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Effect of a 1-hour single bout of moderate-intensity exercise on fat oxidation kinetics

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Abstract

The present study aimed to examine the effects of a prior 1-hour continuous exercise bout (CONT) at an intensity (Fat_{max}) that elicits the maximal fat oxidation (MFO) on the fat oxidation kinetics during a subsequent submaximal incremental test (IncrC). Twenty moderately trained subjects (9 men and 11 women) performed a graded test on a treadmill (Incr), with 3-minute stages and 1-km·h^{-1} increments. Fat oxidation was measured using indirect calorimetry and plotted as a function of exercise intensity. A mathematical model (SIN) including 3 independent variables (*dilatation*, *symmetry*, and *translation*) was used to characterize the shape of fat oxidation kinetics and to determine Fat_{max} and MFO. On a second visit, the subjects performed CONT at Fat_{max} followed by IncrC. After CONT performed at 57% ± 3% (means ± SE) maximal oxygen uptake ($\dot{V}o_{2max}$), the respiratory exchange ratio during IncrC was lower at every stage compared with Incr ($P < .05$). Fat_{max} (56.4% ± 2.3% vs 51.5% ± 2.4% $\dot{V}o_{2max}$, $P = .013$), MFO (0.50 ± 0.03 vs 0.40 ± 0.03 g·min^{-1}, $P < .001$), and fat oxidation rates from 35% to 70% $\dot{V}o_{2max}$ ($P < .05$) were significantly greater during IncrC compared with Incr. However, *dilatation* and *translation* were not significantly different ($P > .05$), whereas *symmetry* tended to be greater in IncrC ($P = .096$). This study showed that the prior 1-hour continuous moderate-intensity exercise bout increased Fat_{max}, MFO, and fat oxidation rates over a wide range of intensities during the postexercise incremental test. Moreover, the shape of the postexercise fat oxidation kinetics tended to have a rightward asymmetry.

1. Introduction

During aerobic exercise, carbohydrate (CHO) and fat are the 2 main sources of energy; and their relative utilization depends largely on the exercise intensity [1]. Briefly, absolute fat oxidation rates increase from low to moderate exercise intensities and then markedly decline at high intensities, which implies an exercise intensity (Fat_{max}) at which the rate of fat oxidation is maximal (MFO) [2,3]. A *Fat_{max} zone* has also been defined as the range of exercise intensities with fat oxidation rates within 10% of MFO [2]. To determine fat oxidation rates over a wider range of intensities, graded exercise protocols have been proposed [2,4]; and a sinusoidal mathematical model (SIN) has been developed to provide an accurate description of the pattern of fat oxidation kinetics and to determine Fat_{max}, Fat_{max} zone, and MFO [5]. The SIN model includes 3 independent variables (ie, *dilatation*, *symmetry*, and *translation*) that account for the main expected modulations of the curve and can be adjusted separately to accommodate the data (ie, fat oxidation rates measured during the graded exercise). These variables therefore precisely characterize and quantify the shape of the different fat oxidation kinetics that could occur. Moreover, *dilatation* appeared to be a sensitive marker of the ability to oxidize fat because this variable is correlated with Fat_{max} and MFO [5].

Exercise that elicits MFO does not appear to exceed a moderate intensity. In a study conducted on 300 healthy men and women, Venables et al [4] reported an average Fat_{max} at 48% (range, 25%-77%) of maximal oxygen uptake ($\dot{V}o_{2max}$); on the other hand, others [2,6] have measured MFO at an exercise intensity between 62% and 64% $\dot{V}o_{2max}$ in moderately trained subjects. The intensity corresponding to Fat_{max} may have important clinical relevance when

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considering an exercise prescription for weight management, for instance. It has been shown that a training program of continuous exercise performed at Fat_{max} efficiently increased fat oxidation and insulin sensitivity in obese persons [7] and in patients with the metabolic syndrome [8].

Relative substrate utilization also depends on the exercise duration. Indeed, prolonged moderate-intensity exercise has been shown to result in a time-dependent increase in fat oxidation and a decrease in CHO oxidation [1,9]. Moreover, muscle glycogen content seems to influence substrate utilization; and low muscle glycogen content results in a decrease in the respiratory exchange ratio (RER) and an increase in free fatty acids (FFA) and fat oxidation during exercise [9,10]. Fat metabolism during exercise may be influenced by a prior bout of exercise [11,12]. Stich et al [12] have shown that lipolysis during aerobic exercise of moderate intensity is enhanced when an exercise bout is preceded by exercise of similar intensity and duration performed 1 hour before. In addition, when 2 bouts of 30-minute exercise at 60% $\dot{V}o_{2max}$ separated by a 20-minute rest period are performed, FFA and epinephrine responses are significantly greater and plasma insulin concentrations are lower in the second bout of exercise, which lead to an enhanced lipolysis [11]. These results show that a prior exercise bout of endurance exercise increases fat metabolism during a subsequent second bout of exercise. However, to our knowledge, no study has been carried out to date to determine the impact of a single bout of moderate-intensity exercise on the shape of fat oxidation kinetics.

The aim of the study was therefore to examine the effects of a 1-hour continuous exercise bout at an exercise intensity corresponding to Fat_{max} on the fat oxidation kinetics during a subsequent incremental test. It was hypothesized that the prior continuous exercise would increase postexercise MFO and Fat_{max} and would therefore increase the *dilatation* and shift the top of fat oxidation kinetics to the right (ie, rightward asymmetry).

2. Methods

2.1. Subjects

Twenty healthy adult subjects (9 men and 11 women) 22 to 59 years of age, with various physical activity backgrounds, were recruited to participate in this study, which was approved by the local research ethics committee. All volunteers considered for the experimental protocols were of normal weight according to the World Health Organization (body mass index <25 $kg \cdot m^{-2}$), nonsmokers, and disease-free; in addition, they were not taking any medications and were screened for the absence of electrocardiographic abnormalities at rest and during exercise. All test procedures, risks, and benefits associated with the experiment were fully explained; and written informed consent was obtained from all subjects before their participation.

2.2. General design

Each subject performed an incremental test to exhaustion on a treadmill. The average fat and CHO oxidation rates obtained during the last minute of each submaximal work stage were determined by indirect calorimetry and were plotted as a function of exercise intensity. The SIN model, with its independent variables of *dilatation*, *symmetry*, and *translation* [5], provided a mathematical description of fat oxidation kinetics and determined the parameters of Fat_{max}, Fat_{max} zone, and MFO. On a second visit, the volunteers performed a 1-hour continuous exercise bout at an exercise intensity corresponding to the individual Fat_{max}, followed by a submaximal incremental test to determine the postexercise fat oxidation kinetics. The experimental trials were performed 4 to 7 days apart.

2.3. Experimental design

Before the start of the experiment, subjects were familiarized with equipment and procedures. Volunteers were asked to fill in a 1-day food diary on the day before their first test and to repeat this diet before the subsequent trial. Furthermore, subjects were asked to refrain from vigorous exercise and drinking alcohol or caffeine for the 24 hours preceding each experimental day. Subjects reported to the laboratory after a minimum 6-hour fast period and always at the same time to avoid circadian variance.

On the first visit, body mass and stature were measured; and body composition (body fat mass and percentage of body fat) was estimated from skin-fold thickness measurements at 4 sites, according to the methods of Durnin and Womersley [13]. Subsequently, the subjects performed an incremental exercise test (Incr) on a treadmill (Saturn HP Cosmos, Traunstein, Germany). After a 3-minute rest period, the volunteers walked for a 5-minute warm-up at a speed of 3 $km \cdot h^{-1}$ and gradient of 1%, followed by an increase of 1 $km \cdot h^{-1}$ every 3 minutes until the speed of 7 $km \cdot h^{-1}$ was reached. Subjects were asked to walk for 3 minutes at this speed and then to start running for another 3 minutes, after which the speed was increased by 1 $km \cdot h^{-1}$ every 3 minutes until RER reached 1.0. At this point, the speed was increased by 1 $km \cdot h^{-1}$ every minute until exhaustion to obtain a measure of $\dot{V}o_{2max}$ and corresponding velocity ($v\dot{V}o_{2max}$) within a short time. The first part of Incr (ie, until RER = 1.0) was used to determine fat oxidation kinetics, Fat_{max}, and MFO. Oxygen uptake ($\dot{V}o_2$) was considered to be maximal when at least 2 of the following 3 criteria were met: (1) a leveling of $\dot{V}o_2$ (defined as an increase of no more than 2 $mL \cdot kg^{-1} \cdot min^{-1}$) during the latter stages of the exercise test, (2) a heart rate (HR) greater than 90% of the predicted maximum (220 beats per minute − age), and (3) an RER greater than 1.1. $\dot{V}o_{2max}$ was calculated as the average $\dot{V}o_2$ over the last 20 seconds of the last stage of the test.

The ventilatory threshold 1 (VT_1) was determined as described in the literature using the ventilatory method of Wasserman et al [14] and was supported using the ventilatory

method of Beaver et al [15]. Two blinded and independent investigators determined VT_1.

On a second visit, after a 3-minute rest period and a standardized 5-minute warm-up at 3 $km \cdot h^{-1}$, the subjects performed a 1-hour continuous exercise bout (CONT) at an intensity corresponding to the individual Fat_{max} determined during Incr. After CONT and a 10-minute seated resting period, the volunteers performed a subsequent submaximal incremental test (IncrC), as described in the first visit, until an RER of 1.0 was reached. During the 10-minute resting period, volunteers were allowed to drink water ad libitum.

During all test procedures, HR was recorded continuously by an electrocardiogram (GE Cardiosoft Corina; GE Medical Systems, Freiburg, Germany); and breath-by-breath measurements were performed throughout exercise using an Oxycon Pro gas analysis system (Jaeger, Würzburg, Germany). Before each test, the gas analyzers were calibrated with gases of known concentration (16.0% O_2 and 5.02% CO_2); and the volume was automatically calibrated at 2 different flow rates (0.2 and 2 $L \cdot s^{-1}$).

2.4. Indirect calorimetry and calculations

During the incremental tests, average values for $\dot{V}o_2$ and CO_2 output ($\dot{V}co_2$) were calculated over the last minute of every stage, during which the RER was less than or equal to 1.0. Fat and CHO oxidation rates were calculated using stoichiometric equations [16] and appropriate energy equivalents, with the assumption that the urinary nitrogen excretion rate was negligible:

$$\text{Fat oxidation rate } \left(g \cdot min^{-1}\right) = 1.67\ \dot{V}o_2\left(L \cdot min^{-1}\right) - 1.67\ \dot{V}co_2\left(L \cdot min^{-1}\right)$$

$$\text{CHO oxidation rate } \left(g \cdot min^{-1}\right) = 4.55\ \dot{V}co_2\left(L \cdot min^{-1}\right) - 3.21\ \dot{V}o_2\left(L \cdot min^{-1}\right)$$

Energy expenditure (kilocalories per minute) from fat and CHO was calculated by multiplying the fat and CHO oxidation rates by the energy equivalents of fat (1 g = 9 kcal) and CHO (1 g = 4 kcal). For each subject, the results of the graded exercise tests, until an RER of 1.0, were used to calculate fat oxidation rates over a wide range of exercise intensities. The SIN model [5] and its independent variables (ie, *dilatation*, *symmetry*, and *translation*) were used to model the fat oxidation kinetics:

$$\%MFO = SIN\left\{\left[\frac{\pi^{\frac{1}{s}}}{\pi + 2d}\left(K \cdot \%\ \dot{V}o_{2max} + d + t\right)\right]^{s}\right\},$$

where d, s, and t were, respectively, the variables of *dilatation*, *symmetry*, and *translation* (Fig. 1) and K, the constant of intensity, corresponded to (π/100).

The basic values of 0 for *dilatation*, 1 for *symmetry*, and 0 for *translation* determine a symmetric curve that has intersections with the abscissa axis at (0, 0) and (100, 0) (ie, 0% and 100% $\dot{V}o_{2max}$). This basic curve could therefore be modulated to fit the experimental data by independently changing the values of these 3 variables (Fig. 1). The variables of the SIN model were determined with an iterative procedure by minimizing the sum of the mean squares of the differences between the estimated energy derived from lipid (E_{lipid}) based on the mathematical models and the measured values of E_{lipid}.

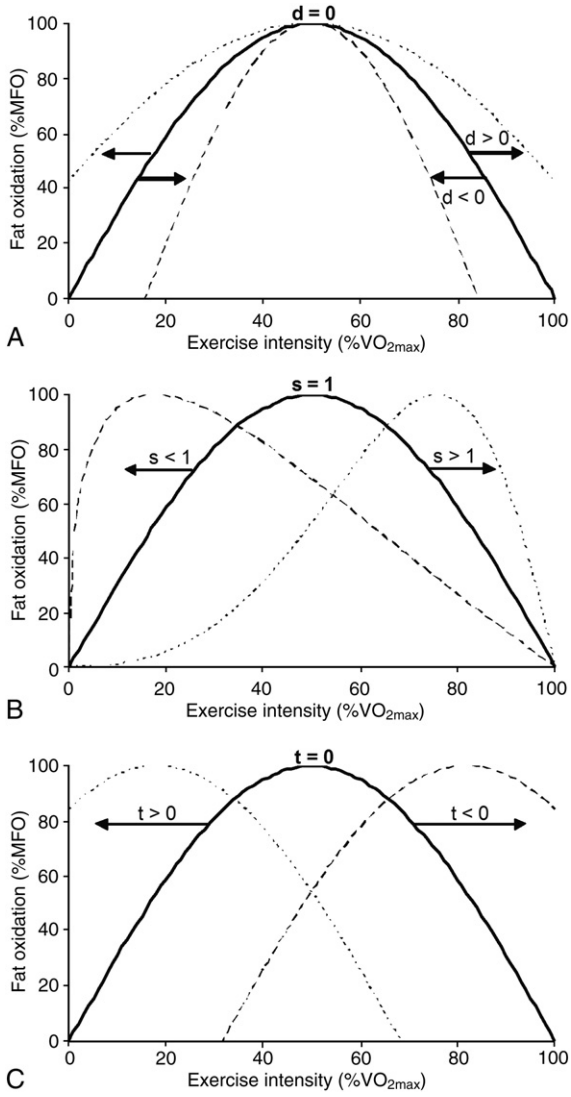

Fig. 1. Graphic representation of the impact of the 3 independent variables (*dilatation* [d], *symmetry* [s], and *translation* [t]) of the SIN model on the basic symmetric curve (solid lines; d = 0, s = 1, and t = 0). Changes in d (A), s (B), and t (C) and corresponding modifications in the SIN curve (dashed and dotted lines).

For each subject, Fat_{max} was calculated by differentiation of the SIN model equation; and fat oxidation rate was determined every 5% $\dot{V}o_{2max}$ between 20% and 85% $\dot{V}o_{2max}$. The Fat_{max} zone was determined as the range of exercise intensities with fat oxidation rates within 10% of MFO [2], with the lower limit referred to as Fat_{max} $zone_{min}$ and the upper limit as Fat_{max} $zone_{max}$.

Table 1
Subject physical characteristics

	n = 20
Age, y	29.2 ± 1.7
Height, cm	172.6 ± 1.8
Body mass, kg	67.1 ± 2.5
BMI, $kg \cdot m^{-2}$	22.4 ± 0.6
Body fat, %	22.5 ± 1.1
FM, kg	15.0 ± 0.9
FFM, kg	52.1 ± 2.2

Values are means ± SE. n indicates number of subjects; BMI, body mass index; FM, fat mass; FFM, fat-free mass.

During CONT, the values of RER and exercise intensity ($\%\dot{V}o_{2max}$) and fat oxidation rates were averaged for 3 minutes every 20 minutes (ie, at 20, 40, and 60 minutes). During the standardized 5-minute warm-up of CONT and IncrC, ventilation ($\dot{V}_E$), $\dot{V}co_2$, and arterial carbon dioxide partial pressure ($PaCO_2$) responses were determined and compared to test the stability of the plasma bicarbonate pool. Moreover, $PaCO_2$ was averaged during the last minute of each submaximal stage of Incr and compared with IncrC. Based on mass balance considerations, $PaCO_2$ was calculated as:

$$PaCO_2 = \frac{863}{\left(\frac{\dot{V}_E}{\dot{V}co_2}\right)\left(1 - \frac{V_D}{V_T}\right)},$$

where 863 is the product of barometric pressure, temperature, and water vapor correction (ie, the factors needed to express $\dot{V}_E$ at BTPS [body temperature pressure saturated], $\dot{V}co_2$ at STPD [standard temperature pressure dry], and CO_2 as a partial pressure). V_D is physiologic dead space, and V_T is tidal volume.

2.5. Statistical analysis

Data are expressed as means ± SE for all variables. One-way repeated-measures analysis of variance (ANOVA) (or Friedman repeated-measures ANOVA on ranks for nonparametric values) was performed to determine the evolution of $\%\dot{V}o_{2max}$, RER, and fat oxidation rates during CONT. A 2-way (exercise intensity × experimental trial) repeated-measures ANOVA test was used to determine differences in HR, RER, and substrate oxidation during Incr compared with IncrC. When the assumption of normality of distribution or the equality of variance was violated, paired *t* test or Wilcoxon signed rank test for nonparametric values was used to compare these parameters at each exercise intensity and to determine differences in the parameters (eg, Fat_{max}, MFO, and RER_{Fatmax}) and variables (ie, *dilatation*, *symmetry*, and *translation*) of the shape of fat oxidation kinetics obtained during Incr and IncrC. For all ANOVAs, significance was located with post hoc analysis using the Tukey test. Finally, Pearson product moment correlations or Spearman rank order correlations were performed to establish the relationships between the changes in characteristics of the shape and parameters of fat oxidation kinetics. For all statistical analyses, significance was accepted at $P < .05$.

3. Results

3.1. Subject characteristics

Physical characteristics of the study participants (n = 20) are listed in Table 1. Table 2 presents maximal performance values (ie, $\dot{V}o_{2max}$, $v\dot{V}o_{2max}$ and maximal HR [HR_{max}]) and VT_1 determined during the maximal incremental test.

3.2. Preincremental test (Incr)

Fig. 2A shows the mean fat oxidation kinetics, represented as a function of exercise intensity ($\%\dot{V}o_{2max}$), obtained during the incremental tests and modeled with the SIN model. During Incr, the subjects performed 10.6 ± 0.4 submaximal 3-minutes stages (ie, until RER = 1.0), which represent 33.8 ± 1.2 minutes. Fat oxidation rates increased with increasing exercise intensities, up to a maximum of 0.40 ± 0.03 $g \cdot min^{-1}$ (range, 0.22-0.63), which occurred at an intensity of 51.5% ± 2.4% $\dot{V}o_{2max}$ (range, 30.3%-65.1%). The Fat_{max} zone was located from 37.6% ± 2.0% $\dot{V}o_{2max}$ to 65.5% ± 2.6% $\dot{V}o_{2max}$. Fig. 2B provides a graphical representation of the mean relative fat oxidation kinetics, expressed as %MFO. Characteristics of the mean fat oxidation kinetics (ie, Fat_{max}, MFO, Fat_{max} zone, and SIN variables) and parameters (ie, RER_{Fatmax}, $\%HR_{max}$, and $\%\ v\dot{V}o_{2max}$) obtained at Fat_{max} during Incr are presented in Table 3.

3.3. Continuous exercise

Characteristics of CONT and energy expenditure during exercise are shown in Table 4. Because exercise intensity was related to individual Fat_{max}, 6 subjects performed CONT while walking at a speed of 5.5 ± 0.2 $km \cdot h^{-1}$ (range, 5.0-6.3), whereas 14 volunteers ran at 9.0 ± 0.3 $km \cdot h^{-1}$ (range, 7.4-11.3) during the 1-hour exercise bout. Continuous exercise was performed at a mean intensity slightly higher than the intensity corresponding to Fat_{max} determined during Incr (Table 3, $P = .002$) and lower than the intensity of VT_1

Table 2
Maximal incremental test (Incr)

	n = 20
$\dot{V}o_{2max}$, $mL \cdot kg^{-1} \cdot min^{-1}$	50.3 ± 1.9
HR_{max}, $beats \cdot min^{-1}$	185 ± 3
$v\dot{V}o_{2max}$, $km \cdot h^{-1}$	14.8 ± 0.6
VT_1, $mL \cdot kg^{-1} \cdot min^{-1}$	32.7 ± 1.6
VT_1, $\%\dot{V}o_{2max}$	65.2 ± 1.3
vVT_1, $km \cdot h^{-1}$	9.1 ± 0.4

Values are means ± SE. vVT_1 indicates velocity at ventilatory threshold 1.

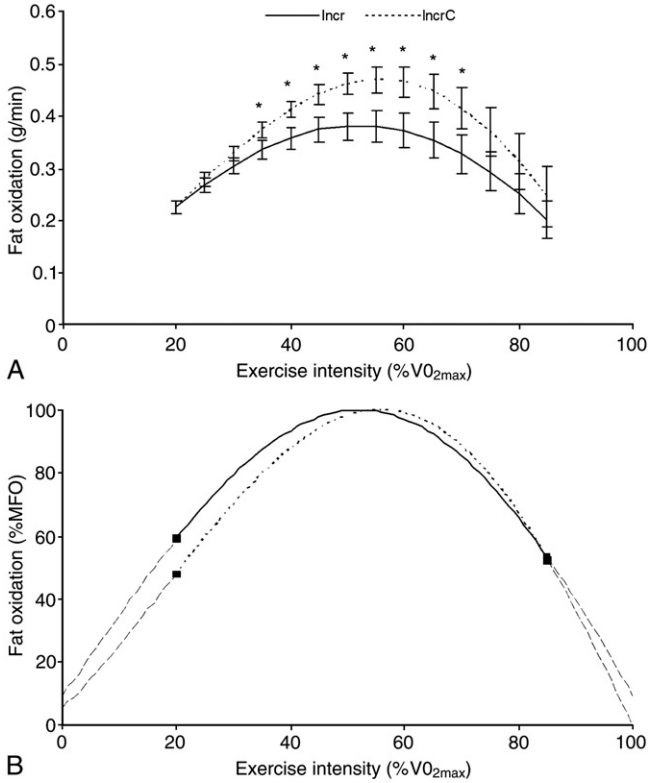

Fig. 2. Mean fat oxidation kinetics (n = 20), in absolute (A) and relative (B) values, constructed with the SIN model during the Incr and IncrC. Data at both extremities of the relative fat oxidation kinetics (B) were extrapolated from the SIN model (dashed lines). Incr, solid lines; IncrC, dotted lines. Values are means ± SE; n indicates number of subjects. *Significant differences in fat oxidation rates between Incr and IncrC; $P < .05$.

(Table 2, $P = .030$). On the other hand, no significant differences were found for the mean RER and mean fat oxidation rate during CONT compared with RER_{Fatmax} and MFO determined during Incr (Table 3, $P > .05$).

During CONT, the relative exercise intensity did not alter throughout exercise ($P > .05$), whereas RER progressively fell during the 1-hour exercise bout (0.90 ± 0.01, 0.89 ± 0.02, and 0.88 ± 0.02 at 20, 40, and 60 minutes, respectively), with significant differences between 20 minutes and 40 or 60 minutes ($P < .05$). Concomitantly, fat oxidation increased throughout exercise; and the fat oxidation rates at 40 and 60 minutes (0.38 ± 0.05 and 0.41 ± 0.06, respectively) were significantly higher than the rate at 20 minutes (0.31 ± 0.04, $P < .001$).

Table 3
Characteristics of fat oxidation kinetics during Incr compared with IncrC

	Incr	IncrC
Fat_{max}, $\%\dot{V}o_{2max}$	51.5 ± 2.4	56.4 ± 2.3*
MFO, $g \cdot min^{-1}$	0.40 ± 0.03	0.50 ± 0.03*
Fat_{max} $zone_{min}$, $\%\dot{V}o_{2max}$	37.6 ± 2.0	45.3 ± 2.6*
Fat_{max} $zone_{max}$, $\%\dot{V}o_{2max}$	65.5 ± 2.6	70.7 ± 2.7*
RER_{Fatmax}	0.87 ± 0.01	0.86 ± 0.01
% HR_{max}	64.2 ± 2.0	71.9 ± 2.1*
% $v\dot{V}o_{2max}$	48.0 ± 2.3	52.1 ± 2.2
Dilatation	0.1 ± 0.1	0.1 ± 0.1
Symmetry	1.1 ± 0.1	1.3 ± 0.1†
Translation	0.0 ± 0.1	0.1 ± 0.1

Values are means ± SE.
* Significant difference from Incr; $P < .05$.
† $P = .096$.

Table 4
Characteristics of the 1-hour CONT

	CONT
Exercise intensity, $\%\dot{V}o_{2max}$	57.7 ± 3.1
RER	0.89 ± 0.01
Fat oxidation rate, $g \cdot min^{-1}$	0.37 ± 0.04
CHO oxidation rate, $g \cdot min^{-1}$	1.58 ± 0.15
EE_{TOTAL}, kcal	576.1 ± 40.3
EE_{FAT}, %EE	35.3 ± 4.1
EE_{CHO}, %EE	64.7 ± 4.1

Values are means ± SE. EE indicates energy expenditure.

3.4. Effects of CONT on IncrC

During the standardized 5-minute warm-up preceding CONT and IncrC, no significant differences were found in $\dot{V}_E$, $\dot{V}o_2$, and $PaCO_2$ between both exercises (Table 5, $P > .05$). In addition, $\dot{V}o_2$ at rest (5.2 ± 0.3 vs 5.6 ± 0.2 $mL \cdot kg^{-1} \cdot min^{-1}$) was similar between Incr and IncrC ($P > .05$). The exercise duration during IncrC was similar as compared with Incr (33.7 ± 1.1 minutes, $P = .841$).

Fig. 3 shows the linear relationships between RER and exercise intensity during both incremental tests. Respiratory exchange ratio was significantly lower at every exercise intensity (ie, from 20% to 85% $\dot{V}o_{2max}$) in IncrC compared with Incr ($P < .05$). Maximal fat oxidation (range, 0.34-0.81 $g \cdot min^{-1}$) and absolute fat oxidation rates from 35% to 75% $\dot{V}o_{2max}$ were significantly higher during IncrC compared with Incr (Table 3, $P < .001$ and Fig. 2A, $P < .05$, respectively). Moreover, Fat_{max} (range, 36.5%-73.2% $\dot{V}o_{2max}$) and the Fat_{max} $zone_{min}$ were located at a higher exercise intensity during IncrC than Incr (Table 3; $P = .013$ and $P = .003$, respectively), whereas the Fat_{max} $zone_{max}$ tended to occur at a higher intensity ($P = .068$). However, the Fat_{max} zone was not significantly greater from Incr (25.5% ± 1.1% vs 27.9% ± 1.1% $\dot{V}o_{2max}$, $P > .05$, respectively). In addition, Δ (ie, the difference between Incr and IncrC) MFO was positively correlated with Δ Fat_{max} ($r = 0.53$, $P = .016$).

Table 5
Ventilation, carbon dioxide output, and arterial carbon dioxide partial pressure during warm-up in CONT and IncrC

	CONT	IncrC
$\dot{V}_E$, $L \cdot min^{-1}$	21.2 ± 2.2	20.1 ± 0.8
$\dot{V}co_2$, $mL \cdot min^{-1}$	673.9 ± 91.0	585.1 ± 33.3
$PaCO_2$, mm Hg	35.8 ± 1.0	35.0 ± 0.9

Values are means ± SE.

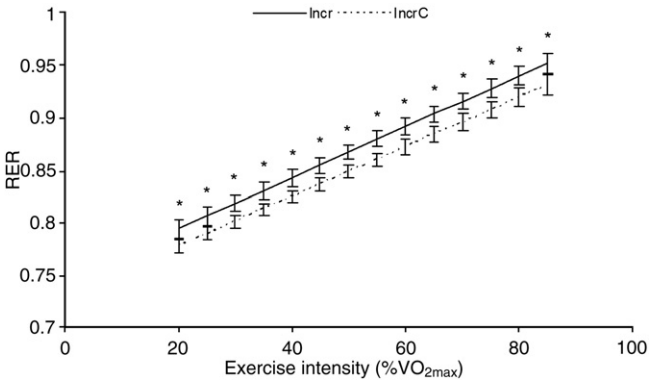

Fig. 3. Mean RER during the Incr and IncrC. Incr, solid line; IncrC, dotted line. Values are means ± SE. *Significant differences in RER between Incr and IncrC; $P < .05$.

The mean fat oxidation kinetics during IncrC were projected upward compared with Incr when fat oxidation rates were expressed in absolute values (Fig. 2A). The variables of *dilatation* and *translation* were not significantly different (Table 3, $P > .05$); however, the *symmetry* tended to be greater (ie, rightward asymmetry) (Fig. 2B, $P = .096$). At the same time, Δ Fat_{max} was correlated with Δ *dilatation* ($r = 0.79$, $P < .001$) and Δ *symmetry* ($r = 0.52$, $P = .020$), whereas significant correlations have been found between Δ Fat_{max} zone and Δ *dilatation* ($r = 0.70$, $P < .001$). Finally, HR during IncrC was significantly higher from 20% to 65% $\dot{V}o_{2max}$ compared with Incr (Fig. 4, $P < .05$).

4. Discussion

The aim of the present study was to examine the effects of a 1-hour continuous exercise bout, performed at an intensity corresponding to Fat_{max}, on the fat oxidation kinetics during a subsequent incremental test. The main finding of this investigation indicated that the prior continuous exercise bout led to an increase of Fat_{max}, MFO, and fat oxidation rates from 35% to 75% $\dot{V}o_{2max}$ during the postexercise graded test. Moreover, the shape of the postexercise fat oxidation kinetics tended to have a rightward asymmetry, with no significant modifications in *dilatation* and *translation*. This partially supported our preliminary hypothesis.

In the present study, fat oxidation kinetics and parameters such as Fat_{max} and MFO were determined with the SIN model. Recently, Chenevière et al [5] have shown that the SIN model presented the same precision as other methods currently used (eg, measured values [2] or polynomial curves [17]) in determination of Fat_{max} and MFO. In addition, the fat oxidation kinetics constructed with the SIN model were as accurate as polynomial curves in fitting experimental data of fat oxidation rates obtained during incremental tests [5], supporting the accuracy of the SIN model to provide descriptions of fat oxidation kinetics and to determine Fat_{max}, Fat_{max} zone, or MFO. The healthy subjects of the present study performed incremental tests with 3-minute stage duration. A recent study has shown that longer exercise steps are preferable with sedentary patients because steady state is not likely to be obtained during the 3-minute period [18]. However, our protocol was adapted from a previously validated one [2] in which the authors concluded that, in healthy subjects, when stage duration was reduced from 5 to 3 minutes, no significant difference was found in Fat_{max} and fat oxidation rates. Moreover, the mean RER and mean fat oxidation rate during CONT were similar as predicted during Incr (ie, RER_{Fatmax} and MFO), further supporting the validity of the present incremental protocol. During the incremental tests, which were of similar duration until RER reached 1.0, Fat_{max} was determined at an exercise intensity of 52% $\dot{V}o_{2max}$ in Incr and 56% $\dot{V}o_{2max}$ in IncrC. Previous studies using treadmill incremental protocols found mean Fat_{max} occurring between 48% $\dot{V}o_{2max}$ for a large group of healthy volunteers [4] and 60% $\dot{V}o_{2max}$ in trained men [6]. The values of the present study are therefore in line with above-mentioned investigations.

During CONT, RER gradually decreased throughout exercise, whereas fat oxidation concomitantly increased. After 40 minutes of exercise, fat oxidation rate was already significantly higher compared with the rate at 20 minutes. Both a decrease in RER and an increase in fat oxidation during continuous moderate-intensity exercise have already been shown by numerous studies [10,19,20]. Febbraio and Dancey [9] observed that RER progressively fell during the first 60 minutes of exercise performed at 93% of the blood lactate threshold (~65% $\dot{V}o_{2max}$), whereas FFA oxidation increased. The RER decline during a long bout of endurance exercise indicates that a greater proportion of the energy is supplied by lipid oxidation [20]. This enhanced fat oxidation is probably due to stimulation of adipose tissue lipolysis, which leads to an increased plasma FFA concentration [10,20]. Increased lipolysis during exercise seems to be mainly due to a high sympathoadrenergic activity in combination with decreased insulin inhibition [19]. Norepinephrine and epinephrine have been

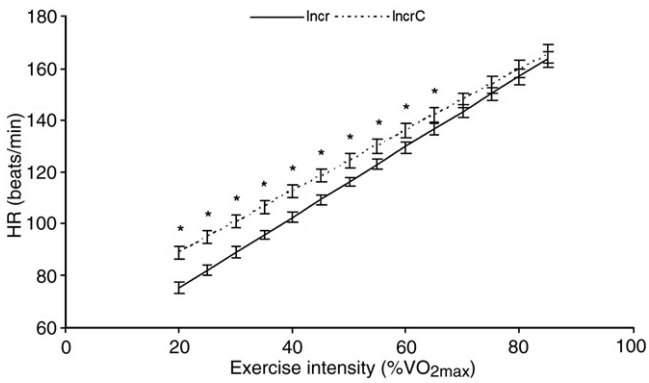

Fig. 4. Mean HR during the Incr and IncrC. Incr, solid line; IncrC, dotted line. Values are means ± SE. *Significant differences in HR between Incr and IncrC; $P < .05$.

shown to play an important role in lipolysis regulation [21]. Indeed, during exercise, lipid mobilization is stimulated by increased catecholamine production, leading to an acceleration of the β-adrenoceptor–mediated lipolysis rate in fat cells [21]. Moreover, intramuscular triacylglycerol also represents a large available source of energy for skeletal muscle activity during moderate-intensity exercise [22]. Therefore, the increasing whole-body fat oxidation found in the present study during CONT might also be related to an increased intramuscular triacylglycerol lipolysis throughout exercise.

Fat metabolism increased during the postexercise submaximal incremental test. Indeed, RER values during IncrC were found to be lower at each exercise intensity compared with Incr. Concomitantly, MFO and absolute fat oxidation rates from 35% to 75% $\dot{V}o_{2max}$ were increased during IncrC, whereas Fat_{max} occurred at higher exercise intensity than in Incr. These results support the assertion that a prior bout of exercise can potentially lead to an increased fat oxidation during a subsequent exercise [11,12,23]. Stich et al [12] showed that lipolysis during exercise of moderate intensity is enhanced when an exercise bout is preceded by exercise of the same intensity and duration performed 1 hour before. In addition, when 2 bouts of 30-minute exercise at 60% $\dot{V}o_{2max}$ separated by a 20-minute rest period were performed, the FFA and epinephrine responses were significantly greater and plasma insulin concentrations were lower in the second bout of exercise, which lead to an enhanced lipolysis [11]. Therefore, combinations of increased epinephrine and decreased insulin concentrations might augment exercise-induced lipolysis in the repeated exercise. Goto et al [11] also observed a remarkable increase in FFA, acetoacetate, and 3-hydroxybutyrate concentrations during the 20-minute rest period after the first bout of exercise; and they hypothesized that the cessation of exercise may create an imbalance between the supply and demand of fatty acids, causing a rapid elevation of FFA concentration. Moreover, these authors found lower plasma glucose during the second half of exercise, which might also be involved in the increased lipolytic action. Besides epinephrine and insulin, the atrial natriuretic peptide (ANP) may play a relevant role in the control of exercise-induced lipolysis, independent of the activation of the sympathetic nervous system. Indeed, a recent study showed the major contribution of ANP in the stimulation of lipid mobilization from subcutaneous adipose tissue during repeated bouts of endurance exercise [23]. These authors suggested that more than 50% of the nonadrenergic lipolysis observed during the repeated bout of exercise was ANP dependent. These results suggest that CONT performed at approximately 60% $\dot{V}o_{2max}$ may have induced an increase in plasma catecholamine concentrations and a decrease in insulin during IncrC, which led to enhanced lipolysis and total fat oxidation during IncrC. Moreover, the higher HR both at rest and during IncrC (ie, from 20% to 65% $\dot{V}o_{2max}$; Fig. 4) compared with Incr may be therefore related to the increase in catecholamine concentrations induced by the prior continuous exercise because these hormones have been shown to stimulate HR [24].

It could be assumed that this enhancement in fat metabolism during the subsequent bout of exercise (Fig. 2A) may have also induced modifications in the shape of the fat oxidation kinetics characterized through the 3 variables of the SIN model [5]. However, contrary to the preliminary hypothesis of the present study, despite increases in Fat_{max}, MFO, and fat oxidation rates during IncrC, the postexercise fat oxidation kinetics only tended to have a greater *symmetry* (ie, rightward asymmetry), with no significant modifications in *dilatation* and *translation* (Fig. 2B). The positive correlation found between Δ Fat_{max} and Δ MFO indicates that the increase of Fat_{max} during IncrC compared with Incr is related to the increase of fat oxidation rates. In addition, Δ Fat_{max} was correlated with Δ *dilatation* and Δ *symmetry*, whereas Δ Fat_{max} zone was significantly linked with Δ *dilatation*. Taken together, these various correlations imply that the characteristics of fat oxidation kinetics are related to the increased parameters, such as MFO, Fat_{max}, or Fat_{max} zone, during the subsequent second exercise. Thus, it could be hypothesized that a longer-duration exercise may increase fat oxidation to a greater extent. Fat_{max} and Fat_{max} zone would be shifted at higher exercise intensities and may therefore induce significant modifications in the shape of the postexercise fat oxidation kinetics. However, the rightward asymmetry detected with the SIN model indicates that, besides higher rates of fat oxidation, the prior exercise also induced a shift of the lipid oxidation toward higher exercise intensities. Indeed, RER values were significantly lower at every exercise intensity (ie, from 20% to 85% $\dot{V}o_{2max}$) during IncrC compared with Incr. Therefore, as RER_{Fatmax} was similar in both tests, Fat_{max} was reached at higher exercise intensity during the postexercise incremental test. This enhanced total fat oxidation during IncrC may be supported by the increased FFA availability induced by higher lipolytic stimulation [11] and a change in the relative partitioning of plasma FFA toward oxidation rather than storage [25].

Some methodological limitations exist and need to be addressed. First, in the present study, substrate oxidation was quantified by indirect calorimetry. Changes in the size of the bicarbonate pool during CONT and recovery period between CONT and IncrC might overestimate the lipid oxidation rate during IncrC (ie, RER < cellular respiratory quotient due to the refilling of muscle and arterial blood bicarbonate pools depleted during CONT) [26]. However, the exercise intensity during CONT was significantly lower than VT_1; and the bicarbonate buffering of the H^+ production might be therefore limited [27]. Indeed, no significant differences in $\dot{V}_E$, $\dot{V}o_2$, and $PaCO_2$ (Table 5) were found during the standardized warm-up preceding IncrC compared with CONT, suggesting the presence of stable plasma lactate and bicarbonate pools at the start of the postexercise incremental test [28]. In addition, the ventilation and

P_{ACO2} (data not shown, $P > .05$) were similar over all stages between Incr and IncrC. Taken together, these results suggest that the use of the mean RER value during IncrC appears to be a valid indicator of substrate partitioning.

Furthermore, as the investigation of sex differences in fat oxidation kinetics was not within the objectives of the present study, neither the phases within the menstrual cycles nor the oral contraceptive has been controlled. However, there is no consensus about the modifications in substrate oxidation during exercise across the menstrual cycle [29,30]. Moreover, the experimental protocol was designed to determine the response of a mixed and heterogeneous group of moderately active individuals to a single moderate-intensity exercise (ie, repeated-measures statistical design). The general results obtained in this study could be applied to gain perspective for exercise prescription, for instance, weight management training.

Finally, the incremental tests (ie, Incr and IncrC) were performed on 2 separate days. It is possible that the effects of CONT were partially counterbalanced by day-to-day variability in RER values, which may occur both at rest and during exercise [31]. However, volunteers filled in a 1-day food diary on the day before their first test and repeated this diet before the subsequent trial.

5. Conclusion

In conclusion, this study shows that a prior 1-hour continuous exercise bout, performed at an intensity corresponding to Fat_{max}, led to an increase in Fat_{max}, MFO, and fat oxidation rates over a wide range of intensities during a subsequent incremental test. Despite the postexercise shift toward lipid oxidation, the *dilatation* and *translation* of the fat oxidation kinetics were not modified. However, the shape of the postexercise kinetics tended to have a rightward asymmetry, which partially supported our preliminary hypothesis. A longer duration of exercise might increase fat oxidation rates, MFO, and Fat_{max} to a greater extent and therefore induce a greater upward and rightward shift of the postexercise fat oxidation kinetics.

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