Is there a diurnal variation of maximal fat oxidation during exercise in young women?

Maximal fat oxidation during exercise in young women: Is there a diurnal variation?

Abstract

Background: Maximal fat oxidation during exercise (MFO) and the intensity that elicits MFO (Fat_{max}) seems to show a diurnal variation in men, which favors an increased performance in the afternoon than the morning. At present, it remains unknown whether the observed MFO and Fat_{max} diurnal variation in men is also present in women. Therefore, the current study examined the diurnal variations of MFO and Fat_{max} in women.

Methods: Nineteen healthy women (age: 26.9±8.7 years, maximum oxygen uptake: 39.8±6.5 ml/kg/min) participated in the study. MFO and Fat_{max} were determined by a graded exercise test in cycloergometer using a cross-over design performed on two separate days, one conducted in the morning (8 am – 11 am) and one in the afternoon (5 pm – 8 pm). Stoichiometric equations were used to calculate fat oxidation rates.

Results: There were no significant differences between MFO-morning and MFO-afternoon (0.24±0.10 vs. 0.23±0.07 g/min, respectively; P=0.681). Similarly, there was no significant differences between Fat_{max}-morning and Fat_{max}-afternoon (41.1±4.7 vs. 42.6±5.5 g/min, respectively; P=0.305). These results persisted after controlling for fat mass percentage (all P>0.5).

Conclusion: In summary, the main finding of the present study was that MFO and Fat_{max} were similar independent of the time-of-day when the exercise test is performed in young healthy women. These results have important clinical implications since they suggest that, in contrast to what was found in men, MFO and Fat_{max} show similar rates during the course of the day in women.

Keywords*:* MFO; Fat_{max}; circadian rhythm, fat oxidation; female.

Introduction

During exercise, energy recruited from fat depots in humans is almost unlimited and the application of training methodologies aiming to improve fat oxidation rates during sport training has been postulated as an interesting strategy to achieve performance increases in endurance disciplines (Gonzalez et al., 2016; Amaro-Gahete et al., 2018; Maunder et al., 2018). Indeed, it has been demonstrated that maximal fat oxidation during exercise (MFO) and the intensity that elicit MFO (Fat_{max}) are strongly associated with Ironman triathlon performance, a discipline characterized by a long duration and low carbohydrate availability (Frandsen et al., 2017). Importantly, MFO and Fat_{max} are also well-recognized health biomarkers, since previous studies have highlighted that greater MFO and Fat_{max} values are positively correlated with insulin sensitivity (Venables and Jeukendrup, 2008), metabolic flexibility (Rosenkilde et al., 2010) and low cardiometabolic risk (Amaro-Gahete et al., 2019e; Montes-de-Oca-García et al., 2020). Therefore, it seems clear that MFO and Fat_{max} are key parameters both in performance and health contexts (Maunder et al., 2018).

Endurance exercise-related metabolic processes show a diurnal variation which favours an increased performance in the afternoon compared to late night and early morning (Ayala et al., 2021). Potential explanations of this important difference could be the optimized body temperature, catecholamine release, neural activation and/or contractile muscle properties observed in response to endurance exercise in the afternoon than in the evening and/or morning (Teo et al., 2011; Ayala et al., 2021). A series of studies have reported that MFO and Fat_{max} values are significantly higher in the afternoon compared with those obtained in the morning in young individuals with normal-weight (Darvakh et al., 2014), patients with obesity (Mohebbi and Azizi, 2011) and endurance-trained athletes (Amaro-Gahete et al., 2019a; Ramírez-Maldonado et al., 2021).

It is therefore, of practical relevance to identify which specific time-point are the most appropriate to obtain an enhanced MFO and Fat_{max} not only in a sport performance setting but also in a health-related setting. Nevertheless, all the above-mentioned studies were conducted solely in men (Mohebbi and Azizi, 2011; Darvakh et al., 2014; Amaro-Gahete et al., 2019a; Ramírez-Maldonado et al., 2021) and therefore, it remains unknown whether MFO and Fat_{max} diurnal variation is also present in women. Considering that (i) scientific knowledge about female responses and adaptations during exercise is markedly less compared to the body of research concerning males, (ii) the growing interest in the physiological responses during exercise in women and (iii) the increased participation of women in sport during the last years - in both recreational and competition levels -, it is of scientific and clinical interest to investigate whether MFO and Fat_{max} vary depending on the time of the day in female individuals. Therefore, the current study examined the diurnal variations of MFO and Fat_{max} in young healthy women. Based on previous work, we hypothesized that MFO and Fat_{max} values would be higher in the afternoon compared with those observed in the morning in our study cohort.

Methods

Participants

Twenty-two young women aged 19 to 40 years were recruited for the present study. Three out of 22 participants were excluded from the final statistical analyses since they did not come to the laboratory meeting the pre-established conditions to evaluate MFO and Fat_{max}. The inclusion criteria were: (i) to have a body mass index ranging from 18 to 27 kg/m^2, (ii) to be a non-smoker, (iii) not to take any medication, (iv) not to suffer from acute/chronic diseases which could be aggravated by exercise, (v) not to be pregnant, and (vi) to have a regular menstrual cycle. Oral and written informed consent were obtained from all subjects before the beginning of data collection. Study procedures were performed strictly following the Declaration of Helsinki (last revision 2013) and were approved by the UFV Research Ethics Committee (18-2020).

Design and methodology

The present experiment was conducted between January and May 2021. The participants came to the laboratory on two different days separated by ~7 days in which MFO and Fat_{max} were assessed at 8 am – 11 am (MFO-morning and Fat_{max}-morning) and at 5 pm – 8 pm (MFO-afternoon and Fat_{max}-afternoon) in random order. They were asked (i) to refrain from moderate and/or vigorous physical activity (24 h and 48h before the trial, respectively), (ii) to come to the laboratory by car or bus (avoiding physical activity), (iii) to eat a personalized and standardized isocaloric diet 24 hours before each trial (i.e., 50% carbohydrates, 30% fat and 20% protein) maintaining the same meal ingestion order, (iv) to fast for 7 – 10 h before each trial, and (v) to avoid stimulants and/or alcohol 24 h prior to the testing days as described in detail in previous studies (Amaro-Gahete et al., 2019b; Ramírez-Maldonado et al., 2021).

Energy requirements were estimated using the Harris-Benedict equation applying a physical activity factor of 1.8 (Harris and Benedict, 1918). Finally, we organized both testing days during the luteal phase of the menstrual cycle (i.e., asking the participants about menstrual cycle length) although it has been shown that MFO and Fat_{max} do not vary across the menstrual cycle (Frandsen et al., 2020).

After the participant' arrival on testing days, a 15-min resting period was established before the starting of the exercise test. Subsequently, they completed a 10-min warm-up on a cyclergometer (Ergoselect 4, Ergoline, Germany) with an intensity of 30% of maximum oxygen uptake (VO_{2max}). On Day 1, we instructed the participants to maintain a cycle cadence of 70 – 90 rpm in order to ensure its replication on Day 2. Subsequently, exercise intensity was increased by 10% of VO_{2max} each 3 min until a respiratory quotient >1 was reached. A breath-by-breath gas analyzer (Ergostik, Geratherm Respiratory, Germany) was used to obtain gas exchange data. Importantly, all trials were performed under controlled environmental conditions (i.e., temperature: 22-24ºC; humidity: 35-45%) and by the same trained staff. Gas analyzer calibrations were completed using a 3-L syringe and 2 standard gas concentrations (i.e., 16.0% for O_2; 5.0% for CO_2, Cortex, Germany) before the beginning of each trial. Fat oxidation rates were calculated from VO_2 - VCO_2 data (Amaro-Gahete et al., 2019c) using the Frayn stoichiometric equation (Frayn, 2016). When a respiratory quotient > 1 was observed, we assumed that the fat oxidation value would be 0.0 g/min. Fat_{max} was defined as the intensity attained in the MFO stage.

One week before the first visit, the participants were invited to the laboratory to perform an anthropometric (i.e., weight and height) and body composition (i.e., fat mass and lean mass) analyses using a Seca electronic column scale and stadiometer (Seca 700, Hammer Steindamm, Hamburg, Germany), and a bioimpedance tool (Tanita InnerScan Dual, RD-901BK36, Japan), respectively, in accordance with the manufacturer' instructions. Thereafter, VO_{2max} was calculated by an incremental exercise test on cyclergometer. The protocol started with a warm-up (10 minutes at 50 W) followed by further increases of 25 W/min until the participant' voluntary exhaustion. VO_{2max} criteria were the following: (i) attaining a steady in VO_2 (i.e., < 2 ml/kg/min differences in the final stage) despite an intensity rise, (b) achieving a maximal heart rate ranging from ±10 bpm of the age-predicted maximal heart rate, and (iii) reaching a respiratory quotient higher than 1.15 (Beltz et al., 2016). If these criteria were not achieved, VO_{2max} was considered as the highest VO_2 value registered during the final step (excluding artefacts). We conducted an individual regression analysis between exercise intensity (W) and VO_2 for each participant to normalize exercise intensity among all participants (i.e., increases of 10% VO_{2max}) on the experimental days.

Statistical analysis

The results are presented as means ± standard deviation, if not stated otherwise. The normality of the study' outcomes was verified through histograms, scatter graphs, and the Shapiro–Wilk test.

Differences between MFO-morning vs. MFO-afternoon and Fat_{max}-morning vs. Fat_{max}-afternoon were analyzed by repeated-measures analysis of variance (ANOVA). A repeated-measures analysis of covariance (ANCOVA) was also conducted to study morning vs. afternoon potential differences adjusting for fat mass percentage and lean mass.

The statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS, v. 21.0, IBM SPSS Statistics, IBM Corporation), whereas graphs were plotted using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). An alfa of $P<0.05$ was set as the level of significance.

Results

Table 1 shows descriptive parameters of the study' participants. Test order was morning-afternoon in a total of 9 participants and afternoon-morning in 10 participants. No significant differences were found in fasting time between the morning and the afternoon test (8.5 vs. 8.3 hrs., respectively, P=0.534).

Insert Table 1

Repeated-measures ANOVA indicated no significant differences between MFO-morning and MFO-afternoon (0.24±0.10 vs. 0.23±0.07 g/min; P=0.681, Figure 1A and 1B, respectively), which remained after adjusting for fat mass percentage (P=0.501). Similarly, there were no significant differences between Fatmax-morning and Fatmax-afternoon (41.1±4.7 vs. 42.6±5.5 % VO_2max-, P=0.305, Figure 1C and 1D, respectively), which persisted after controlling for fat mass percentage and lean mass (all P>0.6).

Insert Figure 1

Discussion

The present study aimed to determine whether MFO and Fat_{max} show a diurnal variation in young healthy women. Our main finding was that MFO and Fat_{max} were similar independently of the time-of-day when endurance exercise is performed in young healthy women. These results have important clinical implications since they suggest that, contrarily to what was found in men, MFO and Fat_{max} show similar rates during the course of the day in women.

The current findings provide additional scientific evidence to the existing knowledge about MFO and Fat_{max} diurnal variation (Mohebbi and Azizi, 2011; Darvakh et al., 2014; Amaro-Gahete et al., 2019a; Ramírez-Maldonado et al., 2021). Our results clearly show that MFO and FATmax rates are similar in the morning and the afternoon, which does not concur with results obtained in prior studies (Mohebbi and Azizi, 2011; Darvakh et al., 2014; Amaro-Gahete et al., 2019a; Ramírez-Maldonado et al., 2021). Concretely, it has been reported that MFO and Fat_{max} are increased in the afternoon compared with the morning in active young men (+7%, and +12%, respectively) (Darvakh et al., 2014), normal-weight and obese men (+9%, and 11%, respectively), and male endurance-trained athletes (+15%, and +8%, respectively) (Amaro-Gahete et al., 2019a; Ramírez-Maldonado et al., 2021), while in this study similar rates of MFO and Fat_{max} were observed in our cohort of healthy young women.

The previously reported diurnal variation of MFO and Fat_{max} has been attributed to specific time-of-day related physiological adaptations occurring during endurance exercise (Kim et al., 2015). Concretely, core body temperature reaches its daily peak in the afternoon leading to a subsequent increment of energy metabolism and enhancing actin-myosin cross cycling and muscle compliance, all of the important factors to optimize fat oxidation rates during endurance exercise (Teo et al., 2011). Furthermore, it is also known that the catecholamine peak in response to exercise is considerably higher in the afternoon than in the morning (Drust et al., 2005; Teo et al., 2011; Ayala et al., 2021). The catecholamines significantly stimulate an increased free fatty acid release and optimize lipolysis processes of both adipose and skeletal muscle tissues (Atkinson et al., 2005; Teo et al., 2011; Ayala et al., 2021), thus promoting higher rates of fat oxidation during exercise. Taking all together, these time-of-day physiological differences in response to exercise may explain the diurnal variation of MFO and Fat_{max} in the above-mentioned studies (Mohebbi and Azizi, 2011; Darvakh et al., 2014; Amaro-Gahete et al., 2019a; Ramírez-Maldonado et al., 2021), but not the results of the present study. Considering the design and methodology of these former studies, it seems reasonable that these discrepancies may be attributed to the participant´ sex.

Most experiments in the field of exercise metabolism have been conducted in men, in part under the consideration that skeletal muscle fiber architecture and enzymatic oxidative activity - among other parameters - are similar in both endurance-trained men and women (Costill et al., 1976; Howald et al., 1985). However, studies suggested that this assumption represents an important sex bias since they found a superior contribution of fat metabolism during endurance exercise in female athletes compared to their male counterparts (Friedlander et al., 1998; Horton et al., 1998). D'Eon et al. concluded that the exogenous administration of estradiol improved fat oxidation rates in response to 60 min of continuous aerobic exercise at moderate intensity in healthy women whose estrogen blood concentrations were previously reduced until postmenopausal levels (D'Eon et al., 2002). Interestingly, these increments in fat oxidation were directly associated with greater free fatty acid concentrations in plasma (D'Eon et al., 2002), which is one of the most important factors that leads to higher levels of MFO (Frandsen et al., 2019). Given that plasma levels of estrogens are relatively elevated during the luteal phase – all women performed exercise tests during this phase of the menstrual cycle -, their subsequent hypothetical increment of free fatty acids blood concentration could be a sufficient stimulus to counteract the potential diurnal variation of MFO and Fat_{max}. Thus, upcoming studies are requested to confirm whether the present findings apply for all phases of the menstrual cycle in which both females' hormones and free fatty acids levels are significantly different.

The present findings should be considered cautiously since there are a number of limitations. Firstly, although we organized all exercise trials during the luteal phase of the menstrual cycle, plasma levels of female sexual hormones (i.e., estrogen and progesterone) and free-fatty acids concentrations were not assessed. Secondly, the present study was conducted in eumenorrheic women and it may not be possible to extrapolate this finding to postmenopausal women. Finally, body temperature and plasma catecholamines were not registered during the exercise test and, therefore, we cannot account for their role for the MFO and Fat_{max} diurnal variation in women.

In conclusion, the main findings of the present study demonstrate that there are no differences in MFO and Fat_{max} rates throughout the day in young healthy women. These findings are of practical and clinical relevance for endurance and conditioning coaches since they can serve to correctly organize the athletes´ schedule for both testing and training tasks. Future studies are needed to elucidate the molecular and physiological processes that explain the observed biological differences between sexes in MFO and Fat_{max} diurnal variation.

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Table 1. Characteristics of the study subjects (n=19).

Age (years)	26.9	±	8.7
Weight (kg)	62.0	±	8.8
Height (m)	166.7	±	6.7
Body mass index (kg/m^2)	22.2	±	2.7
Fat mass (%)	24.3	±	5.5
Lean mass (kg)	44.5	±	5.6
VO_2max (l/min)	2.4	±	0.4
VO_2max (ml/kg/min)	39.8	±	6.5

Values expressed as means ± standard deviation.

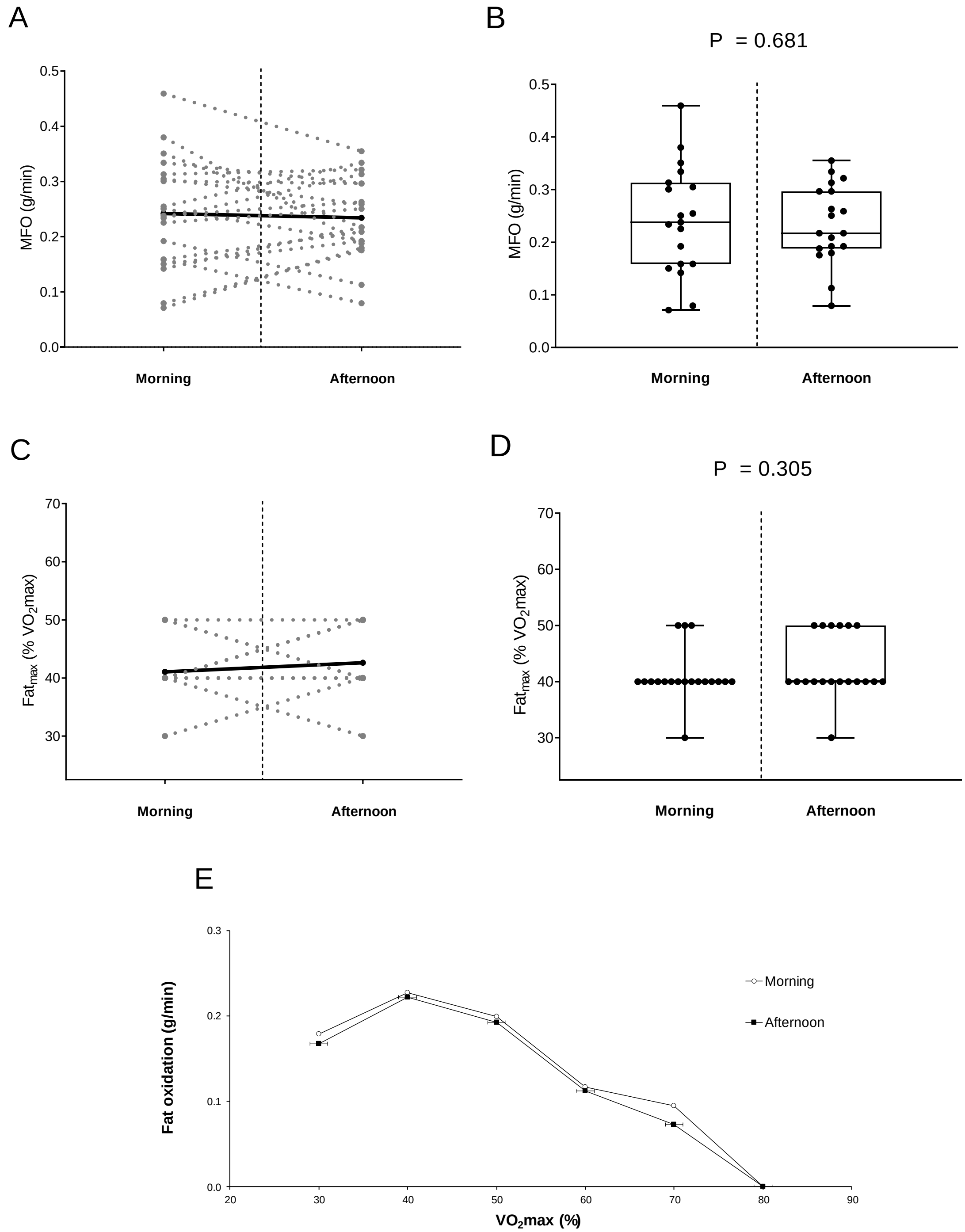

Figure 1. Maximal fat oxidation (MFO) in the morning and in the afternoon, and the intensity which MFO occurs (Fat_{max}) in the morning and in the afternoon. Panel A and C: Individual observations for each subject (grey lines), and the mean for all subjects (black line). Panel B and D: Individual observations for each subject (black dots), standard deviation and minimum/maximum values (box-and-whisker plots). P value obtained by repeated-measures analysis of variance. Panel E: Fat oxidation rate during the incremental exercise test on a cycle ergometer.