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## Reliability of the Metabolic Response During Steady-State Exercise at FATmax in Young Men with Obesity

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### ABSTRACT

**Purpose:** In this study we evaluated the reliability of blood lactate levels (BLa), energy expenditure and substrate utilization during prolonged exercise at the intensity that elicits maximal fat oxidation (FATmax). Furthermore, we investigated the accuracy of a single graded exercise test (GXT) for predicting energy metabolism at FATmax. **Methods:** Seventeen young men with obesity ( $26 \pm 6$  years;  $36.4 \pm 7.2$  %body fat) performed a GXT on a treadmill in a fasted state (10–12 h) for the assessment of FATmax and cardiorespiratory fitness. Afterward, each subject performed two additional prolonged FATmax trials ( $102 \pm 11$  beats·min<sup>-1</sup>; 60-min) separated by 7 days. Indirect calorimetry was used for the assessment of energy expenditure and substrate utilization kinetics whereas capillary blood samples were taken for the measurement of BLa. **Results:** The BLa (limits of agreement (LoA):  $-1.2$  to  $0.8$  mmol·L<sup>-1</sup>;  $p = 1.0$ ), fat utilization (LoA:  $-8.0$  to  $13.4$  g·h<sup>-1</sup>;  $p = 0.06$ ), and carbohydrate utilization (LoA:  $-27.6$  to  $22.4$  g·h<sup>-1</sup>;  $p = 0.41$ ) showed a good agreement whereas a modest systematic bias was found for energy expenditure (LoA:  $-16811$  to  $33355$  kJ·h<sup>-1</sup>;  $p < 0.05$ ). All the aforementioned parameters showed a moderate to good reliability (Intraclass correlation coefficient: 0.67–0.92). The GXT overestimated fat (~46%) and carbohydrate (~26%) utilization as well as energy expenditure (36%) during steady-state exercise at FATmax. Conversely the GXT underestimated BLa (~28%). **Conclusion:** a single GXT cannot be used for an accurate prediction of energy metabolism during prolonged exercise in men with obesity. Thus, an additional steady-state FATmax trial (40–60 min) should be performed for a tailored and precise exercise prescription.

### ARTICLE HISTORY

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### KEYWORDS

Energy metabolism; exercise test; indirect calorimetry; nutrition; physical exercise

The maximal fat oxidation rate (MFO) measured during a submaximal exercise test and its corresponding exercise intensity (FATmax) are physiological biomarkers commonly used for the assessment of metabolic flexibility and exercise prescription (Brun et al., 2022; Chávez-Guevara et al., 2022). Although not universally observed (Amaro-Gahete et al., 2022), previous studies reported that MFO is directly associated to insulin sensitivity in trained men (Robinson et al., 2015) and subjects with obesity (Cancino-Ramírez et al., 2018; Lambert et al., 2017), suggesting that MFO can be used to investigate the role of metabolic flexibility in the pathophysiology of insulin resistance and type 2 diabetes (Chávez-Guevara et al., 2022). In addition, exercise training performed at FATmax improves adipokines levels, body composition, cardiovascular function, glucose homeostasis, muscle oxidative capacity and metabolic flexibility in patients with obesity and type 2 diabetes (Brun et al., 2022; Chávez-Guevara et al., 2020; Romain et al., 2012). Therefore, adequate determination of the FATmax and MFO seems to be a prerequisite in an effective non-pharmacological approach for the prevention and treatment of cardio-metabolic diseases

In this concern, several exercise protocols have been proposed for the accurate assessment of MFO and FATmax, with

the majority of studies using a single graded exercise test (GXT) with short-duration stages (1–5 minutes) (Amaro-Gahete et al., 2019). The reproducibility of such a GXT, however, is debatable, with numerous studies revealing a significant day-to-day variation of MFO (CV: 1–25%) and FATmax (CV: 3–26%) in trained and untrained healthy persons (Brun et al., 2014; Chrzanowski-Smith et al., 2020; Croci et al., 2014; Dandanell et al., 2017; De Souza Silveira et al., 2016; Gmada et al., 2013; Robles-González et al., 2021). According to previous studies, the reliability of MFO and FATmax is mainly dependent on methodological issues rather than biological factors and physical fitness. For example, the day-to-day variation in MFO and FATmax is reduced when the fat oxidation kinetics is computed through a mathematical modeling instead of visual inspection (Chrzanowski-Smith et al., 2020; Croci et al., 2014). The reproducibility of FATmax, however, is similar between men and women and is not affected by objectively measured physical activity, body fatness nor cardiorespiratory fitness in healthy adults (Chrzanowski-Smith et al., 2020).

On the other hand, Schwindling et al. (2014) and Takagi et al. (2014) showed that substrate utilization calculated during each stage of the GXT does not accurately predict substrate utilization during prolonged exercise in healthy men and

trained men. What's more, recent studies have also showed that carbohydrate utilization raises gradually during the initial 15 minutes of steady-state exercise at FATmax, stimulating an exponential decay in fat oxidation that falls below the MFO recorded during the GXT (Chávez-Guevara et al., 2021; Özdemir et al., 2019). According to Chávez-Guevara et al. (2021), such fat utilization decrement during the initial 15 minutes of steady-state exercise at FATmax is associated to lactate-stimulated acidosis, a phenomenon that may reduce fat oxidation through the inhibition of carnitine palmitoyl transferase 1 (Frangos et al., 2023; Spriet, 2014). Indeed, blood lactate levels (BLa) measured at 30-minutes of steady-state exercise at FATmax were higher than those values observed at FATmax in response to a GXT in young men with obesity (Chávez-Guevara et al., 2021). Hence, it seems that short duration stages are not sufficient to reach a steady-state in physiological parameters and a single GXT may not provide an accurate examination of MFO and FATmax, leading to equivocal conclusions regarding metabolic flexibility and the benefits of FATmax training.

Considering the aforementioned evidence, it seems necessary to investigate the day-to-day reliability of substrate utilization during prolonged exercise at FATmax. This is particularly relevant for subjects with obesity since exercising at FATmax during 40 to 60 minutes is an extended recommendation to improve physical fitness and metabolic flexibility in this population (Chávez-Guevara et al., 2023). Therefore, the current study evaluated the reliability of BLa, energy expenditure and substrate utilization during 60-minutes of walking at FATmax. Furthermore, we also investigated the accuracy of a single graded exercise test for predicting energy metabolism during prolonged exercise.

## Materials and methods

### Participants

Seventeen Hispanic men with obesity enrolled in this study. They were recruited from institutional e-mails, circulated pamphlets and posters. Inclusion criteria were: (I) body fat percentage  $\geq 25\%$ ; (II) fat mass index  $\geq 6 \text{ kg.m}^2$ ; (III) resting heart rate  $< 90 \text{ beats.min}^{-1}$ ; (IV) not engaged in structured exercise program or dietary counseling. None of the participants were under pharmacological treatment or reported a clinical background of cardiovascular, metabolic, or respiratory diseases according to a comprehensive health survey (MacDougall et al., 1991). Moreover, they showed a low

physical activity level ( $\leq 600 \text{ METs.min.sem}$ ) based on the short version of the International Physical Activity Questionnaire (Craig et al., 2003).

### Study design

A repeated measures design including five separate sessions for the assessment of metabolic biomarkers and physical fitness was implemented in this study (Figure 1). Session 1: assessment of health status, physical activity level, body composition and dietary phenotype (i.e., energy and macronutrient intake of the diet). Session 2: evaluation of resting metabolic rate (RMR). Session 3: measurement of CRF, MFO, and FATmax by a GXT. Session 4 and 5: steady-state exercise trials at FATmax during 60 min on a motorized treadmill for the assessment of BLa, cardiorespiratory parameters, energy expenditure and substrate oxidation (see below). All the exercise trials were performed after a 7-days wash-out period with the experimental procedures carried out in the morning (08:00–11:00 h) after an overnight fasting (10–12 h) and under controlled environmental temperature (22–24°C). The energy intake and macronutrient content of the diet were assessed by a trained nutritionist by using the 24-h recall method, recording all the food consumed during three non-consecutive days (including one day of the weekend). Then, the subjects were instructed to maintain their habitual diet and physical activity during the entire study. Moreover, the performance of vigorous intensity exercise and the consumption of alcohol, caffeine and stimulants was restricted at least 24 h prior to all the metabolic measurements. The data from self-reported physical activity, dietary phenotype and resting metabolic rate were used to design a control dinner (55% carbohydrates, 30% lipids, and 15% proteins) which provided the 30% of individual energy requirements ( $977 \pm 145 \text{ kcal}$ ) and allow a proper standardization of resting fat oxidation before both steady-state exercise trials at FATmax (Chávez-Guevara et al., 2021).

The general details of the study were provided to all participants, who signed a written informed consent after acceptance. Previously, the study protocol was approved by the Institutional Ethics Committee and all the experimental procedures were carried out in accordance with the Declaration of Helsinki.

### Anthropometric and body composition measurements

Body weight and height were measured to the nearest 0.1 kg or cm by using a calibrated electronic scale (SECA 876,

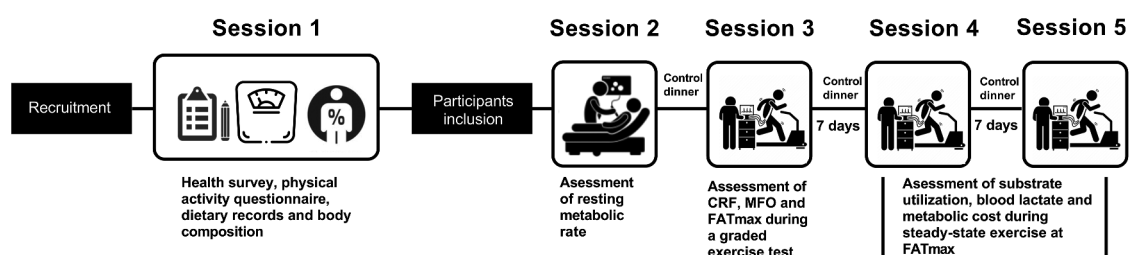


Figure 1. Stepwise study design. CRF, cardiorespiratory fitness; MFO, maximal fat oxidation; FATmax, exercise intensity eliciting maximal fat oxidation.

Hamburg, Germany) and a wall mount stadiometer (SECA BM206, Hamburg, Germany). Fat mass and free fat mass (FFM) were assessed by air displacement plethysmography (BODPOD; Cosmed, Rome, Italy) in accordance with manufacturer guidelines and validated accordingly (Ginde et al., 2005). The guidelines from the American College of Sports Medicine were used for definition of physical fitness (Dumke, 2018). All the measurements were performed with the subjects slightly dressed and barefoot.

### **Metabolic oxidative measurements**

Gas exchange was assessed by indirect calorimetry using a breath-by-breath gas analyzer (Cortex, MetaLyzer 3B, Germany) that provides reliable measurements of oxygen uptake ( $\text{VO}_2$ ) and carbon dioxide output ( $\text{VCO}_2$ ) during physical exercise (Bias =  $0.3 \text{ L}\cdot\text{min}^{-1}$ ; Intraclass correlation coefficient > 0.96) (Meyer et al., 2001). The system was calibrated before each test by using a certified gas mixture of known concentrations (5%  $\text{CO}_2$ , 16%  $\text{O}_2$ , and balanced of  $\text{N}_2$ ; Cortex-Medical) and the flow sensor was calibrated with a 3-L syringe (Hans Rudolph, Shawnee, USA).

### **Resting metabolic rate**

The resting metabolic rate was measured prior to each exercise trial in order to standardize the metabolic condition of the participants. For such assessment, the participants rested in Fowler's anatomical position (semi-sitting) during 15 to 25 minutes while breath-by-breath were continuously recorded. Then, five continuous minutes' steady state ( $\text{RQ} < 5\%$ ) was used to calculate the resting metabolic rate from the Weir equation (Alcantara et al., 2020; Weir, 1949), whereas macro-nutrient oxidation was calculated with Frayn's stoichiometric equations (Frayn, 1983) assuming that nitrogen urinary excretion was negligible.

### **Graded exercise test protocol**

Each subject performed a GXT on a Quinton motor-driven treadmill (TM55, Washington D.C., USA) for the assessment of MFO, FATmax and CRF. The heart rate and BLA were measured at rest before the beginning of the GXT. A brief warm-up (5 min) was carried out by walking at  $4 \text{ km}\cdot\text{h}^{-1}$  with no inclination. Then, the test started at a speed of  $3 \text{ km}\cdot\text{h}^{-1}$  at 1% gradient, followed by speed increments of  $1 \text{ km}\cdot\text{h}^{-1}$  every 3 min until a respiratory exchange ratio of 1.0 was sustained for at least 30 seconds. Thereafter, both speed ( $+1 \text{ km}\cdot\text{h}^{-1}$ ) and gradient ( $+1\%$ ) were simultaneously increased every 3 minutes until volitional exhaustion, following the American College of Sports Medicine general indications for stopping an exercise test (Dumke, 2018). Both gas exchange and heart rate (HR) were continuously measured (Polar Electro F6, Kempele, Finland) while the BLA (see below) and rate of perceived exertion (Borg scale: 0–10) (Borg, 1982) were recorded within the last 30 seconds of each stage.

The peak oxygen uptake ( $\text{VO}_{2\text{peak}}$ ) was calculated by averaging the  $\text{VO}_2$  values recorded over the last 30 s of the test and the CRF of the subjects was defined according to age and sex

by following the ACSM criteria (Dumke, 2018). Furthermore, adhering to analytical procedures previously applied in subject with obesity (Chávez-Guevara et al., 2021; Chávez-Guevara et al., 2023), the  $\text{VO}_2$  and  $\text{VCO}_2$  values registered over the last 120 s of each stage (RER with  $\text{CV} \leq 5\%$ ) were used to calculate substrate utilization and energy expenditure by applying the aforementioned stoichiometric equations (Frayn, 1983; Weir, 1949). Substrate utilization rates and energy expenditure were graphically depicted against exercise intensity (i.e.,  $\% \text{VO}_{2\text{peak}}$ , heart rate) to determine energy metabolism at FATmax (Amaro-Gahete et al. 2019), and these values were used to predict substrate utilization and energy expenditure during prolonged exercise, assuming a steady-state metabolic condition (See Supplementary File 1). Additionally,  $\text{VO}_2$  and HR corresponding to FATmax were used to control the exercise intensity during the two follow-up FATmax trials (see below).

### **Steady-state exercise trials at FATmax**

To standardize the initial metabolic conditions of the participants, a new RMR and resting HR measurements were conducted before this trial. Then, a 5 min warm-up was completed at a speed of  $4 \text{ km}\cdot\text{h}^{-1}$  and a gradient of 0%. Afterward, each patient walked for 60 minutes at their corresponding FATmax HR ( $\pm 5 \text{ beats}\cdot\text{min}^{-1}$ ) previously determined during a GXT. The  $\text{VO}_2$  and  $\text{VCO}_2$  recorded during the last 120 s of each 5 minutes' interval (RER with  $\text{CV} \leq 5\%$ ) were used to calculate energy expenditure (EE) and substrate utilization as described previously. Then, the area under the curve was computed by using GraphPad Prism v. 8.1 (Harvey Motulsky, San Diego, CA, USA) to calculate total fat and carbohydrate utilization as well as total energy expenditure (see Supplementary file 1).

### **Blood lactate assay**

Capillary blood samples were taken during the last 30 seconds of each GXT stage and every 30 min during 60 min of walking at FATmax. The BLA levels were determined by using test strips and the lactate plus meter analyzer (Nova Biomedical, Waltham, MA, USA) that was precalibrated with control lactate solutions (1.0–1.6 and 4.0–5.4 mM). This analyzer uses an electrochemical lactate oxidase biosensor to measure lactate concentration in a  $0.7 \mu\text{L}$  sample and shows a good reliability for the assessment of BLA during physical exercise (Bias =  $-0.5 \text{ mmol}\cdot\text{L}^{-1}$ ) (Hart et al., 2013).

### **Statistical analysis**

The Shapiro–Wilk test, Q–Q, and box plots were used to analyze data distribution. A paired t-test was used to analyze the systematic bias between predicted and measured metabolic parameters (i.e., substrate utilization and energy expenditure). A two-way (time x session) repeated measures analysis of variance was used for evaluating the time-trend variations among the different physiological parameters during steady-state exercise trials at FATmax. The Bonferroni correction was applied for multiple comparisons while the Mauchly's test was

used to test the sphericity of the covariance matrix. If violated, the Greenhouse-Geisser correction was applied ( $\epsilon < 0.75$ ) to test the potential significance of effects (Verma, 2016). The partial ETA squared ( $\eta_p^2$ ) was computed to determine the effect of time and session over substrate utilization and energy expenditure during steady-state exercise by following the criteria proposed by Cohen ( $\eta_p^2 = 0.01$ , *small*;  $\eta_p^2 = 0.06$ , *moderate*;  $\eta_p^2 = 0.14$ , *large*) (Lakens, 2013). Furthermore, a non-parametric Friedman and Wilcoxon test were used to investigate the effect of time and session on BLA levels during both steady-state exercise trials at FATmax.

Intraclass correlation coefficients (ICC), Bland-Altman plots and paired t test were used to examine the reliability of BLA, substrate oxidation and energy expenditure across both steady-state exercise trials at FATmax (Hopkins et al., 2009). Given that substrate utilization was calculated through stoichiometric equations based on  $\text{VO}_2$  and  $\text{VCO}_2$ , the agreement of substrate oxidation during exercise at FATmax was defined according to the measurement error of gas exchange ( $\pm 0.3$  L·min) previously reported for the Metalyzer 3B metabolic cart (Meyer et al., 2001). Likewise, the agreement of BLA during steady-state exercise at FATmax was based on the BLA measurement error previously reported for the Lactate Plus Meter analyzer ( $\pm 0.5$  mmol·L<sup>-1</sup>) (Hart et al., 2013). The criteria proposed by Koo and Li (2016), was considered for the interpretation of reliability based on the ICC values (Moderate: 0.5–0.75; Good: 0.75–0.90; Excellent: 0.90 or higher).

All the analyses were computed in SPSS v. 22 (IBM corporation, NY, USA) while the figures were elaborated in GraphPad Prism v. 8.1. The statistical significance was established at  $p \leq .05$ . Data are reported as mean  $\pm$  SE (graphs),  $\pm 95\%$  CI (Tables), and  $\pm$  SD (text).

## Results

### Participants characteristics and dietary phenotype

The descriptive characteristics of the participants are shown in Table 1. The 76% ( $n = 13$ ) of the enrolled participants showed a poor cardiorespiratory fitness while only 24% ( $n = 4$ ) of the

**Table 1.** Descriptive characteristics of the study participants.

Variable	Mean (95% CI)
<b>Body composition</b>	
Age (years)	26 (23–29)
Body weight (kg)	100.9 (93.2–108.6)
Height (m)	1.74 (1.70–1.78)
Body mass index (kg·m <sup>2</sup> )	33.1 (30.8–35.5)
Body fat (%)	36.3 (32.6–40.0)
Fat free mass (%)	63.6 (59.8–67.3)
Fat mass index (kg/m <sup>2</sup> )	12.2 (10.3–14.2)
<b>Dietary phenotype</b>	
Energy intake (kcal/d)	2242.6 (1804.0–2681.1)
Fat (%)	29.6 (24.7–34.5)
Carbohydrate (%)	49.6 (44.2–55.0)
Protein (%)	20.3 (17.5–23.1)
Fat (g/d)	79.0 (55.4–92.6)
Carbohydrate (g/d)	277.3 (220.4–334.2)
Protein (g/d)	116.0 (87.0–144.9)
<b>Cardiorespiratory fitness</b>	
Peak oxygen uptake (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	38.7 (35.8–41.6)
Maximum heart rate (beats·min <sup>-1</sup> )	181 (175–187)
Maximum blood lactate (mmol·L <sup>-1</sup> )	5.9 (4.9–6.9)

subjects exhibited a fair cardiorespiratory fitness. In addition, the subjects consumed a diet with a balanced macronutrient content where carbohydrates provided  $49.6 \pm 9.7\%$  of daily energy intake. The metabolic and cardiopulmonary parameters recorded at FATmax during the graded exercise test are reported in Table 2

### Reliability of substrate utilization and energy expenditure during prolonged exercise at FATmax

The  $\text{VO}_2$ ,  $\text{VCO}_2$  and HR measured during both steady-state exercise trials at FATmax showed a low variation (CV < 10% for  $\text{VO}_2$  and  $\text{VCO}_2$ ; CV < 5% for HR) and were not different from those values observed in the GXT (Figure 2a,b). A significant effect of time was observed for fat ( $F = 48.4$ ;  $p < .01$ ;  $\eta_p^2 = 0.76$ ) and carbohydrate utilization ( $F = 71.47$ ;  $p < .01$ ;  $\eta_p^2 = 0.81$ ), with fat utilization kinetics showing an exponential decay between the 5–15 min and a subsequent recovery until reaching the predicted fat utilization rate at  $\sim 30$  min (Figure 2c). Conversely, carbohydrate oxidation increased during the first 15 min of exercise and decreased afterward (Figure 2d). Both, energy expenditure ( $F = 1.15$ ;  $p = .33$ ;  $\eta_p^2 = 0.06$ ; Figure 2e) and BLA ( $p > .05$ ) were sustained during the entire exercise sessions (Figure 2f). Additionally, no time per session interaction was observed neither for substrate utilization nor energy expenditure.

The systematic bias and inter-day reliability of metabolic parameters recorded across both steady-state exercise trials at FATmax are shown in Figure 3. Overall, no systematic bias was observed in BLA and substrate utilization between both exercise trials at FATmax. Nevertheless, energy expenditure showed a moderate systematic bias between the first and second steady-state exercise sessions at FATmax (effect size = 0.64).

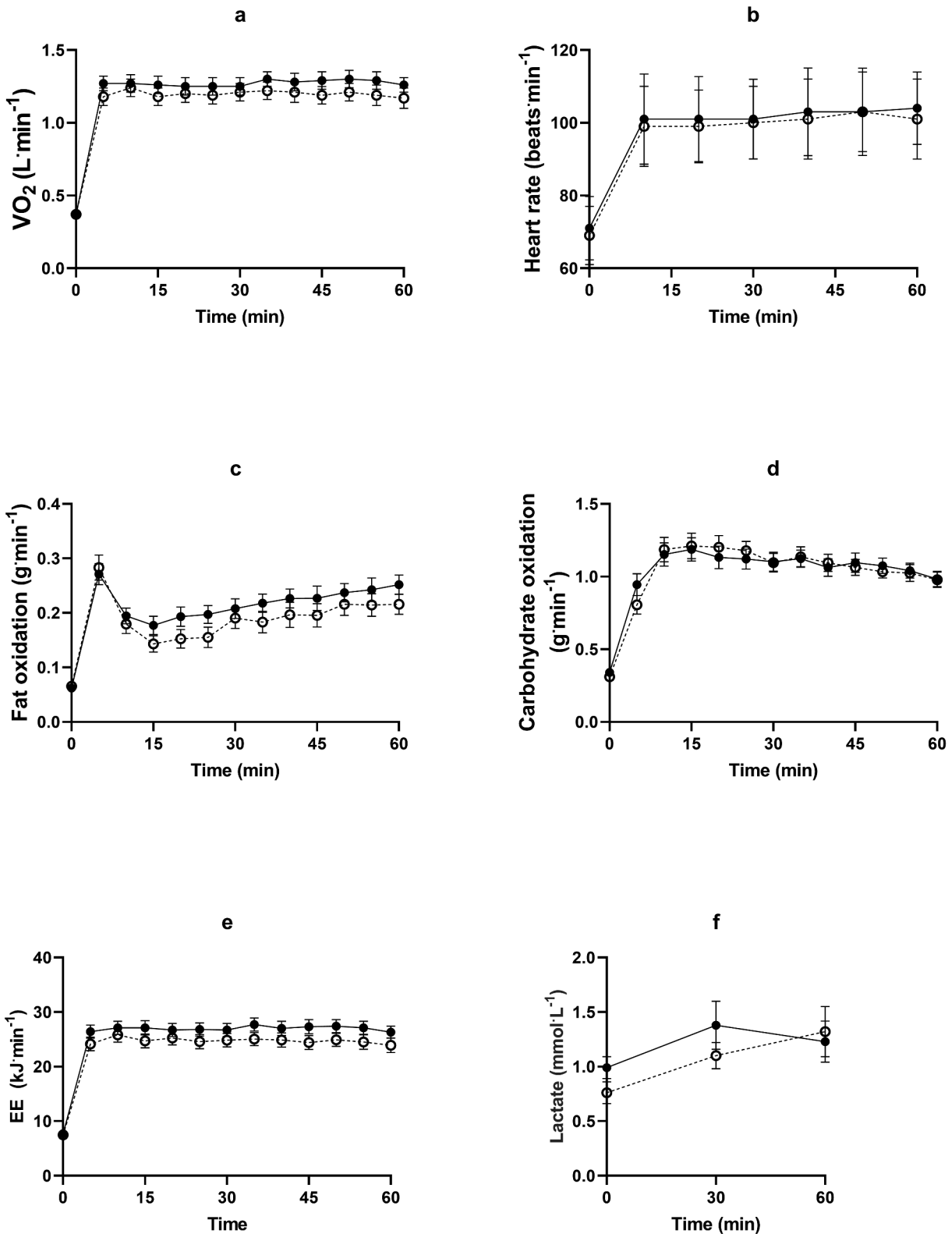
At the individual level, around 50% of the enrolled participants exhibited a reduction in BLA and fat utilization during the second exercise trial at FATmax whereas 75% of the evaluated subjects showed a reduction in energy expenditure. In all the participants, the BLA, energy expenditure and substrate utilization showed a good agreement whereas a moderate to good reliability was observed for all the aforementioned parameters (ICC: 0.67–0.92).

### Accuracy of a single graded exercise test for predicting energy metabolism during prolonged exercise

Fat and carbohydrate utilization during the 60 minutes of steady-state exercise trials were  $\sim 46\%$  and  $\sim 26\%$  lower than values predicted from the GXT, respectively (Supplementary

**Table 2.** Metabolic and cardiopulmonary parameters recorded at FATmax during the graded exercise test.

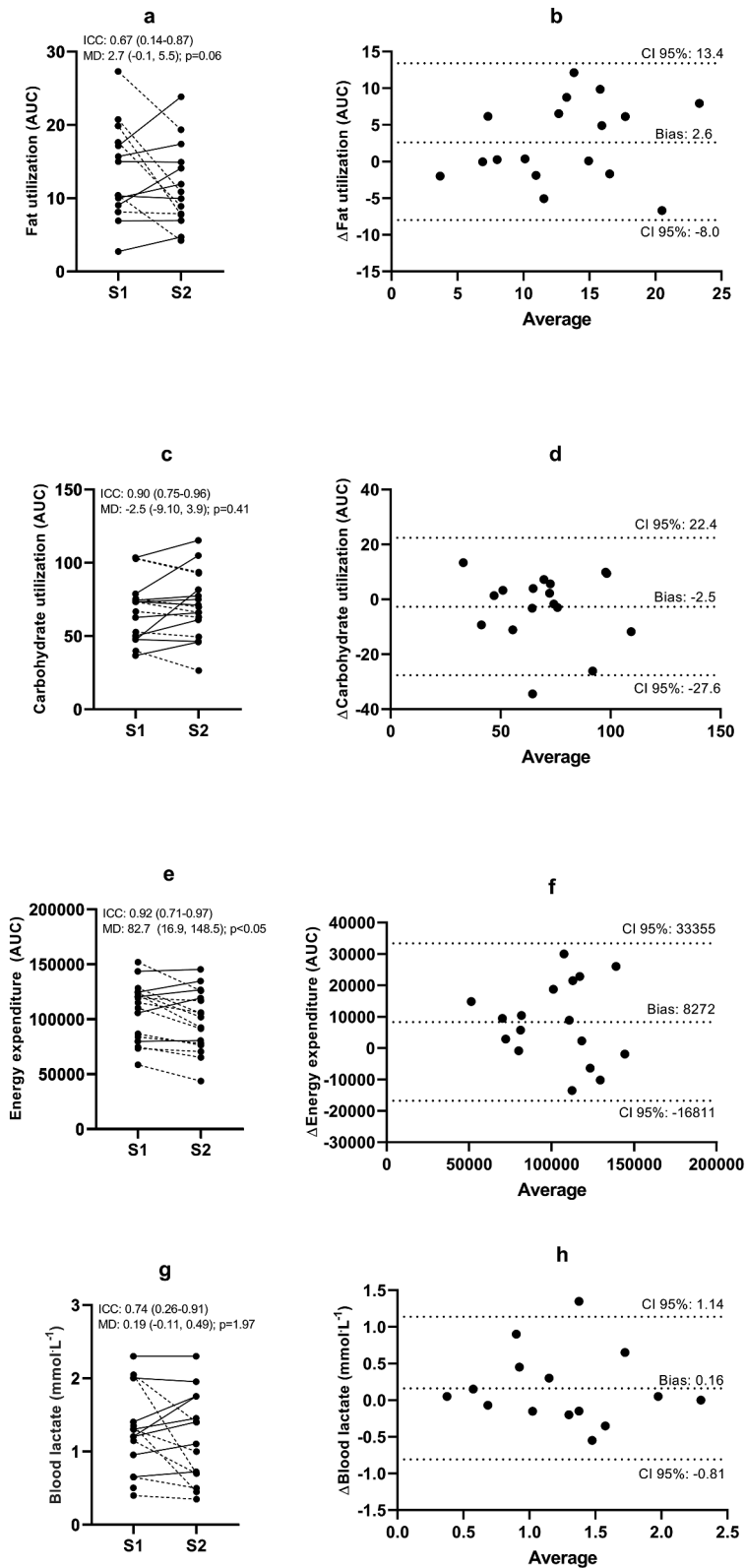
Variable	Mean (95% CI)
Fat oxidation (g·min <sup>-1</sup> )	0.25 (0.21–0.30)
Carbohydrate oxidation (g·min <sup>-1</sup> )	1.02 (0.83–1.19)
Energy expenditure (kJ·min <sup>-1</sup> )	26.94 (24.11–29.77)
Oxygen uptake (L·min <sup>-1</sup> )	1.29 (1.15–1.42)
Heart rate (beats·min <sup>-1</sup> )	102 (96–107)
Blood lactate (mmol·L <sup>-1</sup> )	0.94 (0.69–1.19)
Speed (km·h <sup>-1</sup> )	4.0 (3.6–4.4)



**Figure 2.** Time-trend changes in cardiorespiratory and metabolic indicators during steady state exercise at maximal fat oxidation intensity. Data are presented in 5 min interval during 1 h (each value is expressed as mean  $\pm$  SE).  $VO_2$  (oxygen uptake) (a), heart rate (b), fat (c) and carbohydrate (d) oxidation, energy expenditure (e), and lactate production (e). Session 1 ( $\bullet$ ); session 2 ( $\circ$ ).

file 1). In addition, energy expenditure recorded during steady-state exercise was  $\sim 36\%$  lower than values predicted from the GXT (Supplementary file 1). The average BLA

recorded during steady-state exercise was  $\sim 28\%$  higher than those values observed at FATmax during the GXT ( $0.98 \pm 0.52$  vs.  $1.26 \pm 0.54$   $\text{mmol}\cdot\text{L}^{-1}$ ,  $p < .05$ ).



**Figure 3.** Agreement and reliability of total fat utilization (a,b), carbohydrate utilization (c,d), energy expenditure (e,f) and blood lactate levels (g,h) across the two exercise trials performed at maximal fat oxidation intensity. The participants that showed a decrement on metabolic parameters between the first and the second steady-state exercise trial at FATmax are represented in dotted lines. ICC, intraclass correlation coefficient; MD, mean difference. Data is reported as mean (CI 95%).

## Discussion

The present study evidence that BLA, energy expenditure and substrate utilization show a moderate to good reliability during prolonged exercise at FATmax in young men with obesity. Therefore, a consistent acute-metabolic response can be expected during prolonged FATmax in this population. Our findings corroborate those of Chávez-Guevara et al. (2021) that substrate utilization exhibits a biphasic kinetic during prolonged exercise at FATmax, even though energy expenditure is maintained. Furthermore, we showed that fat and carbohydrate utilization during the 60 minutes of steady-state exercise trials were ~46% and ~26% lower than values predicted from the single GXT, respectively. Our data confirm that a single GXT is not accurate enough for prescribing exercise. A prolonged FATmax trial is the best way to verify substrate utilization. We also found that the MFO value obtained from a single GXT is reached after approximately 40 minutes of steady-state exercise at FATmax. Thus, a minimum of 60 minutes of FATmax exercise may be necessary to achieve a considerable fat utilization for men with obesity.

Previous studies conducted in healthy non-obese individuals suggest that the exponential decay of fat utilization during the initial 25 minutes of exercise might result from the increment of glycolytic flux in skeletal muscle which inhibits fat utilization by different cellular pathways, including the inhibition of carnitine palmitoyl transferase 1 and the increment of mitochondrial FADH<sub>2</sub>, NADH, and acetyl CoA levels (Sahlin, 2009). Nevertheless, although carbohydrate utilization progressively increased during the initial 25 minutes of exercise, we failed to observe an increment in BLA that could explain a consequent reduction in fat utilization. Given that our study only measured blood lactate levels, further research at the molecular level seems to be necessary to fully understand the physiological mechanisms involved in substrate utilization at the beginning of exercise. The same would apply for the observed subsequent recovery in fat utilization, a phenomenon that has been associated to the concomitant augment of adrenaline, growth hormone, glucagon, and intracellular calcium, that promotes the partitioning of triacylglycerols in adipose tissue and skeletal muscle as well as the oxidation of fatty acids into the mitochondrion of muscle fibers (Holloway et al., 2006; Watt et al., 2002).

The present study investigated the reliability of BLA, energy expenditure and substrate utilization during prolonged exercise at FATmax in men with obesity, a population that obtain considerable health benefits by maximizing fat oxidation through exercise (Chávez-Guevara et al., 2020). However, the following limitations ought to be acknowledged: (i) previous investigations have reported a sexual dimorphism in MFO and FATmax (Brun et al., 2020; Chávez-Guevara et al., 2023). Thus, further studies investigating the reliability of energy metabolism during prolonged exercise at FATmax in women with obesity are necessary, (ii) the MFO is determined by age, body mass index and the type of exercise in subjects with obesity (Chávez-Guevara, Amaro-Gahete, Ramos-Jiménez, et al., 2023). Therefore, the findings reported in our work need to be

replicated in adolescents and elderly adults under different exercise conditions (i.e., cycling and rowing), and (iii) although energy intake and macronutrient content were calculated in our study, the discordance between low energy intake and large body fat index in our participants suggests that they may have underestimated their actual dietary intake, a common limitation of the 24-hour recall approach (Waterworth et al., 2022). Additionally, MFO seems to be positively associated with dietary fat in healthy lean individuals (Fletcher et al., 2017; Jurado-Fasoli et al., 2021). Therefore, further studies need to investigate whether the metabolic response to prolonged FATmax exercise differs between individuals consuming a high versus low carbohydrate diet.

## Conclusions

A single GXT cannot predict energy metabolism during prolonged exercise at FATmax. Thus, an additional steady-state exercise trial at FATmax must be used for a tailored and precise exercise prescription. A minimum of 60 minutes of FATmax exercise may be necessary to achieve a considerable fat utilization for men with obesity

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## Disclosure statement

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## Author contribution statement

Investigation, I.A.C.-G.; conceptualization and methodology, A.R.-J. and I.A.C.-G.; formal analysis, F.J.A.-G., I.A.C.-G., R.P.; data curation, R.P. and F.J.A.-G.; writing—original, I.A.C.-G.; writing—review and editing, A.R.-J., F.J.A.-G. and R.P.; visualization, F.J.A.-G. and I.A.C.-G.; supervision and project administration, A.R.-J. All authors have read and agreed to the published version of the manuscript.

## Data availability statement

The dataset supporting the findings from this study is available from the lead author upon reasonable request.



## IRB approval

The study protocol was reviewed and approved by the Ethics Committee of the Autonomous University of Ciudad Juárez (CIBE-2018-1-11) prior study initiation.

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