

# The Effect of Different Intensity Exercise on Energy Consumption and Metabolism Substrate in Obese Adult Women

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## Research Article

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## Abstract

## Background

This study aims to compare differences between obese and normal-weight females in maximal fat oxidation (MFO) and exercise intensity eliciting MFO ( $Fat_{max}$ ), as well as explore the relationship between cardiopulmonary function and  $Fat_{max}$ .

## Methods

Nine obese female (O Group) and nine subjects with normal weight (C Group) participated in this research. Each individual tested by using a MetaMax 3B metabolimeter was performing an incremental graded exercise protocol on an electronic bicycle ergometer to evaluate maximal oxygen uptake ( $VO_{2max}$ ). And, after, exercises at 25%, 35%, 45%, 55%, 65% and 75% of  $VO_{2max}$  were conducted on a separate day to assess MFO and  $Fat_{max}$ .

## Results

The results showed that, in exercises of the same intensity ( $\%VO_{2max}$ ),  $VO_2/kg$  and MET were significantly lower in O Group than these in C Group, while EE/kg was significantly lower. Moreover, the O group had lower  $VO_{2max}/kg$  ( $P < 0.01$ ),  $AT-VO_2/kg$  ( $P < 0.05$ ), and  $Fat_{max}-VO_2/kg$  ( $P < 0.05$ ), the obese women reached MFO (0.22-0.44g/min) at 44.67–59.40% of  $VO_{2max}$  more than C group.

## Conclusions

In conclusion, the application of the graded exercise protocol during this study can get accuracy on MFO and  $Fat_{max}$  estimation, and ensure that the use of  $Fat_{max}$  can be applied to improve cardiorespiratory fitness.

## Introduction

Obesity is a major health problem in many countries of the world because of its association with cardiovascular disease, hypertension, and diabetes mellitus[1, 2], which is a condition characterized by an increased fat accumulation, according to the World Health Organization (WHO) over 650 million of subjects were obese in 2016[3].

One of the best approaches to reduce body weight is an healthy lifestyle which should combine physical activity (PA) with a balanced diet[4, 5]. To our knowledge, several studies have described the relationship between exercise intensity and fat oxidation[6, 7], and focused on the evaluation and characterization of which exercise intensity produced maximal fat oxidation (MFO) is termed  $Fat_{max}$ [8, 9], the Finding, however, is so far controversial. Some studies have observed a lower MFO during exercise and a lower  $Fat_{max}$  in individuals with obesity compared with normal weight individuals[8–10]. Nevertheless, recent studies reported higher MFO and  $Fat_{max}$  in obese people compared with their lean counterparts[11]. This may be no guideline for estimation of MFO and  $Fat_{max}$ .

Different approaches have been proposed to determine the MFO and  $Fat_{max}$ . First, one approach is to perform minimal exercise intensities arbitrarily selected (ie, walking on treadmill at 4.3km/h at the 0%, 3% and 6% slope) and too long exercise duration (ie, 15 minutes at six different workloads) [12, 13]. Secondly, since 2002, four to six continuous prolonged exercise intensities at the increments of 35W are performed on separate days used in the graded exercise test with 3-minute stages[9]. Thirdly, to reach a steady-state gaseous exchange, individuals with low levels of cardiorespiratory fitness need longer time periods to accurately measure substrate metabolism by indirect calorimetry[14, 15]. Next, the main graded exercise protocol variations involved alteration in stage durations (eg, stage duration of 1 minute vs 10 minutes)[16, 17] or exercise intensities (eg, increments of 35W vs 20W)[8, 18]. Lastly, a recent study suggested that graded exercise protocols using a 4-minute stage duration can be enough to attain steady-state gaseous exchange in low cardiorespiratory fitness individuals[19]. Nowadays, there is no consensus on the issue which is the best stage duration to measure MFO and  $Fat_{max}$ , which is consistent with Amaro-Gahete FJ's opinion[20].

Nevertheless, MFO and  $Fat_{max}$  varies with training status. To date, scientific evidence had showed that a constant workload with individual anaerobic threshold levels as the target intensity was feasible to estimate  $Fat_{max}$ [21, 22]. Well-trained individuals reached MFO at 45%-75% of maximal oxygen uptake ( $VO_{2max}$ )[9, 13, 23, 24], and then, sedentary individuals reached MFO at around 30%-50% of  $VO_{2max}$ [25–28]. In addition, despite various graded exercises employed for  $Fat_{max}$  in the obese, to our knowledge, no protocol was developed specifically for the obese nor have been validated against  $Fat_{max}$  determined from continuous exercise at different intensities[29–31]. Consequently,  $Fat_{max}$  have not been accurately determined.

Therefore, when showing the relationship between oxygen uptake and MFO during exercises of  $Fat_{max}$ , the main purpose of this study is to assess differences in MFO and  $Fat_{max}$  between obese and adult women with small workload increments and long stage durations. It is hoped that this study will provide a theoretical basis for the further research in MFO and  $Fat_{max}$  assessment.

## Methods

### Participants.

This research project was conducted in an university, in accordance with the ethical code of the World Medical Association and written approval had also been obtained from the Human Research Ethics Committee of the university. During the study, 10 subjects dropped out due to various reasons such as poor compliance to physical activity treatment, or missing data. Therefore, the study was completed with 18 adults (nine obese female and nine subjects with normal weight ) as subjects for this study. The procedures and purpose of the study, including the right to freely withdraw, were explained to the participants, and their informed consent had also been obtained. (Exclusion criteria were cardiovascular and metabolic diseases that might affect the sports activity.) Participants were assigned to C Group (Control Group,  $18.5\text{kg}\cdot\text{m}^{-2} \leq \text{BMI} < 24\text{kg}\cdot\text{m}^{-2}$ ) and O Group (Obese Group,  $28\text{kg}\cdot\text{m}^{-2} \leq \text{BMI}$ )[32, 33]. The anthropometric characteristics between the two groups are summarized, as shown in Table 1.

Note: compared to control group, \* means  $P < 0.05$ ; \*\* means  $P < 0.01$ .

### Methods and Tests.

All subjects were tested in the morning (between 9 and 12 AM) after an overnight fast (12h) on 2 different days, and under similar environmental conditions (temperature 20–25° C; humidity 50–60%). Subjects had their usual breakfast at least two hours before the test session.

#### Anthropometric and body composition measurements

Body weight and height were measured using a scale and a stadiometer to the nearest 0.1 kg and 0.1 cm, respectively. Body composition was measured by bioelectrical impedance method (X-SCAN PLUS II body composition analyzer, JAWON Company, Korea) while the subjects wore light clothing without shoes.

A gas analyzer (MetaMax 3B, Cortex Biophysik, Germany) was used for the acquisition of signals, including  $VO_{2max}$ , maximal heart rate ( $HR_{max}$ ) and maximal ventilation ( $VE_{max}$ ).

Measurement method of aerobic endurance: the increasing exercise load method was used. The subjects were required to maintain a 60 rpm rotational speed on the bicycle ergometer (Ergoselect 100, Ergoline, Germany). Both the initial load and increasing rate were 25 W for males and 20 W for females. The load of each level lasted for 2 min. After 2 min, the load of next level was applied without rest. The load was increased continuously at this rate until the subjects were exhausted. The gas analyzer (MetaMax 3B, Cortex Biophysik, Germany) was used in the whole process for acquisition of signals, including  $VO_{2max}$ ,  $HR_{max}$ ,  $VE_{max}$  and anaerobic threshold (AT).

All of the subjects were told to maintain a light diet and to avoid any physical activity 48 hours before the test. Each participant was tested individually, while performing a graded exercise protocol on an electronic bicycle ergometer (Ergoselect100, Ergoline, Germany). The test program for aerobic endurance was conducted by the following steps: (1) five-minute warm up at speed of 30 W; (2) resting on the cycle ergometer for more than 5 minutes, until return to quiet state; (3) riding bicycle at 60 rpm rotational speed on the bicycle ergometer and increasing the intensity every two minutes by 20 W. The criteria for reaching  $VO_{2max}$  is as follows<sup>21</sup>: respectively, the respiratory exchange ratio (RER) was  $> 1.15$ , the subjects were exhausted, a plateau in  $VO_{2max}$  (defined as a change of no more than  $2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  with increasing the load).

After the results from aerobic endurance exercise, exercise at 25%, 35%, 45%, 55%, 65%, 75% of  $VO_{2max}$  was conducted on a separate day (1–2 days in between), which were randomized. Test program for maximal fat oxidation (MFO) was conducted in the following steps: (1) determination of fat oxidation at rest and six-minute continuous recording at each intensity; (2) resting between different intensity exercises until return to a quiet state (that is, quiet heart rate, relative oxygen uptake  $\leq 3 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ , respiratory exchange ratio  $\leq 0.8$ ).

### Data analysis.

Data of  $VO_2$  (L/min) and carbon dioxide output ( $VCO_2$ ) were sampled in ten-second intervals during the tests. Even though six-minute data were recorded, only the data from third to fifth minutes were used for analysis, to ensure the accuracy and stability of the data.

Fat oxidation rates were calculated using the equations described by Frayn(1983), with the assumption that urinary nitrogen excretion was negligible. Based on the data recorded every ten seconds, combined with the modified energy consumption formula (international version), the calculation equations adopted in this research were as follows[16–19] :

$$\text{Fat oxidation [g/min]} = 1.695 \times VO_2 \text{ [L/min]} - 1.701 \times VCO_2 \text{ [L/min]}$$

$$\text{Glucose oxidation [g/min]} = 4.585 \times \text{VCO}_2 \text{ [L/min]} - 3.226 \times \text{VO}_2 \text{ [L/min]}$$

$$\text{Energy expenditure [kcal/min]} = \text{fat oxidation [g/min]} \times 9 + \text{glucose oxidation [g/min]} \times 4$$

For each subject, the results of the aerobic endurance exercise test were used to compute the relationship between fat oxidation rate and the relative exercise intensity (%VO<sub>2max</sub>), and a second-degree, polynomial regression was used to determine Fat<sub>max</sub>, MFO and VO<sub>2</sub> for each subject individually.

### Statistical analyses.

Statistical analysis was performed using SPSS19.0 (SPSS Inc., Chicago, USA). In all cases, all values are mean and standard deviation (SD), and values of  $P < 0.05$  were considered statistically significant. A one-way ANOVA was conducted to compare the indexes between different intensities. Moreover, a two way ANOVA for repeated measures (2 groups  $\times$  6 exercise intensity) assessed both main effects and interactions in two groups on VO<sub>2</sub>/kg, HR, MET and RER. In the analysis, Pearson's correlation test was done between VO<sub>2max</sub>/kg, AT-VO<sub>2</sub>/kg and Fat<sub>max</sub>-VO<sub>2</sub>/kg exercise intensity, and the correlation coefficient R and P value were calculated.

## Results

Comparative analysis of related indexes of cardiopulmonary function between the two groups

Note: VO<sub>2</sub> = oxygen uptake expressed in l/min, VO<sub>2</sub>/kg = oxygen uptake expressed in ml/kg/min, VCO<sub>2</sub> = carbon dioxide output expressed in l/min, HR = Heart rate, MET = Metabolic equivalent (MET = VO<sub>2</sub> expressed in ml/kg/min divided 3.5), RER = Respiratory exchange ratio. Data are presented as mean  $\pm$  SD. Compared to 25%VO<sub>2max</sub>, □ means  $P < 0.05$ ; □□ means  $P < 0.01$ , compared to 35%VO<sub>2max</sub>, means  $P < 0.05$ ; means  $P < 0.01$ , compared to 45%VO<sub>2max</sub>, \* means  $P < 0.05$ ; \*\* means  $P < 0.01$ , compared to 55%VO<sub>2max</sub>, Δ means  $P < 0.05$ ; ΔΔ means  $P < 0.01$ ; compared to 65%VO<sub>2max</sub>, # means  $P < 0.05$ ; ## means  $P < 0.01$ .

Table 2 shows the determination of Oxygen pulse, VO<sub>2</sub>, VO<sub>2</sub>/kg, VCO<sub>2</sub>, HR, MET and RER values in individuals with C Group (a) and O (b) Group. With increasing exercise intensity, there were statistically significant differences in Oxygen pulse, VO<sub>2</sub>, VO<sub>2</sub>/kg, VCO<sub>2</sub>, HR, MET in two groups, and no significant differences were found between 25% and 35% of VO<sub>2max</sub>. RER values had no significant differences for individuals with both groups during 25%, 35%, 45% and 55% of VO<sub>2max</sub>.

Note: \* means  $P < 0.05$ , \*\* means  $P < 0.01$ .

**Table 2.** Determination of Oxygen pulse, VO<sub>2</sub>, VO<sub>2</sub>/kg, VCO<sub>2</sub>, HR, MET and RER in individuals with C Group (a) and O (b) Group.

(a) individuals in C Group

characteristic	25%VO <sub>2max</sub>	35%VO <sub>2max</sub>	45%VO <sub>2max</sub>	55%VO <sub>2max</sub>	65%VO <sub>2max</sub>	75%VO <sub>2max</sub>
Oxygen pulse, ml	4.79 $\pm$ 1.30	5.84 $\pm$ 1.26	7.42 $\pm$ 1.39□□	8.25 $\pm$ 1.68□□	8.77 $\pm$ 1.73□□	9.52 $\pm$ 1.81□□
VO <sub>2</sub> , l.min <sup>-1</sup>	0.48 $\pm$ 0.88	0.65 $\pm$ 0.11	0.87 $\pm$ 0.12□	1.09 $\pm$ 0.20□□	1.28 $\pm$ 0.21□□ **	1.54 $\pm$ 0.26□□ **ΔΔ#
VO <sub>2</sub> /kg, ml.kg <sup>-1</sup> .min <sup>-1</sup>	9.30 $\pm$ 1.40	12.52 $\pm$ 1.88	16.92 $\pm$ 2.19□□	21.20 $\pm$ 3.12□□ *	24.85 $\pm$ 3.51□□ **	29.97 $\pm$ 4.28□□ **ΔΔ##
VCO <sub>2</sub> , l.min <sup>-1</sup>	0.41 $\pm$ 0.09	0.55 $\pm$ 0.11	0.76 $\pm$ 0.11□□	0.98 $\pm$ 0.18□□ *	1.17 $\pm$ 0.20□□ **	1.48 $\pm$ 0.26□□ **ΔΔ##
HR, beats.min <sup>-1</sup>	104.74 $\pm$ 16.50	112.45 $\pm$ 14.46	118.43 $\pm$ 13.43	133.43 $\pm$ 10.46□□	146.83 $\pm$ 9.26□□ **	162.93 $\pm$ 10.20□□ **ΔΔ
MET	2.82 $\pm$ 0.51	3.72 $\pm$ 0.64	5.07 $\pm$ 0.72□□	6.36 $\pm$ 0.78□□	7.46 $\pm$ 1.19□□ **	8.98 $\pm$ 1.37□□ **ΔΔ#
RER	0.83 $\pm$ 0.07	0.85 $\pm$ 0.07	0.88 $\pm$ 0.07	0.90 $\pm$ 0.07	0.92 $\pm$ 0.06	0.96 $\pm$ 0.05□□

(b) individuals in O Group

characteristic	25%VO <sub>2max</sub>	35%VO <sub>2max</sub>	45%VO <sub>2max</sub>	55%VO <sub>2max</sub>	65%VO <sub>2max</sub>	75%VO <sub>2max</sub>
Oxygen pulse, ml	6.25±0.71	6.93±0.79	7.58±0.94□□	8.81±0.89□□ *	9.71±0.63□□ **	10.79±0.64□□ **△△
VO <sub>2</sub> , l.min <sup>-1</sup>	0.59±0.06	0.69±0.08	0.83±0.13□□	1.05±0.16□□ *	1.27±0.17□□ **△	1.55±0.16□□ **△△##
VO <sub>2</sub> /kg, ml.kg <sup>-1</sup> .min <sup>-1</sup>	7.17±0.69	8.58±1.08	10.34±1.58□□	13.16±2.35□□ *	15.79±2.28□□ **△	19.31±2.40□□ **△△##
VCO <sub>2</sub> , l.min <sup>-1</sup>	0.46±0.05	0.53±0.08	0.67±0.12□□	0.87±0.17□□	1.11±0.20□□ **△△	1.42±0.21□□ **△△##
HR, beats.min <sup>-1</sup>	94.88±5.73	100.14±7.04	109.71±10.56□□	119.66±11.31□□	130.32±12.37□□ **	143.46±10.70□□ **△△
MET	2.14±0.22	2.53±0.40	3.71±1.13□□	4.13±0.77□□	4.96±0.75□□ *	6.06±0.80□□ **△△#
RER	0.77±0.06	0.76±0.08	0.80±0.07	0.82±0.07	0.88±0.07□	0.91±0.08□□ *

In Fig. 1, VO<sub>2</sub>/kg (a), HR (b), MET (c), RER(d) were plotted against exercise intensity (% maximal oxygen uptake) for the short continue exercise during the C Group (open square) and O Group (filled square). Significant main effects of obesity class on VO<sub>2</sub>/kg ( $F_{1,16}=46.26$ ,  $P \leq 0.01$ ,  $\eta^2 = 0.74$ ), HR ( $F_{1,16}=9.50$ ,  $P = 0.007$ ,  $\eta^2 = 0.37$ ), MET ( $F_{1,16}=32.02$ ,  $P \leq 0.01$ ,  $\eta^2 = 0.67$ ) and RER ( $F_{1,16}=5.50$ ,  $P = 0.032$ ,  $\eta^2 = 0.25$ ) controlling for age were observed. Significant exercise intensity  $\times$  obesity class interactions on VO<sub>2</sub>/kg ( $F_{2,20,35,11}=25.34$ ,  $P \leq 0.01$ ,  $\eta^2 = 0.61$ ) and MET ( $F_{1,76,28,08}=10.66$ ,  $P = 0.001$ ,  $\eta^2 = 0.40$ ) controlling for age were observed. Moreover, significant main effects of exercise intensity on VO<sub>2</sub>/kg ( $F_{2,20,35,11}=373.07$ ,  $P \leq 0.01$ ,  $\eta^2 = 0.96$ ), HR ( $F_{1,97,31,53}=137.66$ ,  $P \leq 0.01$ ,  $\eta^2 = 0.90$ ), MET ( $F_{1,76,28,08}=203.04$ ,  $P \leq 0.01$ ,  $\eta^2 = 0.93$ ) and RER ( $F_{2,53,40,52}=33.53$ ,  $P \leq 0.01$ ,  $\eta^2 = 0.68$ ) controlling for age were observed.

Figure 1 shows that VO<sub>2</sub>/kg and MET were significantly lower in O Group than in C Group. Except for 25% and 45% VO<sub>2max</sub>, HR in C Group was significantly higher than O Group. During the 35%, 45% and 55% VO<sub>2max</sub> intensity, indeed, RER of the C Group was significantly higher than that of O Group.

Comparative analysis between the energy expenditure and substrate oxidation for the two groups

Note: CHO = carbohydrate oxidation, CHO/kg = carbohydrate oxidation expressed in mg.min<sup>-1</sup>.kg<sup>-1</sup>, FAT/kg = fat oxidation expressed in mg.min<sup>-1</sup>.kg<sup>-1</sup>, fat oxidation rate = ratio of energy contribution from fat, EE = energy expenditure expressed in cal.min<sup>-1</sup>, EE/kg = energy expenditure expressed in cal.min<sup>-1</sup>.kg<sup>-1</sup>. Data are presented as mean  $\pm$  SD. Compared to 25%VO<sub>2max</sub>, □ means  $P < 0.05$ ; □□ means  $P < 0.01$ , compared to 35%VO<sub>2max</sub>, means  $P < 0.05$ ; means  $P < 0.01$ , compared to 45%VO<sub>2max</sub>, \* means  $P < 0.05$ ; \*\* means  $P < 0.01$ , compared to 55%VO<sub>2max</sub>, △ means  $P < 0.05$ ; △△ means  $P < 0.01$ ; compared to 65%VO<sub>2max</sub>, #means  $P < 0.05$ ; ## means  $P < 0.01$ .

In Table 3, with exercise intensity increasing, CHO and CHO/kg increased significantly, 25% and 35% VO<sub>2max</sub> excepted, carbohydrate oxidation increased dramatically during 75% of the VO<sub>2max</sub> compared with 65% of the VO<sub>2max</sub> in C Group.

**Table 3.** EE/kg in individuals with C Group (a) and O Group (b).

(a) individuals in C Group

characteristic	25%VO <sub>2max</sub>	35%VO <sub>2max</sub>	45%VO <sub>2max</sub>	55%VO <sub>2max</sub>	65%VO <sub>2max</sub>	75%VO <sub>2max</sub>
CHO,g.min <sup>-1</sup>	0.30±0.19	0.43±0.23	0.70±0.29	0.97±0.35□□	1.25±0.39□□ *	1.83±0.48□□ **△△##
CHO/kg , mg.min <sup>-1</sup> .kg <sup>-1</sup>	5.79±3.72	8.44±4.56	13.61±5.36	18.90±6.85□□	24.49±7.95□□ *	35.53±9.20□□ **△△#
fat oxidation,g.min <sup>-1</sup>	0.13±0.06	0.16±0.07	0.17±0.12	0.18±0.13	0.17±0.14	0.09±0.16 *△##
FAT/kg, mg.min <sup>-1</sup> .kg <sup>-1</sup>	2.56±1.22	3.13±1.37	3.37±2.25	3.57±2.51	3.30±2.76	1.75±3.03 *△##
fat oxidation rate, %	52.67±24.15	47.33±22.43	35.89±23.36	30.44±22.03	23.78±19.16	10.44±16.23□□
EE , kcal.min <sup>-1</sup>	2.38±0.46	3.18±0.59	4.35±0.57□□	5.54±1.01□□	6.56±1.07□□ **	8.12±1.38□□ **△△##
EE/kg, cal.min <sup>-1</sup> .kg <sup>-1</sup>	46.18±7.95	61.90±10.30	84.82±10.51□□	107.72±16.07□□ *	127.68±18.51□□ **	157.91±22.71□□ **△△##

(b) individuals in O Group

characteristic	25%VO <sub>2max</sub>	35%VO <sub>2max</sub>	45%VO <sub>2max</sub>	55%VO <sub>2max</sub>	65%VO <sub>2max</sub>	75%VO <sub>2max</sub>
CHO ,g.min <sup>-1</sup>	0.18±0.15	0.18±0.26	0.39±0.25	0.60±0.42	1.01±0.51□□ *	1.50±0.60□□ **△△
CHO/kg , mg.min <sup>-1</sup> .kg <sup>-1</sup>	2.28±2.03	2.39±3.45	4.89±3.43	7.64±5.71□	12.74±6.89□□ *	18.80±7.94□□ **△△
fat oxidation , g.min <sup>-1</sup>	0.23±0.07	0.27±0.11	0.27±0.10	0.30±0.15	0.26±0.16△	0.21±0.20△
FAT/kg, mg.min <sup>-1</sup> .kg <sup>-1</sup>	2.81±0.83	3.37±1.27	3.34±1.15	3.72±1.70	3.14±2.01△	2.65±2.46△
fat oxidation rate, %	74.00±22.42	77.89±30.59	62.22±21.22	55.22±25.92	38.00±22.64□	26.11±24.54□□ *
EE , kcal.min <sup>-1</sup>	2.77±0.27	3.20±0.40	3.99±0.64□	5.12±0.88□□ *	6.36±0.96□□ **△	7.94±0.97□□ **△△##
EE/kg, cal.min <sup>-1</sup> .kg <sup>-1</sup>	34.44±2.90	39.39±5.32	49.64±8.41□	64.04±13.12□□	79.26±13.63□□ **△	99.03±14.46□□ **△△##

while fat oxidation and FAT/kg, during 55% of VO<sub>2max</sub>, fat oxidation was more than in other workloads, but there was no statistical significance during 25%,35% and 45% of VO<sub>2max</sub>. Compared to 25% and 35% of VO<sub>2max</sub>, the percentage of energy supplied from fat during 75% of the VO<sub>2max</sub> was very significantly lower. With exercise intensity increasing, EE and EE/kg increased significantly and simultaneously.

In O Group, with exercise intensity increasing, carbohydrate oxidation and CHO/kg increased very significantly, but in 25%,35% and 45% of VO<sub>2max</sub>. When VO<sub>2max</sub> was 55%, the fat oxidation and FAT/kg were significantly higher than fat oxidation during other workloads. Although exercise intensity during 55% of VO<sub>2max</sub> had higher fat oxidation, other exercise intensities had no dramatic differences. With exercise intensity increasing, fat oxidation rate was reduced progressively and EE and EE/kg increased very significantly.

Comparative analysis of related indexes of cardiopulmonary fitness and fat oxidation between the two groups

The following are the results in Table 4:

Note: AT = Anaerobic Threshold. Data are presented as mean ± SD. Compared to C Group, \* means  $P < 0.05$ ; \*\* means  $P < 0.01$ . Compared to VO<sub>2max</sub>/kg, △△ means  $P < 0.01$ . Compared to AT-VO<sub>2</sub>/kg, ◆◆ means  $P < 0.01$ .

Note: Data are presented as mean ± SD (95%CI of mean). CI: confidence interval of 95%. Compared to C Group,  $P < 0.05$ .

Results of ANOVA indicated that VO<sub>2max</sub>/kg ( $P \leq 0.01$ ), AT-VO<sub>2</sub>/kg ( $P = 0.003$ ), Fat<sub>max</sub>-VO<sub>2</sub>/kg ( $P = 0.024$ ) and MFO ( $P = 0.010$ ) expressed as g/min of O Group were significantly lower than those of C Group. Moreover, in both group Fat<sub>max</sub>-VO<sub>2</sub>/kg was lower at MFO than AT.

In Fig. 2, fat oxidation (e) and FAT/kg (f) are plotted against exercise intensity (% maximal oxygen uptake) for the short, continued exercise for C Group (real line) and O Group (broken line).

In O group, for  $VO_{2max}/kg$  values there was significant positive correlation in  $AT-VO_2/kg$  ( $R = 0.827, P = 0.011$ ) and  $Fat_{max}-VO_2/kg$  ( $R = 0.767, P = 0.016$ ) values. However, during C Group,  $VO_{2max}/kg$  values had no correlation with  $AT-VO_2/kg$  and  $Fat_{max}-VO_2/kg$  values, furthermore, the value of  $AT-VO_2$  had found relationship with MFO expressed as g/min ( $R = 0.884, P = 0.002$ ) and MFO expressed as g/min/kg ( $R = 0.861, P = 0.003$ ), and a positive correlation had found  $AT\%VO_{2max}$  and MFO expressed as g/min ( $R = 0.803, P = 0.009$ ), and MFO expressed as g/min/kg ( $R = 0.814, P = 0.008$ ). However, it did not found during O Group. As shown in Fig. 2(e) and Fig. 2(f), Significant main effects of exercise intensity on fat oxidation ( $F_{2,01,32,08}=3.96, P = 0.03, \eta^2 = 0.20$ ) and  $FAT/kg$  ( $F_{2,14,34,25}=4.15, P = 0.02, \eta^2 = 0.21$ ) controlling for age were observed. with exercise intensity increasing, fat oxidation at different exercise intensities were increased first, then decreased.

In both groups, EE and EE/kg had positive correlations with oxygen pluse ( $P \leq 0.01$ ),  $VO_2$  ( $P \leq 0.01$ ),  $VO_2/kg$  ( $P \leq 0.01$ ),  $VCO_2$  ( $P \leq 0.01$ ), HR ( $P \leq 0.01$ ), MET ( $\leq 0.01$ ) and RER ( $P \leq 0.01$ ), But EE and EE/kg had negative correlation with fat oxidation rate ( $P \leq 0.01$ ). Moreover, There were negative correlations between fat oxidation expressed as g/min or g/min/kg and RER ( $p \leq 0.01$ ).

## Discussion

The main objective of this study was to describe and examine the differences in MFO and  $Fat_{max}$  between obese and normal-weight females, and also to do further research on the issues between cardiopulmonary function and substrate metabolism in individuals with obesity.

With exercise intensity increasing, the values of the oxygen pulse,  $VO_2$ ,  $VCO_2$ , HR and MET of the C and O groups were significantly increased, for which a possible explanation for the findings is that skeletal muscles contracted faster, blood circulation accelerated, as well as pulmonary ventilation, cardiac output and HR were increased simultaneously. And these created an excellent condition for gas exchange between lungs and capillaries[34]. Previous published studies concluded that, according to the classification of physical workload of Pate, low exercise intensity was less than 3 METs; moderate intensity was less than 3–6 METs; high intensity was more than 6 METs. Thus, the intensity ranging from 45–75% of  $VO_{2max}$  was moderate for people, suffering from obesity, during cycling[35, 36]. In contrast to the same exercise intensity, the values of  $VO_2/kg$ , HR, MET and RER of O group were lower than C Group. It was likely that a difference in body-composition between the two groups in the statistical analysis contributed to the different results in the value of  $VO_{2max}$ . Furthermore, with the same exercise intensity, it obtained a palpable difference among those two groups in physiological respond, which was that C Group could undergo higher exercise intensity with oxygen requirement increasing. It can be possibly attributed to the kind of exercise obese females choose, taking into account their lower mechanical efficiency (for instance, which will be impacted by the weight of body parts)[10].

With exercise intensity increasing, total energy expenditure in the both group gradually increased, while the ratio of energy material was changing. For example, while on one hand fat oxidation rate was reducing, on the other hand, the ratio of carbohydrate for energy supply was escalating (to satisfy the energy demands of the body). Based on the fact there were systematic differences between C Group and O Group, the values of EE/kg and carbohydrate consumption of females from C Group were higher than the ones from O Group, while the values of fat oxidation and fat oxidation rates were lower than in the obesity group were dramatically observed. Moreover, with regard to total energy expenditure, the trend towards higher absolute intensity, total energy consumption energy-supplied ratio from carbohydrate from C Group was observed under same exercise intensity( $\%VO_{2max}$ ). Besides, in terms of fat mobilization and oxidation efficiency, the exercise load of O Group was lower and the major metabolic pattern for aerobic metabolism and fat oxidation rate was higher as well. The present study reported that there was close association between those changes and bodily output power[34, 37]. During exercise intensity from 25% of  $VO_{2max}$  to 55% of  $VO_{2max}$ , fat oxidation significantly escalated due to increase in exercise intensity; however, its value decreased from 55% of  $VO_{2max}$  to 75%  $VO_{2max}$ . One possible reason is that, when exercise intensity surpasses a set range, blood lactate concentration will be sharply rise and, then, the lipid hydrolysis process will be influenced. Another reason is that when the blood which flows through adipose tissue is reduced, then, the transportation of fatty acid will be also affected. Thus, these two processes will affect fat oxidation, mobilization and utilization in common. With exercise intensity increasing, fat oxidation rate must reach a peak during the process, which we call " $Fat_{max}$ " in the field of biochemistry. This means that the fat oxidation rate reaches maximal value with appropriate exercise intensity[38–40]. For as much, the factor of weight excluded, the maximal value of fat oxidation of C and O Group were reached with exercise at 41.76–65.97% and 44.67–59.40% of  $VO_{2max}$ , which highlights the possibility to use the exercise intensity for daily routine training. moreover, compared with C Group, the value of MFO was 0.22–0.44 g/min higher in O Group. Obviously, this result is in accordance with present studies (MFO ranging from 0.25 to 0.4 g/min)[41, 42]. Ara et al. Showed that MFO during exercise in obese subjects was increased compared to normal body weight subjects[20]. Van Loon carried out  $Fat_{max}$  exercises at around 57% of  $VO_{2max}$  for outstanding bicyclists[43]. Recently, Stisen showed that when female athletes and ordinary people were cycling,  $Fat_{max}$  was the same at around 55%  $VO_{2max}$ . In the current paper, it has proved that middle exercise intensity can promote the body reaching the highest fat oxidation rate for carbohydrates, by C.Maffei's intervening for 24 adolescent obese boys in middle exercise intensity[44], this demonstrated that fat oxidation rate could reach maximum during middle exercise intensity again. As expected, with the increase of exercise intensity, the lipolytic hormone response is enhanced, and fat mobilization is increased. However, when exercise intensity is increased to a certain extent, on the one hand, it can lead to an increase in lactic acid concentration, which has a strong inhibitory effect on lipolysis, on the other hand, decrease blood flow in adipose tissue affecting transport of fatty acid, both of which affect fat mobilization and utilization. In summary, the related physiological and biochemical regulation mechanisms of fat and sugar involved in energy supply need further research.

The correlation between fat oxidation and cardiopulmonary function was observed. The values of  $VO_{2max}/kg$  and  $AT-VO_2/kg$  and  $Fat_{max}-VO_2/kg$  of C and O Group had high-positive correlations, while fat oxidation and RER had a negative correlation. Emerenziani GP demonstrated that the differences between IVT and MFO were negligible since they are not relevant applications in obese women[45]. This suggests that further studies are needed to develop a detailed relationship for AT and MFO and there was a high to excellent correlation between fat oxidation rate and the ability of the body to use oxygen. We can't simply rely on HR or  $VO_2$  to determine fat oxidation, and must combine with RER for a determination. As was also indicated in previous studies, Belli A. found that while we always used oxygen consumption to determine individual metabolic capacity, during bicycle riding, the minimization of energy expenditure was mainly determined by pedal speed, and no significant difference was observed between oxygen consumption and EE[46]. According to Keytel LR's study, which was the prediction of energy consumption based on heart rate under sub-maximal exercise, we know that it is fairly accurate to calculate individual EE on the basis of HR after adjusting the factors such as age, gender and BMI[47]. From the data obtained it can be concluded that the physiological and biochemical regulation mechanisms of fat and carbohydrate in energy supply need to be further studied.

This is because on the basis of previous studies(20), we contributed to measure and analyze the energy consumption and substrate metabolism of normal-weight and obese women using a graded protocol with a 6-minute stage duration and six exercise intensities.

But Our study has a few limitations. First, we acknowledge the small sample size, which may limit the applicability of our findings. Therefore, a larger sample size can provide stronger evidence, future research can concentrate on this. However, our results found that there was no significant in  $Fat_{max}-\%VO_{2max}$  value. therefore, they can encourage people to choose a regular exercise with same intensity to lose fat. Second, we did not compare the results with those in subjects with different genders. At this point, the further research in this field is still ongoing and a lot of work remain to be done, such as whether sex, age, and health status play a huge role in this.

## Conclusion

In summary, this study demonstrated individuals with obese or normal-weight might have different MFO, obese females ranging in age from 20–39 years old should do exercise at around 44.67–59.40% of their  $VO_{2max}$ . Moreover,  $Fat_{max}-VO_2/kg$  was lower at MFO than AT, which is also an appropriate exercise intensity for daily routine training, in order to lose body fat and maintain metabolic health. For obese females, the reflects the connection between fat oxidation and the ability of the body to utilize oxygen, rather than merely basing it on HR or oxygen consumption to determine fat oxidation.

## Declarations

### Ethics approval and consent to participate

This study was approved by the Ethics Committee of Wuhan sports University (2015006). All experiments were performed in accordance with the university's set guidelines and regulations. All participants signed an informed consent form to take part in this study.

### Consent for publication

"not applicable" for that section.

### Availability of data and materials

The data generated and analyzed during the current study are available from the corresponding author on reasonable request.

### Competing Interests

All the authors have no competing interest. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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### Authors' Contribution

Li Jin and Qinbo Xue was involved in the study design, data collection, drafting of the manuscript, contributed equally to this work. Xiaoning Zhu and Qinbo Xue were involved in the study analysis, interpretation of the data, drafting of the manuscript. All authors read and approved the final manuscript.

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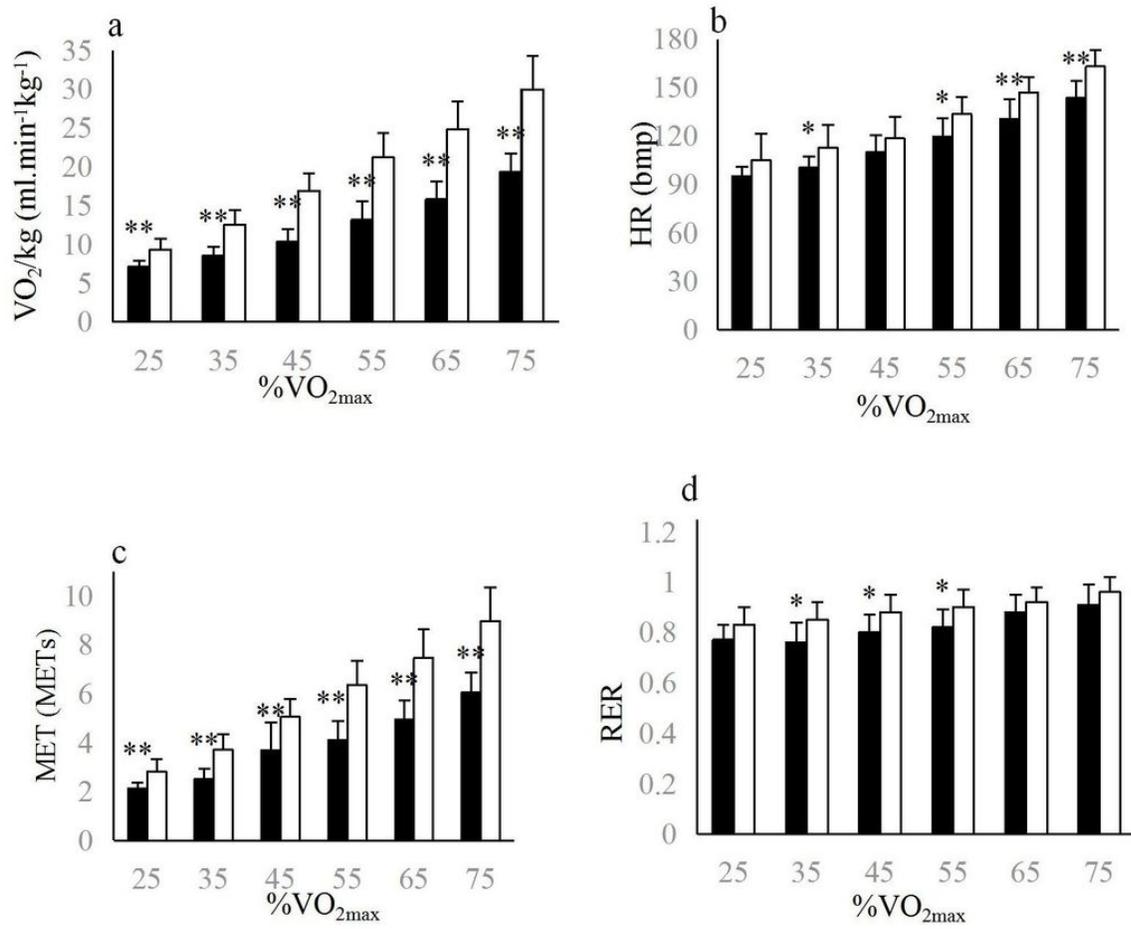
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## Tables

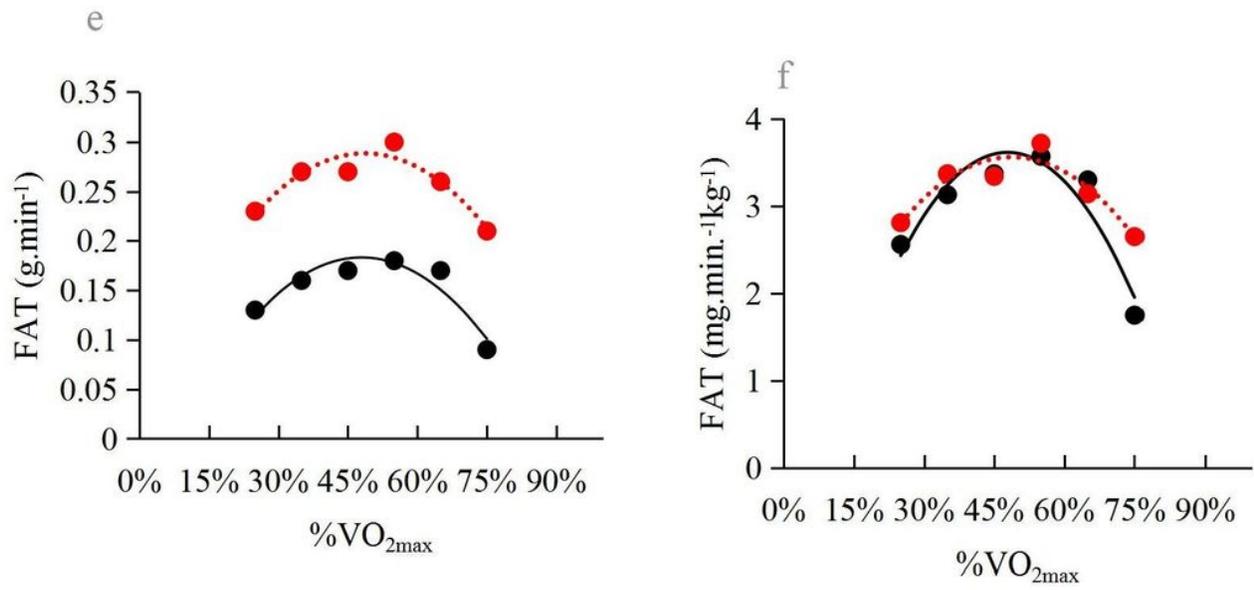
Tables 1 and 4 are available in the Supplementary Files section.

## Figures



**Figure 1**

VO<sub>2</sub>/kg (a), HR (b), MET (c), RER (d) were plotted against exercise intensity during the C Group (open square) and O Group (filled square).



**Figure 2**

fat oxidation (e) and FAT/kg (f) are plotted against exercise intensity for C Group (real line) and O Group (broken line).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.xls](#)
- [Table4.xls](#)