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RESEARCH ARTICLE

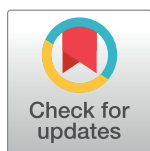
Exercise before breakfast increases 24-h fat oxidation in female subjects

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Abstract

Background

Exercise performed in a postprandial state does not increase 24-h fat oxidation of male and female subjects. Conversely, it has been shown in male subjects that exercise performed in a postabsorptive state increases 24-h fat oxidation compared with that in sedentary control and that with exercise trials performed after breakfast, lunch, or dinner. There is a paucity of study evaluating the effect of exercise performed in a postabsorptive state in female subjects.

Method

Nine young female subjects participated in indirect calorimetry measurement over 24-h using a room-size metabolic chamber in which subjects remained sedentary or performed 60 min exercise before breakfast at 50% of $\dot{V}O_{2\max}$. Exercise was accompanied by an increase in energy intake to ensure that subjects were in a similar state of energy balance over 24 h for the two trials.

Findings

Compared with the sedentary condition, exercise performed before breakfast increased 24-h fat oxidation (519 ± 37 vs. 400 ± 41 kcal/day). Time courses of relative energy balance differed between trials with transient negative energy balance observed before breakfast. The lowest values of relative energy balance observed during the 24-h calorimetry, i.e., transient energy deficit, were greater in exercise trials than in sedentary trials. The transient deficit in carbohydrate balance was also observed before breakfast, and magnitude of the deficit was greater in exercise trial compared to that of sedentary trial.

Interpretation

Under energy-balanced conditions, exercise performed in a post-absorptive state increases 24-h fat oxidation in female subjects. The effect of exercise performed before breakfast can be attributed to nutritional state: a transient deficit in energy and carbohydrate at the end of exercise.

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Introduction

Fat oxidation increases during exercise, and its determinants are well-characterized; exercise intensity [1,2], exercise duration [3], training status [4,5], nutritional state [6,7], and gender are the factors affecting fat oxidation during exercise. Fat oxidation during exercise is expected to be increased by prolonged exercise at moderate intensity performed in a post-absorptive state and to be greater in trained individuals and females.

To understand the implications for body weight regulation, the effect of exercise on energy metabolism during the post-exercise recovery period should be considered as well. Several studies evaluated the effect of exercise on fat oxidation over 24 h (24-h fat oxidation) in an energy-balanced condition; as planned by the experimental design, exercise was accompanied by an increase in energy intake to achieve an energy-balanced condition because over- and under-feeding have profound effects on nutrient oxidation [8,9]. Consensus in the literature states that differences in exercise intensity seems to play little role in determining 24-h fat oxidation [10–14].

The effect of exercise on 24-h fat oxidation seems to depend on when it is performed. Exercise performed in a postprandial state does not increase 24-h fat oxidation of male and female subjects [11,12,15,16]. Conversely, we have shown in male subjects that exercise performed in a postabsorptive state, i.e., before breakfast, increase 24-h fat oxidation compared with that in sedentary control and exercise trials performed after breakfast, lunch, or dinner [16–18]. Of note, there is a paucity of study evaluating the effect of exercise performed in a postabsorptive state in female subjects. Physiology and pathophysiology differ between male and female beyond reproductive function [19], and several lines of evidence warrant further studies with female subjects. First, there are gender differences in substrate utilization during and after exercise; females oxidize more fat during exercise, while they oxidize less fat during post-exercise period when compared to males [20–22]. Second, skeletal muscle oxidative activity of males and females responds differently to training. Endurance exercise undertaken in the overnight fasted state was more effective for increasing citrate synthase and 3-hydroxy-CoA dehydrogenase activity in skeletal muscle of males, while exercise after breakfast was more effective in females [23]. Third, there are gender-based differences in content of intermyocellular triacylglycerol (IMTG) and gene expression responsible for fat oxidation. Content of IMTG in female skeletal muscle is higher than that of males [24,25], and female skeletal muscle has abundant protein and mRNA of carnitine palmitoyltransferase 1 [26], FAT/CD36, and hormone sensitive lipase [27] compared to males.

The aim of the present study was to determine whether exercise performed in the postabsorptive state increase 24-h fat oxidation in female subjects. To this end, 24-h indirect calorimetry was performed on two occasions with either a 60 min exercise session before breakfast or sedentary control condition. Both experimental trials were designed to be energy-balanced over 24 h.

Materials and methods

Subjects characteristic

Nine young female subjects were recruited to the present study after providing written informed consent. They were moderately physically active, but none of them engaged in endurance training. Subjects had no current medical conditions, and none were taking any medications, including oral contraceptives, at the time of the study. Until one week before the first experiment, subjects spent a night in the metabolic chamber to become familiarized to the measurement condition. This study was approved by the ethics committee of the University of Tsukuba.

Pre-study evaluation

All subjects performed a graded exercise test comprised of submaximal and maximal tests using a cycle ergometer to determine workload corresponding to 50% of individual maximal oxygen uptake ($\dot{V}O_{2\max}$) [16].

Experimental protocol

The study was a randomized, repeated measures design comprised of two 24-h calorimetry trials with or without exercise sessions performed before breakfast. To standardize conditions between trials, 24-h calorimetry trials were carried out in the early follicular phase of the menstrual cycle, and all experiments were completed within 2 months. Subjects were asked to maintain their body weight throughout the study, with no significant difference in body weights observed between individual calorimetry trials ($P > 0.40$).

On the day prior to 24-h calorimetry, subjects entered the metabolic chamber (day 1, 22:00), and 24-h energy expenditure and nutrient oxidation were measured from 6:00 on day 2 to 6:00 on day 3. Once in the metabolic chamber, subjects slept for 7 h from 23:00 to 6:00. On day 2, 3 meals (breakfast at 8:00, lunch at 12:00, and dinner at 18:00) were provided, and subjects exercised at 50% of $\dot{V}O_{2\max}$ for 60 min using a cycle ergometer beginning at 6:30 (exercise trial) or remained in a sedentary position (sedentary trial). Subjects were instructed to remain awake and maintain a sedentary position except when performing prescribed exercise sessions and to sleep only at times specified by the protocol.

Experimental meals were designed to achieve individual energy balance assuming a resting metabolic rate of 22.1 kcal/kg/day according to estimated energy requirements for Japanese individuals [28]. Physical activity factor was assumed to be 1.75 ($2,248 \pm 65$ kcal/day) on day 1, 1.60 ($1,991 \pm 55$ kcal/day) in trials with exercise sessions, and 1.24 in sedentary trials ($1,637 \pm 42$ kcal/day) on day 2. Expressed as percentages of total energy intake, experimental meals consisted of 15% protein, 25% fat, and 60% carbohydrate. The contributions of breakfast, lunch, and dinner to total 24-h energy intake were 33%, 33%, and 34%, respectively.

Measurements

Energy metabolism was measured using a room-size metabolic chamber (Fuji Medical Science, Chiba, Japan). The airtight chamber measured $2.00 \times 3.45 \times 2.10$ m, with an internal volume of 14.49 m^3 . The temperature and relative humidity of in-coming air was controlled at $25.0^\circ\text{C} \pm 0.5^\circ\text{C}$ and $55.0\% \pm 3.0\%$, respectively. Concentrations of O_2 and carbon dioxide (CO_2) in out-going air were measured using an online process mass spectrometer (VG Prima δB , Thermo Electron, Winsford, UK). At every 5 min, O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) rates were calculated using an algorithm providing improved transient response [29]. Urine was collected during the indirect calorimetry, and urinary nitrogen concentration was measured using a chemiluminescent nitrogen analyzer (TN-100, Mitsubishi Chemical Corp., Kanagawa, Japan). Macronutrient oxidation and energy expenditure were calculated from $\dot{V}O_2$, $\dot{V}CO_2$ and urinary nitrogen excretion [30]. Energy and nutrient balance relative to the start of 24-h calorimetry were estimated as the difference between input (meal consumption) and output (oxidation). Relative energy balance was defined as a function of time (t) from 6:00 on day 2.

Relative energy balance (t) = accumulated energy intake (t) – accumulated energy expenditure (t)

Non-exercise activity was estimated, using a wrist watch-like device (ActiGraph, Ambulatory Monitoring, NY, USA), as the number of times the activity signal crossed the zero

reference point per minute [31]. Body composition was measured using the bioimpedance method (BC-118E, TANITA Co., Tokyo, Japan), which estimates fat free mass highly correlated to that determined by dual energy X-ray absorptiometry ($r = 0.973$) [32].

Statistical analyses

Data in the main text and figures are presented as means \pm SE. Time course of energy expenditure, carbohydrate oxidation and fat oxidation were compared using repeated measures two-way ANOVA for sedentary and exercise trials. Mean values were compared using the paired t-tests for exercise trial and sedentary control trial. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SPSS statistical software (Version 22, IBM Japan, Tokyo, Japan).

Results

Physical characteristics of subjects were 23.9 ± 1.3 year of age, 161.4 ± 1.6 cm of height, 57.8 ± 1.6 kg of body weight and $26.9 \pm 1.2\%$ of body fat. Their maximal oxygen uptake ($\dot{V}O_{2\max}$) was 43.6 ± 1.7 ml/kg/min. Habituation of weekly exercise was 2.6 ± 1.0 (h/wk). Work load, relative intensity of the exercise and average heart rate during 60-min exercise session were shown in Table 1. As intended, relative intensity of the exercise was close to 50% of $\dot{V}O_{2\max}$.

Time course of energy metabolism during 24 h indirect calorimetry was shown in Fig 1. Compared to the sedentary control trial, energy expenditure and oxidation of carbohydrate and fat were higher during the exercise (6:30–7:30). Prior to exercise (6:00–6:30 due to preparation for exercise) and immediately after exercise (7:30–8:00 due to wiping off sweat and changing clothes), energy expenditure and nutrient oxidation were higher in exercise trials compared to those of control trials, although difference in fat oxidation immediately after the exercise was not statistically significant. Accumulated energy expenditure and carbohydrate and fat oxidation over 24 h were higher for the exercise trial than those of the sedentary trial. No significant differences were observed in 24-h protein oxidation ($P = 11$) (Table 2).

Time courses of relative energy balance differed between trials with transient negative energy balance observed before breakfast (Fig 2). The lowest values of relative energy balance observed during the 24-h calorimetry, i.e., transient energy deficit, were greater in exercise trials (-507 ± 20 kcal) than in sedentary trials (-127 ± 4 kcal, $P < 0.01$). As planned by experimental design, relative energy balance of both trials eventually converged to a value similar to the initial value by the end of the study (6:00 of the day 3), and there were no significant differences in 24-h energy balance between sedentary (122 ± 34 kcal/day) and exercise trials (67 ± 45 kcal/day, $P = 0.20$). The transient deficit in carbohydrate balance was also observed before breakfast, and magnitude of the deficit was greater in exercise trial (-339 ± 23 kcal) compared to that of sedentary trial (-69 ± 3 kcal, $P < 0.01$). Similarly, transient deficit in fat balance was also observed (exercise, -144 ± 13 ; sedentary, -39 ± 6 kcal, $P < 0.01$). Differences in time course of carbohydrate balance between the two experimental conditions gradually decreased,

Table 1. Energy metabolism during exercise.

Work load (W)	87 \pm 5
Relative intensity (% $\dot{V}O_{2\max}$)	52.1 \pm 2.9
HR (beats/min)	129 \pm 7

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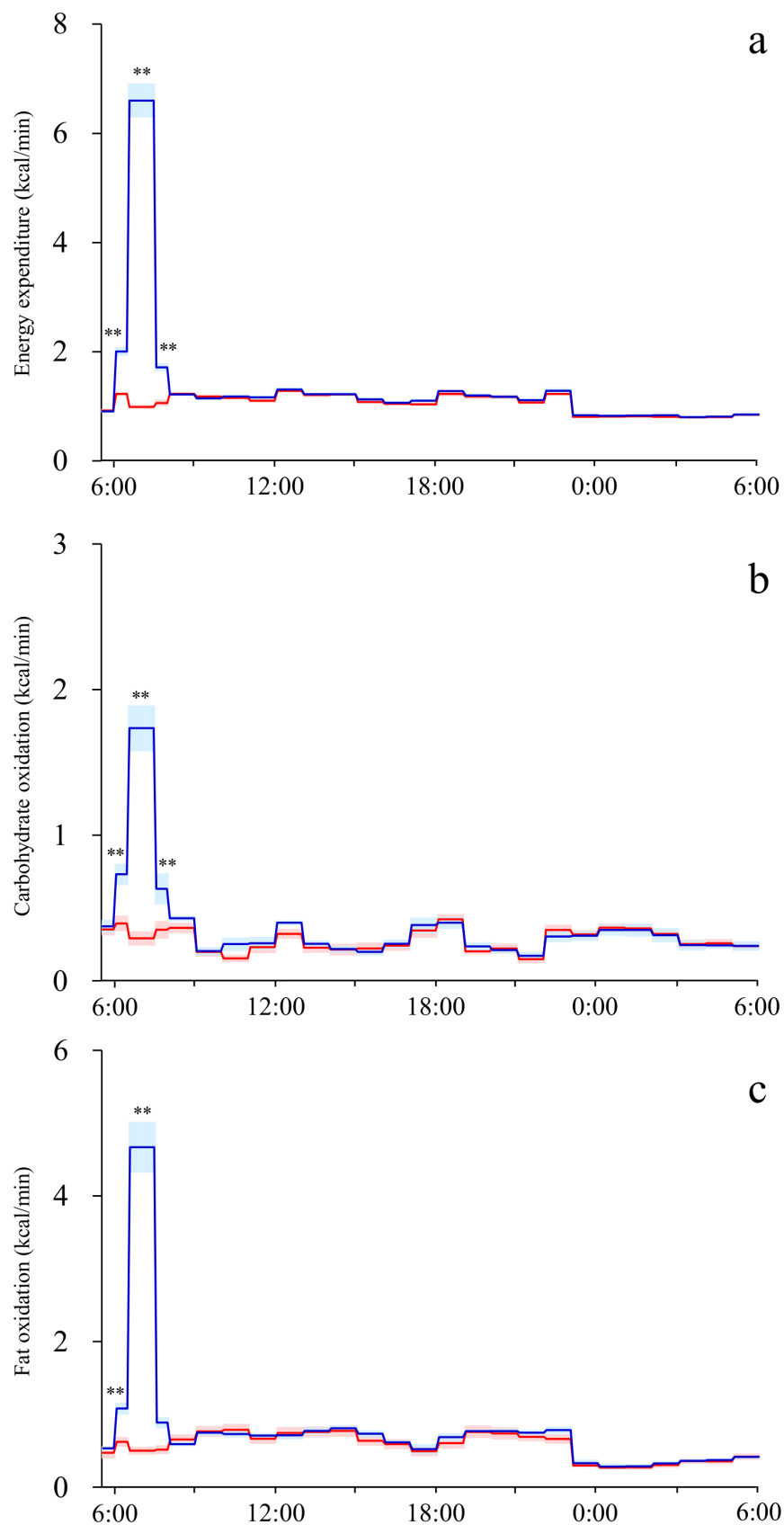


Fig 1. Time course of energy expenditure (a), carbohydrate oxidation (b) and fat oxidation (c) for sedentary (red) and exercise trials (blue). Mean \pm SE values plotted at 1-h intervals except for 6:00–6:30 and 7:30–8:00; 6:00–6:30. Significant differences between exercise and sedentary trials: *, $P < 0.05$; **, $P < 0.01$.

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and there was no significant difference at the end of 24 h calorimetry ($P = 0.96$). Similarly, the differences in time course of relative fat balance became statistically insignificant ($P = 0.45$).

No significant differences in non-exercise physical activity were observed between sedentary (97 ± 8 counts/min) and exercise trials (105 ± 10 counts/min, $P = 0.61$).

Discussion

The main finding of the present study was that exercise performed after overnight fasting increases 24-h fat oxidation in female subjects. Previous studies evaluating in an energy-balanced condition demonstrated that exercise performed in a postprandial state does not increase 24-h fat oxidation in male and female subjects [11,12,15,16]. Conversely, exercise performed before breakfast increased 24-h fat oxidation more than exercise performed after breakfast, lunch, or dinner, or in a sedentary control trial in male subjects [16–18]. Taken together, it is suggested that exercise performed in a postabsorptive state but not that performed in a postprandial state increases 24-h fat oxidation in male and female subjects.

The effect of exercise performed before breakfast may be attributed to the nutritional state or circadian nature of the early morning hours. In our previous studies with male subjects, the effect of exercise performed before breakfast was compared with exercise performed after breakfast, lunch, or dinner, and non-exercise control, and transient energy/carbohydrate deficit was negatively correlated with 24-h fat oxidation [16–18]. In the present study with female subjects, exercise performed in a postabsorptive state induced bigger transient energy/carbohydrate deficit compared with sedentary control trial. Carbohydrate pool size is the smallest among the macronutrients stored in the body, and the metabolic response to changes in carbohydrate storage is more sensitive than the response to fat and protein [8]. Hence, the transient carbohydrate deficit has been indicated as a factor; glycogen shortage induces nuclear translocation of AMPK and upregulation of genes responsible for fat oxidation in skeletal muscle [33], and hepatic glycogen shortage activates a liver–brain–adipose neural axis to enhance lipolysis [34].

It is worth mentioning that additional energy intake for the exercise trials was provided as a proportion of the overall diet as opposed to direct replacement of the substrates oxidized to support exercise. Possibility that mismatch of additional nutrients and those oxidized during the exercise affect 24-h fat oxidation remains to be evaluated. Alternatively, the effect of exercise performed before breakfast can be attributed to the circadian nature of the early morning hours since circadian rhythmicity can be seen in many physiological processes, which include body temperature, activity, sleep, metabolism, and hormone and neurotransmitter secretion

Table 2. Energy metabolism during 24 h.

	Sedentary	Exercise
Energy expenditure (kcal/24h)	1516 \pm 40	1925 \pm 43*
Carbohydrate oxidation (kcal/24h)	892 \pm 35	1126 \pm 49*
Fat oxidation (kcal/24h)	400 \pm 41	519 \pm 37*
Protein oxidation (kcal/24h)	225 \pm 34	279 \pm 46

*: $P < 0.05$

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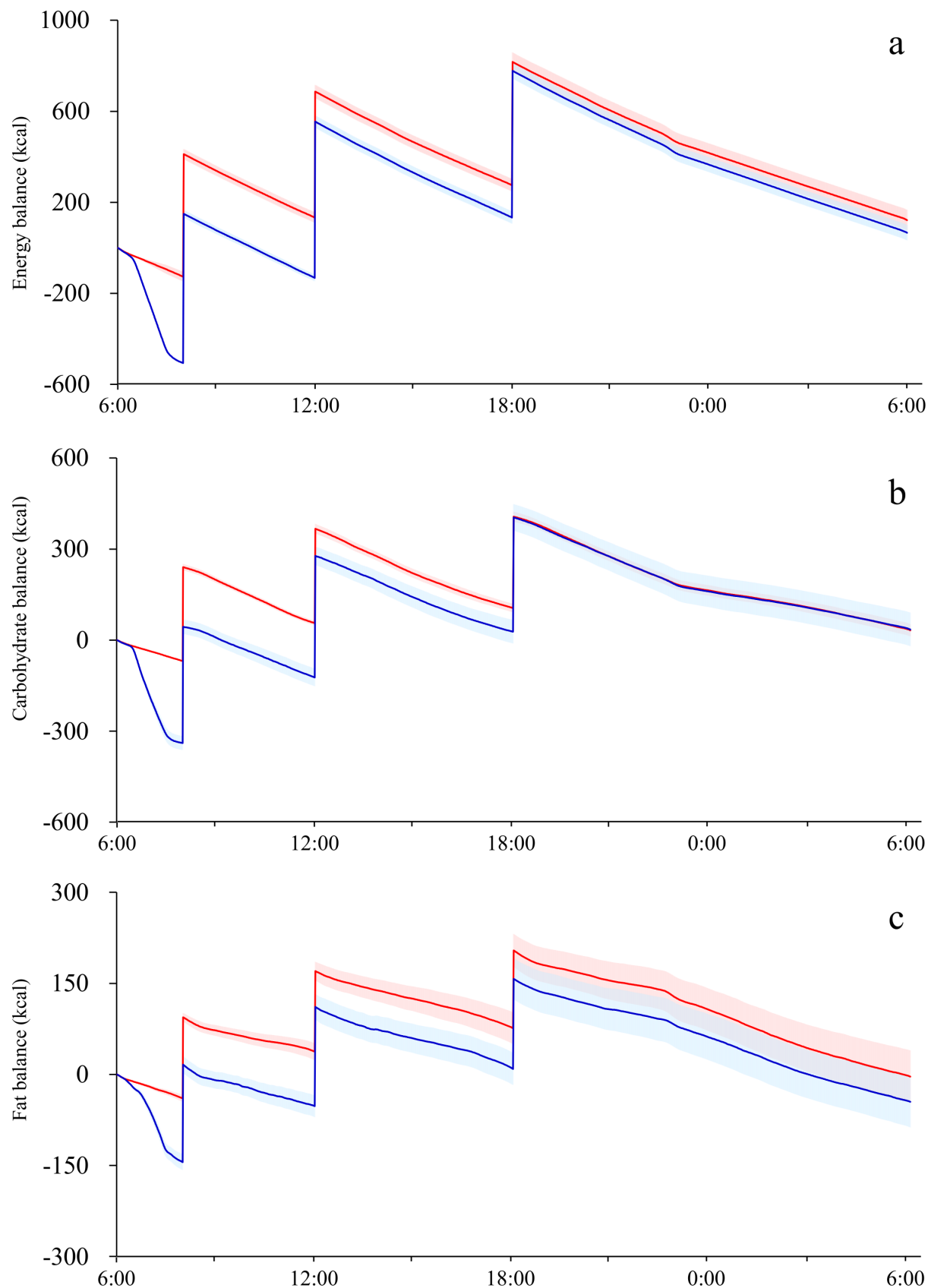


Fig 2. Time course of relative energy balance (a), carbohydrate balance (b) and fat balance (c). Mean \pm SE values are plotted at 30-min intervals for exercise trials (blue) and sedentary trials (red).

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[35]. However, the literatures do not support this possibility. First, fat oxidation during exercise in the morning (9:00 h) and evening (17:00 h) was not different when evaluated 3 h after consumption of an identical meal [36]. Second, only a 4-h difference in exercise timing affected 24-h fat oxidation; compared to exercise performed after breakfast (10:30–11:30 h), exercise performed before breakfast (06:30–07:30 h) oxidized significantly more fat [17].

In the present study, the indirect calorimetry was performed in early follicular phase, which is consistent with literature; in many of the metabolic studies with female subjects, follicular phase is often adopted to standardize conditions. It has been reported that fat oxidation during mid-luteal phase is higher compared to early follicular phase, and this is attributed to the difference in the circulating level of sex steroids, such as 17- β -estradiol [37]. Since glycogen utilization during endurance exercise is influenced by the menstrual cycle [38], an additional study is necessary to assess the effect of exercise on 24-h fat oxidation during luteal phase. In addition, indirect calorimetry itself does not allow us to evaluate sub-spices of substrates: glucose and glycogen for carbohydrate, plasma free fatty acid, triacylglycerol, and IMTG for fat. To understand the metabolic response to exercise performed at different times of day, further studies with other experimental approaches are warranted. For example, insight into tissue metabolites using magnetic resonance spectroscopy or tissue biopsy and/or use of tracers to evaluate substrate kinetics would be valuable.

In terms of the translational potential of the present study, some considerations are required. As a part of the lifestyle diversity in our society, there is wide variation in the time of day that individuals choose to exercise. Recent surveys on time use in the US and Japan have revealed that most people exercise after work, while a few individuals exercise before work on weekdays [39,40]. It requires cautious consideration to decide whether habitual exercise performed in the morning and that performed in the afternoon or early evening have different effects on 24 h fat oxidation. The effect of a single bout of exercise on 24-h fat oxidation cannot be extrapolated to the reduction of body fat with chronic exercise. Compared to training in the fed state, the fasted state suppressed body weight gain from a hypercaloric fat-rich diet in one study in male subjects [41]. However, in other studies with normal diet, no difference in weight change was observed between the training in the fed and fasted state in male and female subjects [23,42–46]. One of the aforementioned studies analyzed weighed food records; average daily intake of energy, protein, and carbohydrate significantly increased during the training, but these increases were not significantly different between the fed and fasted training groups [23]. Evaluating the effect of training performed in fed and fasted state on body composition may require a longer intervention period, which would make it difficult to accurately control or monitor dietary habits during the entire study.

Exercise performed in a postabsorptive state increases 24-h fat oxidation in female subjects. Together with previous studies [11,12,15,16], the present study suggests that effect of single bout of exercise on 24-h fat oxidation depends on when it is performed. Comparison of the chronic effects of exercise performed at different time of day on body fat remains to be performed.

Author Contributions

Conceptualization: KI R. Kawabuchi YN NO MS KT.

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Methodology: KI R. Kawabuchi KT.

Project administration: KT.

Supervision: YN MS.

Writing – original draft: KI R. Kawabuchi KT.

Writing – review & editing: YN MS.

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