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ORIGINAL ARTICLE

Metabolic and muscular factors limiting aerobic exercise in obese subjects

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Abstract

Purpose The aim of the present study was to understand the role of central (cardiovascular O_2 delivery) and peripheral factors (muscle level) in limiting the maximal aerobic performance in obese (OB) subjects.

Methods Fifteen OB (mean age ± SD 25 ± 7 years; BMI 43 ± 7 kg/m^2) and 13 lean sedentary subjects (CTRL, age 27 ± 7 years; BMI 22 ± 3 kg/m^2) participated in this study. Oxygen uptake ($\dot{V}O_2$), hearth rate (HR) and cardiac output (CO) were measured during cycle ergometer (CE) and knee extension (KE) incremental tests. Maximal voluntary contractions (MVCs) of knee extensor muscles were performed before and immediately after the two tests.

Results $\dot{V}O_2$peak, HR peak and CO peak were significantly higher in CE than KE (+ 126%, + 33% and + 46%, respectively, $p < 0.001$), both in OB and CTRL subjects, without differences between the two subgroups. Maximal work rate was lower in OB than CTRL (191 ± 38 vs 226 ± 39 W, $p < 0.05$) in CE, while it was similar between the two subgroups in KE. Although CE and KE determined a reduction of MVC in both subgroups, MVC resulted less decreased after CE than KE exercises (− 14 vs − 32%, $p < 0.001$) in OB, while MVC decrements were similar after the two exercises in CTRL (− 26% vs − 30%, $p > 0.05$, for CE and KE, respectively).

Conclusions The lower muscle fatigue observed in OB after CE compared to KE test suggests that central factors could be the most important limiting factor during cycling in OB.

Keywords Obesity · Exercise tolerance · Knee extension · Small muscles exercise · Aerobic function

Abbreviations

$a - \bar{v}O_2$ diff	Arteriovenous oxygen difference
BM	Body mass
BMI	Body mass index
CE	Cycle ergometer
CTRL	Control group
CO	Cardiac output
ΔMVC	Maximal voluntary contraction changes in percentage
FM	Fat mass
FFM	Fat-free mass
HR	Heart rate
KE	Knee extension exercise
MVC	Maximal voluntary contractions of the knee extensor muscles
MVC_end	MVC immediately after the end of incremental exercise
OB	Obese
Tlim	Time to exhaustion
$\dot{V}CO_2$	CO_2 output
$\dot{V}O_2$	Pulmonary O_2 uptake
Vol_{TM}	MUSCLE thigh volume

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Introduction

Obesity is associated with some cardiovascular diseases, such as coronary heart disease and abnormalities in heart rate properties (Poirier et al. 2006; López-Jiménez and

Cortés-Bergoderi 2011; Alpert et al. 2016; Ortega et al. 2016). At rest, obese subjects are reported to have greater blood and systolic volumes and cardiac output than lean controls subjects. These alterations, which are required to supply the increased metabolic demands at rest, might lead to deleterious consequences, including the development of left ventricle hypertrophy and of abnormalities in diastolic and systolic phases (López-Jiménez and Cortés-Bergoderi 2011; Ortega et al. 2016; Alpert et al. 2016; Poirier et al. 2006). Severe obesity (BMI $\geq$ 35 kg/m^2) is strongly related to higher risk of cardiovascular disease incidence and mortality (Ortega et al. 2016). Blessedly, physical exercises and nutritional programs are able to reduce this risk and even reverse obesity (Donnelly et al. 2009). Physical exercises, in particular those aimed at improving the cardiorespiratory fitness level, are reported to be extremely effective (Ortega et al. 2016). Although both sedentary obese and lean subjects have similar peak aerobic power (VO_2peak) (Lazzer et al. 2013; Vella et al. 2012), sedentary obese subjects show greater peak stroke volumes (SV) and cardiac output (CO) values than the lean individuals (Vella et al. 2012). By contrast, obese subjects do not reach the same maximal exercise work load of their lean counterparts (Lazzer et al. 2013).

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

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

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

Subjects and methods

Subjects

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

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

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

Experimental protocol

Before the start of the study, the subjects were familiarized with the equipment and the procedures. Subjects were asked to avoid strenuous exercises the day before

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

Anthropometric characteristics and body composition

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

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

$$V = \cdot 0.866 \cdot \left\{ \left(\frac{L}{12\pi} \right) \cdot (C1 + C2 + C3) - \left[\frac{S - 0.4}{2} \right] \cdot L \cdot \left[\frac{C1 + C2 + C3}{3} \right] \right\} - 1750, \quad (1)$$

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

Table 1 Physical and functional characteristics of subjects

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

All values are presented as mean ± standard deviation

BMI body mass index, Vol_{TM} muscle thigh volume

p: Significance by means of Student's *t* test for unpaired data

Maximal voluntary contraction and isometric fatigue

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

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

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

Incremental exercises

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

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

"Peak" values of the investigated variables were calculated during the last ~ 20 s of the exhausting work rate. Heart rate (HR) was recorded by a dedicated device (Polar Electro, Oulu, Finland). O_2 uptake (VO_2) and CO_2 output (VCO_2) were determined by means of a metabolic portable unit (K5, Cosmed, Italy). Expiratory flow measurements were performed by a turbine flowmeter calibrated before each experiment by a 3 L syringe at three different flow rates. Calibration of O_2 and CO_2 analyzers was performed before each experiment by utilizing gas mixtures of known composition (16.00% O_2; 4.00% CO_2). The gas exchange ratio (R) was calculated as $VCO_2 \cdot VO_2^{-1}$.

Cardiac output (CO) was monitored continuously by bioimpedance method (PhysioFlow, Manatec, France), following the procedure described in a previous study (Charloux et al. 2000). This method has been validated during maximal incremental exercises (Richard et al. 2001), and also used in overweight (Palmieri et al. 2006) and obese subjects (Vella et al. 2011; 2012; Charloux et al. 2000). PhysioFlow and metabolimeter values were synchronized and mediated every 10 s. At the end of every step, the last two measurements were taken in account and mediated. Arteriovenous oxygen difference ($a - vO_2$ diff.) was estimated by dividing CO (l min^{-1}) into VO_2 (l min^{-1}) and multiplying by 100. The $a - vO_2$ diff. above 19 ml l00 ml^{-1} were considered not physiological and excluded with the correspondent CO values.

Statistical analyses

Statistical analyses were performed using SPSS 20.0 software (IBM, Chicago, USA), with significance set at $p < 0.05$. All results were expressed as means and standard deviation (SD). The differences between subgroups (OB vs. CTRL) on anthropometric characteristics, body composition, MVC, Tlim (time to exhaustion) were compared by means of a Student's t test for unpaired data. Further, Student's t test for unpaired data, was used to compare sex on MVC. The peak exercise values (VO_2, CO, $a - vO_2$ diff, Load Peak) and MVC_end in percentage of the pre-values, that representing the muscle fatigue immediately after exercise, were analyze by two-ways analysis of variance (ANOVA) between-within factors and sex by covariate. Post hoc comparisons were made using Bonferroni procedure for significant differences.

The effect of group, sex, intensity and interactions on the submaximal values (VO_2, CO and $a - vO_2$ diff) during the two exercises were analyzed with linear mixed, multilevel, growth model, fit by maximal likelihood, taking into account groups and intensity as fixed effect and subjects and intercept as random effects. Bonferroni post hoc was applied as appropriate. For this analysis, statistical power was checked, and the results was still reliable only if eight or more subjects per subgroup were presented. Then the post hoc procedures were made, and the figures show, the steps until at least eight subjects per groups were still present.

The trends of the MVCs recovery (percentage of the pre-exercise values) were analyzed by ANOVA for repeated measure, within-between subjects taking in account MVC_end (MVC immediately after the exercise in percentage of pre-values) and sex by covariates.

Results

Physical characteristics of OB and CTRL are shown in Table 1. OB had significantly greater BM (+ 90%), BMI (+ 95%), Vol_{TM} (+ 19%), FFM (+ 30%), FM (+ 309%) and %FM (+ 122%) than CTRL.

Absolute MVC was not significantly different between the two subgroups (OB 242 ± 75 Nm; CTRL 236 ± 80 Nm; $p = 0.850$), while MVC normalized by Vol_{TM} was lower in OB (34.84 ± 7.26 Nm L L^{-1}) than CTRL (43.66 ± 10.79 Nm L^{-1}), $p = 0.020$.

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

Peak values of the main variables determined during CE and KE tests are given in Table 2. ANOVA procedures shows that VO_2peak, HR peak and CO peak were not significantly different between the two subgroups, being, however, significantly higher in CE than KE (+ 126%, + 33% and + 46%, respectively, $p < 0.001$).

In CE, VO_2peak normalized by FFM was 32% lower in OB compared with CTRL (0.038 ± 0.003 vs. 0.056 ± 0.007 L min^{-1} kg^{-1}; $p = 0.001$). By contrast, VO_2peak normalized by Vol_{TM} was not significantly different between the two subgroups in KE (0.184 ± 0.447 vs. 0.192 ± 0.442 L min^{-1} L^{-1}; $p = 0.657$). CO peak normalized by FFM tended to be lower in OB during CE (OB 0.312 ± 0.100 vs. CTRL 0.386 ± 0.061 L min^{-1} kg^{-1}; $p = 0.066$), while it was not significantly different between the two subgroups during KE (OB 2.59 ± 0.96 vs. CTRL 2.29 ± 0.49 L min^{-1} L^{-1}; $p = 0.379$).

$a - vO_2$ Diff peak (Table 2) was greater in CE (+ 44%, $p < 0.001$) than KE. The post hoc analysis found lower values in OB than CTRL in CE (− 22%, $p = 0.012$), no differences being found in KE ($p = 0.510$).

Although the two subgroups reached similar VO_2 peak in CE, work load peak was 15% lower in OB than CTRL ($p = 0.021$), while it was not significantly different between the two subgroups in KE (Table 2).

The trends of VO_2, HR, CO and $a - vO_2$ diff during the incremental tests are shown in Fig. 1**.** Only VO_2 during CE was significantly higher in OB at each step by average (36%, $p < 0.001$, Fig. 1a). No significant differences were found between the two subgroups on VO_2, HR, CO and $a - vO_2$ during the incremental tests on KE (Fig. 1b, d, f, h). In addition, all parameters increased significantly as a function of work load.

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

In addition, the recovery trends of MVC after CE and KE incremental exercises are shown in Fig. 3.

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

Discussion

The main results of the present study are: (1) in OB, the reduction of MVC immediately after the end of the incremental exercise on CE was lower than on KE, while it was similar in CTRL subjects, (2) both in CE and KE, peak values of absolute VO_2, HR and CO did not differ between OB and CTRL, (3) the recovery from fatigue after the end of the two incremental exercises (CE and KE) was not significantly different between OB and CTRL.

Immediately after the completion of the two incremental exercises, MVC decreased by 14 and 32% in OB (respectively, after CE and KE tests, $p < 0.001$), and by 26 and 30% in CTRL (respectively after CE and KE tests, $p > 0.05$). These values were similar to those found by Millet (2011) in their review about fatigue in ultra-marathon races. Millet showed that the lower limb MVC decreases as a function of the duration of the running exercise: i.e., the longer

Table 2 Peak values determined during the incremental exercises on a cycle-ergometer (CE) and knee-extension ergometer (KE)

	CE		KE		Significance		
	Obese	Controls	Obese	Controls	*G*	*E*	*G* × *E*
VO_2 (L min^{-1})	2.68 ± 0.68	2.93 ± 0.65	1.26 ± 0.51	1.15 ± 0.26	0.598	< 0.001	0.18
HR (bpm)	164 ± 15	177 ± 11	131 ± 22	125 ± 15	0.441	< 0.001	0.031
CO (L min^{-1})	20.10 ± 5.42	20.61 ± 4.04	15.77 ± 5.89	12.60 ± 2.45	0.522	< 0.001	0.017
$a - vO_2$ diff (mL L^{-1})	12.05 ± 3.05	15.42 ± 2.26*	9.65 ± 4.71	10.02 ± 1.26	0.040	< 0.001	0.017
Load peak (W)	191 ± 38	226 ± 39*	62 ± 13	58 ± 14	0.082	< 0.001	0.012

All values are presented as mean ± standard deviation

Significance by two-way analysis of variance (ANOVA) between-within factors: *G* group effect, *E* exercise effect, *G* × *E* group × exercise (interaction) effect

VO_2 oxygen consumption, *CO* cardiac output, $a - vO_2$ *diff* arteriovenous oxygen difference

*Significantly different between obese and control ($p < 0.05$) by Bonferroni procedures

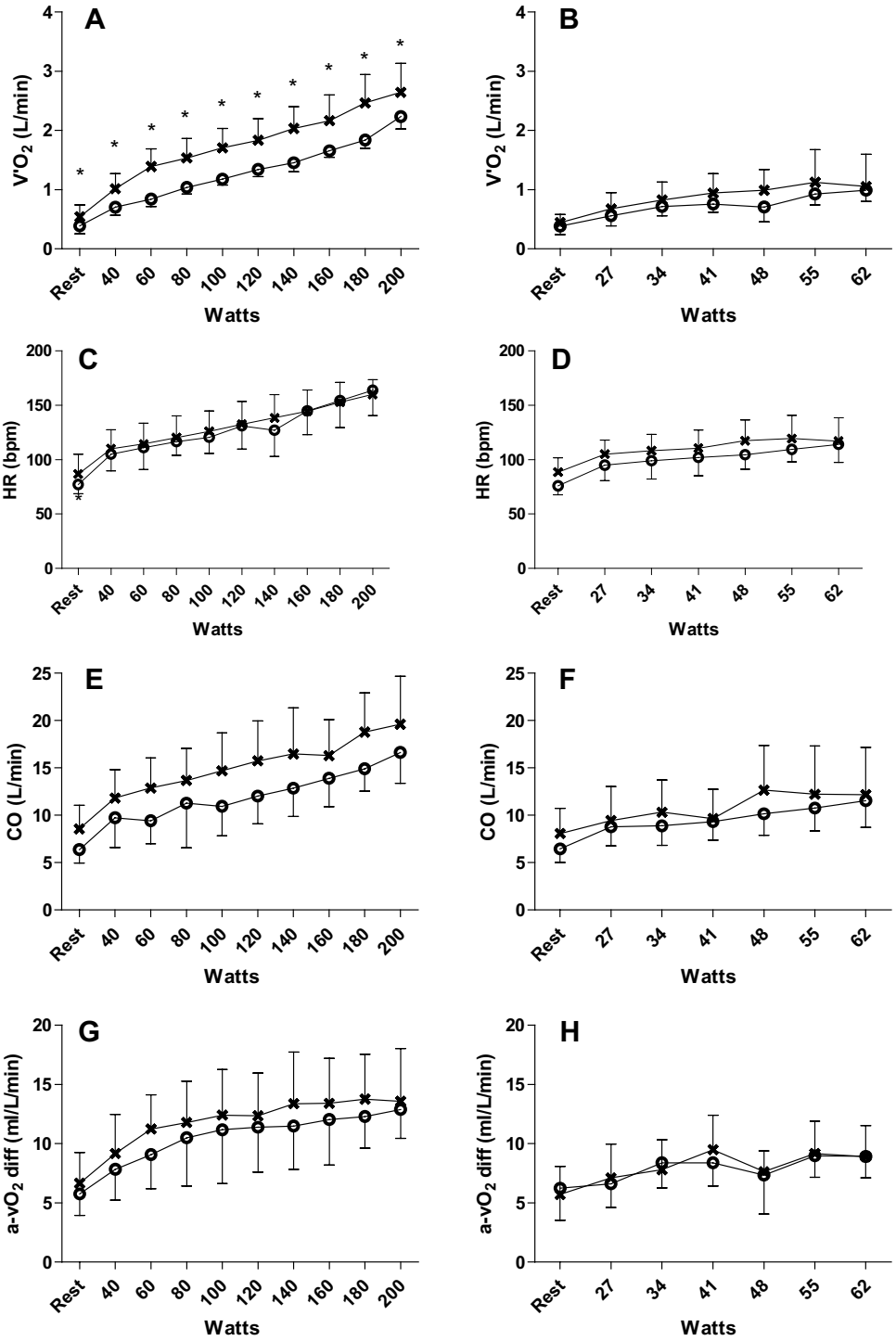

Fig. 1 Submaximal values during the incremental exercises on the cycle-ergometer (**a**, **c**, **e**, **g**) and knee-extension ergometer (**b**, **d**, **f**, **h**) as a function of work load (W) in OB (-x-) and CTRL (-o-) subjects. All values are presented as mean ± standard deviation. VO_2 oxygen consumption, *HR* heart rate, *CO* cardiac output, $a - vO_2\ diff$ arteriovenous oxygen difference. *Significantly different between obese and control ($p < 0.001$). The post hoc procedures were made. The figures show the data of at least eight subjects per subgroups, while Table 2 reports the average of all the subjects (see statistical paragraph for more details)

the duration of the exercise, the greater the decline in knee extensor muscles force ability. However, the two variables do not follow a linear relationship. Indeed, muscle force production does not overcome the specific threshold of 30–40% MVC loss, even in extremely long race; this finding suggests that some muscle force ability level needs to be preserved, maybe to avoid neuromuscular dysfunction and mobility limitations after exercise. This strategy that is supposed to be implicated in the management of fatigue after exhausting running exercises might be also present after other types of fatiguing exercise tasks, such as KE and CE. Indeed, MVC decrements observed in the present study after the end of the two exercises in CTRL, and after the completion of KE in OB, lied on the lower margin of the fatigued threshold noticed by Millet's group. On the contrary MVC loss experienced by OB after the end of CE was lower (− 14%). This

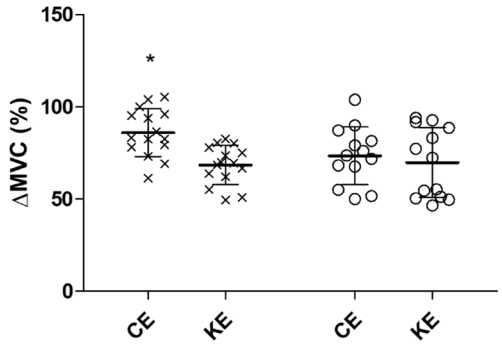

Fig. 2 Maximal voluntary contraction changes in percentage (ΔMVC, %) of pre-values, obtained immediately after the end of the incremental exercises on cycle ergometer (CE) and knee-extension ergometer (KE), in OB (x) and CTRL (o) subjects. All values are presented as mean ± standard deviation. *Significantly different between CE and KE exercises ($p < 0.001$)

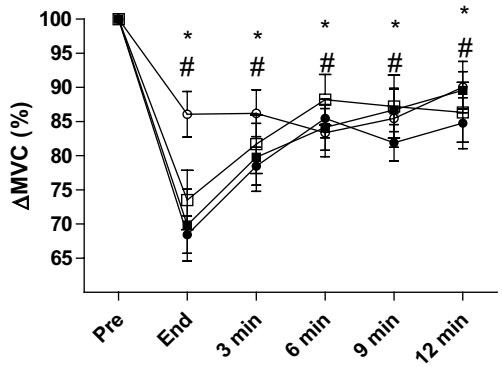

Fig. 3 Maximal Voluntary Contraction changes (ΔMVC, %) after 3, 6, 9 and 12 min the end of incremental exercises on cycle ergometer in OB (-o-) and CTRL (-□-) subjects, and on knee-extension ergometer in OB (-●-) and CTRL (-■-) subjects. All values are presented as mean ± standard deviation. *Significantly different from pre values obtained after incremental exercises on cycle ergometer in obese subjects. #Significantly different from pre values obtained after incremental exercises on knee-extension ergometer in control subjects. See statistical paragraph for more details

finding suggests that muscle function was preserved in OB and hence other factors might have limited the performance during whole body aerobic exercise, in agreement with results from previous studies (Noakes et al. 2004; Noakes 2012; Blain and Hureau 2017). The fact that CTRL showed similar MVC decrements after the end of the two exercises might be due to the important roles that both central and peripheral factors play in the management of exercise tolerance in normal weight individuals (Blain and Hureau 2017).

During incremental exercise on CE, VO_2 peak, HR peak, CO peak and $a - vO_2$ diff were greater than in KE in both subgroups, as previously observed (Lazzer et al. 2013; Esposito et al. 2010; Salvadego et al. 2013), thus confirming the existence of a cardiac reserve in single leg KE exercise. Blomstrand and coworkers (Blomstrand et al. 1997) showed that in the quadriceps muscle, Krebs cycle and oxygen uptake are more active and pronounced during single leg KE than during CE exercises. These findings confirm that KE exercise could be a better way to evaluate possible peripheral muscle functions avoiding central or cardiovascular constrictions.

In the present study, absolute values of VO_2peak, HR peak and CO peak, during incremental exercise on CE were not significantly different between OB and CTRL, in agreement with previous studies (Lazzer et al. 2013; Salvadori et al. 1999; Vella et al. 2012). However, submaximal VO_2 values in OB were significantly higher than CTRL for the same mechanical load, as previously reported (Salvadori et al. 1992, 1999; Vella et al. 2011, 2012; Lazzer et al. 2013). Lafortuna and colleagues hypothesized that the greater O_2 cost in obese during cycling was mainly related to the extra work required to move legs (Lafortuna et al. 2008). In fact, although the gross mechanical efficiency was lower in obese compared with lean individuals, the net one was similar (Salvadori et al. 1992). This might explain why the exercise tolerance was impaired in obese. Nevertheless, other factors could be involved. Looking at our data, when VO_2peak was normalized by FFM during CE, the maximal aerobic power in OB was lower than that observed in CTRL. This finding might further impair exercise tolerance, as demonstrated by the lower peak work rate described in OB during CE (Lazzer et al. 2013). Moreover, also the oxygen cost of breathing, significantly higher in resting obese individuals (Kress et al. 1999), may contribute to the increased metabolic cost of cycling in this population. Finally, the fraction of VO_2 related to the work performed by respiratory muscles has been shown to rise disproportionately in obese subjects during exercise (Alemayehu et al. 2018; Salvadego et al. 2017). Indeed, these researchers (Salvadego et al. 2015; 2017; Alemayehu et al. 2018) found that respiratory muscles were overloaded during exercise in severely obese people, being associated with an increase O_2 cost and a reduced exercise tolerance (Dempsey et al. 2006).

In the present study, CO peak values were not significantly different between OB and CTRL during CE exercise. However, CO peak normalized by FFM was lower in OB than CTRL, suggesting a lower blood and O_2 delivery at muscle level, which could limit the muscles' ability to extract O_2 in OB. These findings are partially in disagreement with the results from previous works in this field. In fact, some researchers (Ferrero et al. 1996; Salvadori et al. 1999; Vella et al. 2011, 2012) showed higher CO at rest in obese than lean subjects, which is probably related to the greater muscle mass owned by the former ones. While Vella and colleagues (Vella et al. 2011, 2012) observed higher CO peak (and submaximal CO) in obese compared with lean

controls during CE incremental exercise; by contrast, Salvadori and colleagues (Salvadori et al. 1999) showed and opposite behavior of CO in the two subgroups. The discrepancies between these studies could be explained by the different measurement methods and the different degrees of obesity between the study populations.

To the best of our knowledge, there are very few studies measuring $a - vO_2$ diff in obese and it is not so clear whether the $a - vO_2$ diff peak in this population is a limiting factor or not. In the present study, $a - vO_2$ diff peak was lower in OB than CTRL on CE exercise. This finding might support the conjecture of a central limitation experienced by OB participants during the physical effort. As discussed above, for the same oxygen consumption, OB had higher O_2 cost devoted to the respiratory muscles (Salvadego et al. 2015, 2017; Alemayehu et al. 2018) compared with lean counterparts. This finding means that during exercise, the locomotory muscles of OB subjects have potentially less circulating O_2 available. So, even though the two subgroups have the same VO_2peak and CO peak during CE, the central circulation could represent a constraining factor in OB individuals. Moreover, fatigued respiratory muscles might induce sympathetic vasoconstriction of the locomotor muscles by activation of metaboreflex pathway, and hence they might contribute to limit the performance (Dempsey et al. 2006). This mechanism enhanced in obese (Salvadego et al. 2015, 2017; Alemayehu et al. 2018) could preserve muscle function and might explain lower muscle fatigue and $a - vO_2$ diff peak in OB after CE. The $a - vO_2$ diff values reported for OB individuals in the present study were similar to the those found by Vella and coworkers (Vella et al. 2011, 2012), where $a - vO_2$ diff was lower compared with the lean subgroup. This finding seems inconsistent with those collected by other researchers (Rowland et al. 2003) reporting the presence of a similarity between $a - vO_2$ diff values in the two subgroups of subjects. $a - vO_2$ diff depends on the capacity of the muscle to uptake oxygen from blood circulation. This capacity might be limited by muscle blood perfusion, oxygen diffusion or mitochondrial respiratory function. Limberg and colleagues (Limberg et al. 2010) did not find differences between OB and normal weight subjects in blood flow at the muscle level, measured during single leg KE and single forearm flexion exercises. Similarly, capillary/muscle fiber ratio, which is correlated with muscle oxygen diffusion (Howlett et al. 2003, 2009), was not significantly different between OB and lean subjects (Gavin 2004). Concerning the mitochondrial respiratory function, Konopka et al. (2015) showed that the oxidative capacity recorded in obese individuals was comparable to that observed in lean sedentary subjects, while obese exhibited a greater uncoupled mitochondrial respiratory function. Hence, obese required more oxygen to phosphorylate the same amount of ADP in ATP compared with the lean counterpart. Although insufficient to draw firm conclusion on this argument, the data from literature might support the lack of differences in $a - vO_2$ diff between obese and lean subjects.

In KE exercise, OB and CTRL exhibited similar maximal external work load, VO_2, CO and $a - vO_2$ diff. Similarly, when VO_2peak was corrected for the thigh muscle volume (VO_2/Vol_{TM}), no differences have been detected in the two subgroups. This finding suggests that muscle peripheral impairments could have affected both subgroups indistinctively, once central or cardiovascular limitation was ruled out.

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

Finally, the capacity to recover from muscle fatigue was not affected by obesity status. This finding is in agreement with data from previous works (Maffiuletti et al. 2007; Garcia-Vicencio et al. 2015). Recovery was similar between the two subgroups across the different time points in both the exercises. MVC remained impaired even twelve minutes after the end of the exercises.

In conclusion, MVC decreased less after the end of CE than KE exercises in OB subjects. On the contrary, MVC decreased to a similar extent after the end of both exercises in CTRL. This finding might suggest the occurrence of a muscle function preservation during incremental cycling exercise to exhaustion in OB, but not in CTRL.

In KE, peak values of absolute VO_2, HR and CO did not differ between OB and CTRL. This result might indicate that both subgroups reached similar metabolic status at the muscle level. Finally, similar muscle function recovery from fatigue has been observed in OB and CTRL subgroups after the end of both the exercises. Hence, it might be inferred

that muscle function recovery was not be impaired in obese individuals.

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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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