

Research article

Effects of Different Intensities of Endurance Exercise in Morning and Evening on the Lipid Metabolism Response

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Abstract

To study the effects of different exercise intensity performed at different exercise times on lipid metabolism response during prolonged exercise. Nine young men performed endurance exercise at different exercise intensities (60%VO₂max or Fatmax) in the morning (9 am to 10 am) or evening (5 pm to 6 pm); blood samples were collected before exercise and immediately and one and two hours after exercise completion. Expired gas was analyzed from the start of exercise until two hours after exercise completion. There were no significant changes in catecholamine (adrenaline and noradrenaline) and free fatty acid levels between morning and evening trials for each endurance exercise intensity. However, the morning and evening trials both exhibited significantly higher lipid oxidation at Fatmax than that at 60%VO₂max. These results suggest that exercise at Fatmax offers greater lipid oxidation than that at 60%VO₂max, regardless of exercise timing.

Key words: Exercise timing, prolonged exercise, exercise intensity, Fatmax, lipid oxidation, catecholamines.

Introduction

Exercise is a fundamental way to oxidize lipids in order to prevent or mitigate metabolic syndrome. In recent years, maximal fat oxidation (MFO; i.e., the maximum value of fat oxidation) and Fat_{max} (Fat_{max}=workload where greatest % of fat is oxidized to an incremental exercise load) have been investigated in a number of studies (Achten and Jeukendrup, 2003b; Sakamoto et al., 2012; Venables et al., 2005). It has been reported that MFO and Fat_{max} are both below the ventilator threshold or approximately the same as the lactate threshold (Jeukendrup, 2004; Venables et al., 2005). Numerous factors such as sex, age, body and muscle composition, diet, training habits, physical activity, and lipid oxidation-related enzyme activity of skeletal muscle, all influence lipid metabolism and possibly affect fat utilization (Achten and Jeukendrup, 2003a; Brandou et al., 2006; Nordby et al., 2006; Stisen et al., 2006; Venables et al., 2005). Fat_{max} can also be represented at the exercise intensity before carbohydrate % increases which is different than the workload shown by MFO. Exercise at Fat_{max} is thought of as the intensity for the most useful workload for weight loss and lipid metabolism during training (Jeukendrup and Achten, 2001; Sakamoto et al., 2012; Venables et al., 2005). However,

Fat_{max} determined with the incremental exercise test is not necessarily the exercise intensity at which maximum percentage of lipid oxidation is attained during prolonged exercise. In fact, a previous study that compared lipid oxidation during prolonged exercise above, below, and at Fat_{max} found no differences in lipid oxidation between exercise intensities, suggesting that Fat_{max} may not be the optimal exercise intensity for maximal lipid oxidation (Schwindling et al., 2014).

Exercise intensity and duration also determine energy consumption. Exercise performed for the same duration at different intensities results in more energy consumption during the higher-intensity segments. A previous study reported increased lipid oxidation at +10%VO_{2peak} than that at Fat_{max} (Takagi et al., 2014). However, the exercise duration in the previous study was constant; it is likely that more energy was consumed during +10%VO_{2peak} than at Fat_{max}; thus, the study may have overestimated lipid oxidation during exercise. Additional research with controlled energy consumption during exercise is necessary to assess lipid oxidation at different exercise intensities.

Exercise intensity affects the secretion of hormones associated with energy substrate oxidation (Hansen et al., 2012; Van Loon et al., 2001; Wideman et al., 2002; Zouhal et al., 2008). Secretion of catecholamines that promote lipolysis, increases to a greater extent at higher relative exercise intensities; however, catecholamine secretion is not always increased at Fat_{max} because exercise intensity is typically relatively low at this point (Hansen et al., 2012; Mohebbi et al., 2015; Van Loon et al., 2001; Zouhal et al., 2008). This does not mean that all lipids in blood, which are increased by hormones, are oxidized; rather, the amount of lipid oxidation varies according to relative exercise intensity (Klein et al., 1994; Romijn et al., 1993). Previous studies reported that carbohydrates are the main energy substrate in high-intensity exercise, compared with lipids being utilized more in low-to-moderate intensity exercise with the relative amount of lipids peaking at MFO and decreases during moderate intensity exercise (Romijn et al., 1993; Van Loon et al., 2001). Nonetheless, "moderate or lower intensity" represents a broad range of values, and it is possible that the substrate metabolism response also varies according to the exercise intensity within this range (Horowitz and Klein, 2000). A previous study has shown that the proportion of lipids used as the energy substrate in exercise

significantly decreases above 60%VO_{2max} (Friedlander et al., 1999) and they noted that fat oxidation rate decreased and switched more to carbohydrate. Therefore, in the present study, we comparatively examined exercise at Fat_{max}, at which the highest proportion of lipids is utilized, and at 60%VO_{2max}, at which the utilization of lipids significantly decreases.

It is possible that the levels of lipid oxidation are higher during exercise performed in the evening than the levels during exercise performed in the morning. A previous study on lipid oxidation at Fat_{max} determined by using an incremental exercise test reported significantly higher oxidation levels in the evening than in the morning (Mohebbi and Azizi, 2011). However, it is unclear if lipid oxidation is greater for exercise performed at Fat_{max} in the evening than the levels for exercise performed in the morning. Therefore, it is important to determine the exercise intensity and timing that result in maximal lipid oxidation during prolonged exercise.

In the present study, we investigated how differences in exercise intensity affect the blood hormone response and energy substrate oxidation during exercise performed in the morning and evening.

Methods

Participants

The study subjects were nine healthy young men who did not exercise regularly (Table 1). Before participation, all subjects received a full explanation outlining the present study and its safety, and provided their written consent to participate. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the ethics committees of Waseda University.

Table 1. Baseline physical characteristics (n = 9). Values are mean ± SE

Age (years)	25.6 (.6)
Height (m)	1.71 (.01)
Weight (kg)	67.5 (2.5)
%fat	15.6 (1.6)

Measurement of VO_{2max}

All subjects underwent an incremental exercise test to calculate their VO_{2max}. Measurements were randomly performed twice in each subject between 9:00 am to 10:00 am and 5:00 pm to 6:00 pm. The trials were conducted at least 1 week apart. The exercise test used a treadmill (MAT-2700, Fukuda Denshi) and the Bruce protocol, in which the incline and the speed are increased every 3 min. The amount of expired gas during the exercise test was analyzed with an expired gas analyzer (Aero Monitor AE300s, Minato Medical Science), with mean values for every 30 s calculated by using the breath-by-breath method. VO_{2max} was defined when at least two of the following three conditions were met: 1) plateaued oxygen intake, 2) respiratory exchange ratio (RER) of 1.1 or higher, and 3) heart rate (HR) at 90% of the age-specific maximum predicted HR (220 – age) (Pillard et al., 2010).

Determination of Fat_{max} and MFO

Fat_{max} is defined as the exercise intensity with the MFO. MFO is defined as the maximum amount of lipid oxidation observed during the incremental exercise test. Lipid oxidation rates were calculated through indirect calorimetry from gas exchange measurements by using Frayn's equations. For each participant, the rates were calculated after smoothing the curve plotting lipid oxidation as a function of exercise intensity. A digital filter (40 s) was used to smooth the breath-by-breath fluctuations with a width that depended on the amount of noise in the data (Takagi et al., 2014). MFO and Fat_{max} were determined for each participant in the morning and evening.

Experimental protocol

The subjects were instructed to abstain from strenuous exercise and from consuming alcohol or caffeine from 2 days before the experiment. They were also instructed to eat a provided meal 3 h before the start of the experiment, and were prohibited from consuming food or drink other than those provided. The meal was equivalent to 2430 kJ, and 34.4% of the energy was derived from fat, 59.9% from carbohydrate, and 5.7% from protein. This experiment consisted of four different exercise conditions—a morning and an evening trial at 60%VO_{2max} and Fat_{max}—and all subjects performed all four trials. The four trials were randomized cross-comparison tests and had an at least 1 week interval between them. Exercise was started at 9:00 am and 5:00 pm for the morning and evening trials, respectively, at exercise intensities of 60%VO_{2max} and Fat_{max}.

The exercise duration for the 60%VO_{2max} trial was 1 h. On the other hand, the duration of the Fat_{max} (morning: 101.33 ± 4.71 min, evening: 96.85 ± 5.91 min) trial was determined for each subject such that the amount of energy consumed during exercise was equal to that of the 60%VO_{2max} trial. Energy consumption was calculated according to the guidelines of American College of Sports Medicine (David and Brian, 2007). Energy consumption during exercise was calculated using the following method before 60%VO_{2max} and Fat_{max} exercise. First, we calculated the 60%VO_{2max}, based on the VO_{2max} obtained from the incremental exercise test. We then estimated the value of the energy consumption during 60 minutes of exercise from the oxygen uptake at 60%VO_{2max}, with the equation below. Next, in order to obtain values consistent with energy consumption during 60 minutes of exercise at 60%VO_{2max}, we used the same equation to estimate exercise duration at Fat_{max} from the oxygen uptake at Fat_{max}.

$$\text{Energy expenditure (kcal)} = \text{VO}_2 (\text{L/min}) \times 5 \text{kcal} \times \text{time (min)}$$

Moreover, the energy consumption in each trial was calculated from the amount of respective substrate oxidation, with carbohydrate set as 4 kcal/g and fat as 9 kcal/g.

The exercise intensity was changed by adjusting the incline of the treadmill at 5–10 min after the start of loading. The 60%VO_{2max} and Fat_{max} of the morning and evening trials were set according to the VO_{2max} from the

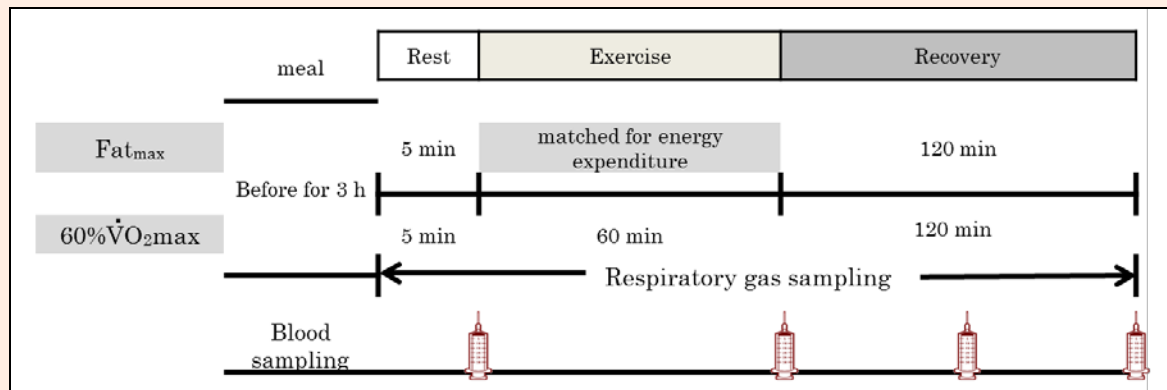


Figure 1. Protocol for exercise and blood sampling for Fat_{max} and $60\%VO_{2max}$ trials.

respective morning and evening incremental exercise tests. The amount of expired gas was analyzed, and HR was measured during 5 min of rest before commencing the exercise trial, during the exercise, and at 120 min after completing the exercise. These measurements were used to calculate the oxygen uptake (VO_2), carbon dioxide output (VCO_2), RER, and lipid/carbohydrate oxidation. Blood samples collected during rest and immediately, 1 h, and 2 h after exercise completion were used to measure the blood hormone levels (Figure 1).

Measurement of energy substrate oxidation

Expired gas was collected over time, during rest, during exercise, and after the completion of exercise, and an expired gas analyzer (Aero Monitor AE300S, Minato Medical Science) was used to measure VO_2 and VCO_2 . After completing the exercise, the subjects were asked to sit on a chair, where they maintained a seated position during the 2-h post-exercise recovery phase. These data were the basis for finding, in 30-s intervals, the RER during the 5-min seated pre-exercise rest, during the exercise, and at 120 min after the completion of the exercise. The rates of lipid and carbohydrate oxidation (per minute) were also calculated according to the following formulas (Achten and Jeukendrup, 2003b):

$$\text{Lipid oxidation level} = 1.67 \times VO_2(L) - 1.67 \times VCO_2(L)$$

$$\text{Carbohydrate oxidation level} = 4.55 \times VCO_2(L) - 3.21 \times VO_2(L)$$

Blood collection and analysis

Blood was collected from a cubital vein before each trial and immediately, 1 h, and 2 h after completing the exercise trial. After collection, blood for serum analysis was allowed to stand for 30 min at room temperature, whereas blood for plasma analysis was immediately centrifuged at 3500 rpm for 10 min (KUBOTA). After centrifugation, serum and plasma were extracted from the respective blood collection tubes, and refrigerated (4°C) or frozen (-80°C) until measurement.

Plasma levels of adrenaline and noradrenaline were analyzed through high-performance liquid chromatography. Cortisol levels were analyzed through solid-phase radioimmunoassay (RIA).

The serum levels of free fatty acid (FFA) were measured by using enzymatic methods, whereas the serum levels of growth hormones were analyzed through solid-phase RIA.

Plasma and serum concentrations measured immediately, 1 h, and 2 h after the completion of exercise were corrected for the rate of change in post-exercise plasma volume; these corrected values were used for statistical analysis. The hemoglobin and hematocrit values were obtained by using an automated cell counter (Sysmex K-2000, Kobe, Japan). Plasma volume was calculated according to the methods of Dill and Costill (1974). The plasma concentrations of adrenaline, noradrenaline, growth hormone, cortisol, and FFA were adjusted to account for changes in plasma volume.

Statistical analysis

All measurements are shown as means \pm standard errors. Two-way factorial analysis of variance was used in all measurement items. The Bonferroni procedure was used for the post hoc test. Statistical processing was performed by using PASW Statistics for Windows, version 18.0, and the level of significance was set to $<5\%$.

Results

VO_{2max} , MFO, and Fat_{max}

None of the subjects exhibited significant differences in VO_{2max} , MFO, or Fat_{max} between the morning and evening incremental exercise tests (Table 2).

Table 2. Maximal oxygen uptake and MFO during the graded exercise to exhaustion protocol. Values are mean (\pm SE).

	Morning	Evening
VO_{2max} (ml/kg/min)	47.5 (1.6)	47.5 (1.0)
MFO (mg/min)	414.0 (50.2)	457.0 (38.4)
Fat_{max} ($\%VO_{2max}$)	36.1 (1.7)	38.2 (2.2)

MFO, maximal fat oxidation, Fat_{max} , the intensity at which MFO occurred.

Energy consumption

The total energy consumption from before exercise to the recovery phase did not exhibit a significant difference in the Fat_{max} trial and $60\%VO_{2max}$ trial in the morning and in the evening (Table 3).

Table 3. Total energy utilized, CHO oxidation and Fat oxidation during exercise and post exercise in each trials. Value are mean (\pm SE).

Kcal	Morning		Evening	
	Fat _{max}	60%VO _{2max}	Fat _{max}	60%VO _{2max}
Total energy	787.2 (35.8)	816.6 (27.5)	814.5 (30.8)	818.2 (31.5)
Energy from CHO	533.7 (35.6) *	681.9 (28.7)	566.3 (12.1) #	683.1 (23.4)
Energy from Fat	235.5 (30.1) \$	134.7 (12.4)	248.2 (27.6) †	135.0 (13.2)

CHO, Carbohydrate. * $p < 0.01$ Fatmax vs 60%VO_{2max} in Morning, # $p < 0.01$ Fatmax vs 60%VO_{2max} in Evening. \$ $p < 0.01$ Fatmax VS 60%VO_{2max} in Morning, † $p < 0.001$ Fatmax VS 60%VO_{2max} in Evening

Table 4. Oxygen uptake and HR in the morning and evening. Value are mean (\pm SE).

	Morning			Evening		
	Pre	Post	Post 2 h	Pre	Post	Post 2 h
	60%VO_{2max}					
Oxygen consumption (ml/min)	271 (9)	2081 (77)	292 (17)	268 (12)	2055 (86)	296 (13)
HR (beat/min)	72 (3)	160 (3)	84 (4)	67 (3)	161 (4)	82 (4)
	Fat_{max}					
Oxygen consumption (ml/min)	258 (6)	1266 (104)	292 (23)	278 (13)	1427 (90)	293 (18)
HR (beat/min)	70 (3)	123 (7)	77 (3)	69 (5)	126 (7)	73 (6)

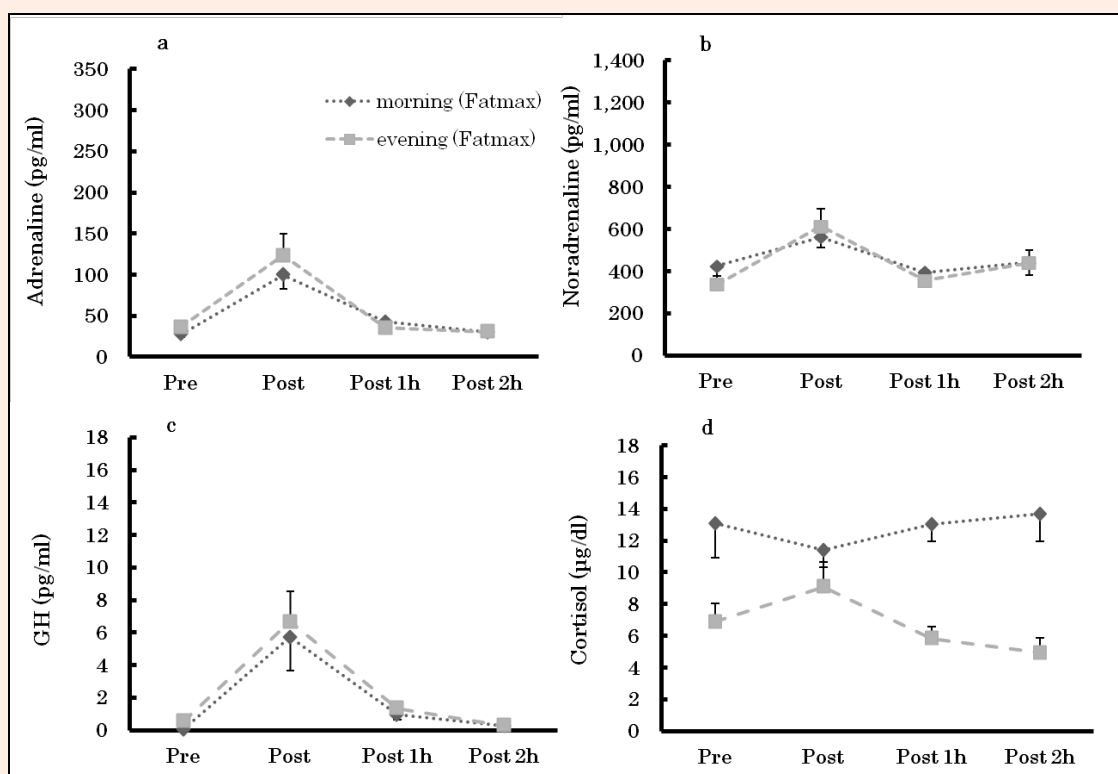
VO₂ and HR

The VO₂ and HR before, immediately after, and 2 h after the completion of exercise did not differ significantly between morning and evening for both Fat_{max} and 60%VO_{2max} (Table 4). VO₂ values during exercise at 60%VO_{2max} were $1,967.74 \pm 65.45$ mL/min in the morning and $1,945 \pm 83.01$ mL/min in the evening; values during exercise at Fat_{max} were $1,242.55 \pm 83.90$ mL/min in the morning and $1,325.14 \pm 82.25$ mL/min in the evening. Include here that there was not a statistical difference

between the morning and evening values.

Hormone response

There were no significant associations between adrenaline, noradrenaline, growth hormone, or cortisol levels before the exercise and immediately after, 1 h after, or 2 h after the completion of exercise at either of the exercise intensities; similarly, there were no significant differences in Fat_{max} (Figure 2) or 60%VO_{2max} (Figure 3) between the morning and evening trials.

**Figure 2.** Changes in plasma hormone levels during morning and evening Fat_{max} trials. Plasma concentrations of adrenaline (a), noradrenaline (b), growth hormone (c), and cortisol (d) before (Pre), immediately after (Post), 1 h after (Post 1h), and 2 h after (Post 2h) exercise are shown.

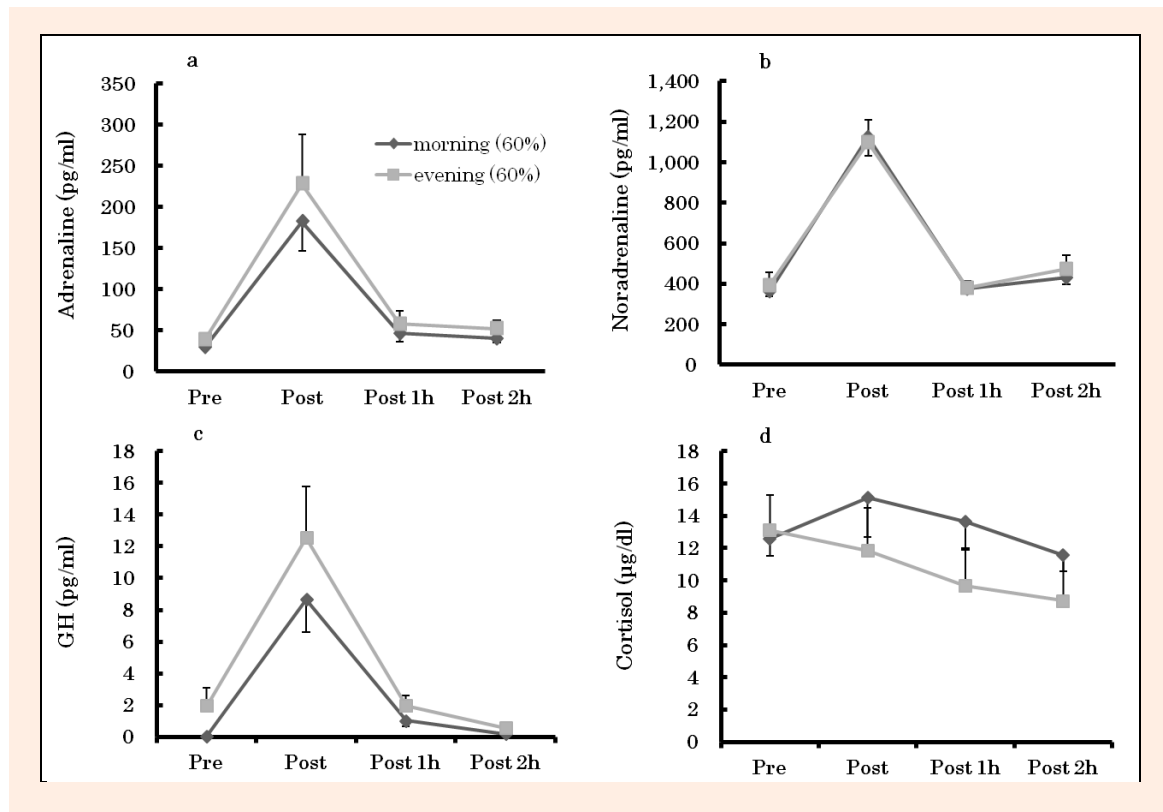


Figure 3. Changes in plasma hormone levels during morning and evening trials at 60%VO_{2max}. Plasma concentrations of adrenaline (a), noradrenaline (b), growth hormone (c), and cortisol (d) before (Pre), immediately after (Post), 1 h after (Post 1 h), and 2 h after (Post 2 h) exercise are shown.

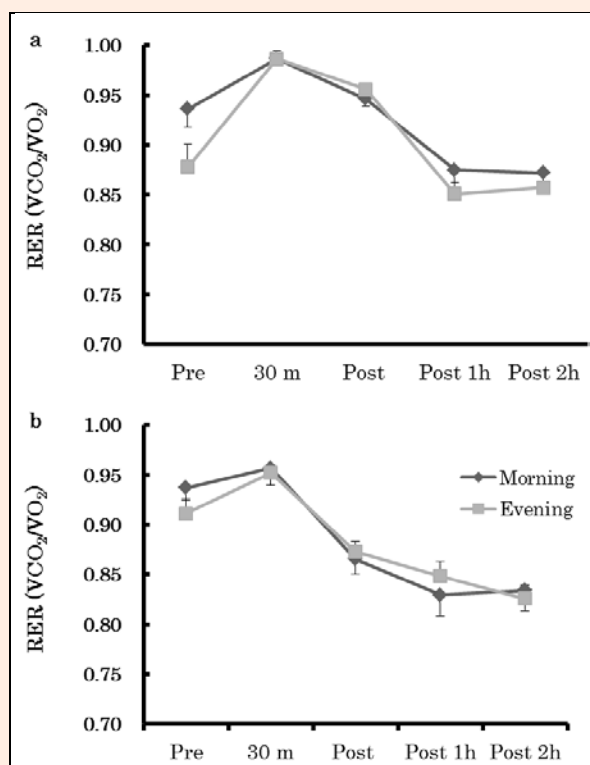


Figure 4. Respiratory exchange ratio (RER) during and after exercise for the 60%VO_{2max} (a) and Fat_{max} (b) in the morning and evening trial.

RER

RER was not significantly associated with exercise timing in the Fat_{max} or 60%VO_{2max} trials (Figure 4). However, RER was significantly associated with exercise intensity at both Fat_{max} and 60%VO_{2max} for both morning and evening trials; in the morning trial, Fat_{max} was significantly lower immediately and 1 h after the completion of exercise than the levels in the 60%VO_{2max} trial (Figure 5a). The level for the evening Fat_{max} trial was significantly lower 30 min after the start of exercise and immediately after the completion of exercise than the levels in the 60%VO_{2max} trial (Figure 5b). In addition, RER change during exercise showed a low value compared to the sustained 60%VO_{2max} at Fat_{max} (Table 5).

Lipolysis and lipid oxidation level

The FFA concentration did not differ significantly between morning and evening trials at Fat_{max} or 60%VO_{2max} (Figure 6). Similarly, energy substrate oxidation was not significantly associated with exercise timing and intensity. However, lipid oxidation was significantly higher at Fat_{max} than at 60%VO_{2max} in both the morning and evening trials (Figure 7a). The 60%VO_{2max} also had significantly higher carbohydrate oxidation than the levels at Fat_{max} for both the morning and evening trials (Figure 7b).

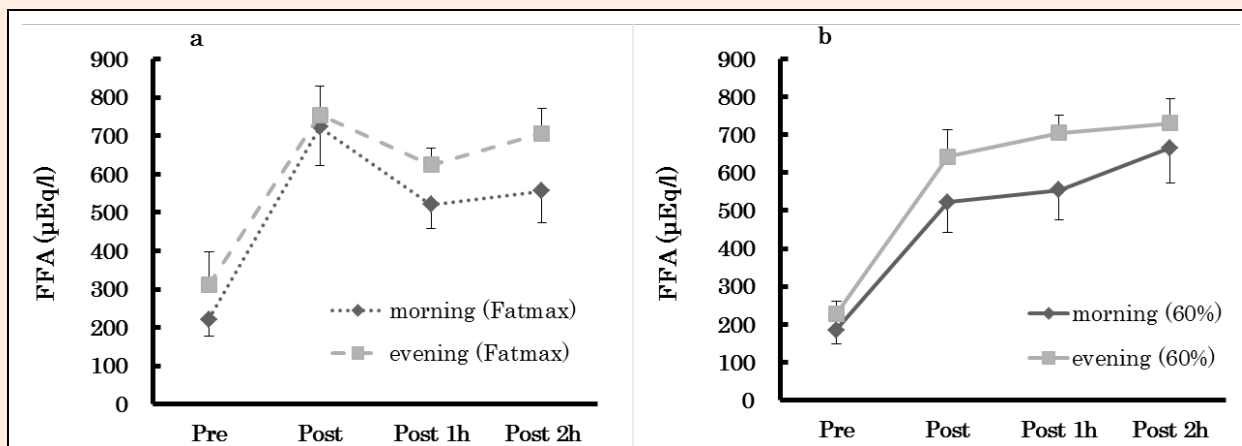
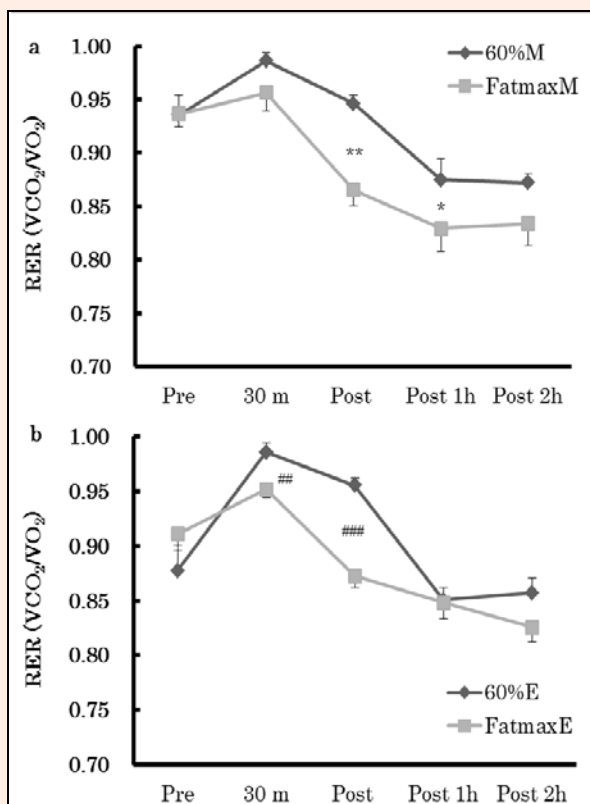
Discussion

In the present study, we focused on exercise timing (morning vs. evening) and exercise intensity (60%VO_{2max}

Table 5. Respiratory exchange ratio during exercise. Value are mean (\pm SE).

	RER	10 min	20 min	30 min	40 min	50 min	60 min	70 min	80 min
60%VO_{2max}	Morning	1.02 (.01)	1.00 (.01)	.98 (.01)	.97 (.01)	.95 (.01)	.95 (.01)		
	Evening	1.00 (.01)	.99 (.01)	.99 (.01)	.98 (.01)	.97 (.01)	.96 (.01)		
Fat_{max}	Morning	.97 (.01)	.96 (.01)	.96 (.01)	.94 (.01)	.91 (.01)	.90 (.01)	.88 (.01)	.88 (.01)
	Evening	.97 (.01)	.97 (.01)	.96 (.01)	.94 (.01)	.93 (.01)	.91 (.01)	.90 (.01)	.88 (.01)

RER, respiratory exchange ratio.

**Figure 6.** Changes in serum free fatty acid (FFA) levels during morning and evening exercise trials at Fat_{max} (a) and 60%VO_{2max} (b). Serum concentrations of FFA before (Pre), immediately after (Post), 1 h after (Post 1 h), and 2 h after (Post 2 h) exercise are shown.**Figure 5.** Respiratory exchange ratio (RER) during and after exercise at 60%VO_{2max} and Fat_{max} in the morning (a) and evening (b) trials. Data represent the means \pm standard errors. * $p < 0.05$, ** $p < 0.01$, significantly different from the 60%VO_{2max} values of the morning Fatmax trial. ## $p < 0.01$, ### $p < 0.001$, significantly different from 60%VO_{2max} values in the evening Fatmax trial.

vs. Fat_{max}) to investigate how blood hormone response and energy substrate oxidation are affected by transient endurance exercise. The principal result of the present study was that Fat_{max} exhibited higher total lipid oxidation than did 60%VO_{2max}, irrespective of exercise timing. The exercise intensity and blood hormone response also did not differ significantly between the morning and evening exercise trials.

These results suggest that, compared with 60%VO_{2max}, Fat_{max} may represent a better exercise intensity goal for lipid oxidation during prolonged exercise. A previous study did not observe a significant difference in a comparison of energy substrate oxidation at exercise intensities other than Fat_{max}, indicating that there remains some uncertainty about the usefulness of Fat_{max} as an effective measure of exercise intensity that optimizes exercise-associated lipid oxidation (Schwindling et al., 2014). However, the authors did not standardize the amount of energy consumed in each exercise trial, and the exercise duration was constant for all trials. As the total amount of energy consumed increases with higher exercise intensities, this may have affected the assessment of energy substrate oxidation in this previous study. In contrast, in the current study, we standardized the energy consumption. The results showed that Fat_{max} resulted in higher total lipid oxidation than 60%VO_{2max} in both the morning and evening trials. Thus, standardized energy consumption may have resulted in increased lipid oxidation per unit time during prolonged exercise at Fat_{max}.

It is possible that lipid oxidation varies with exercise intensity owing to differences in fatty acid mobilization. FFAs play an important role as an energy source during prolonged exercise (Desk et al., 1976). The source

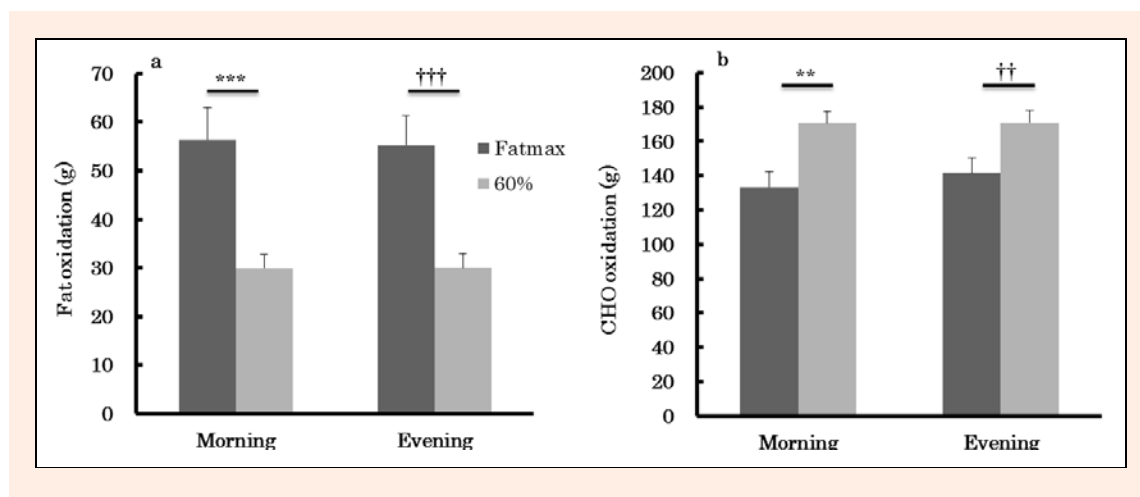


Figure 7. Fat (a) and carbohydrate (CHO) oxidation (b) for each trial. * $p < 0.01$, *** $p < 0.001$ Fatmax vs 60%VO_{2max} in the morning, †† $p < 0.01$, ††† $p < 0.001$ Fatmax VS 60%VO_{2max} in the evening.

of FFA oxidized during exercise changes depending on exercise intensity (Romijn et al., 1993; Van Loon et al., 2001). Moreover, in exercise at 60%VO_{2max} or higher, mobilization of FFA to the muscle cells is slightly inhibited and lipid oxidation is decreased (Achten and Jeukendrup, 2004; Stephens et al., 2007). Furthermore, accumulation of blood lactate involves the reduction of muscle pH, for reducing the activity of carnitine palmitoyltransferase-1 (CPT-1), which is responsible for the uptake of fatty acids into the mitochondria, lowering lipid oxidation (Bezaire et al., 2004; Starritt et al., 2000). In the present study, an examination of blood lactate concentration at Fat_{max} and 60%VO_{2max} during prolonged exercise and following the exercise load trials revealed no significant differences between the trials; however, lactate concentration tended to be higher at 60%VO_{2max} than at Fat_{max} (morning: 1.17 ± 0.07 , 1.74 ± 0.24 mmol/L, $p = 0.06$; evening: 1.06 ± 0.10 , 1.61 ± 0.26 mmol/L, $p = 0.09$). Therefore, the exercise intensity-dependent inhibition of the mobilization of FFAs to skeletal muscle may be lower at Fat_{max} (morning: $36.1 \pm 1.7\%$ VO_{2max}, evening: $38.2 \pm 2.2\%$ VO_{2max}) than at 60%VO_{2max}. Therefore, variations in lipid oxidation may have been due to differences in FFA mobilization to skeletal muscles at different exercise intensities.

The proportion of fat as the energy substrate due to exercise differs according to exercise intensity (Venables et al., 2005). Previous studies showed that the proportion of fat as the energy substrate was higher in low- to moderate-intensity exercise than in high-intensity exercise (Sidossis et al., 1997; Thompson et al., 1998; Venables et al., 2005). In this study, the energy consumption was equal at Fat_{max} (approximately 37% VO_{2max}) and 60%VO_{2max}. We think that because the Fat_{max} was observed at lower-intensity exercise as compared with 60%VO_{2max}, the utilization rate of fat as an energy substrate was higher and fat oxidation was greater.

The lipid oxidation during exercise in the present study did not differ significantly between the morning and evening trials at Fat_{max} and 60%VO_{2max}. Previous studies showed the relationship among various blood hormones such as catecholamines and growth hormones in energy

substrate metabolism (Hansen et al., 2012). The blood concentrations of these hormones increase in response to exercise, which promotes lipid metabolism (Gibney et al., 2003; Zouhal et al., 2008). However, in this study, no marked fluctuations were observed for catecholamines and growth hormones in the morning and evening. In addition, the energy consumption and oxygen intake in the morning and evening were the same at the same exercise intensity. Thus, we do not believe that any difference exists in energy substrate oxidation between morning and evening.

The blood hormone levels during prolonged exercise were unaffected by exercise timing in the present study. The levels of hormones that affect energy substrate oxidation, such as catecholamines, reportedly change with exercise intensity. The levels of adrenaline and growth hormones during prolonged exercise are also reportedly higher in the evening than in the morning (Kim et al., 2015). Similar results were expected in the present study; however, no significant differences were observed between the morning and evening exercise trials. Previous studies have shown variable catecholamine responses with changes in posture, activity, or during rest or activity cycles (Carmen et al., 1987; Schöfl et al., 1997). Fluctuations in catecholamine levels have also been shown to be associated with psychological factors such as stress or anxiety (Ward et al., 1983). Therefore, it is possible that fluctuations in the response to exercise in the present study were affected by changes in activity cycle or psychological factors between trials. The subjects in the present study were instructed to maintain similar activity patterns between trials, to the greatest extent possible, and each trial was conducted under the same laboratory conditions to minimize the above-mentioned potential effects; however, those factors were not rigorously evaluated and cannot be definitively ruled out. Additional investigations that adjust for lifestyle differences such as physical activity and sleep time may show significant differences in catecholamine response according to exercise timing.

This study has several limitations. First, the subjects were healthy young men, and thus our findings are not generalizable to other populations such as obese men

and women, or athletes. Second, it is necessary to take into consideration the various time periods at which the trials were conducted. In the present study, we examined differences in lipid oxidation and blood hormone response to exercise only in the morning and evening. However, various blood hormones, which influence substrate metabolic response, have been shown to fluctuate during the day, suggesting the possibility that levels differ during different time periods. Third, it is necessary to perform an examination of the various intensities of exercise. Exercise within the range of moderate intensity is widely recommended to prevent or mitigate obesity; however, it is possible that even within the range of low to moderate intensity exercise, the substrate metabolic responses differ. Fourth, although there are previous studies that have examined fluctuations in $\text{VO}_{2\text{max}}$ during the day, no clear consensus has been reached (Atkinson and Reilly, 1996; Faria and Drummond, 1982). In the present study, no significant differences between the morning and evening trials were noted. However, it has been shown that $\text{VO}_{2\text{max}}$ is influenced by lifestyle (Hill et al., 1988). Therefore, because it is possible that different lifestyles influence $\text{VO}_{2\text{max}}$ and energy substrate metabolism, a more detailed examination that considers lifestyle is needed. Fifth, biopsies may be necessary in order to identify the reason for decreased fat oxidation due to increased exercise intensity. Blood lactate concentration is not a direct indicator of fat oxidation in this study. Although increased blood lactate concentrations inhibit the mobilization of fatty acids, biopsy investigations of CPT-1 will be necessary to clarify the effect on fat oxidation. Finally, it is necessary to take the sample size into consideration. Although no statistically significant differences concerning adrenaline and growth hormones were noted, a trend for higher absolute values were observed in the evening than in the morning. Therefore, taking the sample size into consideration may allow observing more definite fluctuations.

Conclusion

The results of this investigation on the effects of different exercise intensities at different exercise times on metabolic response revealed higher lipid oxidation at Fat_{max} than that at 60% $\text{VO}_{2\text{max}}$ in both the morning and evening exercise trials with match energy expenditure. The results of this study also suggest that time of day of the exercise did not affect lipid oxidation in prolonged exercise.

Acknowledgments

This study was supported by Grants-in-Aid Scientific Research (B) 24300238 and a Grant-in-Aid from the Japan Society for the Promotion of Science Fellows (2015). All authors declare no conflicts of interest.

References

- Achten, J. and Jeukendrup, A. (2003a) The effect of pre-exercise carbohydrate feedings on the intensity that elicits maximal fat oxidation. *Journal of Sports Science* **21**, 1017-1024.
- Achten, J. and Jeukendrup, A. (2004) Optimizing fat oxidation through exercise and diet. *Nutrition* **20**, 716-727.
- Achten, J. and Jeukendrup, A. (2003b) Maximal Fat Oxidation During Exercise in Trained Men. *International Journal of Sports Medicine* **24**, 603-608.
- Atkinson, G. and Reilly, T. (1996) Circadian variation in sports performance. *Sports Medicine* **21**, 292-312.
- Bezaire, V. and Heigenhauser, G.J.F., Spriet, L.L., Heigenhauser, G.J.F. and Lawrence, L. (2004) Regulation of CPT I activity in intermyofibrillar and subsarcolemmal mitochondria from human and rat skeletal muscle. *American Journal of Physiology. Endocrinology and Metabolism* **1**, 85-91.
- Brandou, F., Savy-Pacaux, A., Marie, J., Brun, J. and Mercier, J. (2006) Comparison of the type of substrate oxidation during exercise between pre and post pubertal markedly obese boys. *International Journal of Sports Medicine* **27**, 407-414.
- Carmen, O.G., Curtis, G.C., Zelnik, T., Mccann, D., Roth, T., Guire, K. and Hubber-Smith, M. (1987) Circadian fluctuation of plasma epinephrine in supine humans. *Psychoneuroendocrinology* **12**, 41-51.
- David, S. and Brian, L. (2007) Exercise Prescription- A case study approach to the ACSM guidelines-second edition.
- Desk, R., Williams, L. and Health, K. (1976) Fatty Acids as Substrates for Heart and Skeletal Muscle. *Circulation Research* **38**, 459-463.
- Dill, D.B. and Costill, D. (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology* **37**, 247-248.
- Faria, I.E. and Drummond, B. (1982) Circadian changes in resting heart rate and body temperature, maximal oxygen consumption and perceived exertion. *Ergonomics* **25**, 381-386.
- Friedlander, A.L., Casazza, G.A., Horning, M.A., Usaj, A., Brooks, G.A., Anne, L., Casazza, G.A., Horning, A., Usaj, A. and Endur-, G.A.B. (1999) Endurance training increases fatty acid turnover, but not fat oxidation, in young men. *Journal of Applied Physiology* **86**, 2097-2105.
- Gibney, J., Healy, M.L., Stolinski, M., Bowes, S.B., Pentecost, C., Breen, L., Millan, C.M.C., Sonksen, P.H. and Umpleby, A. (2003) Effect of Growth Hormone (GH) on Glycerol and Free Fatty Acid Metabolism during Exhaustive Exercise in GH-Deficient Adults. *The Journal of Clinical Endocrinology and Metabolism* **88**, 1792-1797.
- Hansen, D., Meeusen, R., Mullens, A. and Dendale, P. (2012) Effect of acute endurance and resistance exercise on endocrine hormones directly related to lipolysis and skeletal muscle protein synthesis in adult individuals with obesity. *Sports Medicine* **42**, 415-431.
- Hill, D.W., Cureton, K.J., Collins, M.A. and Grisham, S.C. (1988) Diurnal variations in responses to exercise of "morning types" and "evening types". *The Journal of Sports Medicine and Physical Fitness* **28**, 213-219.
- Horowitz, J.F. and Klein, S. (2000) Lipid metabolism during endurance exercise. *The American Journal of Clinical Nutrition* **72**, 558S-563S.
- Jeukendrup, A. and Achten, J. (2001) Fatmax: A new concept to optimize fat oxidation during exercise? *European Journal of Sport Science* **1**, 1-5.
- Jeukendrup, A. (2004) Relation Between Plasma Lactate Concentration and Fat Oxidation Rates Over a Wide Range of Exercise Intensities. *International Journal of Sports Medicine* **25**, 32-37.
- Kim, H.-K., Konishi, M., Takahashi, M., Tabata, H., Endo, N., Numao, S., Lee, S.-K., Kim, Y.-H., Suzuki, K. and Sakamoto, S. (2015) Effects of Acute Endurance Exercise Performed in the Morning and Evening on Inflammatory Cytokine and Metabolic Hormone Responses. *PLoS One* **10**, e0137567.
- Klein, S., Coyle, E.F. and Wolfe, R.R. (1994) Fat metabolism during low-intensity exercise in endurance-trained and untrained men. *American Journal of Physiology* **267**, E934-E940.
- Mohebbi, H. and Azizi, M. (2011) Maximal fat oxidation at the different exercise intensity in obese and normal weight men in the morning and evening. *Journal of Human Sport and Exercise* **6**, 49-59.
- Mohebbi, H., Nourshahi, M., Ghasemikaram, M. and Safarimosavi, S. (2015) Effects of exercise at individual anaerobic threshold and maximal fat oxidation intensities on plasma levels of nesfatin-1 and metabolic health biomarkers. *Journal of Physiology Biochemistry* **71**, 79-88.
- Nordby, P., Saltin, B. and Helge, J.W. (2006) Whole-body fat oxidation determined by graded exercise and indirect calorimetry: A role for muscle oxidative capacity? *Scandinavian Journal of Medicine and Science in Sports* **16**, 209-214.
- Pillard, F., Wymelbeke, V. Van, Garrigue, E., Moro, C., Crampes, F., Guillard, J., Berlan, M. and Gliszinski, I. (2010) Lipid

oxidation in overweight men after exercise and food intake. *Metabolism* **59**, 267-274.

- Romijn, J.A., Gastaldelli, A., Horowitz, J.F., Endert, E. and Wolfe, R. (1993) Regulation in relation of endogenous fat and carbohydrate to exercise intensity and duration metabolism. *American Journal of Physiology. Endocrinology and Metabolism* **265**, E380-E391.
- Sakamoto, S., Konishi, M., Kim, H.K., Endoh, N., Takahashi, M., Takagi, S. and Midorikawa, T. (2012) Exercise prescription for fat metabolism disorder - From the viewpoint of fat oxidation rate. *The Journal of Physical Fitness and Sports Medicine* **1**, 499-504.
- Schöfl, C., Becker, C., Prank, K., von zur Mühlen, A. and Brabant, G. (1997) Twenty-four-hour rhythms of plasma catecholamines and their relation to cardiovascular parameters in healthy young men. *European Journal of Endocrinology* **137**, 675-683.
- Schwindling, S., Kindermann, W. and Meyer, T. (2014) Limited Benefit of Fatmax -Test to Derive Training Prescriptions. *International Journal of Sports Medicine* **35**, 280-285.
- Sidosiss, L.S., Gastaldelli, A., Klein, S. and Wolfe, R. (1997) Regulation of plasma fatty acid oxidation during low- and high-intensity exercise. *American Journal of Physiology* **272**, E1065-E1070.
- Starritt, E.C., Howlett, R.A., Heigenhauser, G.J.F., Spriet, L.L., Emma, C., Howlett, R.A., George, J.F. and Spriet, L. (2000) Sensitivity of CPT I to malonyl-CoA in trained and untrained human skeletal muscle. *American Journal of Physiology. Endocrinology and Metabolism* **278**, 462-468.
- Stephens, F.B., Constantin-Teodosiu, D. and Greenhaff, P. (2007) New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *The Journal of Physiology* **581**, 431-444.
- Stisen, A.B., Stougaard, O., Langfort, J., Helge, J.W., Sahlin, K. and Madsen, K. (2006) Maximal fat oxidation rates in endurance trained and untrained women. *European Journal of Applied Physiology* **98**, 497-506.
- Takagi, S., Sakamoto, S., Midorikawa, T. and Konishi, M. (2014) Determination of the exercise intensity that elicits maximal fat oxidation in short-time testing. *Journal of Sports Sciences* **32**, 175-182.
- Thompson, D.L., Townsend, K.M., Boughey, R., Jr, Patterson, K. and Bassett, D. (1998) Substrate use during and following moderate- and low-intensity exercise: Implications for weight control. *European Journal of Applied Physiology* **78**, 43-49.
- Van Loon, L., Greenhaff, P., Constantin-Teodosiu, D., Saris, W. and Wagenmakers, A. (2001) The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of Physiology* **536**, 295-304.
- Venables, M.C., Achten, J. and Jeukendrup, A. (2005) Determinants of fat oxidation during exercise in healthy men and women: a cross-sectional study. *Journal of Applied Physiology* **98**, 160-167.
- Ward, M.M., Mefford, I.N., Parker, S.D., Chesney, M. a, Taylor, C.B., Keegan, D.L. and Barchas, J. (1983) Epinephrine and norepinephrine responses in continuously collected human plasma to a series of stressors. *Psychosomatic Medicine* **45**, 471-486.
- Wideman, L., Weltman, J.Y., Hartman, M.L., Veldhuis, J.D. and Weltman, A. (2002) Growth hormone release during acute and chronic aerobic and resistance exercise: recent findings. *Sports Medicine* **32**, 987-1004.
- Zouhal, H., Jacob, C., Delamarche, P. and Gratas, A. (2008) Catecholamines and the Effects of Exercise , Training and Gender. *Sports Medicine* **38**, 401-423.

Key points

- It is important to consider exercise intensity when evaluating lipid oxidation.
- Few studies have investigated the effects of the intensity of exercise on lipid oxidation in the morning and evening.
- Fat_{max} exhibited greater total lipid oxidation compared to that of 60%VO_{2max} when energy expenditure was equated, but time of day did not affect lipid oxidation in prolonged exercise.

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