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Original empirical article

EFFECT OF TIME OF DAY ON MFO AND FATMAX DURING EXERCISE IN OBESE AND NORMAL WEIGHT WOMEN

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Abstract. *Circadian rhythms regulate some of the metabolic and hormonal variables that affect fat oxidation. Thus, the purpose of this study was to investigate the effect of time of day on maximal fat oxidation (MFO) and the exercise intensity at which MFO occurred (FATmax) in obese and normal weight women. The MFO and Fatmax were measured in 10 normal weight and 10 obese women during an incremental running exercise test with 3 min stages on the treadmill by means of the indirect calorimetry method. The student's t-test and the one-way ANOVA with repeated measures were used to analyze the variables. We found that fat oxidation and MFO in both groups were higher in the evening than the morning, but there were no significant differences in the MFO between the obese and normal group. In addition, the fat oxidation rates during low intensity exercise (<60% VO2max) in obese and normal weight groups were similar, but during higher exercise intensity, among normal weight women were significantly higher than among obese women. Our results suggest that exercise in the evening with Fatmax intensity is better for weight loss purposes.*

Key words: *maximal fat oxidation, time of day, obese and normal weight women.*

INTRODUCTION

Obesity has become a serious and growing public health problem. Previous ways of combating obesity have failed, and new approaches need to be taken (Wyatt, Winters & Dubbert 2006). The biological clock regulates the expression and/or activity of enzymes and hormones involved in the metabolism. However, recently, studies have indicated that metabolism, food consumption, timed meals, and some nutrients feed back to entrain circadian clocks. Moreover, the disruption of circadian rhythms leads to metabolic and hormonal disorders (Froy, 2010).

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Studies involving human participants indicate that heart rate (Garet, Kirkendall, 1999), gastric emptying, gut absorption rate and gastro-intestinal enzyme activity, all peak at certain times during the day (Goo et al., 1987). Also, body temperature, oxygen consumption (Weinert & Waterhouse, 2007) and catecholamine levels (Ayako & Keiichi, 1995) reach peak in the afternoon. Thus, we hypothesize that changes in these variables can alter substrate oxidation.

Chwalibog et al. (2002) indicated that during the day, carbohydrates are the major oxidative fuel, while, during the night, changes in the energy status are accommodated by increasing fat oxidation (Chwalibog & Thorbek, 2002). Salata et al. (1988) reported greater release of ACTH in the afternoon than in the morning (Salata et al. 1988). But another study reported that metabolic and hormonal responses to exercise can be assessed with equal reliability in the morning and evening (Galliven et al. 1997). In a previous study, we reported that energy expenditure and oxygen consumption during a 30-min treadmill exercise at an intensity of 65% of VO_2max, were higher at 18:00 than 08:00 (Rahmani-nia, Azizi & Mohebe, 2009).

Although effect of time of day on the components of sport performance have all been documented (Scheen et al. 1998), it is not known whether metabolic and hormonal responses to exercise vary according to the time of day, because almost all of the detailed studies have been performed in the morning (Scheen et al. 1998). Therefore the aims of the present study include determining the following: Does time of day influence fat and carbohydrate oxidation? And does different exercise intensity in the morning and evening alter substrate oxidation?

During whole-body exercise, substrate utilization is mainly influenced by exercise intensity, the duration of the exercise, the muscle substrate stores and the state of training (Galbo, 1983; Nordby et al. 2006). When exercise intensity is increased, there is a progressive increase in the utilization of carbohydrates, derived both from the plasma glucose and muscle glycogen (Kristiansen et al. 2000). In contrast, whole-body fat oxidation reaches its peak at moderate exercise intensities normally ranging between 55% and 65% of maximal oxygen uptake (Galbo, 1992; Achten et al. 2002).

The highest rate of fat oxidation in sedentary, obese subjects during exercise is not well documented. Several lines of evidence indicate that obese subjects may have an impaired capacity to oxidize fat (Kim et al. 2000; Pérez-Martin et al. 2001) compared with lean individuals. However, Steffan et al. (1999) compared rates of fat oxidation in obese and normal weight women with similar VO_2max values ($ml \cdot kg^{-1} \cdot LBM^{-1} \cdot min^{-1}$) and found no difference in substrate utilization between the two groups (Steffan et al., 1999). Also Ranneries et al. (1998) found no difference in fat oxidation between formerly obese women and normal weight women at 50% VO_2 max (Ranneries et al., 1998). But, Goodpaster et al. (2002) demonstrated that fat oxidation during cycle exercise (50% of VO_2max) in obese participants was higher than in lean participants (Goodpaster et al. 2002). In addition, in a previous study we observed that energy expenditure and oxygen consumption during treadmill exercise (65% of VO_2max) in the obese group was higher than the lean group (Rahmani-nia, Azizi & Mohebe, 2009).

However, due to inconsistencies in the previous results, there are no quantitative results concerning potential differences in substrate oxidation at different exercise intensities among obese and normal weight women. In addition, the effect of time of day on fat and carbohydrate oxidation in obese and normal weight women is unknown. Therefore, the purpose of this study was to determine the effect of time of day on maximal fat oxidation (MFO) and the exercise intensity at which the fat oxidation rate was maximal (Fatmax) during exercise in obese and normal weight women.

MATERIAL AND METHODS

Twenty two healthy, untrained (VO_2max <50 ml/min/kg and less than three sessions per week of physical activity) women between the ages of 19 and 26 voluntary participated in this study. The participants numbered 10 obese (BMI >30 kg/m^2) and 10 normal weight women (BMI 20-25 kg/m^2). Self-administrated questionnaires determined the participants to be non-smokers, free from diabetes, hypertension, and coronary heart disease, and that they were not taking medications known to influence metabolic responses. All of the participants had been weight-stable (±1 kg) at least 3 months before our study. The participants were instructed not to engage in any strenuous exercise on the day preceding the experimental test, and they participated in 5 separate trials spaced between 5 to 7 days from each other. The order of trials was randomized and followed a counterbalanced format. An institutional ethics review board at the University of Guilan approved this study, and all the participants provided written informed consent forms prior to participation.

Body composition

The body mass index was calculated as mass (in kilograms) divided by height squared (in square meters). Whole-body fat mass and fat-free mass (FFM) were measured by the bioelectrical impedance method (In Body 3.0, Korea) approximately 1 week before the first admission.

Testing protocol

The exercise protocol used here was adapted from a previously described and validated protocol (Achten et al., 2002) in which it was concluded that an incremental exercise test with stages which were 3-min in duration could be used to determine both MFO and Fatmax (Achten et al. 2002). The subjects started exercising at a speed of 3.5 km/h and at a gradient of 1%. The speed was increased by 1 km/h every 3 min until a speed of 6.5 km/h was reached. At this point, the gradient was increased by 2% every 3 min until an RER of 1 was reached. Finally, the speed was increased every minute until exhaustion. The aim of the final section of the exercise test was to obtain a measure of VO_2max within a short time. Breath-by-breath measurements were taken throughout the exercise by using an open circuit gas analysis system (COSMED, Quark b2, s.r.l. Rome, Italy). As a result of the familiarization sessions, minimal adjustments were required during the experiment. Exercise in the morning session began at 08:00 after 8-12 h of fasting (Kanely et al. 2001), and in the evening session, exercise began at 18:00 after 5-6 h of fasting (Thuma et al. 1995).

Indirect calorimetry and calculations

Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were averaged over the last 2 min of each exercise stage where the RER was <1.0. For each of these stages, fat and carbohydrate oxidation were calculated by using stoichiometric equations (Frayn, 1983), with the assumption that the urinary nitrogen excretion rate was negligible. Fat and CHO oxidation rates were then plotted as a function of exercise intensity, expressed as a percentage of maximal oxygen uptake (VO_2 max). From each fat oxidation curve, several features were identified according to a previously described procedure (Achten & Jeukendrup, 2003): 1) MFO, the peak rate of fat oxidation measured over the entire range of exercise intensity, 2) Fatmax, the exercise intensity at which the fat oxidation rate was maximal, and 3) Fatmin: the exercise intensity where the fat oxidation rate reached zero (i.e., where RER $\geq$ 1.0).

Statistical analysis

The results are reported as the mean ± SD. The student's t-test was used to compare the participants' characteristics. A two-way ANOVA (obese vs. normal weight) with one factor repeated measure (time) was used to evaluate fat and CHO oxidation between groups during the exercise. For all of the statistical procedures, the significance level was set at $p \leq 0.05$.

THE RESULTS

The characteristics of obese and normal weight participants are shown in Table 1. Weight, the Body mass index (BMI), Fat mass, fat percent and fat free mass (FFM) were significantly higher in the obese than in normal weight women ($p \leq 0.05$). Absolute VO_2max was significantly higher in the obese than normal weight group ($p \leq 0.05$); however, VO_2max was similar when adjusted for FFM between obese and normal weight women.

Table 1. The characteristics of obese and normal weight women.

	Obese (n=10)		Normal weight (n=10)	
Age (year)	23.8 ± 2.3		23.6 ± 1.8	
Height (cm)	163 ± 4.3		161 ± 4.5	
Weight (kg)	82.04± 3.2†		59.6 ± 3.05	
BMI (kg/m^2)	31.1 ± 1.01†		22.8 ± 0.97	
Fat mass (kg)	28.7 ± 2.8†		17 ± 1.6	
Fat (%)	36.08 ± 1.08†		28 ± 2.6	
FFM (kg)	53.3 ± 2.8†		42.49 ± 3.3	
HR maximum (beat/min)	196.2 ± 1.6		196.4 ± 1.8	
	PM	AM	PM	AM
VO_2max (ml/min/FFM)	47 ±1.8	46.49 ±2.6	46.44 ±2.1	45.9±1.9
VO_2max (ml/min/kg)	33.07 ±2†	32.86 ±2.9†	37.29±2.4	36.89±1.7
MFO (mg/min/kgFFM)	8.54 ±1.5*	8.01 ±1.2	8.89 ±1.9*	7.51± 1
FATmax (%VO_2max)	44.6 ±6*	40.9 ±4.4	49.9 ±5.1*	43±5.9

Values are means ± SD. FFM, Fat-free mass; BMI, Body mass index; HR, heart rate; MFO, maximal fat oxidation.
†: Significantly different from normal weight women ($p \leq 0.05$).
*: Significantly different from morning values ($p \leq 0.05$).

Our results show that fat oxidation rates (mg/min) at all exercise intensities in the evening were higher than in the morning, and were significantly higher in the evening than morning at 40%, 50% and 60% VO_2max in both groups ($p \leq 0.05$) (Figures 1 and 2). There were no significant differences in CHO oxidation in the morning and evening for both groups. The findings of Borg scales show that the rate of perceived exertion (RPE) in both groups in the evening was lower than in the morning (Figure 3).

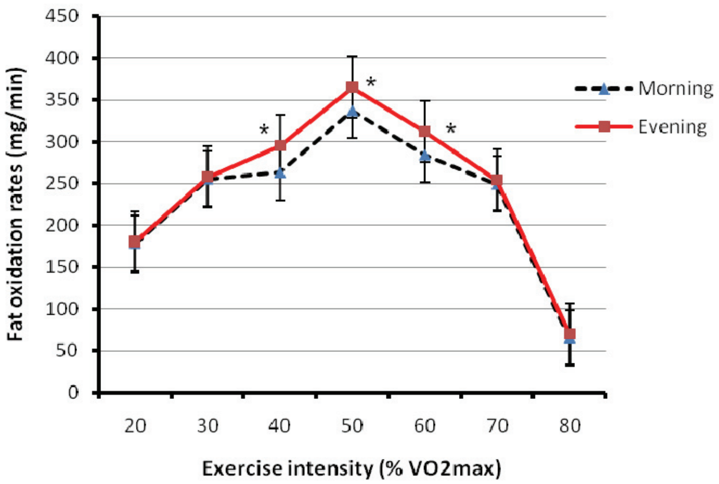

Fig. 1. A comparison of fat oxidation rates in the evening and morning in normal weight women ($p \leq 0.05$). ∗: Significantly different from morning values ($p \leq 0.05$).

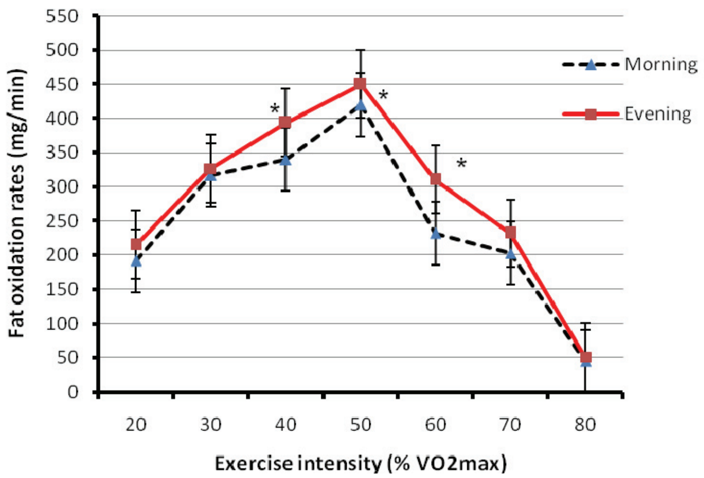

Fig. 2. A comparison of fat oxidation rates in the evening and morning in obese women ($p \leq 0.05$). ∗: Significantly different from morning values ($p \leq 0.05$).

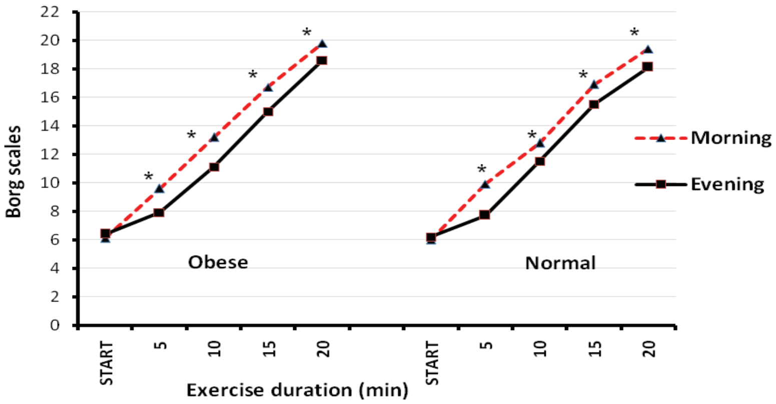

Fig. 3. A comparison of RPE in the evening and morning in obese and normal weight women ($p \leq 0.05$). ∗: Significantly different from evening values ($p \leq 0.05$).

In the case of the obese participants, the MFO (mg/min/kgFFM) in the evening (8.54± 1.5) was significantly higher than in the morning (8.01±1.2) ($p \leq 0.05$). In addition, in the normal weight participants, the MFO was significantly higher in the evening (8.9±1.9) than in the morning (7.51±1) ($p \leq 0.05$). There were no significant differences in the MFO between the obese and normal weight group in the evening and in the morning.

Fatmax occurred at an exercise intensity of around 44.6 ± 6% and 40.9 ± 4.4% VO_2max in the obese and 49.9 ±5.1% and 43 ± 5.9% VO_2max in normal weight group in the evening and morning respectively, and it was significantly higher in evening than in the morning for both groups ($p \leq 0.05$).

We found that fat oxidation rates (mg/min/kgFFM) at 60%, 70% and 80 % VO_2max in the morning and in the evening in the normal weight group were significantly higher than in the obese group (p≤0/05) (Figures 4 and 5).

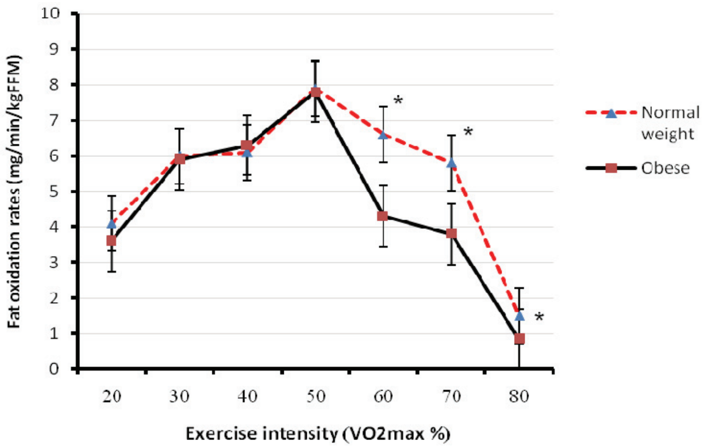

Fig. 4. A comparison of fat oxidation rates in obese and normal weight women in the morning ($p \leq 0.05$). *: Significantly different from obese women ($p \leq 0.05$).

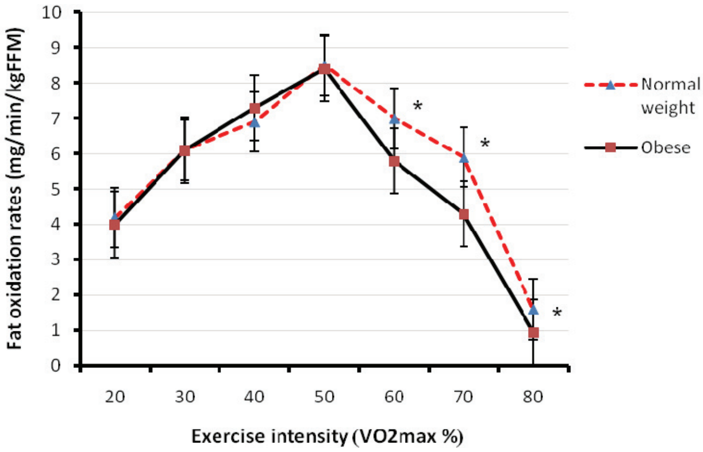

Fig. 4. A comparison of fat oxidation rates in obese and normal weight women in the morning ($p \leq 0.05$). *: Significantly different from obese women ($p \leq 0.05$).

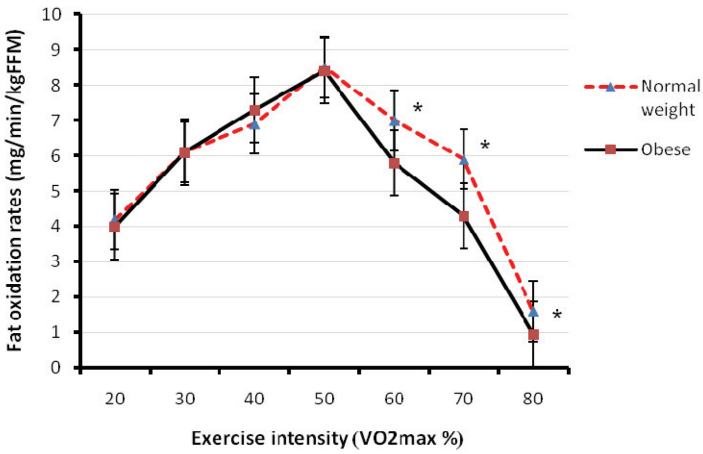

Fig. 5. A comparison of fat oxidation rates in obese and normal weight women in the evening ($p \leq 0.05$). *: Significantly different from obese women ($p \leq 0.05$).

Fatmin in the morning were found at ($\geq$ 90.2 ± 1.3% VO_2max) in the normal weight and ($\geq$ 88.5 ± 2.7% VO_2max) in the obese group, and Fatmin in the evening were found at ($\geq$ 89.3 ± 2.4% VO_2max) in the normal weight and ($\geq$ 91.4 ± 1.7% VO_2max) in the obese group.

Discussion

Previous studies demonstrated that training status had a significant effect on substrate utilization (Van loon et al. 1999). We found that there are no significant differences in VO_2max (ml/min/kgFFM) between obese and normal weight groups. Thus training status cannot affect the comparison of the results between the groups.

Our results show that fat oxidation rates (mg/min) in the evening were higher than in the morning. Previous studies indicate that body temperature, mean power and peak power (Souissi et al., 2004), aerobic power (Hill et al., 1992), time to exhaustion, peak oxygen consumption and aerobic system response (Hill, 1996), in the evening were higher than in the morning. In addition, peak catecholamine levels were observed in the evening (Ayako, 1999). Thus the increase in these metabolic and hormonal variables can elevate fat oxidation in the evening compared to the morning in both groups. The findings of the Borg scale show that RPE in the evening was lower than in the morning in both groups, that indicates that the participants did not feel as much stress in the evening, that maybe facilitated more fat oxidation in the evening than the morning in both groups. In a previous study we reported that EE and VO_2 in the evening were higher than in the morning, and RER was lower (Rahmani-nia, Azizi & Mohebe, 2009). That maybe shows more fat oxidation in the evening than in the morning. However, because there is not enough information about fat oxidation in obese and normal weight participants during different intensity exercise in the evening and morning, the interpretation of the results is difficult and needs future studies.

The concept of Fatmax has received a great deal of attention in recent years (Achten et al., 2002, 2003; Venables et al., 2005). This is due to an effort to recognize that facilitation of fat metabolism is of importance for both aerobic performance and health-related benefits of exercise. Fatmax was defined as the intensity where the greatest Fat oxidation was observed. In the current study, we found that Fatmax occurred around 44.6 ± 6% and 40.9 ± 4.4% VO_2max in the obese and 49.9 ±5.1% and 43 ± 5.9% VO_2max in the normal weight group in the evening and morning respectively. The mean Fatmax found in this study is close to what was reported by Bergman and Brooks (1999), who showed that Fatmax occurred at 40% VO_2peak in trained individuals, where as untrained individuals reached their Fatmax at 59% VO_2peak. The current Fatmax, however, is much lower than that reported by Knechtle et al. (2004), who have observed that the greatest fat oxidation took place at 75% VO_2peak in highly trained individuals (Knechtle et al., 2004). It appears that Fatmax is a measure that is fitness dependent. The impact as to whether and how aerobic training would affect Fatmax has yet to be determined.

We show that there were no significant differences in the MFO (mg/min/kgFFM) between the obese and normal weight group in the evening and morning. Also during low and moderate exercise intensity (≤ 60%VO_2max) there were no significant differences in fat oxidation (mg/min/kgFFM) between the obese and normal weight group. Rahmani-nia et al. (2009) reported that obesity did not have a significant effect on fat and CHO oxidation during moderate exercise. In addition, Schiffelers et al. (2001) found that a heparin injection (that increases plasma free fatty acids) led to a similar decrease in RER in lean and obese groups. This indicates that maybe fat oxidation capacity is equal in lean and obese people, and differences in fat oxidation are due to a deficiency of obese people in terms of fat mobilization. Geerling et al. (1994) reported that body fatness was not related to the proportion of fat oxidized during exercise. In contrast, Goodpaster et al. (2002) reported that during submaximal exercise, obese participants derived a greater proportion of their energy from fatty acid oxidation fat than lean participants. The contradiction in the results could be due to the differences in duration and intensity of the exercise.

Also, we have shown that at high exercise intensity (> 60% VO_2max) fat oxidation in the normal weight group was significantly higher than in the obese group. Wade et al. (1990) found that heavier men oxidized less fat during exercise than lean men. Lazzer et al. (2007) reported that at different steps of exercise intensity, fat oxidation rates tended to be higher in obese than in non-obese adolescents for an exercise intensity lower than 40% VO_2max and tended to be lower for exercise intensity higher than 40% VO_2max.

However, studies show that obesity is associated with a diminished capacity to oxidize fat. Impairments in the ability to mobilize fatty acids from adipose tissue and to oxidize fatty acids in skeletal muscles have been reported in obese participants during exercise (Blaak & Wim, 2002). In another study, the increase in arterial FFA concentration may be blunted during high exercise intensity in obese participants, resulting in lower circulating FFA concentrations in obese as compared to lean participants (Mandarini et al. 1996). Additionally, the capacity to increase muscle blood flow during high intensity exercise may be stunted in obese participants, possibly also contributing to a lowered FFA supply to the muscle cells (Blaak & Wim, 2002).

A better understanding of substrate utilization during exercise and the mechanisms regulating fuel selection provides an interesting background to define individual optimal exercise intensity. In fact, in many standard programs of exercise training, obese participants exercise above their crossover point and their maximal fat oxidation rate point, i.e. at an intensity level where the fat oxidation rate is quite low. Thompson et al. (1998) have shown that low-intensity long duration exercise results in greater total fat oxidation than moderate intensity exercise of similar energy expenditure (Thompson et al., 1998). We suggest that in obese women, exercise intensity prescription could be done at the maximal fat oxidation rate point. At these power outputs, fat utilization remains sustained, whereas it breaks down at higher intensities. To analyze if exercise with Fatmax intensity is more successful than standardized exercise prescription, we have to compare exercise at the maximal fat oxidation rate point level, versus standardized training programs.

In conclusion, our data suggest that Fatmax occurred around 44.6 ± 6% and 40.9 ± 4.4% VO_2max in the obese and 49.9 ±5.1% and 43 ± 5.9% VO_2max in normal weight group in the evening and morning, respectively. Fat oxidation was similar in obese and normal weight women at an exercise intensity lower than 60% VO_2max and tended to be higher in normal weight than obese participants for an exercise intensity higher than 60% VO_2max. At all exercise intensities, fat oxidation rates were higher in the evening than in the morning. Thus we suggest that exercise in the evening with Fatmax intensity is better for weight loss purposes in obese and normal weight women.

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UTICAJ DOBA DANA NA MFO I FATMAX TOKOM VEŽBANJA KOD GOJAZNIH ŽENA I ŽENA SA NORMALNOM TEŽINOM

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Dnevni ritam tela reguliše neke metaboličke i hormonske varijable koje utiču na oksidaciju masti. Cilj ovog istraživanja bio je da se istraže posledice doba dana na maksimalnu oksidaciju masti (MFO) i intenzitet vežbe pri kom je došlo do MFO (FATmax) u slučaju gojaznih žena i žena normalne težine. MFO i Fatmax mereni su na uzorku od 10 žena sa normalnom težinom i 10 gojaznih žena tokom testa trčanja na pokretnoj traci od po 3 min uz upotrebu indirektne kalorimetrijske metode. T-test i jednostruka ANOVA sa ponovljenim merama korišćeni su da bi se analizirale varijable. Ustanovili smo da su oksidacija masti i MFO u slučaju obe grupe bili veći u večernjim časovima nego ujutru, ali da nije bilo značajnih razlika u MFO između ispitanica koje su bile gojazne i onih koje nisu. Pored toga, stopa oksidacije masti tokom vežbi nižeg intenziteta (<60% VO_2max) u slučaju gojaznih ispitanica i onih sa normalnom težinom bila je slična, ali tokom vežbanja većeg intenziteta, među ispitanicama sa normalnom težinom bila je značajno veća nego u slučaju gojaznih ispitanica. Naši rezultati ukazuju na to da vežbanje u večernjim časovima pri Fatmax intentzitetu doprinosi gubitku težine.

Ključne reči: *maksimalna oksidacija masti, doba dana, gojazne žene i žene sa normalnom težinom.*