



Effects of exercise before breakfast on plasma free fatty acid profile and 24-h fat oxidation



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ABSTRACT

Background: Free fatty acids (FFAs) are an important source of energy, and also serve as signaling molecules to regulate gene expression. Exercise performed in a post-absorptive state, in contrast to that performed in a postprandial state, increases 24-h fat oxidation under an energy-balanced condition. The primary aim of the present study was to clarify whether the effects of exercise on the concentration and composition of plasma FFAs, which may underlie distinct effects of exercise on 24-h fat oxidation, depend on the nutritional state of the individual when performing the exercise.

Methods: Ten healthy young men underwent 3 trials of indirect calorimetry in a metabolic chamber. The subjects performed exercise at 60% of VO_{2max} for 60 min in either a post-absorptive or postprandial state, or remained sedentary without an exercise session (control). All trials were designed to be energy balanced over 24 h. Blood samples were collected immediately before and after exercise.

Results: Fat oxidation over 24 h was increased only when exercise was performed in a post-absorptive state (control, 531 ± 60 ; post-absorptive, 779 ± 70 ; postprandial, 569 ± 37 kcal/24 h). The increase in the 24-h fat oxidation was related to the magnitude of the transient carbohydrate deficit after exercise. The plasma FFA concentration after exercise was higher in the post-absorptive trial (0.38 ± 0.04) than in the control (0.13 ± 0.01) and postprandial (0.15 ± 0.02 mM) trials. The ratio of unsaturated to saturated (U/S) fatty acids after exercise was higher in the post-absorptive trial (1.76 ± 0.06) than in the control (1.56 ± 0.07) and postprandial (1.53 ± 0.08) trials. On the other hand, the plasma FFA concentration after exercise in a postprandial state did not differ significantly from that in the control trial.

Conclusion: Exercise performed in a post-absorptive state effectively increased the plasma FFA concentration and U/S ratio to a greater degree than exercise performed in a postprandial state, underlying the increase in the 24-h fat oxidation. The increase in the plasma FFA concentration was related to the transient carbohydrate deficit after exercise.

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1. Introduction

The effects of exercise on energy expenditure and substrate oxidation persist during the post-exercise recovery period. To assess the effect of exercise on the body fat balance, several studies evaluated the effect of endurance exercise on fat oxidation over a

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24-h period (24-h fat oxidation) [1–6]. These studies found that exercise performed in a post-absorptive state increased 24-h fat oxidation in an energy-balanced condition [1–3], whereas exercise performed in a postprandial state did not [4–6]; energy intake and expenditure were balanced by experimental design to eliminate the profound effects of energy balance on substrate oxidation. After overnight fasting, glycogen storage in the body reaches the nadir of its diurnal rhythm [7], and exercise performed before breakfast further decreases glycogen levels [2,3]. Although a diet containing carbohydrates is recommended to replenish glycogen stores after exercise [8], exercise performed in a post-absorptive state leads to a considerable reduction in the glycogen storage that cannot be recovered for at least 4 h after glucose intake [9]. The magnitude of the transient decrease in the glycogen store after exercise negatively correlates with 24-h fat oxidation, suggesting that decreased glycogen storage in the body is a mechanism by which 24-h fat oxidation is enhanced by exercise performed in a post-absorptive state [1–3].

One potential mechanism by which the glycogen state affects fat oxidation is its interaction with AMP-activated protein kinase (AMPK). The decrease in muscle glycogen triggers sequential events, including dissociation of the AMPK-glycogen interaction; enhanced activity and altered intracellular localization of AMPK; and upregulated expression of genes associated with fat oxidation, such as carnitine palmitoyltransferase, fatty acid translocase, and hormone-sensitive lipase [10]. Additionally, decreased glycogen in the liver stimulates lipolysis in adipose tissue through a central nervous system-mediated mechanism [11]. On the basis of these findings together, the underlying mechanisms of exercise performed in a post-absorptive state to increase 24-h fat oxidation seems to be the enhanced availability of free fatty acids (FFAs) and their oxidative capacity.

The role of FFAs as signaling molecules in addition to serving as an oxidized substrate, was recently revealed. Although it is well established that fatty acid derivatives such as eicosanoids have key signaling functions, compelling evidence indicates that fatty acids themselves have signaling functions [12,13]. The expression of several genes is influenced by the plasma fatty acid levels and their composition, and the ratio of saturated fatty acids (SFA) to polyunsaturated fatty acids (PUFA) is associated with the expression of 25 genes related to fat metabolism in peripheral blood mononuclear cells [12]. PUFA inhibits proteolytic processing of sterol regulatory element binding protein-1 (SREBP-1), activates peroxisome proliferator-activated receptors (PPAR) [13], and stimulates fatty acid catabolism. Previous studies demonstrated that the plasma FFA concentration during and after exercise depends on the nutritional state of the individual. Exercise performed in a post-absorptive state for 120 min at 50% [14] and for 60 min at 70% [15] of the maximal power output increases plasma FFA, whereas exercise performed in a postprandial state does not increase plasma FFA concentrations. In addition, the plasma FFA composition is altered by acute exercise. The unsaturated to saturated (U/S) FFA ratio increases immediately after cycle ergometry in an overnight fasted state for 60 min at 50%–55% [16] or 61% [17] of individual maximal oxygen uptake (VO_{2max}), and the FFA concentration positively correlates with the U/S ratio [16].

Taken together, it is possible that exercise performed in a post-absorptive state significantly decreases the glycogen store, which stimulates the supply of FFA from adipose tissue and upregulates the fat oxidation capacity in the skeletal muscle, resulting in increased 24-h fat oxidation. The aim of the present study was to clarify 1) whether exercise performed in a post-absorptive state increases the plasma FFA concentration and modifies its composition, and 2) whether the transient decrease in the glycogen store after exercise is related to an increase in the plasma FFA

concentration. To this end, 24-h indirect calorimetry was performed over 3 trials with a 60-min exercise session before breakfast or after lunch and in a non-exercise condition.

2. Methods

2.1. Subject characteristics

Ten young healthy men were recruited to the present study after providing written informed consent. The subjects had no current medical conditions, were taking no medications at the time of the study, and were non-smokers. This study was approved by the ethics committee of the University of Tsukuba. This trial was registered at <http://www.umin.ac.jp/english/> as UMIN000040638.

2.2. Pre-study evaluation

To determine the workload corresponding to 60% of the individual maximal oxygen uptake, all subjects performed a graded exercise test comprising sub-maximal and maximal tests using a treadmill, as described previously [2]. The relative oxygen uptake and velocity of the treadmill running corresponding to 60% of the individual VO_{2max} were determined by regression analysis. Experimental exercise intensity was set not to exceed that maximizes fat oxidation [18]. This pre-study evaluation preceded the main experimental trials by at least 1 week.

2.3. Experimental protocol

The present study had a randomized, cross-over design comprising four 24-h calorimetry trials with exercise sessions performed in a post-absorptive (morning) or postprandial (afternoon) state, or with no exercise session (control). A washout period of at least 1 week was instituted between each calorimetry trial, and all experiments of each subject were completed within 2 months. Subjects were asked to maintain their body weight throughout the study, and no significant difference in body weight was detected between individual calorimetry trials ($P = 0.18$).

Subjects entered the metabolic chamber on the day prior to the exercise sessions (day 1, 22:00). Once in the metabolic chamber, the subjects slept for 7 h from 23:00 to 6:00. On day 2, 3 meals (breakfast at 9:00, lunch at 13:00, and dinner at 18:00) were provided, and the subjects ran on a treadmill at 60% of VO_{2max} for 60 min beginning at 7:00 (morning) or at 16:00 (afternoon) or remained in a sedentary position (control). The subjects were instructed to remain awake and maintain a sedentary position except when performing the prescribed exercise sessions and to sleep only at the times specified by the protocol. The 24-h energy expenditure and nutrient oxidation were calculated from 6:00 on day 2–6:00 on day 3 and compared among the 3 experimental conditions.

Experimental meals were designed to achieve an individual energy balance assuming a resting metabolic rate of 24.0 kcal/kg/day according to estimated energy requirements for Japanese individuals [19]. The physical activity factor was assumed to be 1.75 (2644 ± 63 kcal/day) on day 1, and 1.85 in trials with exercise sessions (2833 ± 125 kcal/day), or 1.34 in control trials (2052 ± 90 kcal/day) on day 2. Expressed as percentages of the total energy intake, experimental meals comprised 15% protein, 25% fat, and 60% carbohydrate. The contributions of breakfast, lunch, and dinner to the total 24-h energy intake were 33%, 33%, and 34%, respectively. Blood samples were collected into EDTA-coated test tubes at 6:30, 8:30, 15:30, and 17:30 on day 2, and at 6:30 on day 3. The blood was immediately centrifuged at 4 °C, and the plasma was decanted and stored at –80 °C until analysis.

2.4. Measurements

Energy metabolism was measured using a room-sized metabolic chamber with a volume of 14.49 m³ (Fuji Medical Science, Chiba, Japan). The airtight chamber had a port for blood sampling and was furnished with a bed, desk, chair, toilet, and treadmill. The temperature and relative humidity of the in-coming fresh air was controlled at 25.0 ± 0.5 °C and 55.0 ± 3.0%, respectively. Concentrations of oxygen (O₂) and carbon dioxide (CO₂) in the outgoing air were measured using an online process mass spectrometer (VG Prima δB, Thermo Electron, Winsford, UK). Every 5 min, O₂ consumption (VO₂) and CO₂ production (VCO₂) rates were calculated using an algorithm providing improved transient response [20]. Macronutrient oxidation and energy expenditure were calculated from the VO₂, VCO₂, and urinary nitrogen excretion. Energy and nutrient balance relative to the start of the 24-h calorimetry were estimated as the difference between the input (meal consumption) and output (oxidation) [1–3]. For example, 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)

For determining the plasma FFA, 0.5 ml of each sample was extracted with a chloroform - methanol mixture (2:1, v/v), and separated by thin-layer chromatography using plates coated with silica gel 60 (Merck, Darmstadt, Germany). The plates were developed with hexane - diethyl ether - acetic acid (70: 30: 1, v/v/v), and lipid spots were located under ultraviolet light after spraying with primulin. The spots corresponding to FFA were excised into capped glass tubes for the preparation of fatty acid methyl esters. Non-esterified fatty acids were converted to methyl esters using a commercially available mixed solution (NACALAI TESQUE, Kyoto, Japan) and quantified using a gas chromatography mass spectrometer (GCMS-QP2010, SHIMADZU Corp. Kyoto, Japan) equipped with a capillary column (60 m × 0.32 mm I.D., 0.25 mm thick, Agilent Technologies, Santa Clara, CA, USA) [21–23]. Twenty-five FFAs were identified from the mass spectrograms, and heptadecanoic acid (17:0) was used as an internal standard. The SFA, monounsaturated fatty acid (MUFA), and PUFA were calculated from the sum of each identified FFA.

Glucose and β-hydroxybutyrate were measured using an enzymatic method with the Glucose CII-Test Wako kit (Wako Pure Chemical Industries, Osaka, Japan) and β-Hydroxybutyrate Colorimetric assay kit (BioVison, Milpitas, CA, USA), respectively. Insulin was measured by enzyme-linked immunosorbent assay (Insulin ELISA kit, Mercodia, Uppsala, Sweden).

Non-exercise physical activity was estimated using a wrist watch-like device (ActiGraph, Ambulatory Monitoring Inc., Ardsley, NY, USA) as the number of times the activity signal crossed the zero reference point per minute. Data during the entire indirect calorimetry, except 1 h of exercise trial, were used to non-exercise physical activity.

2.5. Statistical analyses

Data in the main text and figures are presented as the mean ± SE. Mean values of pairs of conditions were compared using Student's t-test. For comparisons among 3 trials, 1-way repeated measures analysis of variance (ANOVA) with post hoc pair-wise comparisons using the Bonferroni's correction for multiple comparisons was performed. Changes in the blood parameters among the 3 trials were compared by 2-way repeated measures ANOVA to identify the main effect for trial and time, and interaction of trial and time, with post hoc pair-wise comparisons using Bonferroni's

correction. Correlations between 2 variables were assessed by Pearson's correlation coefficient. Statistical significance was set at P < 0.05. All statistical analyses were performed using SPSS statistical software (Version 24, IBM Japan, Tokyo, Japan).

3. Results

The mean physical characteristics of included subjects were as follows: age, 24.8 ± 1.1 years; height, 173.7 ± 1.7 cm; body weight, 63.9 ± 1.3 kg; and body fat, 14.3% ± 0.5%. The mean maximal oxygen uptake was 58.2 ± 2.9 ml/kg/min. All subjects completed 3 trials, and body mass, body fat, and fat free mass did not differ significantly among the trials.

During the experimental exercise sessions, energy expenditure and carbohydrate oxidation were lower in morning trials (663 ± 31 and 438 ± 17 kcal/60 min, respectively) than in afternoon trials (688 ± 33 and 602 ± 28 kcal/60 min, respectively; P < 0.01), while fat oxidation was higher in morning trials (214 ± 20 kcal/60 min) than in afternoon trials (75 ± 7 kcal/60 min; P < 0.01). Heart rate during exercise was not significantly different between trials (morning, 143 ± 6; afternoon, 144 ± 5 beats/min; P = 0.68). The time course of energy expenditure, and carbohydrate and fat oxidation are shown Fig. 1. The 24-h energy expenditure was significantly higher during trials with exercise sessions (morning, 2833 ± 90; afternoon, 2840 ± 91 kcal/24 h) than during control trials (2020 ± 66 kcal/24 h; P < 0.01). The 24-h energy expenditure did not differ significantly between the morning and afternoon trials. The 24-h fat oxidation was significantly higher in the morning trials (779 ± 70 kcal/24 h) than in the other trials (afternoon, 569 ± 37; control, 531 ± 60 kcal/24 h; P < 0.05). There was no significant difference in 24-h fat oxidation between afternoon and control trials. Accumulated carbohydrate oxidation over 24 h in descending order was as follows: afternoon (1994 ± 67 kcal/24 h), morning (1793 ± 88 kcal/24 h), and control trials (1263 ± 49 kcal/24 h). Urinary nitrogen excretion was not significantly different among the 3 trials (control, 8.8 ± 0.8; morning, 10.2 ± 0.6; afternoon, 10.9 ± 0.6 g/24 h; P = 0.07). The energy and macronutrient balance over 24 h are shown Table 1. The energy balance over 24 h was maintained in all trials as planned by experimental design. The carbohydrate balance over 24 h was significantly different in the afternoon trials compared with the control and morning trials. In contrast to the control and morning trials, the fat balance over 24 h was positive in the afternoon trial. Although the energy balance over 24 h was maintained in all trials, the time course differed among trials (Fig. 2). The nadir of the relative energy balance, i.e., the greatest transient energy deficit, was lower in morning exercise trials than in the control and afternoon exercise trials (morning exercise, -923 ± 38; control, -245 ± 8; afternoon exercise, -251 ± 9 kcal; P < 0.01).

A significant main effect of trial (P < 0.05) and time (P < 0.01), and an interaction of trial and time (P < 0.01) were observed in total FFA (Fig. 3a), SFA (Fig. 3b), MUFA (Fig. 3c), and PUFA concentrations (Fig. 3d). The total plasma FFA concentration was markedly higher after the morning exercise trials (i.e., 08:30 on day 2) than after the other 2 trials (P < 0.01). Similarly, plasma concentrations of SFA, MUFA, and PUFA were significantly increased by exercise in the morning (P < 0.01, Fig. 3). The total FFA and all subclasses of fatty acids were slightly elevated after afternoon exercise (i.e., 17:30) compared with morning exercise, but not in comparison with the control trial (P < 0.05). At 06:30 on the day after exercise, the total FFA, SFA, and PUFA concentrations were significantly lower in the morning trial compared with the control and afternoon trials (P < 0.05). For the U/S ratio, the main effect of time was significant (P < 0.01), but the main effect of trial was not significant (Fig. 4). The trial by time interaction tended to be significant (P = 0.056). The U/S ratio was significantly higher after morning exercise at 8:30

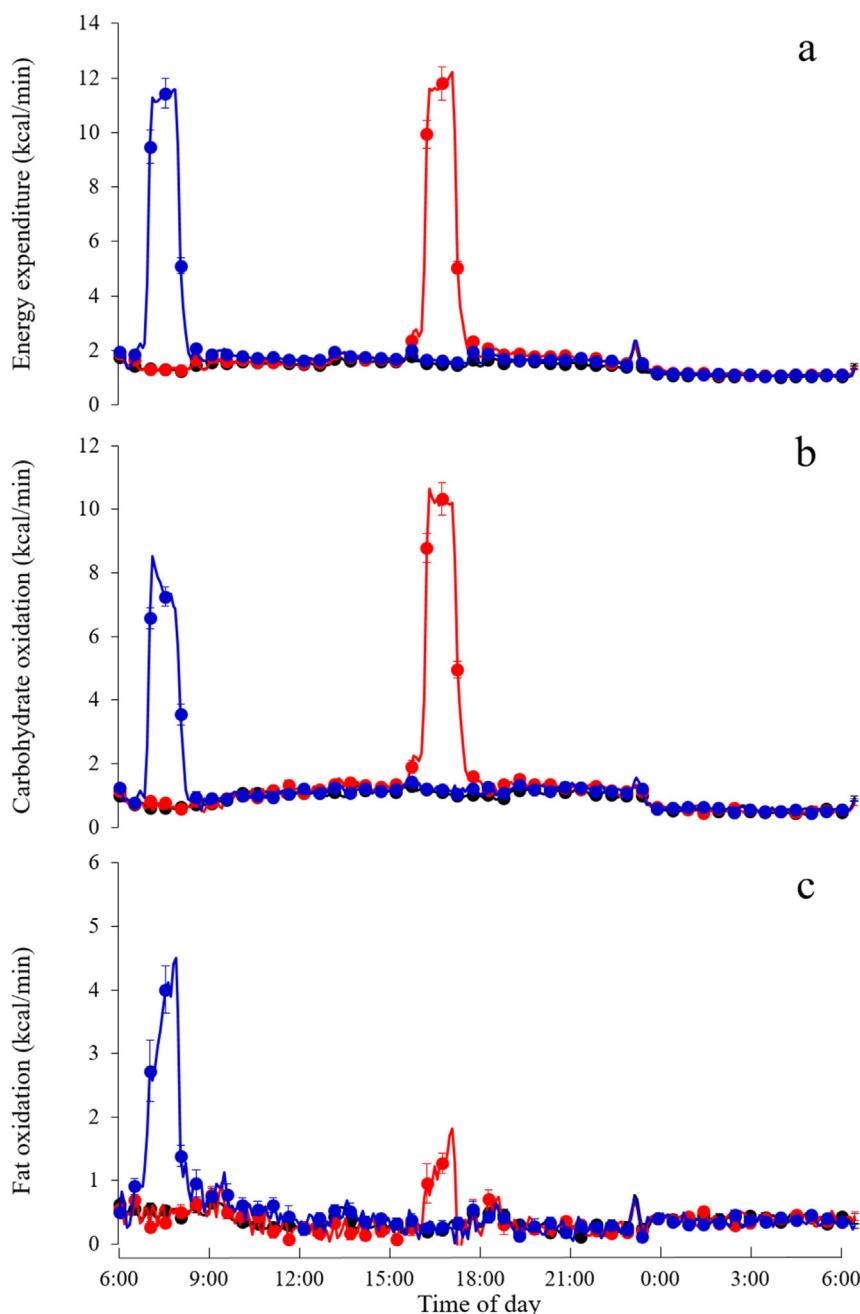


Fig. 1. Time course of energy expenditure and substrate oxidation. Energy expenditure (a), carbohydrate oxidation (b), and fat oxidation (c) are shown for the control (black line), morning (blue line), and afternoon trials (red line). Plots are mean \pm SE at 30 min intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Energy and macronutrient balance during 24-h.

	Control	Morning	Afternoon
Energy balance (kcal/24 h)	32 \pm 47	-1 \pm 55	-8 \pm 56
Carbohydrate balance (kcal/24 h)	-73 \pm 50	-131 \pm 56	-332 \pm 26 ^{a,b}
Fat balance (kcal/24 h)	-36 \pm 50	-76 \pm 75	135 \pm 43 ^b
Protein balance (kcal/24 h)	49 \pm 46	146 \pm 12	129 \pm 13

^a P < 0.05 vs Control.

^b P < 0.05 vs Morning.

than after the afternoon exercise trial (P < 0.05), and tended to be higher than that after the control trial (P = 0.061, Fig. 4), whereas

there were no significant differences in the U/S ratio among the 3 trials after exercise in the afternoon (i.e., at 17:30). The carbohydrate balance relative to the beginning of the calorimetry (06:00) and the plasma FFA concentration relative to the first blood sample at 6:30 were negatively correlated (Fig. 5).

For the plasma glucose concentration, the main effects of trial and time, and the trial by time interaction were not significant (Fig. 6a). For the plasma insulin concentration, there was a significant main effect of trial (P < 0.01), time (P < 0.01), and a significant trial by time interaction (P < 0.05). The plasma insulin concentration was significantly higher in the afternoon trial than in the control and morning trials (P < 0.01), and the difference between

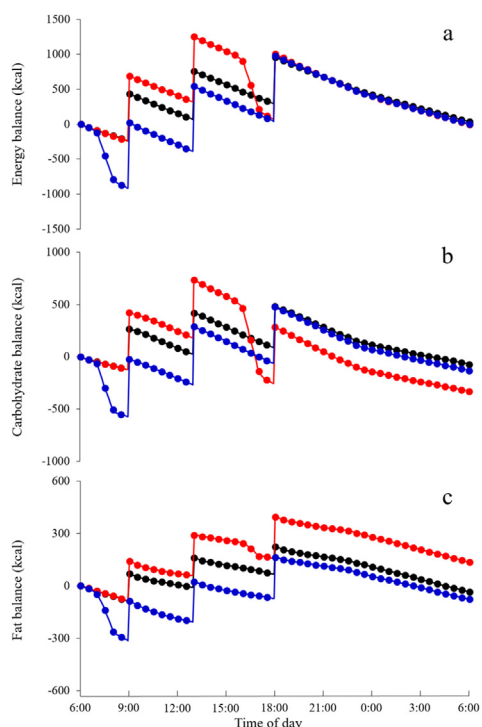


Fig. 2. Time course of relative energy balance and substrate balance. Energy balance (a), carbohydrate balance (b), and fat balance (c) were calculated by setting the initial reference value at 06:00 on day 2. Mean values are plotted at 30-min intervals for the control (black line), morning (blue line), and afternoon trials (red line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the control and morning trials was also statistically significant ($P < 0.05$, Fig. 6b). The plasma β -hydroxybutyrate concentration also had significant main effects for trial ($P < 0.05$, control vs. morning), but the main effects for time and the trial by time interaction were not statistically significant (Fig. 6c). The non-exercise physical activity did not differ significantly among control, morning, and afternoon trials (80 ± 9 , 80 ± 11 , and 83 ± 11 count/min, respectively; $P = 0.92$).

4. Discussion

Exercise performed in the post-absorptive state led to an increase in 24-h fat oxidation, whereas exercise performed in a postprandial state did not, a finding that is consistent with our previous studies [1–3]. The present study was designed to determine the distinct effects of exercise performed in a postprandial or post-absorptive state under an energy-balanced condition on the plasma FFA concentration and FFA composition. Our main findings were that the plasma FFA concentration was increased and its composition changed following exercise performed in a post-absorptive state. Changes in the plasma FFA were negatively correlated with the relative balance of energy and carbohydrate.

In contrast to exercise performed in a postprandial state, exercise performed in a post-absorptive state led to a marked increase in the plasma concentration of SFA, MUFA, and PUFA, which in turn increased the total FFA concentration. The uptake and oxidation of fatty acids is enhanced in skeletal muscle when the blood concentration of fatty acids is elevated [24]. In addition, oxidation of individual fatty acids varies depending on the number of double bonds and/or carbon length [25]. MUFA and PUFA are more easily oxidized than SFA, and less likely than SFA to be stored as body fat.

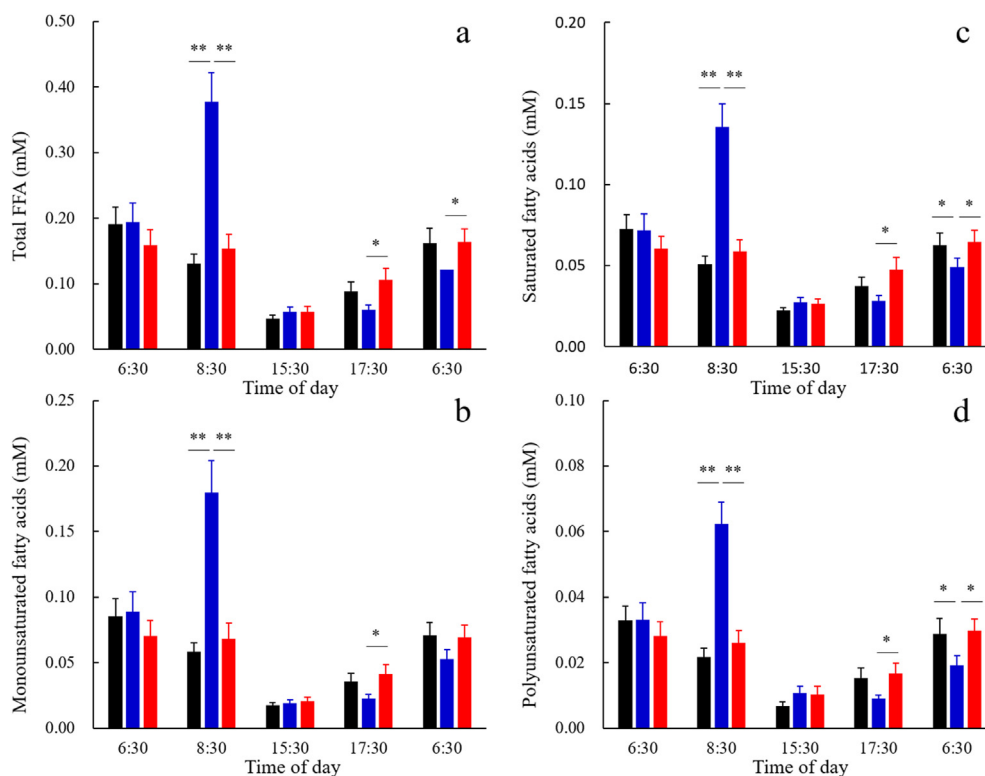


Fig. 3. Plasma concentrations of total FFA (a), saturated fatty acids (b), monounsaturated fatty acids (c), and polyunsaturated fatty acids (d). Control, morning, and afternoon trials are shown as black, blue, and red bars, respectively. Values are mean \pm SE. *; $P < 0.05$, **; $P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

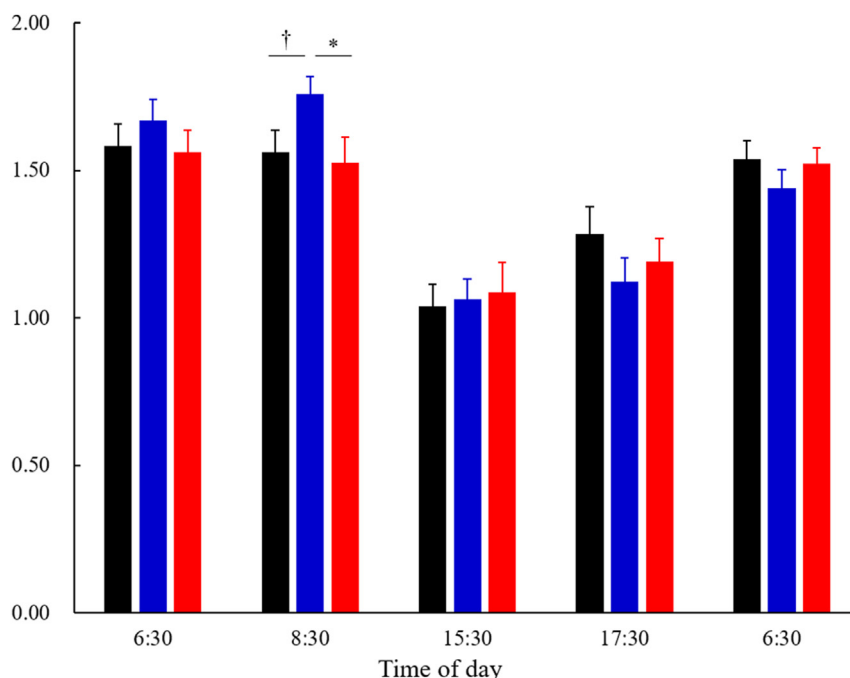


Fig. 4. The ratio of unsaturated-to-saturated FFA (U/S). Control, morning, and afternoon trials are shown as black, blue, and red bars, respectively. Values are mean \pm SE. *: $P < 0.05$, †: $P = 0.06$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

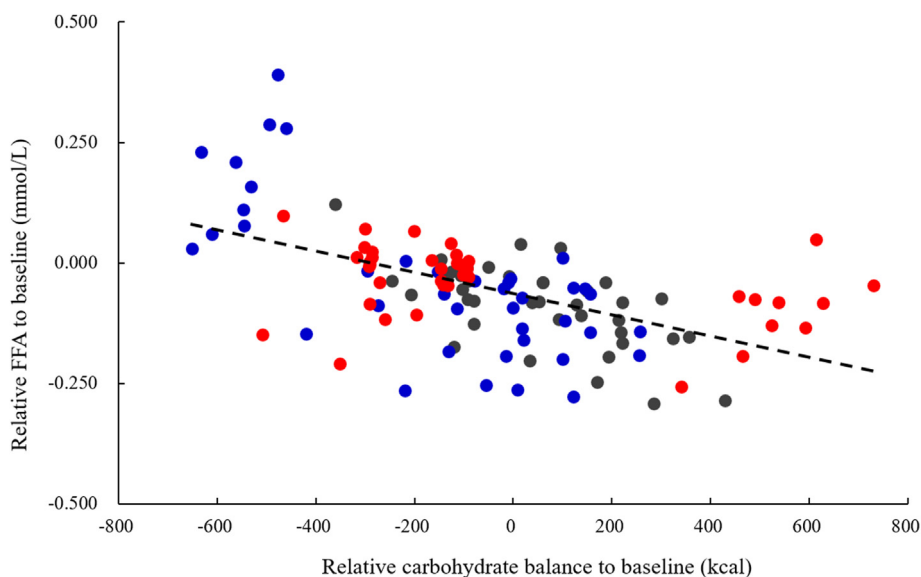


Fig. 5. Carbohydrate balance and circulating FFA level relative to the baseline value. The carbohydrate balance relative to the value obtained at 6:00, and the circulating FFA level relative to the value obtained at 6:30 of day 2 were negatively correlated ($r = -0.55$, $P < 0.01$). Control, morning, and afternoon trials are indicated by the black blue, and red circles, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Changes in the fatty acid concentration and/or composition observed after exercise in the post-absorptive state underlie the mechanisms upregulating fatty acid oxidation in skeletal muscle during and immediately after exercise.

Exercise before breakfast induces a transient decrease in energy and carbohydrate balance [1–3]. Nadirs of energy and carbohydrate balance are negatively correlated with the 24-h fat oxidation [2]. Stored carbohydrate, i.e., glycogen, not only supplies energy through glycogenolysis, but also regulates enzyme activity and gene expression related to carbohydrate/fat metabolism by glycogen degradation per se [10]. The carbohydrate balance relative to baseline values,

which indicates variations in glycogen storage, negatively correlated with changes in FFA relative to the baseline value after an overnight fast (Fig. 5). Therefore, increases FFA in response to glycogen degradation may be a response to a protracted increase in fat oxidation. Above all, PUFA regulates key transcription factors controlling liver fat metabolism; activation of PPAR α to enhance fatty acid oxidation, and suppression of SREBP-1 and ChREBP to inhibit de novo lipogenesis and PUFA synthesis [26]. Fat oxidation remained slightly higher for a while after exercise in the morning trials than that in control trials. It is plausible that elevated circulating PUFA underlies the residual increase in fat oxidation after exercise in the morning trial. On the other

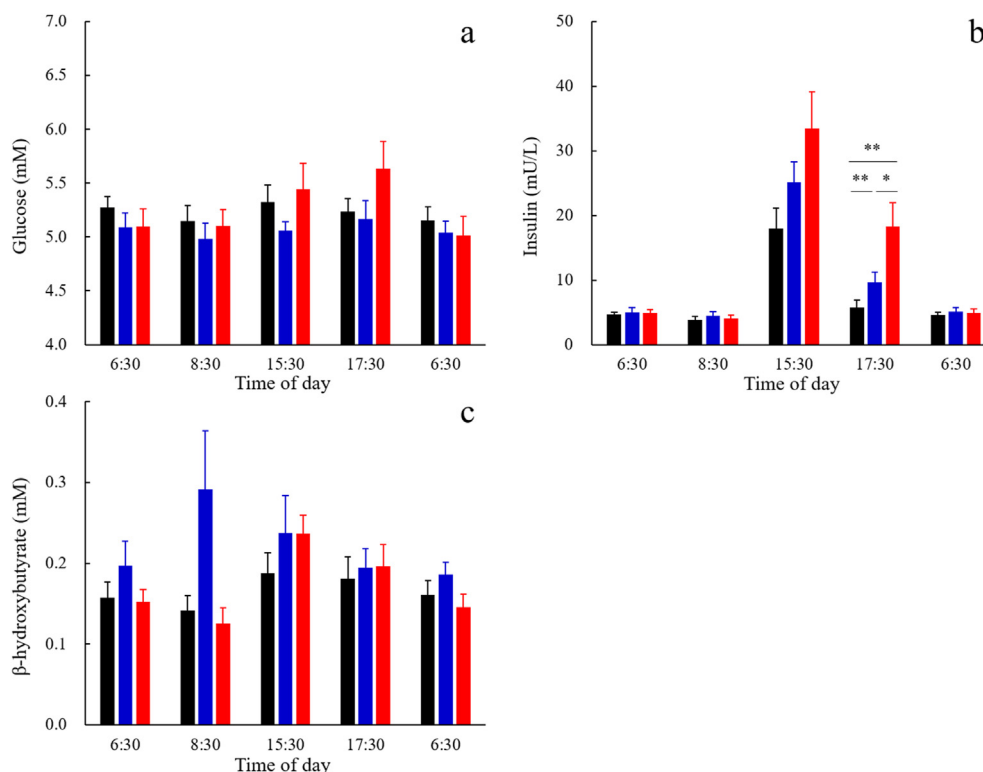


Fig. 6. Plasma glucose (a), insulin (b), and β -hydroxybutyrate (c) concentrations. Control, morning, and afternoon trials are shown as black, blue, and red bars, respectively. Values are mean \pm SE. *: $P < 0.05$, **: $P < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hand, neither an increase in the plasma PUFA concentration nor a residual increase in fat oxidation were observed after the afternoon exercise trial. In addition, the effect of exercise on the U/S ratio of circulating FFA depended on the nutritional state under which exercise is performed, and the U/S ratio was not changed by exercise performed in a postprandial condition. The increase in the U/S ratio of circulating FFA after exercise in the morning trial is consistent with findings from previous studies demonstrating that exercise performed after an overnight fast increases the U/S ratio [16,17]. Hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) are the major lipases in adipocyte lipolysis [27] catalyzing the rate-limiting step in the breakdown of adipocyte triacylglycerol. An increase in the gene expression of HSL and ATGL by exercise for 60 min at 60% of VO_{2peak} is blunted in a fed state compared with a fasted state [28]. Adipose HSL preferentially acts on triacylglycerol carrying PUFA [29], and ATGL exhibits a tendency to carry MUFA or PUFA rather than SFA [30]. Furthermore, U/S ratio of adipose tissue triglyceride are markedly higher than those of plasma FFA. Overall, an increase in adipose tissue-derived circulating PUFA through HSL and ATGL may underlie the increased fat oxidation after exercise in the post-absorptive state.

When glycogen in the muscle and liver is decreased after overnight fasting or prolonged exercise, fatty acids are mobilized from adipocytes, transported to the liver for ketogenesis, and used as an alternative fuel [31]. Production of ketone bodies is an oxygen-requiring metabolic process, and the respiratory quotient of ketogenesis is 0.57, which affects the interpretation of indirect calorimetry, leading to an overestimation of fat oxidation. If ketone bodies are oxidized, however, the respiratory quotient of the whole process, ketogenesis and its oxidation, is similar to that of fat oxidation, i.e., 0.7 [32]. In the present study, the plasma β -hydroxybutyrate concentration was relatively low compared with post-exercise ketosis in a previous study [33]. Therefore, the contribution of ketone bodies to

whole body energy metabolism and their effect on indirect calorimetry were negligible in this study. The insulin concentration at 17:30 on day 2 was higher for both the morning and afternoon trials than the control trial. It is likely that carbohydrate intake at lunch in trials with exercise (141 ± 19 g) was higher than that in the control trial (100 ± 12 g) so that the energy balance could be maintained over 24 h. Despite the increase in the insulin level, the 24-h fat oxidation was greater in the morning trial than in the control trial, which highlights a mechanism other than that mediated by a reduction in glucose and insulin, such as increased delivery of FFAs and an enhanced capacity for FFA oxidation.

A major limitation of this study is that the intervals between blood sampling were not sufficiently frequent to gain insight into the time course of the changes in the plasma FFA concentration after exercise and meals. For example, it would be interesting to investigate whether the residual increase in fat oxidation for several hours after morning exercise is accompanied by elevated FFA levels. The time course of the changes in the FFA concentration and its composition with a higher temporal resolution also warrants further study.

In conclusion, 24-h fat oxidation was increased by exercise only when the exercise was performed in a post-absorptive state under energy-balanced conditions. Exercise performed in a post-absorptive state led to a significant increase in the plasma FFA concentration and U/S ratio compared with that performed in a postprandial state, and the transient carbohydrate deficit after exercise was related to an increase in the plasma FFA concentration.

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CRediT authorship contribution statement

Kaito Iwayama: Conceptualization, Funding acquisition, Investigation, Methodology, Writing - original draft. **Ayane Ogawa:** Conceptualization, Data curation, Investigation, Methodology, Writing - original draft. **Yoshiaki Tanaka:** Investigation. **Katsuhiko Yajima:** Investigation. **Insung Park:** Investigation. **Akira Ando:** Investigation. **Simeng Zhang:** Investigation. **Fumiya Tanji:** and **Yoshiharu Nabekura:** Conceptualization, Supervision. **Kouhei Yamamoto:** and **Kumpei Tokuyama:** Project administration.

Declaration of competing interest

None of the authors has any conflict in relation to the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.metop.2020.100067>.

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