and gas analysers of the system were calibrated using, respectively, a 3-L calibration syringe and calibration gas (16.00% O_2 ; 4.00% CO_2). During the exercise test, an electrocardiogram was recorded continuously and displayed online for visual monitoring, and heart rate (HR) was measured with a dedicated monitor device (Garmin, US). $\dot{V}\text{O}_{2\text{peak}}$ was estimated for each subject considering the last 20 s of the graded exercise tests.

The substrate oxidation rate was calculated from $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ values determined during the last minute of each workload level, according to the protocol of Achten (Achten et al. 2002) and using the following equations (Frayn 1983): Fat oxidation rate (g min^{-1}) = $1.67 \cdot \dot{V}\text{O}_2 \cdot \text{min}^{-1} - 1.67 \cdot \dot{V}\text{CO}_2 \cdot \text{min}^{-1} - 0.307 \cdot P_{\text{oxi}}$,

$$\begin{aligned} \text{Carbohydrate oxidation rate (g min}^{-1}\text{)} \\ &= 4.55 \cdot \dot{V}\text{CO}_2 \cdot \text{min}^{-1} \\ &\quad - 3.21 \dot{V}\text{O}_2 \cdot \text{min}^{-1} - 0.459 \cdot P_{\text{oxi}}. \end{aligned}$$

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):

$$\begin{aligned} \text{Protein oxidation rate (g min}^{-1}\text{)} \\ &= [\text{energy expenditure (kJ min}^{-1}\text{)} \cdot 0.12] \cdot 16.74^{-1} (\text{kJ g}^{-1}). \end{aligned}$$

For each subject, the results of the graded exercise test were used to compute the relationship between fat oxidation rate as a function of exercise intensity, expressed as % $\dot{V}\text{O}_{2\text{peak}}$. The best fit was obtained with a polynomial relationship of the second order. The graded exercise test on the motorized treadmill was performed in the same conditions (speed and incline) in the three different periods.

Cardiovascular function

During the graded exercise test on the motorized treadmill, at rest and during the first step at 1.11 m s^{-1} and 0% slope, stroke volume (SV) and cardiac output (CO) were monitored continuously by bioimpedance method (PhysioFlow, Manatec, France), following the procedure described by Charloux et al. (Charloux et al. 2000). This method has been validated during maximal incremental exercises (Richard et al. 2001) and has been utilized also in overweight and obese subjects (Vella et al. 2011). PhysioFlow and

metabolimeter values were synchronized and mean values were calculated every 10 s. Data obtained during the last 20 s of rest and of the first step were taken in account for further analysis. Arteriovenous oxygen difference ($a-v \text{ O}_2 \text{ diff.}$) was estimated by the following equation (Bloos and Reinhart 2009):

$$a - v \text{ O}_2 \text{ diff. (ml O}_2 \cdot 100 \text{ ml}^{-1}) = V' \text{ O}_2 (\text{ml} \cdot \text{min}^{-1}) \cdot \text{CO}^{-1} (\text{ml} \cdot \text{min}^{-1}) \cdot 100.$$

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

Biopsies and mitochondrial respiration ex vivo

Biopsies were obtained from the *vastus lateralis* muscle by percutaneous excision after an overnight fasting. A microneedle (Tru-cut Histocore, 12 G, Biomed Instrument and product GmbH, Germany) was used to collect the specimens; after anaesthesia of the skin using lidocaine (2%), a small incision was made to penetrate skin and fascia. Specimens were put in preserving BIOPS solution (BIOPS: 10 mM EGTA calcium buffer [free Ca^{2+} concentration 100 nmol l^{-1}], 20 mM imidazole, 20 mM taurine, 50 mM $\text{K}^+ / 4$ morpholinoethanesulfonic acid, 0.5 mM dithiothreitol, 6.56 mM MgCl_2 , 5.77 mM ATP and 15 mM phosphocreatine, pH 7.1), containing 10% (wt vol^{-1}) fatty acid-free BSA and 30% (vol vol^{-1}) DMSO at 4°C , then immediately frozen in liquid nitrogen and stored at -80°C until the analysis (Salvadego et al. 2016).

To measure mitochondrial respiration ex vivo, fibre bundles in ice-cold BIOPS solution were quickly cleaned from the connective and fatty tissues, and separated under magnification (MC170 HD, Leica Microsystems, Switzerland, LTD) with a sharp-ended needle, leaving only small areas of contact, and then incubated with $20 \mu\text{g ml}^{-1}$ saponin for 30 min with a continuous gentle stirring to ensure complete permeabilization. After being rinsed twice in respiration medium MiR05 (0.5 mM EGTA, 60 mM potassium lactobionate, 3 mM MgCl_2 , 20 mM taurine, 10 mM KH_2PO_4 , 20 mM HEPES, 110 mM sucrose and 1 g l^{-1} BSA, pH 7.1), permeabilized fibre bundles were measured for wet weight and immediately transferred into the respirometer chambers for O_2 consumption measurements. Measurements were performed by high-resolution respirometry (Oxygraph-2 k; Oroboros Instruments, Innsbruck, Austria) at 37°C (Pesta and Gnaiger 2012) in duplicate using two specimens for each

subject (2–4 mg tissue wet weight in each 2 ml glass chamber). The oxygen levels in the chambers were maintained above air saturation in the range of 300–450 μM (average O_2 partial pressure 250 mmHg) to prevent oxygen limitation of respiration. To this aim, intermittent re-oxygenation steps were performed by adding 0.3 mM hydrogen perox-

ide (MiR05 was added with 280 U ml^{-1} catalase before the measurements). Measurements were run in the presence of 25 μM blebbistatin to prevent ADP-induced contraction (rigor), particularly evident in small length fibres, such as those obtained from biopsy by microneedles (Hughes et al. 2015). A sequential, multiple substrate-uncoupler-inhibitor titration (SUIT) protocol was applied (Pesta and Gnaiger 2012) to measure different respiratory states and substrate/coupling control ratios. Cytochrome C (10 μM) was added to test for mitochondrial outer membrane integrity during the measurements, and only samples demonstrating a negligible damage (i.e. $< 10\%$ increase in respiration after cytochrome C addition) were taken in consideration (i.e. MICT PRE $n = 6$ and MICT POST $n = 8$; HIIT PRE $n = 6$ and HIIT POST $n = 7$). The main mean characteristics of these subgroups were not significantly different from whole group of subjects.

Data were digitally recorded and analysed using DatLab4 software (Oroboros Instruments). Chemicals and reagents were from Sigma (St. Louis, MO, USA).

Citrate synthase activity (CS)

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

25 °C in triplicate. Activity is expressed as mU (nmole/min) per mg of protein.

Statistical analyses

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

The differences on the training adherence between the two groups were analysed by student’s test for unpaired data. Anthropometric characteristics, body composition, $\dot{V}O_{2peak}$ cardiovascular parameters, data derived from questionnaire and food diary, glycolipid metabolism, and CS and mitochondrial function parameters were analysed with a generalized linear mixed, multilevel, growth model, fit by maximal likelihood, which takes into account the subjects as random effects and group (MICT vs HIIT), and gender, time, time² and interaction group $\times$ time as fixed effects. For CS and mitochondrial function parameters, it was not necessary add ‘time²’ since there has been only two time points. The same analyses were applied to the fat oxidation during the exercise, adding the % of $\dot{V}O_{2peak}$ as fixed factor. As a logistic regression, the overall fit of the model was tested using a Chi-square likelihood test. The smaller its deviance [minus twice the log-likelihood ($-2LL$)], the better is the prediction. The model was implemented by adding the random and fixed effects one by one, until the smallest $-2LL$. If, by adding an effect, the $-2LL$ did not become significantly smaller, the effect was not considered. In case of the interaction and the group effects were not significant, the model considered the two groups as one group (Twisk 2006; Wolman et al. 2012).

If a statistical difference was detected in any of the fixed effects, the multilevel models were performed with partial pooling (Gelman et al. 2012). For instance, to break down the interaction group $\times$ time, the analysis was rerun three times to exclude every time one of the three time points; in this way, it was possible to find in which time point the interaction was located. If the gender distribution of the subjects was balanced in the two groups and no gender differences and no interaction between groups were found in the parameters studied, then male and female subjects were considered together. Finally effect size (ES) has been calculated as proposed by Feingold (2009). $ES < 0.20$ was considered small, < 0.50 medium and > 0.50 large as proposed by Cohen (1988).

Results

Adherence to the training program

During the training period, subjects were involved in 34.4 ± 0.2 and 34.8 ± 0.3 training sessions for MICT and HIIT groups, respectively, ($P = 0.811$) without adverse events. The average of energy expended during the training sessions were 23.8 ± 4.1 and 22.4 ± 2.5 kJ kg⁻¹ of FFM for MICT and HIIT groups, respectively, ($P = 0.256$). The duration of each session was greater for MICT (44.3 ± 7.6 min) than for HIIT (33.6 ± 3.6 min, $P < 0.001$, ES 1.78).

Anthropometric characteristics and body composition

At PRE, no significant differences were found between MICT and HIIT for age (37.3 ± 0.6 and 40.1 ± 0.4 years, group effect $P = 0.334$) and stature (1.72 ± 0.11 and 1.71 ± 0.07 m, group effect $P = 0.895$) (Table 1).

At POST, mean weight loss was 5.84 ± 0.15 kg (time effect $P < 0.001$, ES 0.40), BMI decreased by 2.03 ± 0.05 kg m⁻² (time effect $P < 0.001$, ES 0.46), waist circumference decreased by 4.51 ± 0.17 cm (time effect $P < 0.001$, ES 0.39), hip circumference decreased by 4.75 ± 0.16 cm (time effect $P < 0.001$ ES 0.51), FM decreased by 5.37 ± 0.16 kg (time effect $P < 0.001$, ES 0.56) and FFM did not change significantly in MICT and HIIT groups, without differences between groups (interaction $G \times T$ ranging from $0.331 < P < 0.767$).

After 4 months of follow-up (Table 1), body mass increased by 2.15 ± 0.16 kg (time effect $P < 0.015$, ES 0.12) and BMI increased by 0.71 ± 0.05 kg m⁻² (time effect $P < 0.003$ ES 0.13), in MICT and HIIT without differences between groups (interaction $G \times T$ respectively $P = 0.331$ and $P = 0.587$); the values, however, were still significantly lower than at PRE (time effect $P < 0.001$, ES respectively 0.29 and 0.34). Waist and hip circumference and FM did not change significantly during the follow-up period remaining significantly lower than at PRE (time effect $P < 0.001$, ES 0.76). FFM increased significantly during the follow-up period, by 3.18 ± 0.16 kg (time effect $P < 0.001$ ES 0.26) in MICT and HIIT groups, and were not significantly different than at PRE (Table 1).

Dietary and physical activity habits

Dietary habits

At PRE, no significant differences were found between MICT and HIIT groups in energy intake (9509 ± 763 vs. 7720 ± 522 kJ, group effect $P = 0.061$), and carbohydrates

Table 1 Anthropometric characteristic, physical capacities and physical activity habits before (PRE) and after 3 months (POST) of weight management program, and after 4 months of follow-up in moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) groups

	MICT			HIIT			P		
	PRE (n:16)	POST (n:16)	FOLLOW-UP (n:14)	PRE (n:16)	POST (n:16)	FOLLOW-UP (n:12)	Gr	T	Gr × T
Body mass (Kg)	107.1 ± 4.4	101.2 ± 4.5*	103.8 ± 5.4* [‡]	103.5 ± 2.7	97.8 ± 2.5*	98.0 ± 3.0* [‡]	0.790	0.001	0.331
BMI (kg m ⁻²)	36.1 ± 1.3	33.9 ± 1.2*	35.2 ± 1.5* [‡]	35.1 ± 0.9	33.2 ± 1.0*	32.9 ± 0.9* [‡]	0.638	0.001	0.587
Waist (cm)	113.0 ± 3.5	109.4 ± 4.0*	110.5 ± 4.4*	114.1 ± 2.2	108.8 ± 2.1*	107.4 ± 2.2*	0.406	0.001	0.590
Hip (cm)	123.1 ± 2.8	118.0 ± 2.9*	119.9 ± 3.1*	120.5 ± 1.8	116.1 ± 2.2*	113.9 ± 1.5*	0.875	0.001	0.500
FFM (kg)	69.4 ± 3.9	68.6 ± 4.1	71.0 ± 5.0 [‡]	65.1 ± 2.9	64.7 ± 2.7	69.3 ± 3.9 [‡]	0.386	0.001	0.767
FM (Kg)	37.7 ± 2.7	32.4 ± 2.3*	32.5 ± 3.0*	38.4 ± 2.1	32.9 ± 2.5*	28.8 ± 2.0*	0.970	0.002	0.433
HRpeak (bpm)	180.1 ± 0.9	177.2 ± 1.1*	173.0 ± 1.3*	181.0 ± 0.9	176.0 ± 0.5*	178.2 ± 0.83*	0.141	0.001	0.149
V'O ₂ peak (L min ⁻¹)	3.02 ± 0.05	3.19 ± 0.05* [‡]	2.95 ± 0.06* [‡]	2.88 ± 0.04	3.35 ± 0.05* [‡]	3.32 ± 0.06* [‡]	0.288	0.001	0.001
V'O ₂ peak (mL min ⁻¹ Kg ⁻¹ FFM)	43.58 ± 0.39	46.80 ± 0.39* [‡]	42.02 ± 0.51* [‡]	44.26 ± 0.45	51.51 ± 0.40* [‡]	48.04 ± 0.56* [‡]	0.093	0.001	0.001
IPAQ_TOT (MET-min week ⁻¹)	818 ± 220	1481 ± 292*	1965 ± 552*	766 ± 253	1257 ± 208*	2223 ± 507*	0.954	0.001	0.635
IPAQ_VIG (MET-min week ⁻¹)	190 ± 135	547 ± 281*	928 ± 372*	45 ± 45	310 ± 138*	660.0 ± 285*	0.264	0.003	0.779
IPAQ_MOD (MET-min week ⁻¹)	247 ± 102	596 ± 204	384 ± 136	435 ± 243	431 ± 94	1060 ± 423	0.259	0.090	0.275
IPAQ_WALK (MET-min week ⁻¹)	380 ± 127	337 ± 94	653 ± 294	286 ± 99	517 ± 162	503 ± 121	0.948	0.080	0.936
SF12_P1 (pt)	504 ± 1.9	51.4 ± 2.3	52.4 ± 1.8	50.0 ± 2.2	53.1 ± 1.7	52.6 ± 1.6	0.888	0.161	0.976
SF12_MI (pt)	46.3 ± 2.7	49.1 ± 2.2	45.2 ± 2.6	52.4 ± 1.8	50.8 ± 2.2	51.7 ± 2.4	0.282	0.611	0.984

All values are presented as mean ± standard error

BMI body mass index, FM Fat Mass, FFM Fat-free Mass, IPAQ-TOT International Physical Activity Questionnaire, IPAQ-VIG vigorous activity, IPAQ-MOD moderate-intensity activity, IPAQ-WALK physical activity derived from walking, SF12_P1 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

Gr group effect, T: time effect; Gr × T: groups × time effect. For statistical significance, see paragraph results

Significance by generalized linear mixed model (see statistical paragraph): *Significantly different from PRE, $P < 0.05$

[‡]Significantly different from POST, $P < 0.05$; [‡]Interaction Groups × Time (pre—post) effect $P < 0.05$

(45 ± 2 vs. $45 \pm 1\%$, group effect $P=0.953$), lipids (37 ± 2 vs. $36 \pm 2\%$, group effect $P=0.383$) and proteins (16 ± 1 vs. $16 \pm 1\%$, group effect $P=0.663$) contributions to energy intake.

At POST, mean energy intake decreased significantly by -1689 ± 84 kJ day⁻¹ (time effect $P<0.001$, ES 0.62) without difference between groups (interaction $G \times T$ $P=0.244$). Carbohydrates contribution to energy intake did not change significantly, but lipids decreased by $-4.27 \pm 0.29\%$ (time effect $P<0.05$ ES 0.57) and protein increased by $+3.46 \pm 0.15\%$ (time effect $P<0.001$ ES 1.02) without differences between groups.

After the follow-up period, mean energy intake increased significantly by 600 ± 100 kJ day⁻¹ (time effect $P<0.05$ ES 0.51) without differences between groups ($P=0.215$); the values returned similar to those described at PRE (time effect $P=0.098$). Carbohydrates, lipids and proteins contributions to energy intake did not change significantly from POST to follow-up, but lipids remained lower than at PRE (-4% , time effect $P=0.028$, ES 0.47).

Physical activity habits

Physical activity habits, evaluated by the IPAQ questionnaire, were similar between the two groups (MICT and HIIT) at baseline (group effect P values ranging from 0.259 to 0.954; Table 1) and the interactions $G \times T$ were not significant (P values ranging from 0.275 to 0.936; Table 1).

However, after the training period (POST, Table 2), total (IPAQ_TOT) and vigorous (IPAQ_VIG) physical activities increased by 72 and 264% (time effect, respectively, $P=0.011$, ES 0.62 and $P=0.040$, ES 0.77), respectively, in both groups. But, moderate activity (IPAQ_MOD) and physical activity derived from walking (IPAQ_WALK) did not change significantly (time effect, respectively, $P=0.090$ and $P=0.080$) in both groups.

After the follow-up period (Table 2), total (IPAQ_TOT) and vigorous (IPAQ_VIG) physical activities did not change significantly and remained higher than at PRE by 100 (time effect $P=0.001$, ES 1.38) and 168% (time effect $P=0.004$, ES 1.72) in MICT and HIIT groups, respectively. Also, moderate-intensity activity (IPAQ_MOD) and physical activity derived from walking (IPAQ_WALK) after the follow-up period did not change significantly (time effect, respectively, $P=0.080$ and $P=0.090$) in both groups.

The quality of life assessed by the SF12 questionnaire concerning physical and mental indices showed no differences over time in both groups (Table 2).

Physical capacities

Cardiovascular parameters

At rest, $\dot{V}O_2$, CO, SV, SAP, DAP, SVC and $a-v\bar{O}_2$ diff. values were similar between the two groups (MICT and HIIT) at baseline (group effect P values ranging from 0.132 to 0.748; Table 2) and the interactions $G \times T$ were not significant (P values ranging from 0.318 to 0.866; Table 1).

After the training period (POST), SAP decreased significantly at rest by mean -5% (time effect $P<0.001$, ES 0.55) in MICT and HIIT groups, without significant changes for the other parameters (Table 2).

After the subsequent follow-up period, SAP did not change significantly and remained significantly lower than at PRE by mean -5% (time effect $P=0.003$, ES 0.66) in MICT and HIIT groups. Also, DAP decreased significantly compared to POST, by 4% (time effect $P=0.033$, ES 0.50) in MICT and HIIT groups; finally, $\dot{V}O_2$ increased significantly compared to PRE, by 19% (time effect $P=0.039$, ES 1.28) in MICT and HIIT groups (Table 2), while $a-v\bar{O}_2$ diff increased almost significantly between PRE and POST (time effect $P=0.093$) and between POST and follow-up (time effect $P=0.055$).

During walking at 1.11 m s⁻¹ (4 km h⁻¹) and 0% slope ($\sim 40\%$ of $\dot{V}O_{2peak}$), $\dot{V}O_2$, CO, SV, SAP, DAP, SVC and $a-v\bar{O}_2$ diff values were similar between the two groups (MICT and HIIT) at baseline (group effect P values ranging from 0.195 to 0.951; Table 2) and the interactions $G \times T$ were not significant (P values ranging from 0.354 to 0.781; Table 1). Physical training caused a significant decrease in $\dot{V}O_2$ by -7% (time effect $P=0.039$, ES 1.78) in MICT and HIIT groups, and in SAP by -11% ($P<0.001$, ES 0.65) in MICT and HIIT groups. After the follow-up period, $\dot{V}O_2$ did not change significantly and remained significantly lower than at PRE by 12% (time effect $P=0.011$, ES 0.21) in MICT and HIIT groups. SAP increased significantly but remained significantly lower than at PRE by 6% (time effect $P=0.007$, ES 0.95) in MICT and HIIT groups. Finally, both CO and SVC were significantly lower than at PRE by 19% ($P<0.001$, ES, respectively, 0.46 and 0.13) in MICT and HIIT groups (Table 2).

Peak oxygen uptake

At PRE, no significant differences were found between MICT and HIIT for HR_{peak} and $\dot{V}O_{2peak}$ (Table 1).

At POST, HR_{peak} decreased by 3.90 ± 0.20 bpm (time effect $P<0.001$) in MICT and HIIT groups, without difference between groups (Table 1). Absolute $\dot{V}O_{2peak}$ increased by 0.17 ± 0.01 and by 0.46 ± 0.20 L min⁻¹ (time effect $P<0.001$) in MICT and HIIT groups, respectively, with a significantly lower increase in MICT than HIIT ($+6\%$

Table 2 Cardiovascular parameters before (PRE) and after 3 months (POST) of weight management program, and after 4 months of follow-up in moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) groups

	MICT			HIIT			P		
	PRE (16)			FOLLOW-UP (14)			POST (16)		
	PRE (16)	POST (16)	FOLLOW-UP (14)	PRE (16)	POST (16)	FOLLOW-UP (12)	Gr	T	Gr × T
At rest									
V'O ₂ (L min ⁻¹)	0.35 ± 0.02	0.37 ± 0.02	0.39 ± 0.03*	0.37 ± 0.01	0.37 ± 0.02	0.49 ± 0.08*	0.132	0.012	0.318
CO (L min ⁻¹)	6.71 ± 0.33	5.95 ± 0.31	5.97 ± 0.30	7.16 ± 0.30	7.15 ± 0.30	7.09 ± 0.51	0.345	0.113	0.595
SV (ml)	88.82 ± 4.86	84.10 ± 5.63	84.54 ± 4.35	92.98 ± 4.49	96.05 ± 5.76	88.64 ± 6.69	0.158	0.393	0.817
SAP (mmHg)	132.06 ± 2.66	126.13 ± 2.20*	126.15 ± 1.95*	139.56 ± 3.46	131.56 ± 2.08*	128.75 ± 2.47*	0.620	0.002	0.339
DAP (mmHg)	83.88 ± 2.10	82.69 ± 1.36	80.00 ± 2.21†	87.50 ± 2.42	90.38 ± 1.73	86.08 ± 2.03†	0.439	0.002	0.383
SVC (ml min ⁻¹ mmHg ⁻¹)	67.08 ± 2.71	60.68 ± 3.06	62.76 ± 3.44	69.71 ± 3.34	69.28 ± 3.74	70.85 ± 5.26	0.410	0.118	0.463
a-v O ₂ diff. (mL 100 mL ⁻¹)	5.42 ± 0.32	6.05 ± 0.35	5.66 ± 0.59	5.28 ± 0.30	5.45 ± 0.30	6.52 ± 0.60	0.748	0.039	0.866
Walking at 1.11 m s ⁻¹ , 0% slope									
V'O ₂ (L min ⁻¹)	1.27 ± 0.06	1.17 ± 0.06*	0.05*	1.23 ± 0.05	1.15 ± 0.06*	1.08 ± 0.06*	0.431	0.001	0.384
CO (L min ⁻¹)	13.77 ± 0.61	12.08 ± 0.74	10.85 ± 0.37*†	13.31 ± 0.83	14.23 ± 0.72	11.06 ± 0.44*†	0.951	0.001	0.717
SV (ml)	136.60 ± 5.73	134.50 ± 8.92	120.18 ± 7.81	135.11 ± 8.05	147.38 ± 7.73	123.75 ± 6.88	0.611	0.077	0.781
SAP (mmHg)	143.57 ± 3.76	130.00 ± 1.64*	137.86 ± 3.80*†	149.69 ± 4.24	132.50 ± 1.64*	138.75 ± 3.14*†	0.195	0.001	0.354
DAP (mmHg)	85.00 ± 2.01	1.65	81.57 ± 2.12	87.50 ± 2.54	88.44 ± 1.56	85.00 ± 1.51	0.426	0.443	0.777
SVC (ml min ⁻¹ mmHg ⁻¹)	133.11 ± 5.09	122.66 ± 7.78	108.90 ± 4.24*†	126.83 ± 7.43	139.30 ± 7.44	109.47 ± 5.56*†	0.498	0.012	0.762
a-v O ₂ diff. (mL 100 mL ⁻¹)	9.58 ± 0.65	10.17 ± 0.65	10.33 ± 0.51	9.57 ± 0.46	8.65 ± 0.73	10.47 ± 0.83	0.893	0.073	0.481

All values are presented as mean ± standard error

V'O₂ oxygen consumption, CO cardiac output, SV stroke volume, SAP systolic arterial pressure, DAP diastolic arterial pressure, a-v O₂ diff. arteriovenous difference of O₂, SVC systemic vascular conductance

Gr: group effect; T: time effect; Gr × T: groups × time effect

Significance by generalized linear mixed model (see statistical paragraph); *Significantly different from PRE, P < 0.05

†Significantly different from POST, P < 0.05;

and 16%, interaction $G \times T$ $P < 0.001$, ES 0.44, Table 1; Fig. 1a). $\dot{V}O_{2\text{peak}}$ normalized by FFM increased by 3.3 ± 0.2 and by 7.2 ± 0.3 mL min⁻¹ kg⁻¹ FFM (time effect $P < 0.001$) in MICT and HIIT groups, respectively, with a significantly lower increase in MICT than HIIT (+8%, and +16%, interaction $G \times T$ effect $P < 0.001$ ES 0.80, Table 1 and Fig. 1b).

HRpeak did not change significantly during the follow-up period, and remained significantly lower than at PRE (Table 1, time effect $P < 0.001$ ES 0.42). Absolute $\dot{V}O_{2\text{peak}}$ decreased by 0.19 ± 0.02 and by 0.21 ± 0.01 L min⁻¹ (time effect $P < 0.001$ ES 0.19) in MICT and HIIT groups, returning at PRE values in MICT but remaining higher in HIIT (+8%, time effect $P < 0.001$, Table 1 and Fig. 1a). $\dot{V}O_{2\text{peak}}$ normalized by FFM decreased by 4.79 ± 0.14 mL min⁻¹ kg⁻¹ FFM (time effect $P < 0.001$) in MICT and HIIT groups, returning at PRE values in MICT but remaining higher in HI time effect IT (+5%, time effect $P < 0.001$, Table 1 and Fig. 1b).

Fat oxidation rate

Before the training period (PRE), fat oxidation rate during the incremental test was not significantly different between groups (group effect $P = 0.110$). Maximal fat oxidation rate was observed at $41 \pm 1\%$ of $\dot{V}O_{2\text{peak}}$ in MICT (0.41 ± 0.01 g min⁻¹, Fig. 2a) and at $43 \pm 1\%$ of $\dot{V}O_{2\text{peak}}$ in HIIT (0.43 ± 0.1 g min⁻¹, Fig. 2b) groups. At exercise intensities above $60 \pm 1\%$ of $\dot{V}O_{2\text{peak}}$, fat oxidation rate decreased markedly and the contribution of fat oxidation to energy supply became negligible above $80 \pm 1\%$ of $\dot{V}O_{2\text{peak}}$.

After the training period (POST), there was a significant interaction $G \times T$ ($P = 0.016$); in the MICT group, fat oxidation rates were not significantly different from those at PRE at all the exercise intensities (Fig. 2a). On the other hand, the HIIT group exhibited a greater absolute rate of fat oxidation at 60% (+45%, time effect $P = 0.019$; ES 0.99), 70% (+119%, time effect $P < 0.001$; ES 1.67) and almost significantly greater at 80% (time effect $P = 0.076$) of $\dot{V}O_{2\text{peak}}$ (Fig. 2b), whereas at 40 and 50% of $\dot{V}O_{2\text{peak}}$, the values were not significantly different from those at PRE.

After 4 months of follow-up, in the MICT group, fat oxidation rates were still not significantly different from those at PRE at all the exercise intensities (Fig. 2a). On the other hand, the HIIT group exhibited a greater absolute rate of fat oxidation at 60% (+32%, time effect $P = 0.031$; ES 0.69), 70% (+28%, time effect $P < 0.001$, ES 0.40) and 80% (+80%, time effect $P = 0.047$, ES 0.68) of $\dot{V}O_{2\text{peak}}$ (Fig. 2b), whereas at 40 and 50% of $\dot{V}O_{2\text{peak}}$, the values remained not significantly different from those at PRE.

Mitochondrial respiration ex vivo and citrate synthase activity

Results of citrate synthase (CS) activity assays are reported in Table 3. No significant differences were observed after the weight management program compared to the baseline values in both MICT and HIIT groups. Thus, none of the two protocols of exercise training affected CS activity. Conversely, the weight management program with both protocols affected significantly the intrinsic mitochondrial respiration. A CI+CII SUI protocol was applied in order to obtain conditions of electron transport convergent at the Q-junction corresponding to the operation of the TCA cycle and mitochondrial substrate supply in vivo, thereby measuring the maximal oxidative phosphorylation capacity. Specifically, non-phosphorylating resting mitochondrial respiration was measured in the presence of malate (4 mM) and glutamate (10 mM), and in the absence of adenylates so that O_2 consumption was mainly driven by the back leakage of protons through the inner mitochondrial membrane (complex I state 2 respiration or 'leak' respiration). Saturating ADP (5 mM) was then added followed by succinate (10 mM), thereby achieving the maximal ADP-stimulated mitochondrial respiration sustained by complex I and complex II (complex I+II state 3 respiration). Next, electron transport system capacity (complex I+II ETS) was evaluated by the uncoupler protonophore FCCP titration. Rotenone (1 μ M) was then added to inhibit complex I and to evaluate ETS sustained by complex II (rotenone insensitive) and by complex I (rotenone inhibited). The degree of oxidative phosphorylation coupling for a specific substrate supply (glutamate/malate in this case), referred to as OXPHOS coupling, was determined by calculating the ratio between complex I+II state 3 respiration minus complex I leak respiration and complex I+II state 3 respiration [(state 3 – leak)/state 3] (Pesta and Gnaiger 2012; Salvadego et al. 2016). Data of different respiration states and substrate/coupling control ratios are reported in Table 3. In particular, maximal ADP-stimulated mitochondrial respiration (CI+II state 3 respiration) increased significantly (time effect $P = 0.042$ ES 0.86) with respect to the baseline values after the weight management program (POST) in both MICT and HIIT groups. The increase was +51%, without significant difference between the two groups (interaction $G \times T$ was $P = 0.708$). The data dealing with coupling control ratio (OXPHOS coupling) show that they were not affected by the two training protocols. At baseline, the values of the ratio were within 0.69–0.78 and did not change significantly after the weight management program, for both MICT and HIIT. Finally, the capacity of the electron transport system (ETS) uncoupled from the phosphorylating system augmented after the weight management program. Specifically, maximal ETS sustained by substrates of complexes I and II, glutamate/malate and succinate (ETS_CI+CII) increased

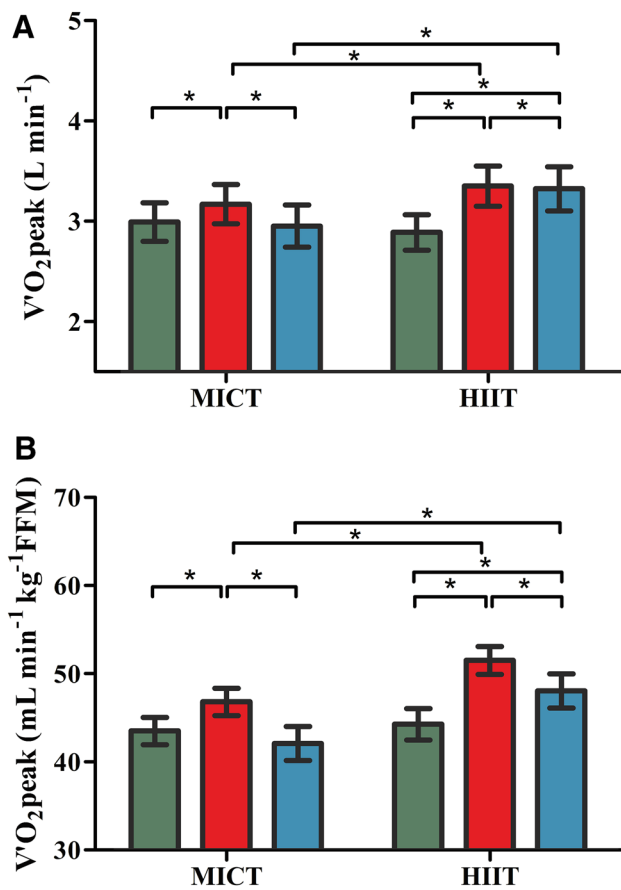


Fig. 1 Absolute peak oxygen uptake ($\dot{V}O_{2peak}$, panel A) and peak oxygen uptake normalized by fat-free mass ($\dot{V}O_{2peak}$ FFM⁻¹, panel B) measured before (PRE, green square) and after 3 months (POST, red square) of weight management program, and after 4 months of follow-up (blue square), in moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) groups. All values are presented as mean $\pm$ standard error. Significance by generalized linear mixed model (see statistical paragraph): *Significantly different, $P < 0.05$

significantly (time effect $P = 0.042$, ES 0.96) as compared with baseline, without significant difference between them (interaction $G \times T$ was $P = 0.953$). In addition, either rotenone-sensitive complex I ETS (ETS_{CI}) or -insensitive complex II ETS (ETS_{CII}) exhibited a pattern similar to that of ETS_{CI} + CII, indicating that both complex I and complex II were upregulated, although their increases vs. the baseline values did not reach the statistical significance.

Discussion

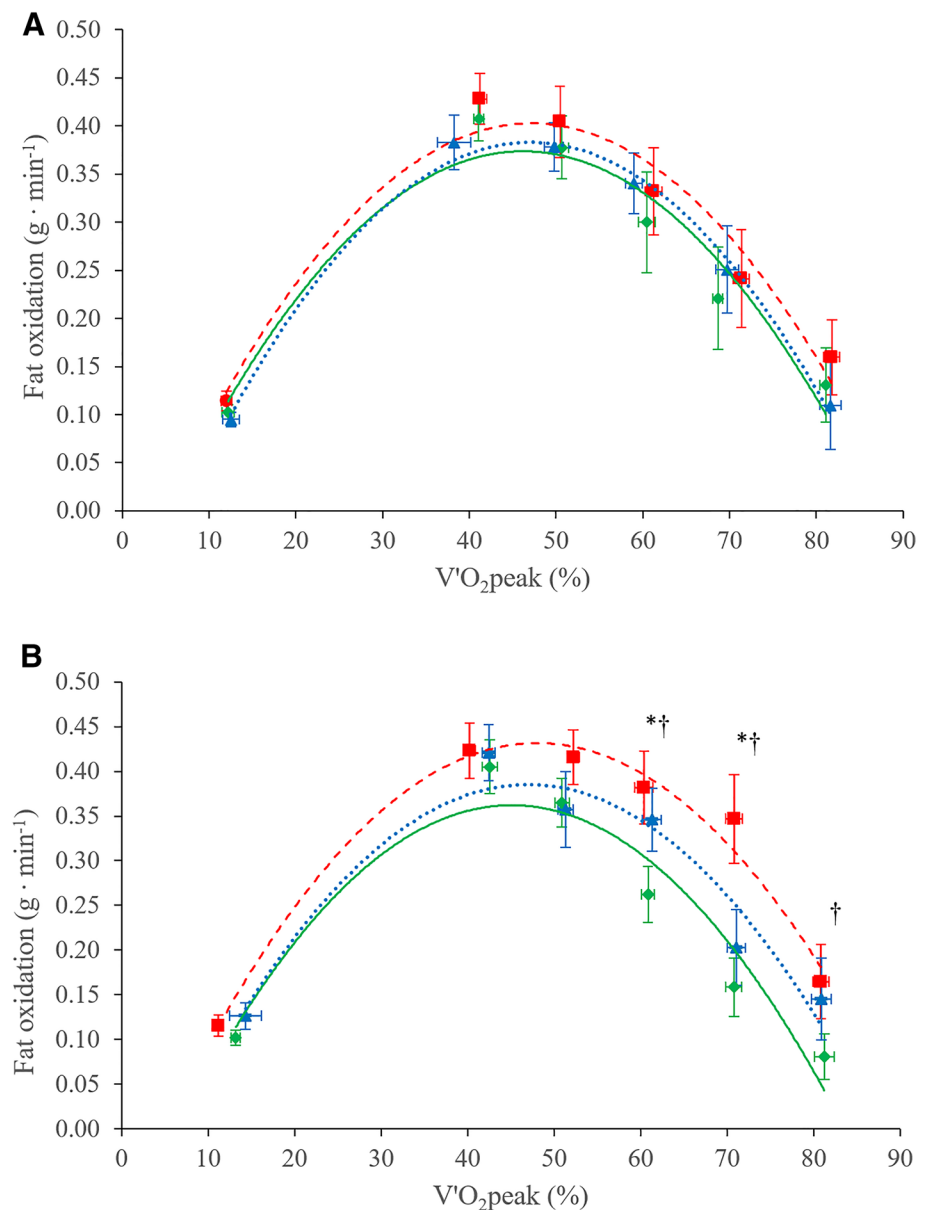
In obese patients, the 3-month weight management program entailing, in terms of exercise, MICT or HIIT, resulted in the following: (1) significant improvement of $\dot{V}O_{2peak}$ in both groups, although more pronounced in HIIT; (2) significant

increase in fat oxidation rate during submaximal exercise, only in the HIIT group; and (3) similar increases, in the two groups, of maximal ADP-stimulated mitochondrial respiration; (4) similar adherence (>90%) without adverse events in the two groups; and (5) after 4 months of follow-up, only in the HIIT group $\dot{V}O_{2peak}$ and fat oxidation rate were still greater compared to the baseline measurements.

HIIT program improving physical capacities and fat oxidation rate

Both MICT and HIIT increased the $\dot{V}O_{2peak}$, although a greater improvement was observed for HIIT, confirming previous research conducted in the general population and in obese people (Lazzer et al. 2017). HIIT would elicit $\dot{V}O_{2max}$ improvements through both central and peripheral adaptations, whereas MICT would elicit $\dot{V}O_{2max}$ improvements mainly through peripheral adaptation (Daussin et al. 2007). In this study, a $\dot{V}O_{2peak}$ increment was measured in the MICT group and mitochondrial respiration (peripheral factor) was improved as well. It must be said also that despite mitochondrial respiration improved in MICT, fat oxidation (peripheral factor) did not. This might suggest that mitochondrial respiration improvement in obese subjects does not contribute to improve also fat oxidation and the $\dot{V}O_{2peak}$ increment is occurred independently from fat oxidation following MICT. The intensity that elicits maximal whole-body fat oxidation is approximately 60% of $\dot{V}O_{2max}$ in well-trained athletes (Achten et al. 2002), 50% in general population (Achten et al. 2002) and 40% in obese patients (Lazzer et al. 2017). During HIIT the most part of the energy comes from carbohydrates (Hetlelid et al. 2015), since above 80% of $\dot{V}O_{2max}$ the contribution of the fat oxidation is almost negligible (Achten et al. 2002; Lazzer et al. 2017). Nevertheless, Hetlelid et al. (2015) found that, by comparing well-trained athletes and moderately active people, the higher performance by the former group during HIIT was mainly explained by their nearly threefold higher rates of fat oxidation capacity, that allows them to keep fat oxidation rate by far higher compared with moderately active people, both during high-intensity exercise and during recovery. In accordance with the Hetlelid results (Hetlelid et al. 2015), the fundamental role of fat oxidation during HIIT might explain why this type of training is more efficient than MICT in improving the fat oxidation rate. Further, previous studies showed that maximal fat oxidation rate was related to $\dot{V}O_{2max}$ (Nordby et al. 2006; Hetlelid et al. 2015), suggesting that a training suited for improving $\dot{V}O_{2max}$ might improve fat oxidation capacity as well. These data regarding HIIT group support this hypothesis but on the contrary the aerobic fitness improvement following MICT seems mainly due to central factors since mitochondrial respiration enhancements are not accompanied by an improvement

Fig. 2 Fat oxidation rate as a function of exercise intensity expressed as percent of peak oxygen uptake ($\dot{V}O_{2peak}$) before (PRE, green diamond continuous line) and after 3 months (POST, red square dashed line) of weight management program, and after 4 months of follow-up (blue triangle dotted line), in moderate-intensity continuous training (MICT, panel **a**) and high-intensity interval training (HIIT, panel **b**) groups. All values are presented as mean $\pm$ standard error. *Significantly different PRE vs. POST, $P < 0.05$. †Significantly different PRE vs. follow-up, $P < 0.05$



of the capacity to utilize fats during the exercise, maybe due to the more pronounced central limitation in obese (Vaccari et al. 2019). On the other hand, carbohydrate utilization during HIIT is not different between well-trained athletes and moderately active people (Hetlelid et al. 2015); moreover, it does not change following HIIT training (Lazzer et al. 2017); this further suggests that lipid oxidation capacity, rather than carbohydrate utilization, is important during HIIT in obese subjects.

$\dot{V}O_2$ and SAP, at rest and during walking at submaximal intensity, decreased in both training groups, with no differences. It is noteworthy that HIIT can improve a clinically relevant parameter, such as SAP, by an amount similar to that described following MICT, despite a lower training time requirement. A previous meta-analysis described a

better ability by HIIT in reducing blood pressure in obese patients and in general, HIIT seems more effective in reducing markers correlated with cardiometabolic risk (García-Hermoso et al. 2016). According to the literature (Sawyer et al. 2016), HIIT might induce different vascular adaptations than MICT; given a similar $\dot{V}O_{2max}$ increment, HIIT would increase arterial dilatation, whereas MICT would increase the resting arterial diameter (Sawyer et al. 2016). These data showed a slight increment on SVC and $a-v \bar{O}_2$ diff during submaximal exercise, without differences between groups, suggesting an improvement of vascular function. According to previous research conducted on non-obese sedentary subjects (Daussin et al. 2007), HIIT improves the maximal performance of the cardiovascular system, whereas

Table 3 Mitochondrial parameters before (PRE) and after (POST) the weight management program in moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) groups

	MICT		HIIT		P		
	PRE (n=6)	POST (n=7)	PRE (n=6)	POST (n=8)	Gr	T	Gr × T
CS	307 ± 25	278 ± 18.10	308 ± 24	268 ± 21	0.996	0.142	0.479
Maximal ADP-stimulated respiration	1.88 ± 0.37	3.13 ± 0.48*	1.93 ± 0.59	2.63 ± 0.47*	0.403	0.042	0.708
ETS_CI+CII	1.97 ± 0.41	2.86 ± 0.48*	1.48 ± 0.37	2.38 ± 0.38*	0.274	0.042	0.953
OXPPOS coupling	0.78 ± 0.09	0.81 ± 0.05	0.69 ± 0.08	0.75 ± 0.08	0.309	0.520	0.835
ETS_CI	1.24 ± 0.23	1.63 ± 0.31	1.01 ± 0.14	1.61 ± 0.27	0.748	0.062	0.606
ETS_CII	0.73 ± 0.20	1.23 ± 0.23	0.48 ± 0.23	0.77 ± 0.17	0.083	0.058	0.610

All values are presented as mean ± standard error

CS: Citrate Synthase activity, expressed as mU/mg protein; the values of CS refer to n=13 for MICT and n=14 for HIIT groups unlike the above indicated. Maximal ADP-stimulated mitochondrial respiration: CI+II state 3 respiration measured in the presence of malate (4 mM), glutamate (10 mM), succinate (10 mM) and saturating ADP (5 mM), normalized by CS and expressed as pmol O₂ s⁻¹ mU⁻¹. ETS_CI+CII: maximal Electron Transport System capacity sustained by CI+II, obtained by stepwise addition of the chemical uncoupler FCCP, normalized by CS and expressed as pmol O₂ s⁻¹ mU⁻¹. OXPPOS coupling: [(state 3 – leak)/state 3], where leak is the non-phosphorylating resting respiration measured in the presence of malate (4 mM) and glutamate (10 mM), and in the absence of ADP (CI state 2/leak respiration); ETS_CI: ETS sustained by CI (rotenone-sensitive ETS), normalized by CS and expressed as pmol O₂ s⁻¹ mU⁻¹. ETS_CII: ETS sustained by CII (rotenone-insensitive ETS), normalized by CS and expressed as pmol O₂ s⁻¹ mU⁻¹

Significance by generalized linear mixed model (see statistical paragraph): *Significantly different from PRE, $P < 0.05$

improvements obtained by MICT would mainly manifest at submaximal intensities.

MICT and HIIT improving oxidative phosphorylation capacity but not efficiency

These results revealed an improvement of maximal ADP-stimulated respiration and maximal ETS capacity after both MICT and HIIT, without changes in oxidative phosphorylation coupling, suggesting that oxidative phosphorylation capacity but not efficiency was enhanced by exercise training. Furthermore, no improvement of mitochondrial content was observed, as estimated by CS activity, after both training. Thus, it may be inferred that the increase in mitochondrial function observed in the present study was due to changes in oxidative phosphorylation complexes activity/assembly, or remodelling of mitochondrial inner membrane. Menshikova et al. (2007) suggested a similar hypothesis in sedentary obese individuals undergoing moderate-intensity physical activity combined with weight loss. In particular, the authors observed an improved enzymatic capacity for oxidative phosphorylation without a significant change in mtDNA content, hypothesizing a mitochondrial cristae remodelling. These data showing an increase in maximal capacity of both complex I and complex II may be in accordance to such hypothesis. Intriguingly, studies on non-obese subjects showed that some markers of mitochondrial biogenesis increase in high-intensity interval running, more than in moderate-intensity continuous running (Wallman et al. 2009), and others showed that HIIT is more effective in

improving markers associated with mitochondrial contents (MacInnis et al. 2016; MacInnis and Gibala 2017). However, the specific training's characteristics to increase mitochondrial content are not yet fully understood (Bishop et al. 2019). The key factor could be the volume, since Granata et al. (2016), did not find any changes in CS and other mitochondrial content markers following three training interventions at different intensities, but on the other hand, MacInnis et al. (2016) reported that sprint interval training increases mitochondrial content to a similar extent to MICT despite a reduced exercise volume. Nevertheless, it is important to consider that all the studies mentioned above refer to normal weight people, while Boyd et al. (2013) studied this topic in obese/overweight subjects and found no difference in the improvement of oxidative capacity of skeletal muscle and in the mitochondrial content following a low volume at low intensity compared to high-intensity high-volume training.

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

In this study, despite both training modalities improved oxidative mitochondrial function, only after HIIT, the

capacity to oxidize lipids during exercise improved. It should be considered that mitochondrial oxidative capacity widely exceeds systemic O_2 delivery (Boushel et al. 2011) and does not seem to be related with total body fat oxidation (Nordby et al. 2006). Looking at these results, the improvement in fat oxidation in HIIT was not associated with changes in CS activity. This suggests that at least for 3 months of training, the improvement of fat oxidation is not due to mitochondrial adaptations, but due to other factors, like improvements in O_2 muscle supply, capillary density and O_2 diffusion. Indeed, endurance athletes, compared with untrained individuals, have higher whole-body maximal fat oxidation which, however, does not correlate with mitochondrial fat oxidation (Nordby et al. 2006); this further suggests that higher O_2 availability might be the main factor increasing the whole-body fat oxidation.

Training adherence

During the weight management programs, no differences in training adherence between MICT and HIIT and no adverse events were observed, in agreement with previous studies (Jung et al. 2015), but in contrast with Lunt et al. (2014). Lunt et al. (2014) showed that, despite the greater potential efficiency of HIIT in improving aerobic capacity compared to MICT, the effectiveness of HIIT could be reduced due to the lower adherence to training prescriptions. In the present study, a lower adherence to the HIIT program was not noticed, despite the training period was quite long (3 months): the number of training sessions (about 35) was the same in the two groups and the actual intensities were quite close to the programmed ones. HIIT was reported to be more enjoyable compared to MICT, at least inside a laboratory setting (Bartlett et al. 2011); this work showed that this is true even outside the laboratory setting and for a relatively long period of time. Given that ‘lack of time’ remains one of the most commonly cited barriers to regular exercise participation (Gillen and Gibala 2014), HIIT could be a time-efficient exercise strategy that warrants consideration for training prescription also in the obese population.

Follow-up

After 4 months of follow-up, $\dot{V}O_{2peak}$ and fat oxidation rate decreased in both groups compared with POST. Only the HIIT group, however, maintained higher values of $\dot{V}O_{2peak}$ and fat oxidation rate than at PRE, even though other cardiovascular and anthropometric characteristics were equally improved in both groups. This suggests a greater long-term efficiency of HIIT in maintaining the cardiovascular fitness and metabolic health, and so a greater ability to reduce cardiovascular risk and insulin sensitivity (Robinson et al. 2015).

Mean BM after follow-up was slightly increased in both groups compared with POST (although being still lower than at PRE). Interestingly, fat mass and waist and hip circumferences were unchanged between the end of the supervised training and the end of the follow-up period; FFM, on the other hand, increased. This suggests that physical activity, particularly vigorous physical activity, maintained after the training period, induced an increase in FFM.

Along with the increase in FFM, improvements were observed in arterial pressure, SVC and $a-v \bar{O}_2$ diff, suggesting a positive effect of the lean mass increase on the cardiovascular system parameters (Pedersen and Febbraio 2012). Further, the hip and waist circumferences did not increase after the follow-up period despite the BM increment, suggesting a reduction in cardiovascular risk (O’Donovan et al. 2009).

CO decreased compared to POST and $a-v \bar{O}_2$ diff tended to increase both at rest and during walking, suggesting an improved oxygen extraction of the peripheral tissues and an improvement in muscle oxidative function (Daussin et al. 2007), as confirmed by the mitochondrial data. On the other hand, in the follow-up, SAP during walking increased compared to POST and SVC decreased, suggesting an increased arterial stiffness (Saladini and Palatini 2017).

Limitations

In the present study, high-resolution respirometry measurements were performed on frozen muscle samples (see METHODS), which could reduce the magnitudes of the ADP-stimulated mitochondrial respiration, as observed previously (Larsen et al. 2012; Meyer et al. 2014). However, the cryopreservation procedure proposed by Kuznetsov et al. (Kuznetsov et al. 2003) and adopted by different laboratories (Wüst et al. 2011; Salvadego et al. 2016) was strictly followed for obtaining a reliable estimation of mitochondrial function. Furthermore, the intactness of outer mitochondrial membrane was verified by administering cytochrome *c* in the measurement chamber and the samples exhibiting a substantial increase in respiration ($> 10\%$) were excluded from these analyses. This procedure allowed the authors to exclude significant damage to outer mitochondrial membrane in the samples considered in this analysis. In addition, the fibres fragility consequent to muscle microneedle biopsy and sample handling reduced the number of samples exploitable for the mitochondrial respiration analysis. Nevertheless, numerically homogeneous populations of samples ($n = 6-8$) were analysed successfully for both the ET and HIIT groups, and the main physiological mean characteristics in these subgroups were not significantly different from whole group of subjects.

Conclusions

In conclusion, MICT and HIIT improved the anthropometric characteristics, some cardiovascular markers and mitochondria intrinsic function at the same extent. However, HIIT was more efficient in improving $\dot{V}O_2$ peak and fat oxidation capacity and in maintaining them after 4 months of follow-up. These results may be relevant for an appropriate prescription of training programs. Since improving aerobic fitness is very important in reducing the mortality risk in this population (Brown and Kuk 2015), HIIT should be designed not only to reduce energy imbalance but also to optimize and maintain aerobic fitness.

Acknowledgements The authors thank the subjects who agreed to participate to the. They thank the nursing staff at the Department of Public Health—A.A.S. n. 3 “Alto Friuli-Collinare-Medio Friuli” Gemona del Friuli—Udine (Italy), for their qualified assistance during the study. The study was supported by the University of Udine research funds.

Author contributions FV and SL conceived the overall study; all the authors contributed to design the research. FV, ADA, FDV, EC, BM and FF and conducted the experiments. FV, AD, JMS, BN, MC, FF, GB and AA analysed the data. FV wrote the manuscript with the help of AP, BM, MC, IM, FF, GB. SL, BG and IM who revised the manuscript. All the authors read and approved this manuscript.

Compliance with ethical standards

Conflict of interest No conflicts of interest, financial or otherwise, are declared by the author(s).

References

- Achten J, Gleeson M, Jeukendrup AE (2002) Determination of the exercise intensity that elicits maximal fat oxidation. *Med Sci Sports Exerc* 34:92–97. <https://doi.org/10.1097/00005768-20021000-00015>
- Achten J, Jeukendrup AE (2004) Optimizing fat oxidation through exercise and diet. *Nutrition* 20:716–727. <https://doi.org/10.1016/j.nut.2004.04.005>
- Ara I, Larsen S, Stallknecht B et al (2011) Normal mitochondrial function and increased fat oxidation capacity in leg and arm muscles in obese humans. *Int J Obes* 35:99–108
- Bartlett JD, Close GL, Maclaren DPM et al (2011) High-intensity interval running is perceived to be more enjoyable than moderate-intensity continuous exercise: implications for exercise adherence. *J Sports Sci* 29:547–553. <https://doi.org/10.1080/02640414.2010.545427>
- Bishop DJ, Botella J, Genders AJ et al (2019) High-intensity exercise and mitochondrial biogenesis: current controversies and future research directions. *Physiology* 34:56–70. <https://doi.org/10.1152/physiol.00038.2018>
- Bloos F, Reinhart K (2009) Venous oximetry. In: Hedenstierna G, Mancebo J, Brochard L, Pinsky MR (eds) *Applied physiology in intensive care medicine*. Springer, Berlin, pp 53–55
- Boushel R, Gnaiger E, Calbet JAL et al (2011) Muscle mitochondrial capacity exceeds maximal oxygen delivery in humans. *Mitochondrion* 11:303–307. <https://doi.org/10.1016/j.mito.2010.12.006>
- Boyd JC, Simpson CA, Jung ME, Gurd BJ (2013) Reducing the intensity and volume of interval training diminishes cardiovascular adaptation but not mitochondrial biogenesis in overweight/obese men. *PLoS ONE* 8:1–8
- Brown RE, Kuk JL (2015) Consequences of obesity and weight loss: a devil's advocate position. *Obes Rev* 16:77–87. <https://doi.org/10.1111/obr.12232>
- Charloux A, Lonsdorfer-Wolf E, Richard R et al (2000) A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: Comparison with the “direct” Fick method. *Eur J Appl Physiol* 82:313–320
- Cohen J (1988) *Statistical power analysis for the behavioral sciences*, 2nd edn.
- Craig CL, Marshall AL, Sjöström M et al (2003) International physical activity questionnaire: 12-Country reliability and validity. *Med Sci Sports Exerc* 35:1381–1395
- Dandanell S, Meinild-Lundby A-K, Andersen AB et al (2018) Determinants of maximal whole body fat oxidation in elite cross-country skiers: role of skeletal muscle mitochondria. *Scand J Med Sci Sports*. <https://doi.org/10.1111/sms.13298>
- Daussin FN, Ponsot E, Dufour SP et al (2007) Improvement of $\dot{V}O_{2\max}$ by cardiac output and oxygen extraction adaptation during intermittent versus continuous endurance training. *Eur J Appl Physiol* 101:377–383
- de Mello AH, Costa AB, Engel JDG, Rezin GT (2018) Mitochondrial dysfunction in obesity. *Life Sci* 192:26–32
- Devarshi P, McNabney S, Henagan T (2017) Skeletal muscle nucleomitochondrial crosstalk in obesity and type 2 diabetes. *IJMS* 18:831. <https://doi.org/10.3390/ijms18040831>
- Donnelly JE, Blair SN, Jakicic JM et al (2009) Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc* 41:459–471
- Feingold A (2009) Trials in the same metric as for classical analysis. *Psychol Methods* 14:43–53. <https://doi.org/10.1037/a0014699>. Effect
- Fisher-Wellman KH, Weber TM, Cathey BL et al (2014) Mitochondrial respiratory capacity and content are normal in young insulin-resistant obese humans. *Diabetes* 63:132–141
- Formentini L, Ryan AJ, Gálvez-Santisteban M et al (2017) Mitochondrial H⁺-ATP synthase in human skeletal muscle: contribution to dyslipidaemia and insulin resistance. *Diabetologia* 60:2052–2065. <https://doi.org/10.1007/s00125-017-4379-z>
- Frayn KN (1983) Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 55:628–634
- García-Hermoso A, Cerrillo-Urbina AJ, Herrera-Valenzuela T et al (2016) Is high-intensity interval training more effective on improving cardiometabolic risk and aerobic capacity than other forms of exercise in overweight and obese youth? A meta-analysis. *Obes Rev* 17:531–540
- Gelman A, Hill J, Yajima M (2012) Why we (usually) don't have to worry about multiple comparisons. *J Res Educ Eff* 5:189–211. <https://doi.org/10.1080/19345747.2011.618213>
- Gillen JB, Gibala MJ (2014) High-intensity interval training: a time-efficient exercise strategy to improve health and fitness? *Appl Physiol Nutr Metab* 39:409–412
- Gnagnarella P, Salvini S, Parpinel M (2015) Food composition database for epidemiological studies in Italy. <https://www.bda-ieo.it/>. Version 1. Accessed 1 Sept 2019
- Granata C, Oliveira RSF, Little JP et al (2016) Training intensity modulates changes in PGC-1 α and p53 protein content and mitochondrial respiration, but not markers of mitochondrial content in human skeletal muscle. *FASEB J* 30:959–970. <https://doi.org/10.1096/fj.15-276907>
- Gray DS, Bray GA, Gemayel N, Karl K (1989) Effect of obesity on bioelectrical impedance. *Am J Clin Nutr* 50:255–260

- Harris J, Benedict F (1918) A biometric study of human basal metabolism. *Proc Natl Acad Sci USA* 4:370–373
- Hetlelid KJ, Plews DJ, Herold E et al (2015) Rethinking the role of fat oxidation: substrate utilisation during high-intensity interval training in well-trained and recreationally trained runners. *BMJ Open Sport Exerc Med* 1:e000047. <https://doi.org/10.1136/bmjsem-2015-000047>
- Hughes MC, Ramos SV, Turnbull PC et al (2015) Mitochondrial bioenergetics and fiber type assessments in microbiopsy vs. Bergstrom percutaneous sampling of human skeletal muscle. *Front Physiol* 6:1–10. <https://doi.org/10.3389/fphys.2015.00360>
- Hwang H, Bowen BP, Lefort N et al (2010) Proteomics analysis of human skeletal muscle reveals novel abnormalities in obesity and type 2 diabetes. *Diabetes* 59:33–42
- International Physical Activity Questionnaire (2005) Guidelines for data processing and analysis of the international physical activity questionnaire (IPAQ)—Short and Long Forms, revised on November 2005. Ipaq 1–15
- Jung ME, Bourne JE, Beauchamp MR et al (2015) High-intensity interval training as an efficacious alternative to moderate-intensity continuous training for adults with prediabetes. *J Diabetes Res*. <https://doi.org/10.1155/2015/191595>
- Kelley DE, Simoneau JA (1994) Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *J Clin Invest* 94:2349–2356. <https://doi.org/10.1172/JCI117600>
- Kras KA, Langlais PR, Hoffman N et al (2018) Obesity modifies the stoichiometry of mitochondrial proteins in a way that is distinct to the subcellular localization of the mitochondria in skeletal muscle. *Metab Clin Exp* 89:18–26. <https://doi.org/10.1016/j.metabol.2018.09.006>
- Kuznetsov AV, Kunz WS, Saks V et al (2003) Cryopreservation of mitochondria and mitochondrial function in cardiac and skeletal muscle fibers. *Anal Biochem* 319:296–303
- Lanzi S, Codecasa F, Cornacchia M et al (2014) Fat oxidation, hormonal and plasma metabolite kinetics during a submaximal incremental test in lean and obese adults. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0088707>
- Larsen S, Wright-Paradis C, Gnaiger E et al (2012) Cryopreservation of human skeletal muscle impairs mitochondrial function. *Cryo Lett* 33:170–176
- Lazzer S, Tringali G, Caccavale M et al (2017) Effects of high-intensity interval training on physical capacities and substrate oxidation rate in obese adolescents. *J Endocrinol Invest*. <https://doi.org/10.1007/s40618-016-0551-4>
- Lowry OH, Rosebrough NJ, Farr LA, Randall RJ (1951) Protein measurement with the folin phenol reagent*. *Anal Biochem* 217:220–230
- Lukaski HC, Bolonchuk WW, Hall CB, Siders WA (1986) Validation of tetrapolar bioelectrical impedance method to assess human body composition. *J Appl Physiol* 60:1327–1332
- Lunt H, Draper N, Marshall HC et al (2014) High intensity interval training in a real world setting: a randomized controlled feasibility study in overweight inactive adults, measuring change in maximal oxygen uptake. *PLoS ONE* 9:e83256
- MacInnis MJ, Gibala MJ (2017) Physiological adaptations to interval training and the role of exercise intensity: training adaptations and the nature of the stimulus. *J Physiol* 595:2915–2930. <https://doi.org/10.1113/JP273196>
- MacInnis MJ, Zacharewicz E, Martin BJ et al (2016) Superior mitochondrial adaptations in human skeletal muscle after interval compared to continuous single-leg cycling matched for total work. *J Physiol* 00:1–14. <https://doi.org/10.1113/JP272570>
- Menshikova EV, Ritov VB, Ferrell RE et al (2007) Characteristics of skeletal muscle mitochondrial biogenesis induced by moderate-intensity exercise and weight loss in obesity. *J Appl Physiol* 103:21–27
- Meyer A, Charles AL, Zoll J et al (2014) Cryopreservation with dimethyl sulfoxide prevents accurate analysis of skinned skeletal muscle fibers mitochondrial respiration. *Biochimie* 100:227–233. <https://doi.org/10.1016/j.biochi.2014.01.014>
- Mootha VK, Lindgren CM, Eriksson KF et al (2003) PGC-1 α -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 34:267–273. <https://doi.org/10.1038/ng1180>
- Nair KS, Irving BA, Lanza IR (2011) Can dietary nitrates enhance the efficiency of mitochondria? *Cell Metab* 13:117–118
- Nordby P, Saltin B, Helge JW (2006) Whole-body fat oxidation determined by graded exercise and indirect calorimetry: a role for muscle oxidative capacity? *Scand J Med Sci Sports* 16:209–214. <https://doi.org/10.1111/j.1600-0838.2005.00480.x>
- O'Donovan G, Thomas EL, McCarthy JP et al (2009) Fat distribution in men of different waist girth, fitness level and exercise habit. *Int J Obes* 33:1356–1362
- Patti ME, Butte AJ, Crunkhorn S et al (2003) Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci USA* 100:8466–8471. <https://doi.org/10.1073/pnas.1032913100>
- Pedersen BK, Febbraio MA (2012) Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat Rev Endocrinol* 8:457–465
- Pesta D, Gnaiger E (2012) High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. In: *Methods in molecular biology*. pp 25–58
- Richard R, Lonsdorfer-Wolf E, Charloux A et al (2001) Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. *Eur J Appl Physiol* 85:202–207
- Ritov VB, Menshikova EV, He J et al (2005) Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. *Diabetes* 54:8–14. <https://doi.org/10.2337/diabetes.54.1.8>
- Ritov VB, Menshikova EV, Azuma K et al (2009) Deficiency of electron transport chain in human skeletal muscle mitochondria in type 2 diabetes mellitus and obesity. *Am J Physiol Endocrinol Metab* 298:E49–E58
- Robinson SL, Hattersley J, Frost GS et al (2015) Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. *J Appl Physiol* 118:1415–1422. <https://doi.org/10.1152/jappphysiol.00058.2015>
- Saladini F, Palatini P (2017) Isolated systolic hypertension in young individuals: pathophysiological mechanisms, prognostic significance, and clinical implications. *High Blood Press Cardiovasc Prev* 24:133–139. <https://doi.org/10.1007/s40292-017-0199-y>
- Salvadeo D, Keramidas ME, Brocca L et al (2016) Separate and combined effects of a 10-d exposure to hypoxia and inactivity on oxidative function in vivo and mitochondrial respiration ex vivo in humans. *J Appl Physiol* 121:154–163. <https://doi.org/10.1152/jappphysiol.00832.2015>
- Sawyer BJ, Tucker WJ, Bhammar DM et al (2016) Effects of high-intensity interval training and moderate-intensity continuous training on endothelial function and cardiometabolic risk markers in obese adults. *J Appl Physiol* (Bethesda, Md: 1985) 121:279–288
- SIO-ADI (2016) Italian's Standard for the treatment of obesity. In: *Società Italiana di Nutrizione Umana*. <https://www.sio-obesita.org/>. Accessed 1 Sept 2019
- Spinazzi M, Casarin A, Pertegato V et al (2012) Assessment of mitochondrial respiratory chain enzymatic activities on tissues and cultured cells. *Nat Protoc* 7:1235–1246
- Srere PA (1969) Citrate synthase. *Methods Enzymol* 3:5
- Thrush AB, Dent R, McPherson R, Harper ME (2013) Implications of mitochondrial uncoupling in skeletal muscle in the development and treatment of obesity. *FEBS J* 280:5015–5029

- Twisk JWR (2006) Applied multilevel analysis: a practical guide. Cambridge University Press, Cambridge
- Vaccari F, Floreani M, Tringali G et al (2019) Metabolic and muscular factors limiting aerobic exercise in obese subjects. *Eur J Appl Physiol*. <https://doi.org/10.1007/s00421-019-04167-w>
- Vella CA, Ontiveros D, Zubia RY (2011) Cardiac function and arteriovenous oxygen difference during exercise in obese adults. *Eur J Appl Physiol* 111:915–923
- Vijgen GHEJ, Bouvy ND, Hoeks J et al (2013) Impaired skeletal muscle mitochondrial function in morbidly obese patients is normalized one year after bariatric surgery. *Surg Obes Relat Dis* 9:936–941
- Wallman K, Plant LA, Rakimov B, Maiorana AJ (2009) The effects of two modes of exercise on aerobic fitness and fat mass in an overweight population. *Res Sports Med* 17:156–170. <https://doi.org/10.1080/15438620903120215>
- Ware JE, Kosinski M, Keller SD (1996) A 12-item short-form health survey: construction of scales and preliminary tests of reliability and validity. *Med Care* 34:220–233
- Wijngaarden MA, van der Zon GC, van Dijk KW et al (2013) Effects of prolonged fasting on AMPK signaling, gene expression, and mitochondrial respiratory chain content in skeletal muscle from lean and obese individuals. *Am J Physiol Endocrinol Metab*. <https://doi.org/10.1152/ajpendo.00008.2013>
- Williams EP, Mesidor M, Winters K et al (2015) Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. *Curr Obes Rep* 4:363–370. <https://doi.org/10.1007/s13679-015-0169-4>
- Woltman H, Feldstain A, MacKay JC, Rocchi M (2012) An introduction to hierarchical linear modeling. *TQMP* 8:52–69. <https://doi.org/10.20982/tqmp.08.1.p052>
- Wüst RCI, Myers DS, Stones R et al (2011) Regional skeletal muscle remodeling and mitochondrial dysfunction in right ventricular heart failure. *Am J Physiol Heart Circ Physiol* 302:H402–H411. <https://doi.org/10.1152/ajpheart.00653.2011>
- Zurlo F, Lillioja S, Esposito-Del Puente A et al (1990) Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *Am J Physiol Endocrinol Metab* 259:E650–E657. <https://doi.org/10.1152/ajpendo.1990.259.5.e650>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.