Enhancement of fat metabolism by repeated bouts of moderate endurance exercise

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ENDURANCE EXERCISE IS A POTENT STIMULUS TO improve cardiovas-

cular fitness and prevent obesity and several obesity-related diseases: diabetes, hypertension, and hyperlipidemia (2, 19). According to recent guidelines published by the American College of Sports Medicine, moderate to vigorous [~45–85% of oxygen uptake (V̇O2) reserve] endurance exercise with a longer training duration (>20 min) is recommended for most individuals (1, 19). In addition, recommendations for treatment of obesity have been made to ensure greater overall energy expenditure, and prolonged exercise (duration of 45–60 min) has been shown to be desirable (1, 10, 25). Therefore, more emphasis has commonly been placed on extension of exercise duration in an exercise prescription for fat reduction or controlling body mass.

The major stimulus for lipolysis during endurance exercise is circulating catecholamine in combination with a low insulin concentration (29). Some researchers have specifically examined hormonal responses to repeated bouts of endurance exercise on the same day. Those results show that the exercise-induced increases in epinephrine (Epi), norepinephrine (NE), and growth hormone (GH) concentrations were higher during the second exercise bout compared with the first bout (12, 22). Conversely, insulin with antilipolytic action showed a lower value during the second bout of endurance exercise (30). It has been demonstrated that the interleukin (IL)-6 response, which stimulates lipolysis (15, 33), was augmented during the second bout of endurance exercise preceded by the same exercise and an intervening 3-h rest (24). In addition, Stich et al. (30) reported that a second bout of endurance exercise causes enhanced elevation of the free fatty acid (FFA) level and fat oxidation [expressed by a lower respiratory exchange ratio (RER)] during the exercise. These results indicate that a prior bout of endurance exercise augments fat metabolism during a subsequent second bout of the exercise. However, most studies (12, 30) have compared metabolic responses between the bouts of exercise (e.g., first bout vs. second bout). To our knowledge, no studies have compared fat metabolism and hormone responses between “a single bout of prolonged exercise” and “repeated bouts of exercise” of the same total exercise duration.

The aim of the present study was to compare the fat metabolism between “a single bout of prolonged exercise (60 min)” and “two repeated bouts of exercise (two bouts of 30-min exercise)” of equivalent intensity [60% maximal oxygen uptake (V̇O2peak)] and total exercise duration (60 min). We hypothesized that repeated bouts of exercise cause greater lipolysis and fat oxidation during the exercise compared with a single bout of prolonged exercise.

METHODS

Subjects. Eight healthy men (means ± SE: 25.3 ± 0.8 yr; height 176.6 ± 1.4 cm, body mass 66.1 ± 1.1 kg, percent fat 17.6 ± 1.5%) participated in this study. All subjects were physically active and accustomed to physical exercise. However, data of one subject were excluded because he was not able to complete all exercise trials (n = 7). Subjects were informed about the experimental procedure and the purpose of this study. Subsequently, their written, informed consent was obtained. The study was approved by the Ethics Committee for Human Experiments of the University of Tsukuba.

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Exercise regimen. Subjects visited the laboratory four times through the experimental period. During the first visit, the subjects’ Vo2 peak was assessed using a graded power test on a cycle ergometer (828E, Monark Exercise). The test began at 60 W; the load was increased progressively at 30-W increments every 2 min until exhaustion. The test was terminated when the subject failed to maintain the prescribed pedaling frequency of 60 rpm or reached the plateau of Vo2. Respiratory gases were collected and analyzed using an automatic gas analyzer (Oxycon-Alpha, Mijnhardt, The Netherlands). The collected data were averaged every 30 s. Each subject’s heart rate (HR) was also measured continuously using a wireless HR monitor (Acculex Plus; Polar Electro Oy). During visits 2–4, the experimental trials (three trials) were conducted.

In a random order, all subjects participated in three trials separated by ~14 days: 1) a trial with a single bout of 60-min exercise (Single); 2) a trial with two repeated bouts of 30-min exercise, separated by a 20-min rest (Repeated); and 3) complete rest (Control) (Fig. 1). These trials were performed between 7:00 and 12:00 after an overnight fast. Endurance exercise was performed with a cycle ergometer at ~60% of subject’s Vo2 peak. Workload at 60% of Vo2 peak was determined using linear regression of data obtained during Vo2 peak testing. The pedaling frequency was set at 60 rpm. In the Single and Repeated trials, the subjects conducted the prescribed endurance exercise for a total of 60 min. Subsequently, the subjects rested on a comfortable chair for 60 min to determine the metabolic response during the recovery period (80–140 min in Fig. 1). In the Control trial, they rested on the same chair throughout the experimental period (0–140 min). In the Repeated trial, the rest period of 20 min between exercise bouts was determined based on the results of pilot study and practical point of view for exercise prescription. The exercise intensity at 60% of Vo2 peak was chosen because metabolic acidosis caused by higher intensity exercise might interfere with the calculation of substrate oxidation pattern using indirect calorimetry (8). In addition, exercise intensity of ~60% of Vo2 peak is commonly recommended in exercise prescriptions for most individuals (1). The endurance exercise bout in each trial was performed at almost the same time of day to avoid diurnal variations of hormone and metabolic responses. In addition, the start time of the second half of 30-min exercise was matched between Single and Repeated trials (at 50-min point in the Fig. 1).

Room temperature was maintained at 23°C throughout the experiment.

Blood and gas analyses. Following overnight fasting, the subjects arrived at the laboratory and rested for 30 min before the first blood collection. Venous blood samples were obtained from an indwelling cannula in the antecubital vein at 15-min intervals during exercise and 30-min intervals during the recovery period. In the Repeated trial, an additional blood sample was collected at the beginning of the second bout of exercise (at 50 min in Fig. 1). In the Control trial, only three blood samples (at 0, 80, 140 min in Fig. 1) were collected.

Blood samples for the measurements of hormones and metabolites were stored frozen at −85°C until analyses. Plasma glucose concentration was analyzed using an enzymatic method; the interassay and intra-assay coefficients of variation (CVs) were 1.0 and 0.7%, respectively. Concentrations of Epi and NE were measured using high-performance liquid chromatography with kits from Tosoh. Sensitivity of these assays, and interassay and intra-assay CVs were 6.0 pg/ml and 2.7 and 2.0% for Epi, and 6.0 pg/ml and 2.4 and 1.3% for NE, respectively. Serum GH concentration was measured using radioimmunoassay with kits from SRL. The GH assay sensitivity was 0.04 ng/ml; the interassay and intra-assay CVs were 4.1 and 3.4%, respectively. Serum insulin concentration was measured using a commercially available kit (Eiken Chemical). The insulin assay sensitivity was 1.01 μU/ml; the interassay and intra-assay CVs were 2.0 and 5.0%, respectively. Serum cortisol concentration was measured using radioimmunoassay with kits from Immunotech. The cortisol assay sensitivity was 1.0 μg/dl, and the interassay and intra-assay CVs were 3.9 and 4.9%, respectively. Serum FFA and ketone body concentrations were measured using enzymatic method (Eiken Chemical, Kainos Laboratory). These interassay and intra-assay CVs were 0.7 and 0.2% for FFA, and 2.9 and 3.5% for ketone body, respectively. Serum glycerol concentration was measured using an enzymatic colorimetric method with kits from Wako Pure Chemical Industries. These interassay and intra-assay CVs were <5.0%. Whole blood lactate concentration and pH were measured immediately after blood collection. The blood lactate concentration was determined using an automatic lactate analyzer (YSI 1500 Sport; Yellow Springs Instrument). Blood pH was also measured using an automatic blood-gas analyzer (model 248; Bayer).

Respiratory gas was collected continuously to determine Vo2, carbon dioxide production, and ventilatory volume throughout the experimental period. All respiratory variables were averaged in each 5-min period at identical time points of blood sampling (Fig. 1) [Repeated trial: 5–0 (baseline), 10–15, 25–30, 45–50, 60–65, 75–80, 105–110, 135–140 min in Fig. 1; Single trial: 15–20 (baseline), 30–35, 45–50, 60–65, 75–80, 105–115, and 135–140 min in Fig. 1]. The RER was determined from Vo2 and carbon dioxide production measurements. It was used to estimate the relative contribution of fat oxidation to the total energy expenditure (%fat contribution) (16). The RER and %fat contribution were estimated without urinary nitrogen analysis because of the negligible contribution of protein to substrate oxidation during exercise (3). Appropriate calibrations of the O2 and CO2 sensors and the volume transducer were conducted before the start of exercise. The HR was monitored continuously throughout the experimental period. During exercise, the ratings of perceived exertion (RPE) were determined every 15 min using a Borg 15-point rating scale (4).

Statistical analysis. Data are expressed as means ± SE for all variables. Because of difference of blood sampling points among the Single (7 points), the Repeated (8 points), and the Control trials (3 points), one-way ANOVA procedures with repeated measures was applied. For comparison of data during the first half (0–30 min in Repeated, 20–50 min in Single) and second half of 30-min exercise (30–80 min) and the 60-min recovery period (80–140 min), a one-way ANOVA procedure with repeated measures was used to determine differences between trials. Post hoc testing was performed using Fisher’s protected least significant difference test. For comparison of data over the experimental period in each trial (vs. baseline values), a one-way ANOVA with repeated measures was applied. P < 0.05 was considered significant.

RESULTS

Circulating metabolites. Figure 2 shows changes in blood lactate and plasma glucose concentrations. Lactate concentration increased significantly during the first half of 30-min exercise in the Single and Repeated trials. During the second
half of 30-min exercise and 60-min recovery period, no significant difference was observed at any time point between trials. During the Control trial, no significant difference was observed over the experimental period. Plasma glucose concentration decreased significantly during the first half of exercise in the Single and Repeated trials ($P < 0.05$). During the second half of exercise, the Repeated trial showed significantly lower glucose concentration than the Single trial ($83 \pm 1$ vs. $98 \pm 2$ mg/dl, $P < 0.05$). During the 60-min recovery period, no significant difference was observed between trials. In the Control trial, the glucose concentration decreased gradually ($P < 0.05$) during the experimental period.

FFA concentration at the beginning of the second half of exercise (=50 min) between Repeated ($0.79 \pm 0.11$ meq/l) and Single trials ($0.34 \pm 0.08$ meq/l, $P < 0.05$). During the second half of exercise, FFA remained at higher values in the Repeated trial. At the 80-min point (the end of exercise), it showed a significantly higher value in the Repeated trial ($0.88 \pm 0.09$ meq/l) than in the Single trial ($0.54 \pm 0.11$ meq/l, $P < 0.05$). During the 60-min recovery period, the FFA remained at significantly higher values in the Repeated trial than in the Single trial ($P < 0.05$). In the Control trial, no significant change was observed during the experimental period.

Glycerol concentration increased significantly during the first half of exercise in the Single and Repeated trials. During the second half of exercise, it showed a greater increase during the last 15-min phase (65–80 min) in the Repeated trial. During the 60-min recovery period, glycerol concentration showed significantly higher values in Repeated trial (at 140-min point: $9.7 \pm 1.2$ mg/dl) than in the Single trial ($6.4 \pm 0.7$ mg/dl, $P < 0.05$). In the Control trial, no significant change was observed during the experimental period.

Figure 3 shows changes in serum FFA and glycerol concentrations. Concentrations of acetoacetate-
tate and 3-hydroxybutyrate did not change during the first half of exercise in the Single and Repeated trials. However, in the Repeated trial, they increased during the subsequent 20-min rest period. Consequently, significant differences were observed in acetoacetate and 3-hydroxybutyrate concentrations at the beginning of the second half of exercise between Repeated (acetoacetate: 91.1 ± 23.5 μmol/l, 3-hydroxybutyrate: 195.7 ± 51.1 μmol/l) and Single trials (acetoacetate: 23.0 ± 4.6 μmol/l, P < 0.05, 3-hydroxybutyrate: 36.1 ± 6.5 μmol/l, P < 0.05). During the second half of exercise, no significant difference was observed between trials. During the 60-min recovery period, Repeated trial showed significantly higher values in 3-hydroxybutyrate concentrations than in the Single trial (P < 0.05). In the Control trial, no significant difference was observed in acetoacetate and 3-hydroxybutyrate concentrations during the experimental period.

Concentrations of NE increased significantly during exercise in the Single and Repeated trials, but no significant difference was observed between the trials, except for values at the beginning of the second half of exercise. In the Control trial, NE concentration was almost constant, but showed a significant increase only at the 80-min point (P < 0.05).

Figure 6 shows changes in serum insulin, GH, and cortisol concentrations. Serum insulin concentration decreased progressively during the first half of exercise in the Single and Repeated trials. During the second half of exercise, insulin concentration was significantly lower in the Repeated (1.8 ± 0.3 μU/ml) than in the Single trial (2.9 ± 0.4 μU/ml, P < 0.05, at 65-min point). During the 60-min recovery period, no significant difference was observed between the trials.

Serum GH concentration increased significantly during exercise in the Single and Repeated trials (P < 0.05), but no significant difference was observed at any points between the
During the recovery period, no significant difference was observed in GH concentrations between the trials. In the Single and Repeated trials, serum cortisol concentration did not change significantly during exercise, except for the value at the end of exercise (80 min) in the Single trial. During the recovery period, cortisol concentration in the Single and Repeated trials decreased, but no significant difference was observed between the trials. In the Control trial, cortisol concentration decreased progressively, and significant differences (vs. preexercise value) were observed at the 80-min and 140-min points.

**Respiratory measurements, HR, and RPE.** No significant difference was observed in V\(_{O2}\) at preexercise between Single and Repeated trials. There was no significant difference in the V\(_{O2}\) between Single and Repeated trials during the first half [Single, 2.15 ± 0.10 vs. Repeated, 2.28 ± 0.09 l/min, not significant (NS)] and second half of exercise bouts (Single, 2.30 ± 0.15 vs. Repeated, 2.33 ± 0.10 l/min, NS). During the recovery period after exercise (105–110 min), V\(_{O2}\) showed a greater value in the Repeated trial (0.34 ± 0.01 l/min) than in the Single trial (0.31 ± 0.02 l/min, \(P = 0.09\)). Before the exercise, relative contribution of fat oxidation to the total energy expenditure (%fat contribution) was slightly, but significantly higher in the Repeated trial than in the Single trial (\(P < 0.05\)). During the exercise, values of %fat contribution showed no significant difference between Single and Repeated trials. However, the Repeated trial showed significantly higher values (76.6 ± 10.0%) than the Single trial (55.9 ± 11.2%, \(P < 0.05\)) during the recovery period (105–110 min). The enhanced fat oxidation in the Repeated trial remained partly, even at 60 min after the exercise (135–140 min, \(P = 0.08\) vs. Single trial).

HR during exercise showed no significant difference between the Single and Repeated trials (values at 80 min, 155 ± 3 beats/min for Single trial, 155 ± 4 beats/min for Repeated trial, NS). In addition, no significant difference was observed during the recovery period.

During the exercise, RPE, which was measured every 15 min, increased gradually in the Single and Repeated trials, but no significant difference was observed at any points between the trials (values at 80 min, 16 ± 2 for Single trial, 16 ± 2 for Repeated trial, NS).

**DISCUSSION**

Concentrations of FFA and glycerol increased progressively during the 60 min of exercise in the Single trial (\(P < 0.05\)). In the Repeated trial, it was interesting that concentrations of FFA, acetoacetate, and 3-hydroxybutyrate increased markedly during the intervening 20-min rest after the first bout of exercise (\(P < 0.05\)). It is possible that the cessation of exercise created an imbalance between the supply and demand of fatty acids (2), causing a rapid elevation of FFA concentration. In addition, the Repeated trial showed pronounced increases in FFA and glycerol concentrations during the final 15 min of the second exercise bout, although no significant difference was observed in respiratory responses during the exercise between the Repeated and Single trials. Therefore, the present results indicate that lipolysis during the second half of exercise is augmented strongly in the Repeated trial. The finding that repeated bouts of exercise showed greater exercise-induced lipolysis than in the single bout of prolonged exercise is novel and has not been reported before.

During the second half of the exercise bout, the Epi response was significantly greater in the Repeated trial than in the Single trial. Conversely, the Repeated trial showed a rapid decrease in insulin concentration during the second exercise bout. Combinations of higher Epi and lower insulin levels might augment exercise-induced lipolysis in the Repeated trial. Moreover, the lower plasma glucose during the second half of exercise might be involved in increased lipolytic action. It is well known that even small changes in blood glucose have great impact on the
Epi and insulin responses during exercise (7, 9, 13). Therefore, the lower glucose level in the Repeated trial might enhance lipolysis during the second half of the exercise bout. The present results, which indicate enhanced Epi and lipolytic responses by repeated bouts of exercise, are consistent with those of previous studies. For example, Stich et al. (30) showed that local glycerol elevation in adipose tissue during repeated bouts of exercise was augmented during a second bout of exercise. Another study with repeated exercise at higher exercise intensity (75% of VO2 peak) demonstrated more pronounced Epi and NE responses to a second bout of exercise on the same day (22).

During the 60-min recovery period, serum FFA and glycerol concentrations were significantly higher in the Repeated trial than in the Single trial. It was also noteworthy that the Repeated trial showed a much elevated 3-hydroxybutyrate concentration during the recovery period, suggesting enhancement of fatty acid oxidation in the liver. Clearly, circulating hormones such as Epi and insulin do not affect the delayed enhancements of FFA and glycerol levels, because concentrations of these hormones decreased completely during the recovery period. Alternatively, a previous study has shown that the GH response to exercise is associated with a postexercise rise in lipolysis (34). However, no significant difference was observed in the magnitude of exercise-induced GH response between Single and Repeated trials, suggesting that GH is not a factor for greater lipolysis during the recovery period in the Repeated trial. In more recent studies, evidence exists that IL-6 stimulates lipolysis in humans (31, 33). In addition, a second bout of endurance exercise on the same day causes a more pronounced IL-6 response than the first bout of exercise (24). Although we were unable to determine circulating IL-6 concentration, there is a possibility that more elevated IL-6 in the Repeated trial is associated with increased lipolysis during the recovery period.

We hypothesized that the Repeated trial would cause greater lipolytic response and concomitant augmentation of fat oxidation during the exercise. However, no significant difference was observed in %fat contribution during the exercise between the trials, even though Repeated trial showed significantly higher FFA and ketone body concentrations at the beginning of the second half of the exercise bout. The results are consistent with those of recent studies (8, 14), which have indicated that elevated FFA concentration caused by prior GH administration had no effect on fat oxidation pattern during subsequent submaximal exercise. Based on these findings, fat oxidation pattern during moderate-intensity exercise might not be strongly affected by a substantial increase in FFA concentration. This finding is also consistent with the concept that carbohydrate oxidation predominates during exercise, even with elevated concentration of fatty acids (5, 8). However, estimation by indirect calorimetry might not be sufficiently sensitive to detect slight changes in substrate oxidation pattern during the exercise.

In contrast, %fat contribution was significantly higher in the Repeated trial than in the Single trial during the recovery period (P < 0.05). In addition, this effect remained partly even at the 60-min point of the recovery period (Repeated trial vs. Single trial, P = 0.08). The increased FFA theoretically facilitates uptake of fatty acids by muscles (20, 21). Therefore, the enhanced fat oxidation during the recovery period might be caused by elevated FFA and ketone body concentrations. Whether the enhanced fat oxidation persists after the 60 min of recovery period remains unclear, but the enhancement of postexercise fat oxidation might play a role in body mass control (27, 28). On the other hand, the interpretation of this result needs precaution, because the preexercise value of %fat contribution was significantly higher in the Repeated trial.

According to the guideline of exercise prescription from the American College of Sports Medicine (1), exercise durations of 45–60 min are a typical target to ensure sufficient energy expenditure for obese people. Although a single bout of prolonged exercise is commonly conducted in exercise prescriptions, programs with repetition of shorter exercise might be preferred when prescribing exercise to sedentary people, such as overweight individuals. In addition, several studies (6, 11, 17, 26) have shown that repeated bouts of shorter exercise produce similar changes in cardiovascular fitness and weight loss compared with a single bout of prolonged exercise. Nevertheless, to our knowledge, this is the first study to compare fat metabolism during exercise between a single bout of prolonged exercise and repeated bouts of exercise of equal total exercise duration. Practical application of the current results could be in the design of exercise regimens for weight management.

In conclusion, repetition of moderate-intensity exercise caused a more pronounced exercise-induced fat mobilization compared with a single bout of prolonged exercise. In addition, the Repeated trial showed augmented FFA, glycerol, ketone body responses, and fat oxidation during the recovery period.

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