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Timing matters: diurnal variation of maximal fat oxidation and substrate oxidation rates in metabolic syndrome-a randomized crossover study

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Keywords:

Carbohydrates oxidation; Circadian variation; FATmax; LIPOXmax; Substrates metabolism.

Abstract

Purpose:

aim of this study was to investigate if diurnal oscillation in maximal fat oxidation (MFO) and substrate oxidation rates during exercise exists in subjects with metabolic syndrome (MetS).

Methods:

In a randomized crossover design, 14 MetS patients were assigned to two graded exercise tests conditions performed in the morning (between 7:00 and 9:00 a.m) and in the afternoon (between 4:00 and 5:00 p.m). MFO was defined as the highest absolute value of fat oxidation obtained from the average of last 2-min stages during an indirect calorimetry test.

Results:

MFO increased by 20.6% from morning to afternoon ($p = 0.0002$, Cohen's $d = 0.52$). There was a significant time of day, ($p < 0.0001$, $\eta^2p = 0.76$) and intensity effect ($p = 0.002$, $\eta^2p = 0.32$) in fat oxidation (Fatox) rates indicating that Fatox was higher in the afternoon than in the morning.

Conclusion:

Our study extends previous findings on the existence of diurnal variation in maximal fat oxidation to MetS patients, highlighting the afternoon as a more favorable time for fat utilization during exercise. These findings have practical implications for optimizing training timing in MetS patients.

Trial registration number:

PACTR202306776991260.

Abbreviations

MFO; Maximal Fat Oxidation; LIPOXmax; the intensity that elicits maximal fat Oxidation; $\dot{V}O_2$; Oxygen consumption; CHO: Carbohydrates; Fatox; Fat Oxidation; CHOox; Carbohydrates Oxidation; EE; Energy Expenditure; EC; Economy.

1. Introduction

Metabolic syndrome (MetS) is an intricate amalgamation of cardio-metabolic risk factors characterized by a shared underlying mechanism – insulin resistance (Alberti et al. 2009). These risk factors encompass elevated blood pressure, dyslipidemia (abnormal lipid profile), central obesity, and impaired glucose regulation.(Alberti et al. 2009) Although, there has been much debate on the etiological role played by insulin resistance in connecting these risk factors, the cluster of cardio-metabolic risk factors is often accompanied and associated to insulin resistance (Alberti et al. 2009). At the molecular level, the insulin resistant state is characterized by an increased preference for glucose oxidation and reduced preference for fat oxidation (Fatox) in postabsorptive state and an altered ability to adapt glucose oxidation to glucose availability in insulin stimulated state (hyperinsulinemic-euglycemic clamp (HEC) as originally coined by the classical limb balance studies of Kelley and Mandarino (2000). This mismatch between substrate availability and substrate oxidation, which is now known under the name of metabolic inflexibility, may lead to ectopic fat deposition and is grossly known as a major etiology in the pathogenesis of insulin resistance (Goodpaster and Sparks 2017).

While indirect calorimetry during HEC testing is widely recognized as the gold standard for assessing metabolic flexibility, several significant caveats are associated with this method. These limitations include the invasive nature of whole procedure, the substantial labor and time required for conducting the tests. Furthermore, the method focusses on measuring substrate oxidation changes in resting conditions, which may not fully capture the dynamic nature of metabolic responses. Additionally, it often overlooks the influence of elevated glucose availability, a common characteristic in insulin-resistant states. (Fernández-Verdejo et al. 2018) An interesting surrogate alternative that gained momentum in last few years was testing metabolic flexibility by measuring substrate oxidation using various exercise protocols. (San-Millán and Brooks 2018; Fernández-Verdejo et al. 2018; Chu et al. 2021; Chu et al. 2018) A 2017 interesting study using an incremental submaximal exercise protocol coupled with indirect calorimetry and lactate measurement has successfully profiled individuals across the metabolic health spectrum. (San-Millán and Brooks 2018) Specifically, the study established that there is an early reliance on carbohydrates oxidation (CHOox) and lower capacity of Fatox during exercise in metabolic syndrome compared to physically active and moderately trained endurance athletes. Similarly, physically active individuals rely earlier on CHOox and have lower fat oxidizing capacity compared to moderately trained individuals(San-Millán and Brooks 2018), ultimately showing that substrate oxidation during exercise could be used as a proxy of metabolic flexibility. Furthermore, a low Maximal Fat Oxidation (MFO) during exercise was associated with a higher clustering of metabolic syndrome risk factors in overweight men. (Rosenkilde et al. 2010)

Emerging evidence also suggests a connection between MFO and 24-hours fat oxidation as well as surrogate measures of insulin sensitivity.(Robinson et al. 2015) Reduced fat oxidation has previously been linked to long-term changes in body weight and composition.(Zurlo et al. 1990; Ellis et al. 2010) For instance, an increased daily respiratory quotient (RQ), indicative of low relative fat oxidation, has been associated with a higher risk of body mass gain and fat mass regain after diet-induced weight loss.(Ellis et al. 2010) Considering that high fat oxidation during exercise can contribute significantly to daily fatox (Robinson et al. 2015), it is not unreasonable to consider targeting higher levels of fatox during exercise advantageous for the maintenance of body mass and body composition and overall metabolic health.

It is becoming increasingly evident that physical performances and various health related parameters including MFO follow diurnal rhythms during rest and exercise.(Mousavian et al. 2014; Amaro-Gahete et al. 2019; Mohebbi and Azizi 2011) MFO, the intensity at which maximal fat oxidation occurs (LIPOX_{max}), and $\dot{V}O_{2max}$ which are very important in both endurance performance and cardio-metabolic health have all been shown to peak in afternoon.(Mousavian et al. 2014; Amaro-Gahete et al. 2019; Mohebbi and Azizi 2011) These findings have significant implications for weight loss programs, as they suggest that scheduling physical exercise to afternoon may be more beneficial for health-related outcomes in general and weight loss in particular. In line with this, a larger-scale study involving over 90,000 men and women have suggested that physical exercise performed in afternoon can reduce risks of premature death more effectively than morning or evening workout sessions.(Feng et al. 2023) Similarly, a 2023 study showed that the glucose lowering of a moderate to vigorous physical activity performed in afternoon was associated with higher improvements in glycemic control in adults with diabetes at 1 year and this effect continued to year 4 of a lifestyle intervention, an effect that was independent of intensity and volume of moderate to vigorous physical activity.(Qian et al. 2023)

These temporal patterns in health-related outcomes in response to exercise suggest that that body's internal clock coordinates physiological function. Indeed, in healthy individuals various metabolic genes; ranging from genes that control mitochondrial respiration and function to genes that control fatty acid metabolism, exhibit a rhythmic activity that follows circadian rhythms.(Christou et al. 2019; Cox and Takahashi 2019) However, emerging evidences suggest that in metabolically compromised individuals, the rhythmic pattern in metabolic gene expression is lost and this contribute to the impaired metabolic phenotype.(Poggiogalle et al. 2018) However, whether this altered rhythmicity in gene expression especially those controlling mitochondrial function in metabolically compromised individuals could translate to whole-body level; and consequently, alter substrate preferences and oxygen consumption is not known. A glimpse into this question comes from study of Mohebbi and Azizi (2011) who showed that individuals with obesity still show oscillation in their daily patterns of substrate preferences during exercise. However, taking into account the role that plays nutritional intake as zeitgeber, these findings could not be ascertained given that dietary intake was not controlled and duration of fasting that separated last meal from each test varied between the morning (8-12h) and afternoon (5-6h) testing sessions, being shorter in afternoon. Therefore, the main aim of this study was to investigate if diurnal oscillation in maximal fat oxidation and substrate oxidation rates during exercise exist in subjects with METs.

2. Materials and Methods

2.1 Participants:

This study was approved by the institutional review board of the Faculty of Medicine of Sousse (Ref: CEFMS 188/2023) and conformed with declaration for Helsinki. The study was registered in pan African clinical trial registry as PACTR202306776991260. Subjects were recruited for this study after providing a written informed consent. A total of 14 MetS patients were recruited for this study 7 Men and 7 women (4 premenopausal and 3 post-menopausal women). Eligible participants (Table1) were recreationally active (undergoing lifestyle changes prescribed by their physician) adults with obesity (both sexes) that met MetS definition (Alberti et al. 2009). Premenopausal women were tested in their early follicular

phase. All participants reported that they undergo recreational physical activity 3 days a week for the last one month.

2.2 Study design:

In a randomized, crossover design, subjects were randomly assigned to 2 experimental conditions: a morning condition and an afternoon condition. A washout period of at least 4 days separated each condition.

2.3 Study protocol

2.3.1 pre-experimental sessions:

Subjects manifesting their preliminary interest were asked to report to the lab after an overnight fast. This first visit was dedicated to explain study procedures and to sign the informed consent, if subjects accepted to participate. From subjects who accepted to participate in the study and signed consent a fasting blood sample to screen for different components of MetS was drawn. Detailed medical history of participants and anthropometric parameters were also collected during this session. Then subjects were asked to report for a second session to measure resting metabolic rate (RMR) and to conduct a graded exercise test. The outcomes from these latter tests were used to calibrate the LIPOXmax protocol during experimental sessions and for dietary and metabolic control before experimental sessions.

2.3.2 Experimental sessions:

Participants were instructed to avoid moderate to vigorous physical activity until the second session. Both morning and afternoon sessions were scheduled for each participant, with morning sessions occurring between 7:00 and 9:00 a.m., and afternoon sessions between 4:00 and 5:00 p.m. All participants were provided with a standardized last meal which represented the lunch in morning session and an early breakfast at 3 a.m in afternoon session.

Regarding nutritional control, one day prior to each experimental condition, participants were given a diet plan that closely matched their estimated daily energy expenditure (EE). To calculate this, resting metabolic rate (RMR) was multiplied by an activity factor of 1.4, which corresponds to sedentary activities since participants were instructed to avoid physical activity; this approach has been shown to be highly correlated with controlled feeding interventions. (Kien and Ugrasbul 2004) The dietary intake was specifically designed to align with the estimated daily EE and was kept consistent across all three experimental conditions. The daily energy intake was divided into the following proportions: 25% for breakfast, 35% for lunch, and 40% for dinner. Additionally, macronutrient composition of meals followed these proportions: 54% carbohydrates (CHO), 30% lipids, and 16% protein.

2.4 Physical tests and Resting and Indirect calorimetry Measurements

2.4.1 $\dot{V}O_2$ peak and Peak Power Output Testing

Subjects reported to the laboratory in the morning to conduct the $\dot{V}O_2$ peak test. The test preceded by a 4 min empty load warm up on a pre-calibrated cycle ergometer (Excalibur, Lode, Groningen, the Netherlands) after which the test started at 20 W for women and 40 for men with 20 W increments per minute until volitional exhaustion. Gas exchanges were collected breath by breath by an automatic gas analysis system (Metasys TR, Brainware SA, La Valette, France). This test was conducted to assess $\dot{V}O_2$ peak and peak power output (PPO). Heart rate and electrocardiographic parameters were monitored continuously throughout the test by standard 12-lead procedure.

2.4.2 Exercise indirect calorimetry (LIPOXmax test)

Subjects performed this test in morning and in afternoon after 12-13 hours fast. The test consisted of six-minute steady-state workloads set at 20, 30, 40, 50, and 60% of Peak power output (PPO) obtained during the $\dot{V}O_2$ peak test. The subjects performed the test on an electromagnetically braked cycle ergometer (Excalibur, Lode, Groningen, the Netherlands). Metabolic and ventilatory responses were assessed breath by breath by an automatic gas analysis system (Metasys TR, Brainware SA, La Valette, France).

2.4.3. Resting metabolic rate

The resting metabolic rate was measured using the indirect calorimetry method ((Metasys TR, Brainware SA, La Valette, France).), using standard procedures (Compher et al. 2006).

2.5 Anthropometric measurements

2.5.1 Height and weight

Height was quantified using a stadiometer in a standing position and after a normal expiration, using (SECA 225, Hamburg, Germany) with a precision of 1 mm. Body mass was assessed using an electronic measuring scale (Polar Electro, Kempele, Finland)

2.5.2 Waist and hip circumference

Waist circumferences were measured using a flexible non elastic tape at the narrowest point of the torso between the lower rib margin and the iliac crest.

2.5.3 Body composition:

The four-site skinfold method of Durnin and Womersley (1973) was used to assess body composition.

2.6 Indirect calorimetry and calculations

For the LIPOXmax test, $\dot{V}O_2$, $\dot{V}CO_2$ values during the last two minutes of each stage were averaged for the determination of non-protein substrate oxidation were used to quantify substrate oxidation (Peronnet and Massicotte 1991): Carbohydrate oxidation: $4.585 \dot{V}CO_2 - 3.225 \dot{V}O_2$ Fat oxidation: $1.646\dot{V}O_2 - 1.7012 \dot{V}CO_2$ Crossover points (Brooks and Mercier 1994) were estimated after calculating absolute (kcal/min) and relative (%) contributions of fat and CHOox to total EE using Atwater factors for fat (9 kcal/g) and CHO (4 kcal/g).

2.7 Blood collection and Biochemical measurements

For serum analysis of triglycerides (TG), total cholesterol (TC), HDL-C, blood was collected in plain tubes and left at room temperature for coagulation. Triglycerides were analyzed following the triglyceridesglycerol-3-phosphate oxidase method. TC was analyzed following cholesterol oxidase method. HDL-C was analyzed directly following a colorimetric enzymatic method. LDL-c was derived from the Friedwald formula. For the analysis of plasma glucose, blood was collected in fluoride/oxalate tubes and was analyzed using the hexokinase method. The IMMAGE Immunochemistry System (Beckman Coulter, USA) was used for measurement of HbA1c by the nephelometry method according to Beckman manufacturers.

2.8 Sample size calculation

Using G*Power version 3.1.9.2 (University of Kiel, Germany), it was estimated that a total sample size of 12 participants would provide 80% statistical power to detect a statistically significant difference between our within-subjects factor (time of day), when this interaction amounted to a standardized effect size (Cohen's d) of 0.88 ($\alpha = 0.05$) as calculated from the data of Amaro-Gahete (Amaro-Gahete et al. 2019). Anticipating a 20% dropout, a total sample size of 14 participants was considered.

2.9 Statistical analysis

The normality of residuals was checked by the Shapiro Wilk test and visual inspection of the distribution. A two-way repeated measures ANOVA was performed to analyze time of day effects in MFO, LIPOXmax and the Crossover points with time of day as a within subject factor and gender as a between subject factor, to analyze whether gender interacts with time of day. A two-way repeated measures ANOVA was performed to analyze time of day effects in $\dot{V}O_2$, $\dot{V}CO_2$, QR, EE, absolute Fatox and CHOox rates and their relative contribution to EE, with time of day and intensity as a within subject factors and gender as a between subject factor. #while not originally designed to assess gender effects, we incorporated gender as a between-subjects factor, to explore potential interference of gender with diurnal variations. Least significant difference Post hoc were used for pairwise comparisons for relevant main or interaction effects. Partial eta-squared (η^2_p) for the magnitude of relevant significant effects of ANOVAs were used and interpreted according to Cohen's scale (small effect: $0.01 < \eta^2_p < 0.06$, medium effect: $0.06 < \eta^2_p < 0.14$, and large effect: $\eta^2_p > 0.14$) were reported. All data are presented as mean \pm SD. Significance level was set at $p < 0.05$.

3. Results

3.1 Subjects characteristics

Fig.1 depicts the participants flow throughout the study. Subjects' characteristics are presented in table 1. 12 Subjects had Rather more a "morning" than an evening chronotype and 2 had a rather more an "evening" than a morning chronotype. Figure S1. shows subjects count per each MetS component.

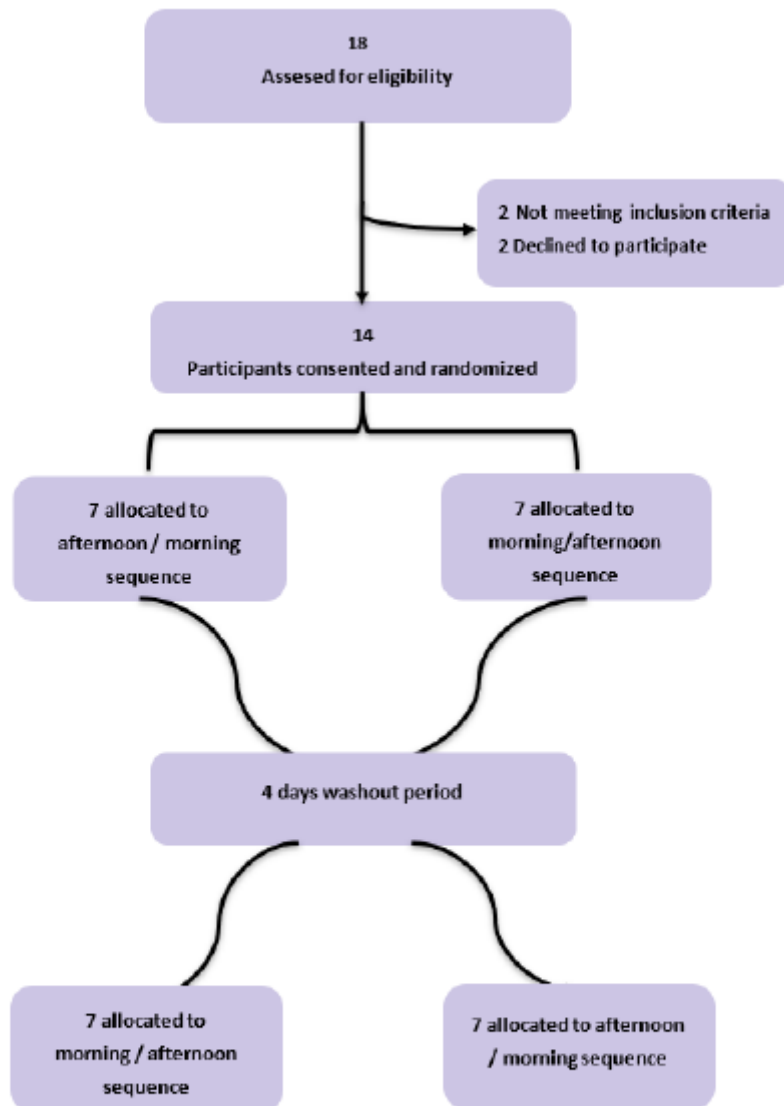


Figure 1

Flow diagram of study participants

3.2 Maximal Fat Oxidation

MFO (Fig.2) increased by 20.56 % from morning to afternoon (time of day, $p=0.0002$, $\eta^2 p = 0,69$) and this was independent of gender (gender*time of day, $p=0.144$), indicating that MFO was higher in the afternoon than in the morning in both males (11.04 %) and females (38.82%).

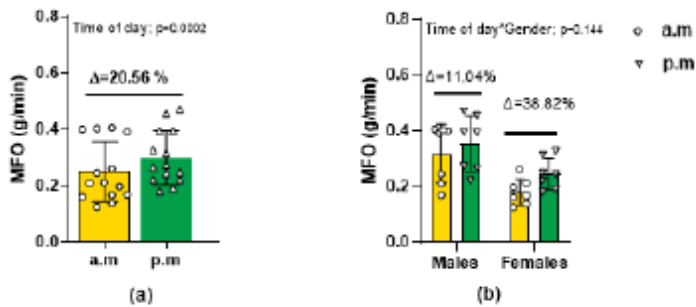


Figure 2

Diurnal variation of maximal fat oxidation (g/min) measured in both sexes (a) and in males and females (b) to illustrate time of day*gender interaction.

3.3. LIPOXmax and Crossover point

A significant time of day effect in LIPOXmax (Fig.3) was also found ($p=0.036$, $\eta^2 p = 0.317$) that occurred at 53.80 % of $\dot{V}O_{2peak}$ in the morning and at 58.5 % of $\dot{V}O_{2peak}$ in the afternoon and this was independent of gender (gender*time of day; $p=0.265$, $\eta^2 p = 0,10$). A similar pattern was also found for the Crossover point (Fig2.b), where it was estimated to occur at 44.07 % of $\dot{V}O_{2peak}$ in the morning and at 53.73 % in the afternoon of $\dot{V}O_{2peak}$ (time of day effect; $p=0.046$, $\eta^2 p=0.293$) and this was also independent of gender (gender*time of day, $p=0.930$, $\eta^2 p=0.001$).

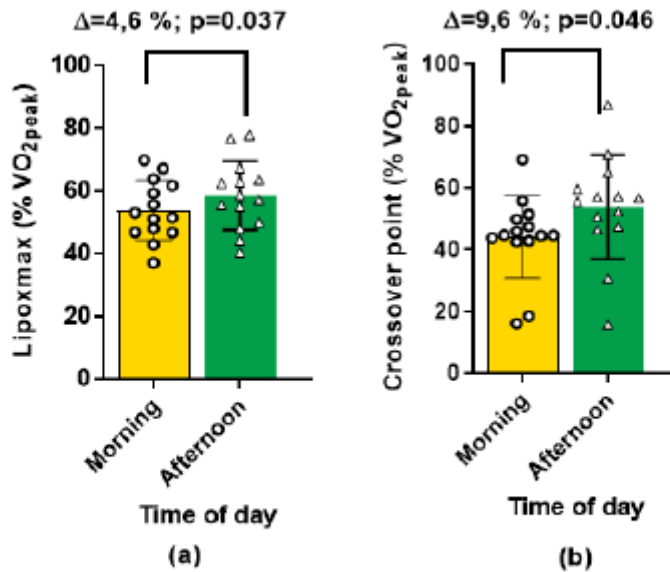


Figure 3

The intensity that elicits maximal lipid oxidation (LIPOXmax) (a) and the Crossover points (b) as expressed in %VO_{2peak} measured in the morning (yellow) and in the afternoon (green).

3.4. Fat Oxidation rates across the ranges of exercise intensity

There was a significant time of day effect in Fatox rates, ($p<0.0001$, $\eta^2 p = 0,81$) and intensity ($p=0.004$, $\eta^2 p = 0,469$) that was independent of gender (time of day*intensity*gender interaction, $p=0.0164$) indicating that Fatox was higher in the afternoon than in the morning in both male and females (Fig. 4a-c). Overall Fat peaked at 30% of PPO then decreased progressively to reach its nadir at 60 % of PPO.

3.5. Carbohydrate Oxidation across the range of exercise intensities

For CHOox (Fig.4d) there was only an effect of intensity ($p<0.0001$, $\eta^2 p = 0.848$) indicating increased oxidation with increasing exercise intensities.

3.6. Energy expenditure across the range of exercise intensities

Absolute EE (Fig.4e) increased over the range of exercise intensities (effect of intensity; $p<0.001$; $\eta^2 p = 0,855$) and was overall higher in afternoon (effect of time of day; $p=0,30$; $\eta^2 p = 0,337$) compared to the morning and this was independent of gender (gender*time of day*intensity interaction; $p=0.376$; $\eta^2 p =0.081$)

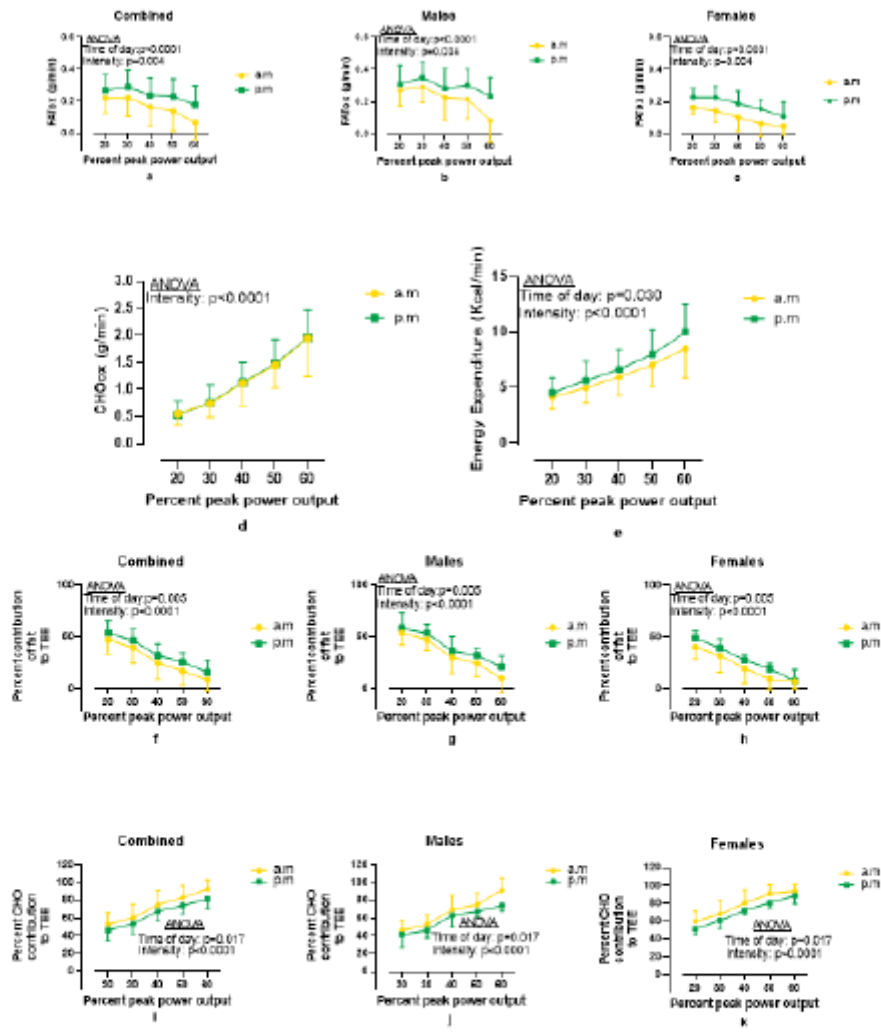


Figure 4

Diurnal variation of substrate oxidation rates, energy expenditure and percent contribution of fat and carbohydrates oxidation to energy expenditure. as a function of exercise intensity. Absolute Fat oxidation (g/min) as a function of exercise intensity (% peak power output) in all (a) patients (male +female), in

males (b), and in females (c). Comparison morning (a.m) vs afternoon (p.m). Absolute carbohydrate oxidation (CHOox) as a function of exercise intensity (% peak power output) measured in the morning (a.m), and in the afternoon (p.m) in all patients (d). Energy expenditure (Kcal/min) as a function of exercise intensity (%Peak power output) measured in the morning (a.m), and in the afternoon (p.m) in all patients (e). Percent fat contribution to Total Energy Expenditure (TEE) across the range of exercise intensities (%Peak power output) in all subjects (f), in males (g), and in females (h) in the morning (a.m) and in the afternoon (p.m). Percent carbohydrates (CHO) contribution to Total Energy Expenditure (TEE) across the range of exercise intensities (%Peak power output) in all subjects (i), in males (j), and in females (k) in the morning (a.m) and in the afternoon (p.m).

3.7. Percent fat contribution to energy expenditure across the range of exercise intensities

The relative contribution of fat to EE was higher in the afternoon (effect of time of day; $p=0.005$, $\eta^2 p =0.494$) and this was independent of gender (gender*time of day* intensity interaction; $p=0.077$, $\eta^2 p = 0.158$) or intensity. Overall Fat contribution to EE was the highest at 20 % of PPO, then decreased to reach its nadir at 60 % of PPO (effect of intensity; $p<0.0008$, $\eta^2 p = 0.837$) (Fig.4f-h).

3.8. Percent carbohydrates contribution across the range of exercise intensities

The relative contribution of CHO (Fig.4i-k). to EE was higher in the morning (effect of time of day; $p=0.011$, $\eta^2 p =0.534$) and this was independent of gender (gender*time of day*intensity interaction; $p=0.312$, $\eta^2 p = 0.121$). Overall CHO contribution to EE increased with increasing exercise intensity to peak at 60 % of PPO (effect of intensity; $p<0.0001$, $\eta^2 p = 0.803$).

3.9. $\dot{V}O_2$ Kinetics across the range of exercise intensities

There was a significant effect of time of day in $\dot{V}O_2$ ($p<0.025$, $\eta^2 p = 0,446$) and intensity ($p=0.0001$, $\eta^2 p = 0,869$) that was independent of gender (gender*time of day* intensity interaction, $p=0.990$, $\eta^2 p = 0.08$) indicating that overall $\dot{V}O_2$ increased with increasing exercise intensities and was higher in the afternoon than in the morning in both males and females (Fig.5a-c).

3.10. $\dot{V}CO_2$ Kinetics across the range of exercise intensities

$\dot{V}CO_2$ (Fig.5d-f) production increased with increasing exercise intensities (effect of intensity; $p<0.0001$, $\eta^2 p = 0.892$) and was overall higher in the afternoon compared to the morning (effect of time of day; $p=0.031$, $\eta^2 p =0.332$) independently of gender (gender*time of day*intensity interaction; $p=0.168$, $\eta^2 p = 0.383$).

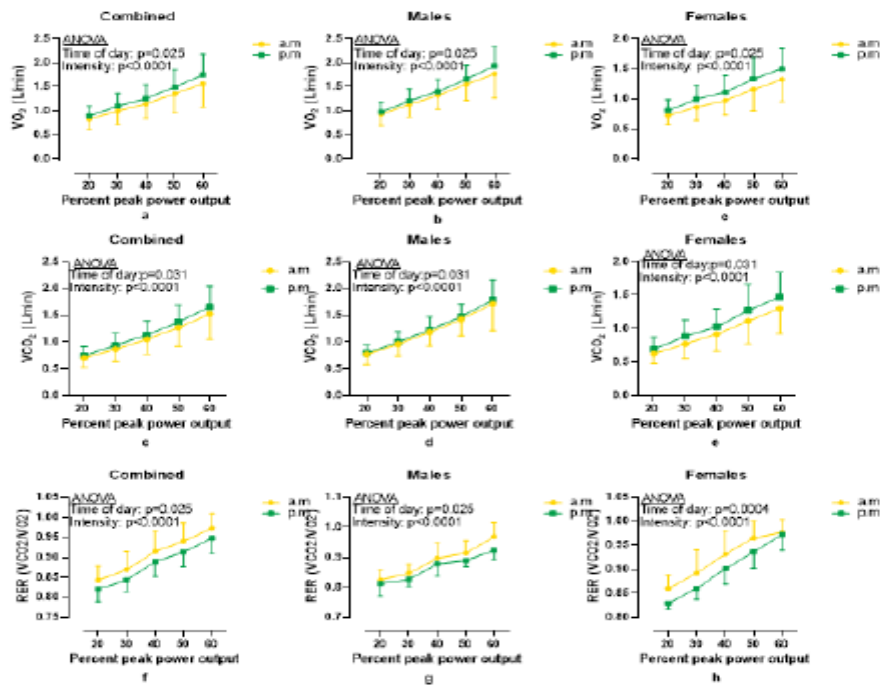
3.11. Respiratory exchange ratio Kinetics across the range of exercise intensities

RER (Fig.5g-i) increased with increasing exercise intensities (effect of intensity; $p<0.0001$, $\eta^2 p = 0.849$). and was overall higher in the morning (effect of time of day; $p=0.0004$, $\eta^2 p =0.652$) independent of gender (gender*time of day*intensity; $p=0.412$, $\eta^2 p = 0.329$).

Figure 5

Diurnal variation of Gaze exchanges and respiratory exchange ratio (RER) as a function of exercise intensity. Oxygen consumption Kinetics ($\dot{V}O_2$) across the range of exercise intensities (%Peak power

output) in all subjects (a), in males (b), and in females (c) in the morning (a.m) and in the afternoon (p.m). Carbon Dioxide production Kinetics (VCO₂) across the range of exercise intensities (%Peak power output) in all subjects (d), in males (e), and in females (f) in the morning (a.m) and in the afternoon (p.m). Respiratory Exchange Ratio (RER) across the range of exercise intensities (%Peak power output) in all subjects (g), in males (h), and in females (i) in the morning (a.m) and in the afternoon (p.m).



3.12. Correlational Analysis

Fig.6 resumes the statistically significant associations between MFO in morning and afternoon with $\dot{V}O_{2peak}$, PPO, body mass and Age.

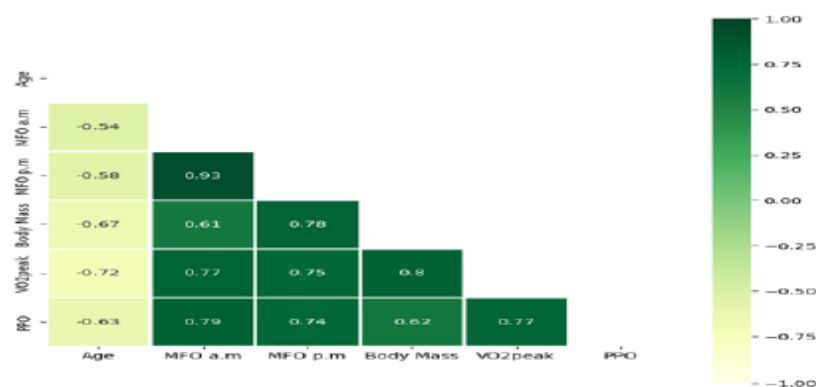


Figure 6

Figure 5. Triangle correlation matrix with the shade of correlation indicating the strength and the direction of correlation as depicted in the right panel bar. PPO; Peak Power Output, MFO a.m; Maximal Fat Oxidation in the Morning, MFO p.m; Maximal Fat Oxidation in the afternoon

4. Discussion

The primary finding of our study indicate that MFO and fat oxidation rates exhibited diurnal variation, with notably higher levels observed during afternoon compared to morning in MetS patients. This temporal pattern also extended to LIPOX_{max}, crossover point, EE, which all were higher in the afternoon compared to the morning. In contrast no diurnal variation in absolute CHO_{ox} was found.

Our findings were in concordance with previous work that demonstrated in non-athlete male students, endurance trained individuals and individuals with obesity, higher MFO, LIPOX_{max}, and $\dot{V}O_2$ peak in the afternoon.(Mousavian et al. 2014; Amaro-Gahete et al. 2019; Mohebbi and Azizi 2011) This similarity in outcomes across different populations suggests that the afternoon may provide a more favorable metabolic environment for promoting fat utilization during exercise.

Such findings could not be interpreted independent of the broader context of circadian physiology of energy metabolism. This diurnal pattern of substrate oxidation during exercise matches at least partly (in the day time) the temporal variations evidenced in substrate metabolism, where diurnal oscillations in EE and substrate oxidation have been observed; with CHO_{ox} starting to progressively increase from the early morning to peak around 11 am and an antiphase pattern of fat oxidation starting from the early afternoon to peak around 8 p.m.(Rynders et al. 2020; Zitting et al. 2018) This shift from preferential reliance on carbohydrates to preferential reliance on fat in the afternoon/evening suggest a Randle cycle given that it coincides with a marked deterioration of glucose tolerance starting from the early afternoon.

Indeed, several studies succeeded to demonstrate that the diurnal peak of non-esterified fatty acids (NEFAs) coincides with the diurnal trough of insulin sensitivity, suggesting a Randle effect and making NEFAs circadian substrates by excellence.(Jha et al. 2015; Yoshino et al. 2014; Morgan et al. 1999; Poggiogalle et al. 2018) The Shift to NEFAs is generally determined by hormones which synch the central circadian clock in the suprachiasmatic nuclei with peripheral metabolism. However, unlike the late nocturnal /early morning peak of fatty acids (Van Cauter et al. 1997), which are known to be induced by Growth Hormone, the hormonal cues underlining the afternoon/evening peak of NEFAs and fat oxidation are less well characterized. But possible candidate mechanisms include the adrenergic stimulation of lipolysis and inhibition of insulin secretion with the latter effect indirectly contributing to induction of lipolysis.(Lee et al. 1992; Jha et al. 2015) An indirect late effect of the morning cortisol peak on insulin sensitivity could also contribute to this metabolic milieu favoring lipolysis and NEFAs partitioning towards oxidation (Plat et al. 1996; Jha et al. 2015).

These diurnal patterns seem also to translate to exercise conditions where adrenaline and Interleukin 6, another lipolytic myokine, responses are amplified by early evening exercise (Kim et al. 2015). Therefore, collectively these findings highlight the importance of considering the timing of exercise and testing protocols in the realm of endurance performance testing. Understanding the influence of circadian rhythms on fat metabolism not only enhances our knowledge of human physiology but also has practical implications for optimizing performance and training strategies in health and disease. Indeed and as mentioned earlier a larger-scale study involving over 90,000 men and women established that afternoon exercise confers superior benefits than morning exercise on the risks of premature death.(Feng et al. 2023) Similarly, afternoon moderate to vigorous physical activity was more efficacious

in optimizing glycemic control in adults with diabetes at 1 year and this effect continued to year 4 of a lifestyle intervention, an effect that was independent of intensity and volume (Qian et al. 2023). However, one may ask; if the organism is biologically pre-determined to oxidize more CHO in morning and more fat in afternoon, how could afternoon exercise be more efficacious in improving glucose control.

One possibility worth considering is that, even though the morning is characterized by a biologically higher relative carbohydrate oxidation, the magnitude of the glucose lowering bout of an exercise would not be high in light of the higher glucose tolerance observed in the morning. However, the efficacy of afternoon exercise in improving long term glucose control may stem from multiple mechanisms; first, supported by our exercise calorimetry findings, it is important to note that, despite fat being relatively the primary contributor to EE in the afternoon, the higher afternoon EE suggests that CHOox levels could be comparable to those in the morning in absolute terms. Consequently, given the biologically lower glucose tolerance in the afternoon, the potential exists for a more substantial reduction in glucose levels following afternoon exercise. Secondly, the heightened fat oxidation observed in the afternoon, particularly in the context of metabolic dysfunction, may prevent non-esterified fatty acids (NEFAs) from being deposited ectopically in the liver and skeletal muscle. (Sabag et al. 2017) This phenomenon could contribute significantly to improved long term metabolic health. Therefore, it could be plausible that afternoon exercise exerts its positive effects on glucose control not only by optimizing blood glucose directly by the acute exercise bout-induced glucose lowering and by its related post exercise increase in insulin sensitivity but also by sparing tissues from the detrimental effects of ectopic NEFA accumulation.

Previous studies have shown a positive association between MFO and insulin sensitivity in healthy young males. (Robinson et al. 2015) Similarly, absolute LIPOXmax (in watts) has been positively associated with insulin sensitivity in non-insulin resistant obese subjects. (Lambert et al. 2017) In context of insulin resistance and T2D, MFO is both lower and shifted toward lower exercise intensities, implying that in these conditions subjects are highly gluco-dependent at lower exercise intensities. Training at the LIPOXmax has been demonstrated to reverse insulin sensitivity and is considered an easy way to prescribe powerful prolonged fat loss interventions to improve glucose metabolism defects in these populations. Additionally, Venables and Jeukendrup (2008) evidenced that continuous training program at the LIPOXmax is superior to moderate intensity interval training in increasing fat oxidation rates in obese adults. Therefore, in light of these studies and diurnal effect in substrate oxidation we found that it might be recommended to prescribe exercise intensities around the LIPOXmax and that exercise should be scheduled to the afternoon, in order to improve the lipid oxidation capacity and ultimately prevent ectopic fat deposition and improve insulin sensitivity. These interpretations should not be taken as an outright prescription against morning exercise. In fact, it's worth noting that engaging in exercise during the morning hours has been also associated with reduced all-cause mortality. However, when compared to afternoon exercise, this latter may be superior in terms of reducing many health-related risks. (Qian et al. 2023; Feng et al. 2023)

Although correlation may not imply causation, the fact that we and others have found that MFO is a strong correlate (see Fig. 5) of $\dot{V}O_{2peak}$ and since $\dot{V}O_{2peak}$ was found to be greater in afternoon in some studies may suggest that a higher MFO may be advantageous to cardiorespiratory fitness. Of note the observed negative correlation between age and both $\dot{V}O_2$ peak and MFO both in morning and afternoon together with the well-established deterioration of cardiorespiratory fitness with ageing may also suggest a causal relationship.

One explanation to this relationship is that a greater reliance on fat may spare CHO from being utilized and consequently delay fatigue that may result from CHO breakdown and their fatigue-inducing metabolites (i.e., H⁺). However, in comparison to glucose oxidation, fat oxidation requires a greater amount of oxygen for the complete breakdown of fats into energy.(Pate et al. 1992) Indeed, in our data an increased $\dot{V}O_2$ underlined increased fat oxidation in afternoon. From an exercise physiology perspective this translates to an increased O₂ cost of work and lower economy thus to higher EE, which are limiting factors of endurance performance. This is incompatible with the well characterized increase in exercise performance in afternoon. (Chtourou and Souissi 2012) It is important to note that such a paradoxical inverse relationship between cycling or running economy, fat oxidation and cardiorespiratory fitness could be traced back in studies dating to as early as 1992,(Pate et al. 1992) and this is all the more true for individuals with higher cardiorespiratory fitness ($\dot{V}O_{2peak}$). In other words, Subjects who have higher cardiorespiratory fitness would also tend to have higher lipid oxidation rates at any submaximal intensity and this would translate to more oxygen consumption, given that fat oxidation releases less energy with each liter of oxygen consumed, and thus an apparent decreased economy would result. Several explanations have been postulated for this paradoxical negative association so far, among which; body mass, age and leg mass, muscle fiber type distribution and exercise relative intensity.

With regard to the two latter explanations, one report showed that correlation and regression coefficients of submaximal $\dot{V}O_2$ and $\dot{V}O_{2peak}$ became higher at velocities reflecting higher relative intensities in subjects with varying cardiorespiratory fitness(Sawyer et al. 2010), mimicking thus the increase in RER with increasing exercise intensity and suggesting that the greater reliance on CHO is the main driver of inverse relationship between running economy and $\dot{V}O_{2peak}$. Beneke and Leithäuser (2017) confirmed these findings and attributed this more directly to increased Anaerobic contribution. At the muscle tissue level this inverse relationship was also confirmed and was attributed to increased less efficient type IIa muscle fibers recruitment.(Beneke and Leithäuser 2017) However, this was not consistent across studies, where Layec et al. (2009) showed no association between EC and muscle and whole body oxidative capacity. An increased oxidative contribution to energy production was, nevertheless, reported in athlete subjects reflecting higher aerobic capacity.(Layec et al. 2009)

Aside the methodological explanations that may underly these discrepancies, such heterogeneity in findings may also challenge our understanding of exercise economy itself. Economy, which is generally defined as a steady state $\dot{V}O_2$ at a given cycling work rate or running velocity do not account of the substrate being used. In fact, $\dot{V}O_2$ is a proxy for the energy rate that can be generated through aerobic metabolism, but it does not consider substrate oxidation, which can result in energy yield variations of up to 7% per unit of $\dot{V}O_2$ in favor of CHO. Indeed, more recently it has been recommended to express economy as aerobic EE(Beck et al. 2018), which account for substrate oxidation. The study of Beneke and Leithäuser (2017) although it showed that the positive relationship between submaximal $\dot{V}O_2$ and $\dot{V}O_{2peak}$ is related to increased CHOox, it does not necessarily suggest that at a fixed cycling work rate or running velocity, subjects with higher $\dot{V}O_2$ will have higher CHOox. This also suggests that at a fixed exercise work rate fitter subjects will have higher contribution of fat oxidation to the total EE of work and this would spare glycogen. In context of diurnal variation of substrate metabolism and performance, the latter suggestion is more logical. Our data also support these postulations because absolute CHOox was similar between the morning and afternoon, however; there was a decrease in the relative contribution of CHO and a relative increase in fatox and EE from morning to afternoon. This suggests an

increased contribution of fat from the morning to the afternoon. Our data should be interpreted with caution, because we did not include measures of lactate to estimate non-oxidative glycolytic (“anaerobic”) contribution to EE, which may increase. In this regard, an interesting study showed that increase in performance during a Wingate anaerobic test from morning to afternoon may be explained by increased aerobic contribution, therefore supporting our results.(Souissi et al. 2007) Besides substrate contribution our data also supports use of aerobic EE as a measure of exercise economy. All in all, further studies are needed to delineate how this biological variation in Fatox interacts with performance and whether such an increase spares glycogen and consequently leads to better rationalization of substrate oxidation in afternoon and ultimately to better performance.

Our findings also revealed that diurnal variation of Fatox is independent of gender as revealed by nonsignificant time of day*gender interaction. In other words, Fatox increased also from morning to afternoon in women. This is in contrast to what has been previously demonstrated by Robles-González et al. (2023). This latter study it appears that no diurnal variation in fatox exists in healthy active young women. The reason for this discrepancy is unclear, but higher $\dot{V}O_{2peak}$ values in this latter study compared to women values in our’s (15.74 vs 39.8 ml/kg/min) may suggest a gender*fitness*time of day interaction. In other terms fitness levels may have reduced or even suppressed amplitude of circadian variation. Therefore, further studies are needed in this regard.

Limitations:

This study, while offering interesting insights, has two notable limitations. First, the presence of 12 participants predominantly with a “rather morning than evening” chronotype may limit the generalizability of these findings to other chronotypes. Second, the reliance on short-duration submaximal exercise tests raises questions about the applicability of these findings to prolonged exercise scenarios often encountered in endurance training scenarios. It is important to acknowledge that the participants, despite having a metabolically compromised profile, were actively engaged in recreational physical activity. This limitation raises the question of whether these findings can be generalized to sedentary MetS patients.

4. Conclusions

In conclusion, our study sheds light on the significant diurnal variations in MFO in individuals with metabolic syndrome. The afternoon exhibited higher MFO, LIPOX_{max}, crossover point, EE, $\dot{V}O_2$ compared to the morning, highlighting the afternoon as a more favorable time for fat utilization during exercise. Understanding the influence of circadian rhythms on fat metabolism enhances our knowledge of human physiology and has practical implications for optimizing training strategies in patients with metabolic syndrome. Further research is needed to explore the relationship between diurnal variations in fat oxidation and exercise performance and to clarify gender and fitness-related interactions in these patterns.

Declarations

Competing Interests:

the authors declare no competing interests

Conflicts of Interest:

The authors declare that they have no competing interests

Submission declaration:

the work described here has not been published previously and is not under consideration for publication elsewhere. If accepted, it will not be published elsewhere in the same form, in English or in any other languages, including electronically without the written consent of the copyright holder.

Author Contributions:

J.M Designed and conducted the study, interpreted the data and drafted the manuscript. M.M.B, helped in conducting the experiments and in drafting the manuscript. T.A, A.EH, I.L, SR, MZ, and S.R helped in drafting and in manuscript revision. A.B, A.O, E.B and J.F.B supervised the study and contributed to the discussion and revised all the aspect of the manuscript. All authors read and approved this final version of manuscript.

Institutional Review Board Statement:

This study was approved by the institutional review board of the Faculty of Medicine of Sousse (Ref: CEFMS 188/2023) and conformed with the declaration for Helsinki. The study was registered in the pan African clinical trial registry as PACTR202306776991260.

Data Availability Statement:

The data supporting the findings of this study will be made available upon scientifically sound requests.

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