

Transient energy deficit induced by exercise increases 24-h fat oxidation in young trained men

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Abstract

Whole body fat oxidation increases during exercise. However 24-h fat oxidation on a day with exercise often remains similar to that of sedentary day, when energy intake is increased to achieve energy-balanced condition. The present study aimed to examine a possibility that time of the day when exercise is performed makes differences in 24-h fat oxidation. As a potential mechanism of exercise affecting 24-h fat oxidation, its relation to exercise-induced transient energy deficit was examined. Nine young male endurance athletes underwent three trials of indirect calorimetry using a metabolic chamber, in which they performed a session of 100 min exercise before breakfast (AM), after lunch (PM) or two sessions of 50 min exercise before breakfast and after lunch (AM/PM) at 65% of $\dot{V}O_{2\max}$. Experimental meals were designed to achieve individual energy balance. Twenty-four-hour energy expenditure was similar among the trials, but 24-h fat oxidation was 1142±97, 809±88 and 608±46 kcal/24-h in descending order of its magnitude for AM, AM/PM and PM, respectively ($P<0.05$). Twenty-four-hour carbohydrate oxidation was 2558±110, 2374±114 and 2062±96 kcal/24-h, for PM, AM/PM and AM. In spite of energy-balanced condition over 24-h, exercise induced a transient energy deficit, magnitude of which was negatively correlated with 24-h fat oxidation ($r=-0.72$, $P<0.01$). Similarly, transient carbohydrate deficit after exercise was negatively correlated with 24-h fat oxidation ($r=-0.40$, $P<0.05$). The time of the day when exercise is performed affects 24-h fat oxidation, and the transient energy/carbohydrate deficit after exercise is implied as a factor affecting 24-h fat oxidation.

Key Words: energy balance, energy flux, EPOC, metabolic chamber

INTRODUCTION

Whole body fat oxidation increases during endurance exercise and the increased fat oxidation persists during post-exercise period (4, 14). One of the earliest studies using a metabolic chamber reported that accumulated fat oxidation over 24-h (24-h fat oxidation) was increased by exercise (4).

However, it is likely that the subjects were in a state of negative energy balance on the day with exercise, which per se would have elevated fat oxidation (24). Negative energy balance over 24-h is associated with increased fat oxidation, i.e. the fuel mix oxidized contains more fat than the mixture supplied by the diet (11, 12, 21). Consensus in the literature states that if exercise is accompanied by increased energy intake to achieve energy-balanced condition, 24-h fat oxidation remains similar to that of sedentary day (22-25).

In addition to energy balance, 24-h fat oxidation may also be affected by energy flux, which refers to the absolute level of energy intake and expenditure under conditions of energy balance (2). In one study, subjects performed exercise at 50% of maximal oxygen uptake ($\dot{V}O_{2max}$) for 60 min or remained sedentary, and energy balance of the two experiments was matched by adjusting energy intake (5). Compared with low energy flux in a sedentary condition, accumulated fat oxidation during 9-h of indirect calorimetry was higher in a high energy flux condition with exercise.

Findings of abovementioned studies can be interpreted that nutritional state when exercise is performed affects 24-h fat oxidation. Exercise has little effect on 24-h fat oxidation when performed after breakfast (22-25), but that performed before breakfast increases fat oxidation during 9-h of post exercise period (5). Compared with exercise performed after breakfast, 24-h fat oxidation was higher when exercise was performed before breakfast in our previous study (35). The mechanism responsible for the increase in 24-h fat oxidation on a day with exercise, if any, remains to be elucidated. However, a link between 24-h fat oxidation and transient energy deficit has been suggested as follows. Even though energy intake and expenditure over 24-h was matched in both experimental conditions, time course of energy balance varied according to the time when exercise was performed. Exercise performed before breakfast, when diurnal variation in energy content of the body reaches its nadir, further decreases body energy, i.e. a transient but large decrease in body energy content (35). Among macronutrients stored in the body, pool size of carbohydrate is the smallest, and metabolic response to changes in carbohydrate storage is more sensitive than those in fat and protein (11, 12). Recent studies suggest an underlying mechanism, which translates

depletion of glycogen in liver and skeletal muscle to a sustained increase in whole body fat oxidation (19, 31). Taken together, available evidence suggests that exercise performed in the postabsorptive state depletes glycogen storage, which in turn increases 24-h whole body fat oxidation.

The objective of the present study was to examine relation between 24-h fat oxidation and exercise induced transient deficit in energy and/or carbohydrate. To this end, 24-h indirect calorimetry was performed on three occasions with a session of 100 min exercise before breakfast (AM), after lunch (PM) or two sessions of 50 min exercise before breakfast and after lunch (AM/PM). All experimental conditions were designed to be energy balanced and matched for energy flux over 24-h. Physiological significance of transient energy deficit affecting 24-h energy metabolism was discussed.

Methods

Subject Characteristics

Nine young male endurance athletes were recruited in this study after giving their written informed consent. This study was approved by the ethics committee of University of Tsukuba. Physical characteristics of subjects were 23.2 ± 2.7 years of age, 168.4 ± 5.7 cm of height, 59.5 ± 1.2 kg of body weight and 11.6 ± 0.6 % of body fat. Their $\dot{V}O_2\text{max}$ was 71.7 ± 6.4 ml/kg/min. All subjects were free from pathological condition and none of them were taking any medications or supplements. One week before the 24-h calorimetry, subjects spent a night in a metabolic chamber in order to get acclimated to the measurement condition.

Pre-study Evaluation

The velocity of treadmill running corresponding to 65% of the individual $\dot{V}O_2\text{max}$ was determined from an incremental exercise test on a motor-driven treadmill (ORK-7000, Ohtake Root Kogyo CO.,LTD, Iwate, Japan), at least 1 week before the main experiment. Initial velocity of the

maximum running test was 200 m/min for 3 min and it was increased by 20 m/min every 2 min until 280 m/min, then it was increased 10 m/min every 1 min until exhaustion. Oxygen uptake ($\dot{V}O_2$) was considered to be maximal when at least 2 of the following 3 criteria were met: 1) $\dot{V}O_2$ reached plateau, 2) heart rate exceeded 90% of the age-predicted maximal value, 3) respiratory exchange ratio increased above 1.10. The highest $\dot{V}O_2$ for consecutive 30 s during the test was taken as $\dot{V}O_{2\text{max}}$ of the subject. Respiratory gas analysis was continuously performed on a breath-by-breath basis using the computerized standard open circuit technique (AE-310s MINATO MEDICAL SCIENCE, Osaka, Japan). Body composition was measured by bioimpedance method (BC-118E, TANITA Co., Tokyo, Japan).

Experimental protocol

The study was a randomized repeated measures design including three 24-h calorimetry measurements comprising AM, PM and AM/PM exercise sessions. A washout period of at least 1 week was inserted between each session. In each trial, subjects stayed in a metabolic chamber for 42-h beginning the day before exercise session (day 1, 22:00) until the day after exercise session (day 3, 16:00). During the daytime before entering the metabolic chamber, subjects were instructed to refrain from performing strenuous exercise and consumption of beverages containing energy, caffeine or alcohol. In the metabolic chamber, subjects slept for 7-h from 23:00 to 6:00, and three meals (breakfast at 8:30, lunch at 12:30 and supper at 18:00) were provided. Subjects were instructed to remain awake and to keep sedentary position other than prescribed exercise session and bedtime by the protocol.

On day 2, subjects performed a session of 100 min exercise beginning at 6:30 (AM), at 16:00 (PM) or two sessions of 50 min exercise at 6:30 and at 16:00 (AM/PM) at 65% of $\dot{V}O_{2\text{max}}$ using a treadmill (T1201, Johnson Health Tech Japan, Tokyo, Japan). In addition to 100 min running, subjects were instructed to perform 15 min of warm-up activity twice a day (6:15 and 15:45). Subjects were allowed to leave the chamber for 30 min to take a shower (22:00 - 22:30). Energy

metabolism during the 30 min break was estimated from metabolic equivalent of taking shower as 2.0 METs and respiratory quotient (RQ) immediately before the break, as previously described (35). On day 3, subjects followed the same protocol of day 2 except the exercise session, and exited the chamber at 16:00. Twenty-four-hour energy expenditure and nutrients oxidation calculated from 6:00 of day 2 to 6:00 of day 3 were compared among the three experimental conditions.

Experimental meals were designed to achieve individual energy balance assuming resting metabolic rate to be 24.0 kcal/kg/day, and physical activity factor to be 1.75 (2464 ± 75 kcal/day) in day 1, 2.48 (3544 ± 127 kcal/day) in day 2 and 1.75 in day 3 (1587 ± 47 kcal for breakfast and lunch), according to the estimated energy requirement for Japanese (26). Expressed as a percentage of total energy, the standardized meals consist of 15 % protein, 25 % fat and 60 % carbohydrate. The contribution of the breakfast, lunch and supper to total 24-h energy intake were 32 %, 34 % and 34 %, respectively.

Measurement

Energy metabolism was measured with a room-size metabolic chamber (Fuji Medical Science, Chiba, Japan). The airtight chamber measures $2.00 \times 3.45 \times 2.10$ m, having an internal volume of 14.49 m^3 . The chamber is furnished with an adjustable hospital bed, desk, chair and toilet. Air in the chamber was pumped out at a rate of 120 L/min. Temperature and relative humidity of in-coming fresh air were controlled at 25.0 ± 0.5 °C and 55.0 ± 3.0 %, respectively. Concentrations of oxygen (O_2) and carbon dioxide (CO_2) in out-going air were measured by an online process mass spectrometer (VG PrimδB, Thermo Electron, Winsford, UK). Precisions of the mass spectrometry, defined as the standard deviation for continuous measurement of calibration gas mixture (O_2 : 15 %, CO_2 : 5 %), were 0.0016 % and 0.0011 % for O_2 and CO_2 , respectively. At every 5 min, $\dot{\text{V}}\text{O}_2$ and $\dot{\text{V}}\text{CO}_2$ production rate ($\dot{\text{V}}\text{CO}_2$) were calculated using an algorithm for improved transient response (37).

Macronutrients oxidation and energy expenditure were calculated from $\dot{\text{V}}\text{O}_2$, $\dot{\text{V}}\text{CO}_2$ and urinary

nitrogen excretion (10). Rates of glucose, fat and protein oxidation were computed from the following equations, assuming urinary nitrogen excretion rate ($\dot{N}$) to be constant during the calorimetry.

$$\text{Glucose oxidation (g/min)} = 4.55 \dot{V}\text{CO}_2 \text{ (L/min)} - 3.21 \dot{V}\text{O}_2 \text{ (L/min)} - 2.87 \dot{N} \text{ (g/min)}.$$

$$\text{Fat oxidation (g/min)} = 1.67 \dot{V}\text{O}_2 \text{ (L/min)} - 1.67 \dot{V}\text{CO}_2 \text{ (L/min)} - 1.92 \dot{N} \text{ (g/min)}$$

$$\text{Protein oxidation (g/min)} = 6.25 \dot{N} \text{ (g/min)}$$

Subsequently, energy production by each nutrient was calculated by taking into account the caloric equivalent of the three substrates, i.e., 3.74 kcal/g glucose, 9.50 kcal/g fat and 4.10 kcal/g protein. It is conceivable that tissue glycogen rather than glucose is the predominant form of carbohydrate being oxidized during exercise. An equation for complete oxidation of glycogen and its caloric equivalent are different from those of glucose. However, estimates of energy expenditure by carbohydrate oxidation in terms of energy expenditure in a unit time (kcal/min) are robust, regardless of whether glucose or glycogen is oxidized in the body (10, 32). Therefore, glucose oxidation was discussed as carbohydrate oxidation in the present study. Energy and nutrient balance relative to the beginning of 24-h calorimetry were estimated as difference between the input and output. For example, relative energy balance was defined as a function of time (t) since 6:00 of day 2.

$$\text{Relative energy balance (t)} = \text{accumulated energy intake (t)} - \text{accumulated energy expenditure (t)}$$

This represents an “apparent” balance, since not all the dietary nutrients are absorbed during the short period (4, 34).

Physical activities including non-exercise activity were estimated using a wrist watch-like device, ActiGraph (Ambulatory Monitoring, NY, USA) with zero crossing mode, as the number of times the activity signal crossed the zero reference point per minute (counts/min).

Statistical analysis

Data in the text and figures were given as means $\pm$ SE of the experimental condition. Statistical

analyses were performed using SPSS statistical software (Version 20, IBM Japan, Ltd., Tokyo, Japan). Mean values of 3 trials were compared using one-way repeated measures ANOVA, with post hoc pair-wise comparisons using Bonferroni correction. Statistical significance was set at the $P < 0.05$ level.

Results

All subjects completed 3 trials, and there were no significant differences in body mass, body fat and fat free mass among the trials. During exercise, energy expenditure was similar, but oxidation of fat and carbohydrate were different among the trials ($P < 0.01$). Fat oxidation during exercise in descending order were exercise performed before breakfast (AM: 481 ± 41), two split sessions before breakfast and after lunch (AM/PM: 279 ± 22) and after lunch (PM: 131 ± 5 kcal/100min). Conversely, carbohydrate oxidation during exercise in descending order were PM (1189 ± 45), AM/PM (992 ± 50) and AM (815 ± 52 kcal/100min) (Fig 1).

Accumulated 24-h energy expenditure was similar among the trials (AM: 3540 ± 124 , AM/PM: 3525 ± 128 , PM: 3487 ± 120 kcal/24-h, $P = 0.15$) and it was balanced with energy intake, i.e. energy balance over 24-h was indistinguishable from 0 and similar among experimental conditions (AM: $+4 \pm 74$, AM/PM: $+19 \pm 60$, PM: $+58 \pm 60$ kcal/24-h, $P = 0.15$). Accumulated 24-h fat oxidation in descending order were AM (1142 ± 97), AM/PM (809 ± 88) and PM (608 ± 46 kcal/24-h), and that of AM was significantly higher than that of AM/PM and PM exercise condition ($P < 0.01$). Contrary, accumulated 24-h carbohydrate oxidation in descending order were PM (2558 ± 110), AM/PM (2374 ± 114) and AM (2062 ± 96 kcal/24-h) ($P < 0.01$). Urinary nitrogen excretion was not significantly different among the 3 trials (AM: 13.1 ± 1.2 , AM/PM: 13.3 ± 1.5 , PM: 12.5 ± 1.3 g/day, $P = 0.39$).

Although energy intake and expenditure over 24-h was matched in all experimental conditions, time course of relative energy balance was different during the first half of day 2 (6:00-18:00, Fig 2). Exercise performed before breakfast decreased relative energy balance to -1460 ± 49 kcal, while the

corresponding value for the trial with exercise performed after lunch was -219 ± 11 kcal. Twenty-four-hour averages of relative energy balance were positive and different among exercise conditions (AM: 100.1 ± 49.4 , AM/PM: 347.7 ± 37.9 , PM: 652.9 ± 42.3 kcal, $P < 0.01$). Nadir ($r = -0.72$, $P < 0.01$) and 24-h average ($r = -0.52$, $P < 0.01$) of relative energy balance were negatively correlated with 24-h fat oxidation (Fig 3). Relative carbohydrate balance during the first half of day 2 in descending order were PM, AM/PM and AM, but it was in opposite order during the second half of day 2, i.e. AM, AM/PM and PM in descending order. Nadir of relative carbohydrate balance was negatively correlated with 24-h fat oxidation ($r = -0.40$, $P < 0.05$), but its average over 24-h was not significantly correlated with 24-h fat oxidation ($r = 0.35$, $P = 0.07$). Time course of fat balance was different throughout 24-h indirect calorimetry, and values in descending order were PM, AM/PM and AM.

The day after exercise session (day 3), subjects followed the same protocol from getting up at 6:00 until exiting the chamber at 16:00. During the 10-h of day 3, total energy expenditure (AM: 915 ± 55 , AM/PM: 923 ± 47 , PM: 910 ± 41 kcal/10h, $P > 0.7$), accumulated carbohydrate oxidation (AM: 565 ± 23 , AM/PM: 557 ± 26 , PM: 532 ± 216 kcal/10h, $P = 0.09$) and accumulated fat oxidation (AM: 175 ± 21 , AM/PM: 191 ± 20 , PM: 216 ± 25 kcal/10h, $P = 0.17$) were not significantly different among 3 trials. Also, urinary nitrogen excretion during the 10-h was not significantly different among 3 trials (AM: 6.9 ± 1.4 , AM/PM: 6.9 ± 0.7 , PM: 6.4 ± 0.4 g/10-h, $P > 0.7$).

There were no significant differences in overall mean heart rate (AM: 69 ± 2 , AM/PM: 70 ± 2 , PM: 69 ± 2 beats/min, $P > 0.4$) and in activity counts (AM: 83 ± 10 , AM/PM: 73 ± 7 , PM: 80 ± 8 counts/min, $P > 0.5$) during the day 2 among the 3 trials.

Discussion

Fat oxidation during exercise is suppressed if carbohydrate is ingested before exercise (3, 7, 8, 9, 18, 27, 38), and the suppressive effect of carbohydrate ingestion on fat oxidation persists at least for 4-h after a meal (27). Consistent with literatures, fat oxidation during exercise was higher when

exercise was performed in the postabsorptive state (AM) compared with that during exercise performed after lunch (PM). However, it was possible that the differences in substrate oxidation during exercise could be offset during post-exercise period as suggested in some experimental condition (24, 25). The main finding of the present study is that 24-h fat oxidation was higher when exercise was performed before breakfast (AM) compared with exercise performed after lunch (PM), and the difference in 24-h fat oxidation between the two exercise protocols (1142 ± 97 vs. 608 ± 46 kcal/24-h, +92 %) was highly significant ($P < 0.01$). When exercise was split into two sessions, before breakfast and after lunch (AM/PM), accumulated fat oxidation was a value between 24-h fat oxidation of the other experimental conditions (809 ± 88 kcal/24-h). In our previous study, difference in 24-h fat oxidation was modest between exercising conditions (+18 %); exercise for 60 min at 50% of $\dot{V}O_2\text{max}$ performed before breakfast and that performed after breakfast (35). Increased exercise volume and/or wider difference in exercise timing in the present study may have highlighted the effect of exercise performed before breakfast on 24-h fat oxidation.

Time course of energy expenditure revealed a prolonged increase in energy expenditure after a bout of exercise. During 3-h from 9:00 to 12:00, energy expenditure in AM trial, i.e. post-exercise period (330 ± 15), was higher than that of PM, i.e. resting metabolic rate (280 ± 12 kcal/3-h, $P < 0.01$). Similarly, energy expenditure from 18:30 to 21:30 was higher in PM trial (342 ± 13) than that of AM (307 ± 13 kcal/3-h, $P < 0.01$). However, energy expenditure during sleep (AM: 471 ± 18 vs. PM: 470 ± 18 kcal/7-h, $P > 0.9$) and the day after the exercise session (915 ± 55 vs. 910 ± 41 kcal/10-h, $P > 0.7$) were indistinguishable between experimental conditions suggesting that there was little residual effect of exercise on energy expenditure.

Exercise performed before breakfast induced transient energy deficit, and it was related to 24-h fat oxidation. Nadir and average of relative energy balance was negatively correlated with 24-h fat oxidation. This finding was strengthened by correlation analysis of pooled data of our previous (35) and present study. Since volume of exercise and energy flux were different between the two studies, 24-h RQ was used as index of 24-h fat oxidation. Combined data clearly showed that lower average

of relative energy balance was related to lower RQ ($r=0.61$, $P<0.01$) (Fig 4). Hence, the transient energy deficit was implied as a factor, which increases 24-h fat oxidation, even when energy intake and expenditure over 24-h is matched.

Nadir of relative carbohydrate balance in descending order was PM (-517 ± 39 kcal), AM/PM (-536 ± 34 kcal) and AM (-879 ± 56 kcal) exercise condition, and the difference was statistically significant ($P<0.01$). The transient decrease in relative carbohydrate balance in AM exercising trial was roughly equivalent to 38% of the whole body glycogen storage, assuming that whole body glycogen store in a postabsorptive state is ~ 2300 kcal (16). Consistently, a study using magnetic resonance spectroscopy reported that exercise (at 70% of $\dot{V}O_{2\max}$ for 83 min) performed before breakfast depleted liver and skeletal muscle glycogen by 51 and 55 %, respectively (6). Modest but significant negative correlation between nadir of relative carbohydrate balance and 24-h fat oxidation ($r=-0.40$, $P<0.05$) suggests that differences in exercise induced glycogen depletion affects accumulated fat oxidation over 24-h.

Cellular energy deficit is sensed by AMP-activated protein kinase (AMPK)(15). When activated, AMPK increases fatty acid oxidation in skeletal muscle (38). AMPK activation by exercise is higher in the fasted state compared with fed state (9), and recent studies provided a potential molecular link between glycogen depletion and AMPK activation (31). Since exercise-induced AMPK activation persists during the post-exercise period for at least 150 min (36), it is plausible that transient energy deficit after exercise performed in a postabsorptive state activates AMPK to increase 24-h fat oxidation.

Although the present study did not address the following issues, fat oxidation is influenced by circulating substrates and hormones, such as glucose (1) and insulin (39). Moreover, higher plasma FFA level in a postabsorptive state favors fat oxidation by increasing substrate availability. Furthermore, a state of negative energy balance such as prolong fasting (29) and exercise (28) increases the unsaturated/saturated ratio of plasma FFA, which also stimulates fat oxidation (33). The role of these circulating factors to mediate interaction between exercise and nutritional status

affecting 24-h fat oxidation remains to be studied.

To evaluate translational potential of the present study, some considerations are required. The present study compared the effect of a single bout of exercise performed at different time of the day on 24-h fat oxidation with energy-balanced condition, and findings of the present study can't be extrapolated to the chronic effects of the postabsorptive exercise to reduce body fat. Since carbohydrate storage capacity of the body is limited, if exercise performed before breakfast oxidizes more fat and saves carbohydrate in energy-balanced condition, positive carbohydrate balance would eventually be counterbalanced by increase in its oxidation. Alternatively, in free-living conditions, the expanded glycogen storage may inhibit subsequent energy intake, since carbohydrate balance is a strong predictor of subsequent ad libitum food intake (13, 30). Finally, in the present study, subjects run 21.0 ± 0.5 km (13.0 ± 0.3 miles), which was the reason young male athletes were recruited. To generalize the present results, studies with subjects of limited capacity to perform endurance exercise, such as middle-aged obese subjects, are required. Furthermore, sex difference in fat oxidation during and after exercise (17) warrants studies with female subjects.

In conclusion, even in energy balanced condition, 24-h fat oxidation is increased if exercise-induced transient energy deficit is significant, which is likely to be observed during exercise performed before breakfast.

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314 **Disclosures**

315 All authors do not have conflicts of interest.

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Figure legends

Fig 1. Time course of energy expenditure and substrate oxidation.

Energy expenditure (a), carbohydrate oxidation (b) and fat oxidation (c) were calculated as kcal/min. Plots are mean $\pm$ SE at every 30 min for trial with a session of 100 min exercise before breakfast ($\circ$), after lunch ($\bullet$) and two sessions of 50 min exercise before breakfast and after lunch ($\odot$).

Fig 2. Time course of relative energy balance and substrate balance.

Energy (a), carbohydrate (b) and fat (c) balance were calculated by setting the initial reference value at 6:00 of day 2. Means for trial with a session of 100 min exercise before breakfast ($\circ$), after lunch ($\bullet$) and two sessions of 50 min exercise before breakfast and after lunch ($\odot$) were plotted at every 30 min.

417

418 Fig 3. Relation between relative energy balance and 24-h fat oxidation.

419 Relations of 24-h fat oxidation to average ($r = -0.52$, $P < 0.01$) and nadir ($r = -0.72$, $P < 0.01$) of
420 relative energy balance were plotted on panel (a) and (b), respectively. All 9 subjects completed 3
421 trials with different exercise protocol; a session of 100 min exercise before breakfast ($\circ$), after lunch
422 ($\bullet$) and two sessions of 50 min exercise before breakfast and after lunch ($\bullet$).

423

**424 Fig 4. Relation between relative energy balance and respiratory quotient in combined data
425 pool.**

426 All 9 subjects in the present study completed 3 trials with different exercise protocol; session of 100
427 min exercise before breakfast ($\circ$), after lunch ($\bullet$) and two sessions of 50 min exercise before
428 breakfast and after lunch ($\bullet$). In our previous study (35), 12 male subjects completed 2 trials with
429 different exercise protocol; session of 60 min exercise at 50% of $\dot{V}O_{2\max}$ before breakfast (Δ) and
430 after breakfast ($\blacktriangle$). Correlation coefficient between 24-h average of relative energy balance and
431 respiratory quotient was 0.61 ($P < 0.01$).







