



# 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

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## 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  $\text{O}_2$  uptake ( $\dot{V}\text{O}_{2\text{peak}}$ ) 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;  $\dot{V}\text{O}_{2\text{peak}}$  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,  $\dot{V}\text{O}_{2\text{peak}}$  (+ 8%) and maximal fat oxidation rate were still higher ( $P < 0.05$ ).

**Conclusions** HIIT was more effective in improving and maintaining  $\dot{V}\text{O}_{2\text{peak}}$  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

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## Abbreviations

a- $\bar{v}\text{O}_2$ 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 <sub>2</sub>	Pulmonary CO <sub>2</sub> output
V'O <sub>2</sub>	Pulmonary O <sub>2</sub> 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'O<sub>2</sub>max) (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'O<sub>2</sub>peak, 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)  $\geq 30$  kg m<sup>-2</sup>. 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  $34 \pm 0.14$  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  $44 \pm 8$  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  $33 \pm 4$  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 ( $\sim 70$ –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  $BM \text{ (kg)} \times \text{stature}^{-2} \text{ (m)}$ . 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 ( $\text{MET} \cdot \text{min} \cdot \text{week}^{-1}$ ) 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 ( $V'O_{2\text{peak}}$ ) 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  $\text{m} \cdot \text{s}^{-1}$  and incline in % followed a sequence: 1.11 (0%), 1.11 (3%), 1.39 (3%), 1.39 (6%), 1.53 (6%), 1.53 (9%), 1.53 (12%), 1.53 (13%), 1.53 (15%), 1.53 (18%), 1.53 (21%) and 1.53 (24%) (Lazzer et al. 2017). During the experiment, ventilatory and gas exchange responses were measured continuously by indirect calorimetry (CPET, Cosmed, Italy). The flowmeter and gas analysers of the system were calibrated using, respectively, a 3-L calibration syringe and calibration gas (16.00%  $O_2$ ; 4.00%  $CO_2$ ). During the exercise test, an electrocardiogram was recorded continuously and displayed online for visual monitoring, and heart rate (HR) was measured with a dedicated monitor device (Garmin, US).  $V'O_{2\text{peak}}$  was estimated for each subject considering the last 20 s of the graded exercise tests.

The substrate oxidation rate was calculated from  $V'O_2$  and  $V'CO_2$  values determined during the last minute of each workload level, according to the protocol of Achten (Achten et al. 2002) and using the following equations (Frayn 1983): Fat oxidation rate ( $\text{g} \cdot \text{min}^{-1}$ ) =  $1.67 \cdot V'O_2 \cdot \text{min}^{-1} - 1.67 \cdot V'CO_2 \cdot \text{min}^{-1} - 0.307 \cdot P_{\text{oxi}}$ ,

$$\begin{aligned} \text{Carbohydrate oxidation rate (g} \cdot \text{min}^{-1}\text{)} \\ &= 4.55 \cdot V'CO_2 \cdot \text{min}^{-1} \\ &\quad - 3.21 V'O_2 \cdot \text{min}^{-1} - 0.459 \cdot P_{\text{oxi}} \end{aligned}$$

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

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

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 % $V'O_{2\text{peak}}$ . 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  $1.11 \text{ m} \cdot \text{s}^{-1}$  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 ( $a-v \bar{O}_2$  diff.) was estimated by the following equation (Bloos and Reinhart 2009):

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

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  $\text{Ca}^{2+}$  concentration  $100 \text{ nmol l}^{-1}$ ], 20 mM imidazole, 20 mM taurine, 50 mM  $\text{K}^+$ /4 morpholinoethanesulfonic acid, 0.5 mM dithiothreitol, 6.56 mM  $\text{MgCl}_2$ , 5.77 mM ATP and 15 mM phosphocreatine, pH 7.1), containing 10% (wt  $\text{vol}^{-1}$ ) fatty acid-free BSA and 30% (vol  $\text{vol}^{-1}$ ) DMSO at  $4^\circ\text{C}$ , then immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  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  $\text{MgCl}_2$ , 20 mM taurine, 10 mM  $\text{KH}_2\text{PO}_4$ , 20 mM HEPES, 110 mM sucrose and  $1 \text{ g l}^{-1}$  BSA, pH 7.1), permeabilized fibre bundles were measured for wet weight and immediately transferred into the respirometer chambers for  $\text{O}_2$  consumption measurements. Measurements were performed by high-resolution respirometry (Oxygraph-2 k; Oroboros Instruments, Innsbruck, Austria) at  $37^\circ\text{C}$  (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  $\mu\text{M}$  (average  $\text{O}_2$  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  $280 \text{ U ml}^{-1}$  catalase before the measurements). Measurements were run in the presence of 25  $\mu\text{M}$  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  $\mu\text{M}$ ) 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  $\text{vol}^{-1}$  in a homogenization buffer containing 250 mM sucrose, 20 mM Tris, 40 mM KCl and 2 mM EGTA with 1:50 vol  $\text{vol}^{-1}$  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  $\mu\text{g}$  of protein was added to each well of a 96-well microplate along with 100  $\mu\text{l}$  of 200 mM Tris-Triton X-100 (0.2% vol  $\text{vol}^{-1}$ ), 20  $\mu\text{l}$  of 1 mM 5,5'-dithiobis-2-nitrobenzoate freshly prepared, 6  $\mu\text{l}$  of 10 mM acetyl-coenzyme A and mQ water to a final volume of 190  $\mu\text{l}$ . Finally, 10  $\mu\text{l}$  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,  $\dot{V}O_2$  peak 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, time<sup>2</sup> and interaction group  $\times$  time as fixed effects. For CS and mitochondrial function parameters, it was not necessary add ‘time<sup>2</sup>’ since there has been only two time points. The same analyses were applied to the fat oxidation during the exercise, adding the % of  $\dot{V}O_2$  peak 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  $34.4 \pm 0.2$  and  $34.8 \pm 0.3$  training sessions for MICT and HIIT groups, respectively, ( $P = 0.811$ ) without adverse events. The average of energy expended during the training sessions were  $23.8 \pm 4.1$  and  $22.4 \pm 2.5$  kJ kg<sup>-1</sup> of FFM for MICT and HIIT groups, respectively, ( $P = 0.256$ ). The duration of each session was greater for MICT ( $44.3 \pm 7.6$  min) than for HIIT ( $33.6 \pm 3.6$  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 ( $37.3 \pm 0.6$  and  $40.1 \pm 0.4$  years, group effect  $P = 0.334$ ) and stature ( $1.72 \pm 0.11$  and  $1.71 \pm 0.07$  m, group effect  $P = 0.895$ ) (Table 1).

At POST, mean weight loss was  $5.84 \pm 0.15$  kg (time effect  $P < 0.001$ , ES 0.40), BMI decreased by  $2.03 \pm 0.05$  kg m<sup>-2</sup> (time effect  $P < 0.001$ , ES 0.46), waist circumference decreased by  $4.51 \pm 0.17$  cm (time effect  $P < 0.001$ , ES 0.39), hip circumference decreased by  $4.75 \pm 0.16$  cm (time effect  $P < 0.001$  ES 0.51), FM decreased by  $5.37 \pm 0.16$  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  $2.15 \pm 0.16$  kg (time effect  $P < 0.015$ , ES 0.12) and BMI increased by  $0.71 \pm 0.05$  kg m<sup>-2</sup> (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  $3.18 \pm 0.16$  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 ( $9509 \pm 763$  vs.  $7720 \pm 522$  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	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	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
BMI (kg m <sup>-2</sup> )	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
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
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
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
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
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
V'O <sub>2</sub> peak (L·min <sup>-1</sup> )	3.02 ± 0.05		3.19 ± 0.05* <sup>†</sup>		2.95 ± 0.06* <sup>†</sup>		2.88 ± 0.04		3.35 ± 0.05* <sup>†</sup>		3.32 ± 0.06* <sup>†</sup>		0.288	0.001
V'O <sub>2</sub> peak (mL·min <sup>-1</sup> ·Kg <sup>-1</sup> FFM)	43.58 ± 0.39		46.80 ± 0.39* <sup>†</sup>		42.02 ± 0.51* <sup>†</sup>		44.26 ± 0.45		51.51 ± 0.40* <sup>†</sup>		48.04 ± 0.56* <sup>†</sup>		0.093	0.001
IPAQ_TOT (MET·min week <sup>-1</sup> )	818 ± 220		1481 ± 292*		1965 ± 552*		766 ± 253		1257 ± 208*		2223 ± 507*		0.954	0.001
IPAQ_VIG (MET·min week <sup>-1</sup> )	190 ± 135		547 ± 281*		928 ± 372*		45 ± 45		310 ± 138*		660.0 ± 285*		0.264	0.003
IPAQ_MOD (MET·min week <sup>-1</sup> )	247 ± 102		596 ± 204		384 ± 136		435 ± 243		431 ± 94		1060 ± 423		0.259	0.090
IPAQ_WALK (MET·min week <sup>-1</sup> )	380 ± 127		337 ± 94		653 ± 294		286 ± 99		517 ± 162		503 ± 121		0.948	0.080
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
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

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

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

At POST, mean energy intake decreased significantly by  $-1689 \pm 84$  kJ day<sup>-1</sup> (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  $600 \pm 100$  kJ day<sup>-1</sup> (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  $a-\bar{v}O_2$  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  $a-\bar{v}O_2$  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<sup>-1</sup> (4 km h<sup>-1</sup>) and 0% slope ( $\sim 40\%$  of  $\dot{V}O_{2peak}$ ),  $\dot{V}O_2$ , CO, SV, SAP, DAP, SVC and  $a-\bar{v}O_2$  diff values were similar between the two groups (MICT and HIIT) at 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<sub>peak</sub> and  $\dot{V}O_{2peak}$  (Table 1).

At POST, HR<sub>peak</sub> decreased by  $3.90 \pm 0.20$  bpm (time effect  $P < 0.001$ ) in MICT and HIIT groups, without difference between groups (Table 1). Absolute  $\dot{V}O_{2peak}$  increased by  $0.17 \pm 0.01$  and by  $0.46 \pm 0.20$  L min<sup>-1</sup> (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							
	POST (16)				FOLLOW-UP (14)				PRE (16)				FOLLOW-UP (12)			
	PRE (16)	POST (16)	FOLLOW-UP (14)	POST (16)	PRE (16)	POST (16)	FOLLOW-UP (12)	POST (16)	Gr	T	Gr × T					
At rest																
V̇O <sub>2</sub> (L·min <sup>-1</sup> )	0.35 ± 0.02	0.37 ± 0.02	0.39 ± 0.03*	0.37 ± 0.02	0.37 ± 0.01	0.37 ± 0.02	0.49 ± 0.08*	0.37 ± 0.02	0.132	0.012	0.318					
CO (L·min <sup>-1</sup> )	6.71 ± 0.33	5.95 ± 0.31	5.97 ± 0.30	5.95 ± 0.31	7.16 ± 0.30	7.15 ± 0.30	7.09 ± 0.51	7.15 ± 0.30	0.345	0.113	0.595					
SV (ml)	88.82 ± 4.86	84.10 ± 5.63	84.54 ± 4.35	84.10 ± 5.63	92.98 ± 4.49	96.05 ± 5.76	88.64 ± 6.69	96.05 ± 5.76	0.158	0.393	0.817					
SAP (mmHg)	132.06 ± 2.66	126.13 ± 2.20*	126.15 ± 1.95*	126.13 ± 2.20*	139.56 ± 3.46	131.56 ± 2.08*	128.75 ± 2.47*	131.56 ± 2.08*	0.620	0.002	0.339					
DAP (mmHg)	83.88 ± 2.10	82.69 ± 1.36	80.00 ± 2.21†	82.69 ± 1.36	87.50 ± 2.42	90.38 ± 1.73	86.08 ± 2.03†	90.38 ± 1.73	0.439	0.002	0.383					
SVC (ml min <sup>-1</sup> mmHg <sup>-1</sup> )	67.08 ± 2.71	60.68 ± 3.06	62.76 ± 3.44	60.68 ± 3.06	69.71 ± 3.34	69.28 ± 3.74	70.85 ± 5.26	69.28 ± 3.74	0.410	0.118	0.463					
a-v̄ O <sub>2</sub> diff. (mL 100 mL <sup>-1</sup> )	5.42 ± 0.32	6.05 ± 0.35	5.66 ± 0.59	6.05 ± 0.35	5.28 ± 0.30	5.45 ± 0.30	6.52 ± 0.60	5.45 ± 0.30	0.748	0.039	0.866					
Walking at 1.11 m s <sup>-1</sup> , 0% slope																
V̇O <sub>2</sub> (L·min <sup>-1</sup> )	1.27 ± 0.06	1.17 ± 0.06*	0.05*	1.17 ± 0.06*	1.23 ± 0.05	1.15 ± 0.06*	1.08 ± 0.06*	1.15 ± 0.06*	0.431	0.001	0.384					
CO (L·min <sup>-1</sup> )	13.77 ± 0.61	12.08 ± 0.74	10.85 ± 0.37*†	12.08 ± 0.74	13.31 ± 0.83	14.23 ± 0.72	11.06 ± 0.44*†	14.23 ± 0.72	0.951	0.001	0.717					
SV (ml)	136.60 ± 5.73	134.50 ± 8.92	120.18 ± 7.81	134.50 ± 8.92	135.11 ± 8.05	147.38 ± 7.73	123.75 ± 6.88	147.38 ± 7.73	0.611	0.077	0.781					
SAP (mmHg)	143.57 ± 3.76	130.00 ± 1.64*	137.86 ± 3.80*†	130.00 ± 1.64*	149.69 ± 4.24	132.50 ± 1.64*	138.75 ± 3.14*†	132.50 ± 1.64*	0.195	0.001	0.354					
DAP (mmHg)	85.00 ± 2.01	1.65	81.57 ± 2.12	1.65	87.50 ± 2.54	88.44 ± 1.56	85.00 ± 1.51	88.44 ± 1.56	0.426	0.443	0.777					
SVC (ml min <sup>-1</sup> mmHg <sup>-1</sup> )	133.11 ± 5.09	122.66 ± 7.78	108.90 ± 4.24*†	122.66 ± 7.78	126.83 ± 7.43	139.30 ± 7.44	109.47 ± 5.56*†	139.30 ± 7.44	0.498	0.012	0.762					
a-v̄ O <sub>2</sub> diff. (mL 100 mL <sup>-1</sup> )	9.58 ± 0.65	10.17 ± 0.65	10.33 ± 0.51	10.17 ± 0.65	9.57 ± 0.46	8.65 ± 0.73	10.47 ± 0.83	8.65 ± 0.73	0.893	0.073	0.481					

All values are presented as mean ± standard error

V̇O<sub>2</sub> oxygen consumption, CO cardiac output, SV stroke volume, SAP systolic arterial pressure, DAP diastolic arterial pressure, a-v̄ O<sub>2</sub> diff. arteriovenous difference of O<sub>2</sub>, 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  $3.3 \pm 0.2$  and by  $7.2 \pm 0.3$  mL  $\text{min}^{-1}$   $\text{kg}^{-1}$  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).

HR<sub>peak</sub> 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  $0.19 \pm 0.02$  and by  $0.21 \pm 0.01$  L  $\text{min}^{-1}$  (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  $4.79 \pm 0.14$  mL  $\text{min}^{-1}$   $\text{kg}^{-1}$  FFM (time effect  $P < 0.001$ ) in MICT and HIIT groups, returning at PRE values in MICT but remaining higher in HIIT (+5%, time effect  $P < 0.001$ , Table 1 and Fig. 1b).

### Fat oxidation rate

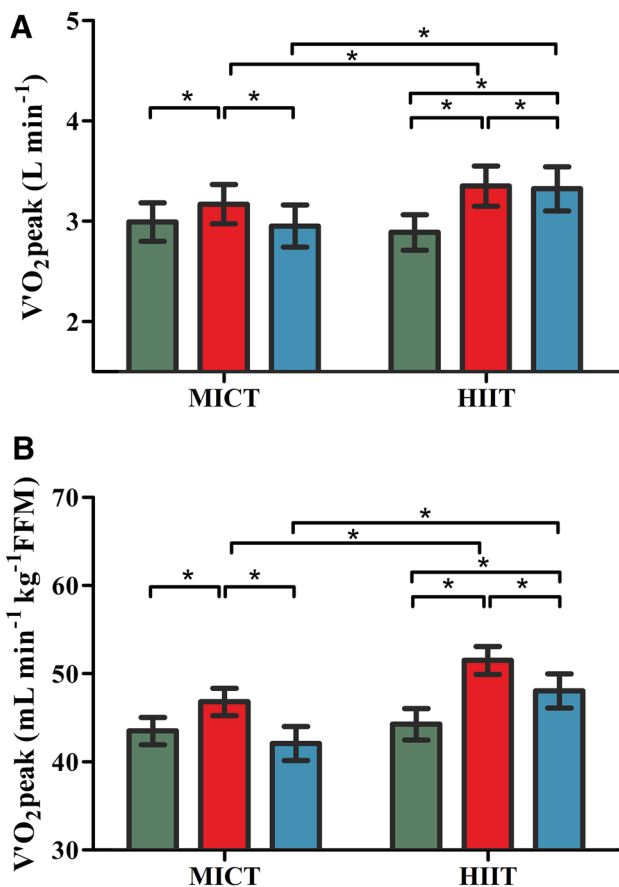
Before the training period (PRE), fat oxidation rate during the incremental test was not significantly different between groups (group effect  $P = 0.110$ ). Maximal fat oxidation rate was observed at  $41 \pm 1\%$  of  $\dot{V}O_{2\text{peak}}$  in MICT ( $0.41 \pm 0.01$  g  $\text{min}^{-1}$ , Fig. 2a) and at  $43 \pm 1\%$  of  $\dot{V}O_{2\text{peak}}$  in HIIT ( $0.43 \pm 0.1$  g  $\text{min}^{-1}$ , 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  $\mu\text{M}$ ) 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<sup>-1</sup>, 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<sub>CI</sub>) or -insensitive complex II ETS (ETS<sub>CII</sub>) exhibited a pattern similar to that of ETS<sub>CI</sub> + 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

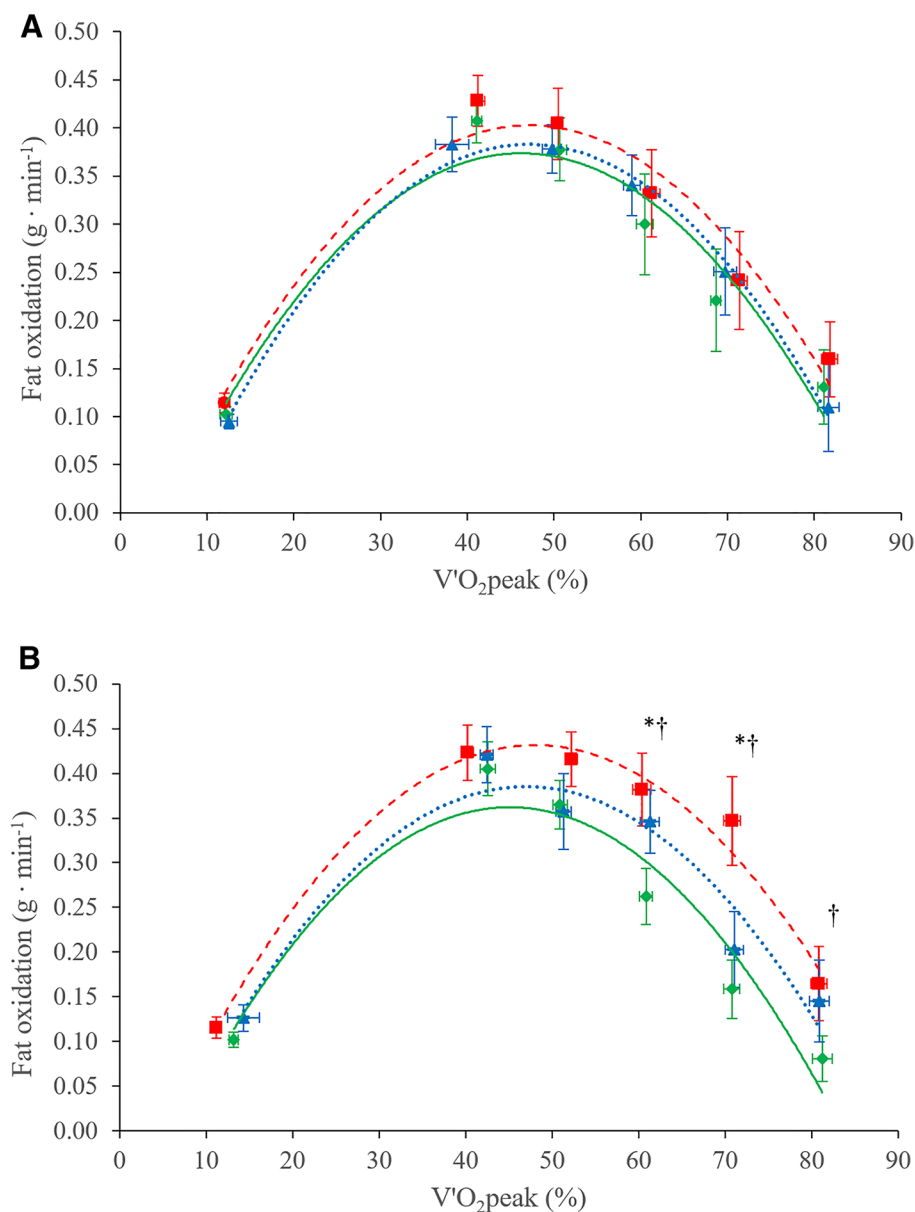
In obese patients, the 3-month weight management program entailing, in terms of exercise, MICT or HIIT, resulted in the following: (1) significant improvement of  $\dot{V}O_{2peak}$  in both groups, although more pronounced in HIIT; (2) significant

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 (Lizzer 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 (Lizzer 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; Lizzer 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 O<sub>2</sub> s<sup>-1</sup> mU<sup>-1</sup>. 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 O<sub>2</sub> s<sup>-1</sup> mU<sup>-1</sup>. 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 O<sub>2</sub> s<sup>-1</sup> mU<sup>-1</sup>. ETS\_CII: ETS sustained by CII (rotenone-insensitive ETS), normalized by CS and expressed as pmol O<sub>2</sub> s<sup>-1</sup> mU<sup>-1</sup>

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 FADH<sub>2</sub> 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.

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## Compliance with ethical standards

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

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