

Effects of Resistance Exercise on Lipolysis during Subsequent Submaximal Exercise

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ABSTRACT

GOTO, K., N. ISHII, S. SUGIHARA, T. YOSHIOKA, and K. TAKAMATSU. Effects of Resistance Exercise on Lipolysis during Subsequent Submaximal Exercise. *Med. Sci. Sports Exerc.*, Vol. 39, No. 2, pp. 308–315, 2007. **Purpose:** This study examined effects of prior resistance exercise on fat metabolism during subsequent submaximal exercise with different recovery periods between exercise bouts. **Methods:** Ten male subjects performed three types of exercise regimens: 1) submaximal endurance exercise only (E), 2) submaximal endurance exercise with prior resistance exercise and 20 min of rest (RE20), and 3) submaximal endurance exercise with prior resistance exercise and 120 min of rest (RE120). Resistance exercise consisted of six exercises, each with three to four sets at 10-repetition maximum. Subjects performed cycle ergometer exercise at 50% of the maximal oxygen uptake for 60 min. **Results:** Prior resistance exercise caused increases in blood lactate, plasma norepinephrine, serum growth hormone (GH), insulin, and glycerol concentrations ($P < 0.01$). Before the submaximal exercise, serum free fatty acid (FFA) concentration was higher in the RE120 than in the RE20 and E trials ($P < 0.01$), although concentrations of plasma norepinephrine, serum GH, insulin, and glycerol were higher in the RE20 than in the RE120 and E trials ($P < 0.05$). Concentrations of FFA and glycerol during the 60-min submaximal exercise were higher in the RE120 and RE20 trials than in the E trial ($P < 0.05$). No significant difference was observed in the acetoacetate and 3-hydroxybutyrate responses. In the RE20 trial, fat oxidation throughout the 60-min submaximal exercise (mean value) was greater than in the E trial ($P < 0.05$), but no significant difference was found between the RE120 and E trials. **Conclusion:** Fat availability during the submaximal exercise was enhanced by prior resistance exercise. However, augmentation of fat oxidation was observed only in the trial with shorter rest between resistance exercise and submaximal exercise bouts (RE20 trial). **Key Words:** FREE FATTY ACIDS, GLYCEROL, GROWTH HORMONE, INSULIN, SUBSTRATE OXIDATION

A training program with combined resistance and endurance exercises is widely recommended to control body weight and to maintain a healthy, independent daily life (14). Some people conduct these two types of exercise modes (bouts) on the same day, with various combinations. However, little is known about the desirable order of combined resistance and endurance exercise bouts to optimize the effect of each type of exercise. Recently, we demonstrated that growth hormone (GH) secretion after a single bout of resistance exercise was strongly attenuated by prior endurance exercise for 60 min (9). Although the role of circulating GH in muscle growth remains unclear, this result suggests that a preceding endurance exercise might impair anabolic processes after subsequent resistance exercise.

Resistance exercise is a potent stimulus for enhancing endocrine activities, resulting in acute enhancements of hormonal secretions (16). Among these hormones, catecholamine and GH have strong lipolytic effects (20,23) and are responsible for lipolysis and the gradual rise of blood concentrations of free fatty acids (FFA) and glycerol during aerobic exercise (28). Although lipolysis is not strongly stimulated during resistance exercise, exercise-induced secretions of catecholamine and GH might enhance lipolysis during the recovery period (22). In fact, resistance exercise alters the energy metabolism and causes postexercise increases in resting oxygen consumption and lipid oxidation lasting approximately 48 h (15).

An enhanced lipolysis after a preceding exercise session might affect metabolic responses during the subsequent submaximal exercise bout when two sessions of exercise are performed successively. Stich et al. (28) have shown that extracellular glycerol concentration in adipose tissue during a submaximal exercise for 60 min was augmented by higher epinephrine and lower insulin levels during exercise when the exercise bout was preceded by the same exercise with intervening 60-min rest. Christmass et al. (6) demonstrated that a 10-min bout of intensive exercise followed by a 45-min rest period modified the use of substrates during a subsequent intermittent intense exercise. Secretions of catecholamine and GH were enhanced during the second bout of exercise when a high-intensity

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endurance exercise was repeated with an intervening rest period of 3 h (26). On the basis of these findings, an exercise program with a preceding exercise bout, especially an intensive exercise such as heavy resistance exercise, would cause augmentation of fat availability during subsequent submaximal exercise. In addition, it is possible that whole-body fat oxidation during submaximal exercise might be enhanced because increased fat availability theoretically upregulates fat oxidation and down-regulates carbohydrate oxidation (24). However, no studies have been conducted of the effects of prior resistance exercise on energy metabolism during the subsequent submaximal exercise. Moreover, information about the optimal length of the recovery period between resistance and endurance exercise bouts is important to maximize the effects of each exercise bout, that is, increasing muscular size and strength and decreasing fat mass.

The purpose of the present study was to examine the impact of prior resistance exercise on lipid metabolism during subsequent submaximal endurance exercise. We hypothesized that prior resistance exercise would increase fat availability and oxidation during subsequent submaximal exercise. In addition, the influence on lipid metabolism of the length of the rest period between these two types of exercise was also investigated.

METHODS

Subjects

Ten healthy men (mean $\pm$ SD: age, 23.3 ± 1.3 yr; height, 174.6 ± 5.8 cm; body mass, 70.6 ± 7.7 kg; % fat, $21.8 \pm 3.0\%$) participated in this study. All subjects were physically active and had experience of recreational exercise training. However, none had been involved in any regular physical training program at the beginning of the study. 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.

Exercise Regimens

Subjects visited the laboratory two times before the experimental trials. During the first visit, their maximal oxygen uptake ($\dot{V}O_{2\max}$) was assessed using a graded power test on a cycle ergometer (828E, Monark, Sweden). The test began at 90 W; the load was increased progressively at 30-W increments every 3 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 $\dot{V}O_2$. Respiratory gas was collected and analyzed with an automatic gas analyzer (Oxycon-Alpha, Mijhardt, Holland) every 30 s. During the second visit, the values of one repetition maximum (1RM) for bench press, lat pulldown, shoulder press, butterfly, arm curl, and squat exercises were measured using weight-stack machines. Before measuring 1RM, the subjects performed

warm-up sets with 10 repetitions at 50 and 70% of the predicted 1RM and stretching of the major muscle groups that were subjected to the exercises. The load was increased until the subjects were unable to perform a lift.

All subjects participated in three trials separated by approximately 7 d in random order: 1) trial with only submaximal endurance exercise (E), 2) trial with submaximal endurance exercise preceded by resistance exercise and 20 min of rest (RE20), and 3) trial with submaximal endurance exercise preceded by resistance exercise and 120 min of rest (RE120) (Fig. 1). These trials were performed between 8:00 a.m. and 12:00 p.m. Sessions of resistance exercise in the RE20 and RE120 trials consisted of six consecutive exercises, each with 10 repetitions for three to four sets (three sets for the shoulder press, butterfly, arm curl and squat, and four sets for bench press and lat pulldown) at 10RM. The subjects were allowed to rest for 1 min among all sets and exercises. This protocol of resistance exercise is a typical method for inducing muscular hypertrophy (10,16). The exercise intensity during the resistance exercise was adjusted to allow the subjects to complete 10 repetitions in each set (approximately 75% of 1RM for the first set). The subjects performed the resistance exercise at the same relative intensity and number of repetitions in each set for RE20 and RE120 trials. In this study, five upper-limb exercises and only one lower-limb exercise were chosen to diminish the effects of muscular fatigue on the performance of subsequent submaximal exercise. Submaximal exercise was performed using a cycle ergometer at approximately 50% $\dot{V}O_{2\max}$ for 60 min. That low intensity was chosen because metabolic acidosis caused by higher-intensity exercise might interfere with the calculation, using indirect calorimetry, of the relative contribution of fat to the total energy expenditure (17). In addition, this exercise intensity has been often recommended for health and reduction of body fat (21). The submaximal exercise in each trial was performed at the same time of day to avoid diurnal variations of metabolic and hormonal responses. The room

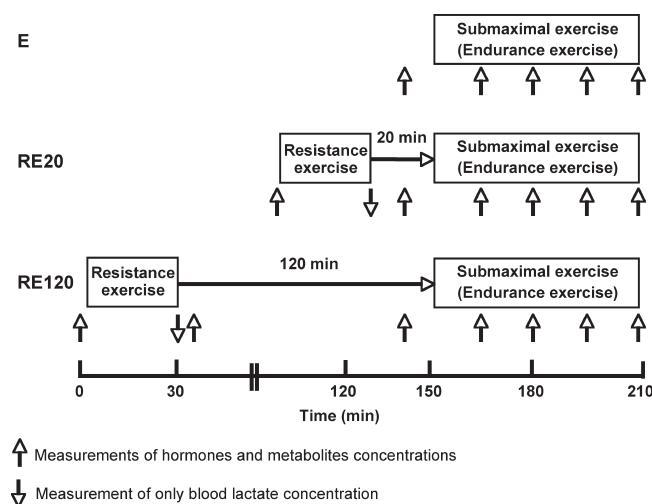


FIGURE 1—Protocols for exercise and blood sampling in three types of exercise regimens.

temperature was maintained at 25–26°C throughout the experiment.

Blood and Gas Analyses

After an overnight fast, the subjects visited the laboratory and rested for 30 min before the first blood collection. Venous blood samples (~10 mL) were obtained from an indwelling cannula in the antecubital vein before the resistance exercise, 0 min (immediately after exercise), 10 min after the resistance exercise (for RE20 and RE120 trials), before the submaximal exercise (for RE120 and E trials), and at the time points of 15, 30, 45, and 60 min during the submaximal exercise (Fig. 1). Regarding blood samples at 0 min after the resistance exercise (for RE20 and RE120 trials), only blood lactate concentration was determined. In the RE20 trial, the blood sample at 10 min after the resistance exercise was regarded as that before the submaximal exercise.

Blood samples for measurements of plasma glucose, epinephrine (Epi) and norepinephrine (NE), serum growth hormone (GH), insulin, free fatty acids (FFA), glycerol, acetoacetate, and 3-hydroxybutyrate concentrations were stored frozen at –85°C until analyses. These concentrations were measured throughout the exercise except for plasma Epi and NE concentrations. Because of the limited plasma sample volume, concentrations of Epi and NE were determined only before the resistance exercise, 10 min after resistance exercise, and before the submaximal exercise (10 min after the resistance exercise in the RE20 trial) in the RE120 and RE20 trials. In the E trial, these were determined only before the submaximal exercise.

Concentrations of Epi and NE were measured with high-performance liquid chromatography using kits from Tosoh Corp., Japan. Sensitivity of these assays, and interassay and intraassay coefficients of variation (CV) were 32.8 pM, 2.7 and 2.0% for Epi; and 0.04 nM, 2.4 and 1.3% for NE, respectively. Serum GH concentration was measured with radioimmunoassay (RIA) using kits from SRL Inc., Japan. The GH assay sensitivity was 0.04 ng·mL⁻¹, and the respective interassay and intraassay CV were 4.0 and 3.4%. Serum insulin concentration was measured with enzymic immunoassay using kits from Eiken Chemical Co., Ltd., Japan. The assay sensitivity was 6.9 pM, and the interassay and intraassay CV were 2.0 and 5.0%. Plasma glucose concentration was measured using an enzymatic method, and the interassay and intraassay CV were 0.6 and 1.2%. Serum FFA and ketone body concentrations were measured using an enzymatic method. These interassay and intraassay CV were 0.2 and 0.9% for FFA and 0.6 and 0.7% for ketone body, respectively. Serum glycerol concentration was measured using an enzymatic colorimetric method with kits from Wako Pure Chemical Industries Ltd., Japan. These interassay and intraassay CV were less than 5.0%. Blood samples were also obtained from the fingertip to measure lactate concentration using an automatic lactate analyzer (YSI 1500 Sport; Yellow Springs Instrument Co., Inc.).

During the entire 60-min submaximal exercise, respiratory gas was collected continuously to determine $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), and ventilatory volume. The respiratory exchange ratio (RER) was determined from $\dot{V}O_2$ and $\dot{V}CO_2$. It was used to estimate the relative contribution of fat oxidation to the total energy production (% fat oxidation) (18). The RER and % fat oxidation were estimated without urinary nitrogen analysis because of the negligible contribution of protein to the substrate oxidation during exercise (3). Data for the respiratory gas analysis were averaged every 30 s. The O_2 and CO_2 analyzer were calibrated with room air and gas of a known CO_2 concentration before each test. A heart rate monitor (Vantage XL; Polar) was used to monitor the heart rate (HR) during the submaximal 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 $\pm$ SE unless otherwise stated. For comparisons of blood parameters during the submaximal exercise, a two-way (trial $\times$ time) analysis of variance (ANOVA) with repeated measures was used, followed by Tukey's *post hoc* test. For oxygen-uptake and substrate-oxidation (% fat) data, a one-way ANOVA with repeated measures and Tukey's *post hoc* test was applied. The areas under the concentration–time curve (AUC) were calculated using a trapezoidal method for 60 min during submaximal exercise. $P < 0.05$ was considered significant.

RESULTS

Circulating Hormones and Metabolites

Figure 2 shows acute changes in plasma glucose and blood lactate concentrations. No significant change was apparent in glucose concentration throughout the exercise bout in all trials, except for after resistance exercise in the RE120 trial ($P < 0.01$). During the submaximal exercise, glucose showed slightly higher concentrations in the RE120 trial, with significant differences at 30- and 45-min points when compared with other trials ($P < 0.01$). Blood lactate concentration showed significant increases immediately after the resistance exercise in the RE20 and RE120 trials ($P < 0.01$). At the beginning of the subsequent submaximal exercise, significant differences were observed between the lactate concentration in the RE20 (6.1 ± 0.5 mM) and those in both the RE120 (0.8 ± 0.1 mM) and the E trials (0.5 ± 0.1 mM, $P < 0.01$). During the submaximal exercise, significant differences remained throughout the 60-min period of exercise between trials ($P < 0.01$).

Plasma Epi and NE concentrations increased 10 min after the resistance exercise in both the RE120 (Epi: 425.3 ± 190.1 (preexercise) to 540.0 ± 86.1 pM (10 after exercise), NE: 1.7 ± 0.2 to 3.8 ± 0.5 nM, $P < 0.01$) and RE20 trials (Epi: 270.8 ± 38.9 to 393.1 ± 68.4 pM, NE: 1.9 ± 0.2 to 3.9 ± 0.4 nM, $P < 0.01$). In the RE120 trial, they

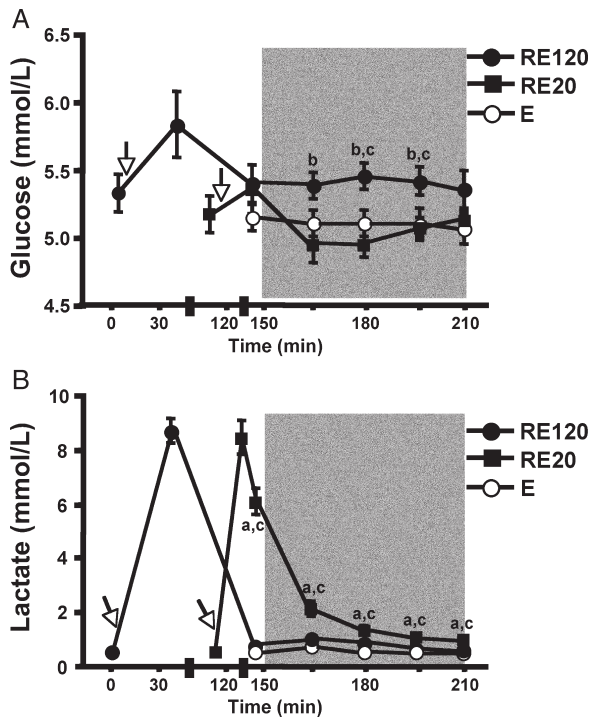


FIGURE 2—Plasma glucose (A) and blood lactate (B) concentrations during the three exercise regimens. Values are means $\pm$ SE. a, $P < 0.05$ compared with RE120 trial; b, $P < 0.05$ compared with RE20 trial; c, $P < 0.05$ compared with E trial. Blood lactate concentration at 10 min after resistance exercise in the RE120 trial was not included in the figure. Arrows in the graph indicate the time points of resistance exercise in the RE120 and RE20 trials. Submaximal exercise is denoted as a shaded area.

reverted to their resting levels during the recovery period. Consequently, significant ($P < 0.01$) differences were found in pre-submaximal exercise concentrations of Epi and NE between the RE20 (Epi: 393.1 ± 68.4 pM, NE: 3.9 ± 0.4 nM) and E trials (Epi: 177.5 ± 26.5 pM, NE: 1.8 ± 0.2 nM).

Figure 3 shows acute changes in serum growth hormone and insulin concentrations. In the RE120 and RE20 trials, GH concentration showed marked increases after a bout of resistance exercise ($P < 0.01$). In the RE120 trial, they returned to their resting levels until the beginning of the subsequent submaximal exercise. Consequently, significant differences were observed in pre-endurance exercise concentrations of GH between the RE20 (18.3 ± 2.9 ng·mL $^{-1}$) and both the RE120 (0.3 ± 0.1 ng·mL $^{-1}$) and E trials (0.6 ± 0.2 ng·mL $^{-1}$, $P < 0.01$). During the submaximal exercise, GH concentration increased gradually in the E trial ($P < 0.01$), although it decreased progressively in the RE20 trial ($P < 0.01$). In the RE120 trial, the response of GH was attenuated significantly when compared with that of the E trial ($P < 0.01$).

Insulin concentration showed marked increases after a bout of resistance exercise in both the RE120 and RE20 trials ($P < 0.01$). Consequently, significant differences were observed in pre-submaximal exercise concentrations of insulin between the RE20 (98.1 ± 15.2 pM) and both the RE120 (59.6 ± 5.5 pM, $P < 0.05$) and E trials (41.7 ± 5.0

pM, $P < 0.01$). During the submaximal exercise, insulin concentrations decreased in all trials, with higher values in the RE120 trial at 30- and 45-min points of exercise when compared with other trials ($P < 0.01$).

Acute changes in serum FFA and glycerol concentrations are shown in Figure 4. In the RE120 trial, the FFA concentration was unchanged immediately after the resistance exercise, but it increased at the beginning of the subsequent submaximal exercise (0.27 ± 0.03 to 0.70 ± 0.10 mM, $P < 0.01$), indicating the activation of lipolysis during the recovery period. Consequently, significant differences were observed in pre-submaximal exercise concentrations of FFA between the RE120 (0.70 ± 0.10 mM) and both the RE20 (0.26 ± 0.02 mM) and the E trials (0.35 ± 0.07 mM, $P < 0.01$). During submaximal exercise, significant differences were found at every time point between the RE120 and E trials ($P < 0.05$) except for the 60-min point of exercise. In the RE20 trial, the concentration of FFA was kept elevated after the 15-min point during the submaximal exercise, and it showed a significant difference between the RE20 (1.02 ± 0.10 mM) and E trials (0.64 ± 0.10 mM, $P < 0.05$) at the 60-min point (after submaximal exercise). The AUC values during the 60-min exercise were significantly greater in the RE120 (44.4 ± 5.0 mM) than in the RE20 (31.3 ± 3.8 mM, $P < 0.05$) and E trials (26.7 ± 4.9 mM, $P < 0.01$).

Glycerol concentration increased significantly after resistance exercise in both the RE120 and RE20 trials ($P < 0.01$). During submaximal exercise, it also increased

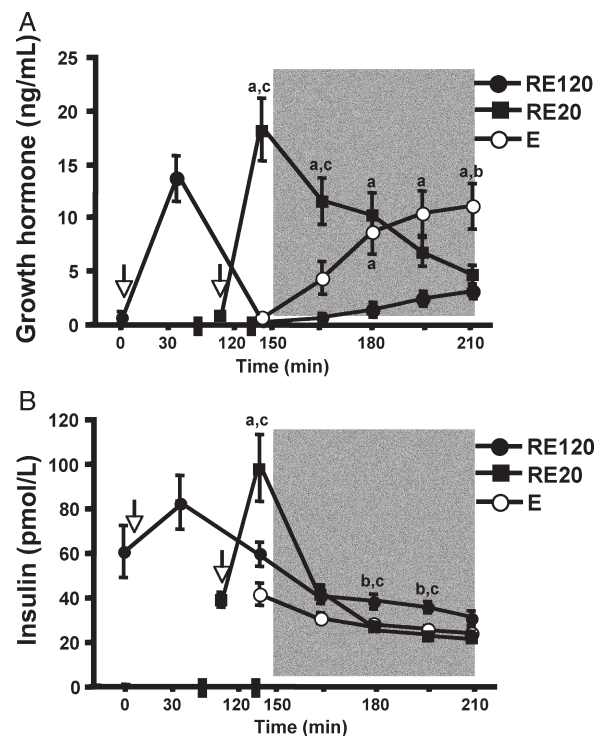


FIGURE 3—Serum growth hormone (A) and insulin (B) concentrations during the three exercise regimens. Values are means $\pm$ SE. a, $P < 0.05$ compared with RE120 trial; b, $P < 0.05$ compared with RE20 trial; c, $P < 0.05$ compared with E trial. Arrows in the graph indicate the time points of resistance exercise in the RE120 and RE20 trials. Submaximal exercise is denoted as a shaded area.

significantly ($P < 0.05$) in all trials, but it showed significantly higher values in the RE20 and the RE120 trials than in the E trial ($P < 0.05$). At the 60-min point of exercise (after submaximal exercise), a significant difference was found between the RE20 (2.6 ± 0.3 mM) and the E trials (1.7 ± 0.3 mM, $P < 0.05$). Also, AUC values during the 60-min exercise were significantly greater in the RE20 trial (103.4 ± 11.3 mM) than in the E trial (65.4 ± 9.9 mM, $P < 0.01$).

Figure 5 shows changes in the serum ketone body (acetoacetate and 3-hydroxybutyrate) concentrations. In the RE120 trial, the concentrations of acetoacetate and 3-hydroxybutyrate did not change immediately after the resistance exercise, but the acetoacetate concentration showed a significant increase (about threefold) at the beginning of the subsequent submaximal exercise (11.0 ± 1.5 to 35.6 ± 8.4 μM , $P < 0.05$). During submaximal exercise, the concentrations of acetoacetate and 3-hydroxybutyrate increased significantly in all trials ($P < 0.05$), with no significant difference among trials. In addition, AUC values during the 60-min exercise showed no significant difference among three trials.

Oxygen Uptake and Substrate Oxidation during Submaximal Exercise

During submaximal exercise, $\dot{V}\text{O}_2$ was significantly higher in the RE20 trial (1.54 ± 0.05 $\text{L}\cdot\text{min}^{-1}$) than in the RE120 trial (1.46 ± 0.05 $\text{L}\cdot\text{min}^{-1}$, $P < 0.05$) during the first

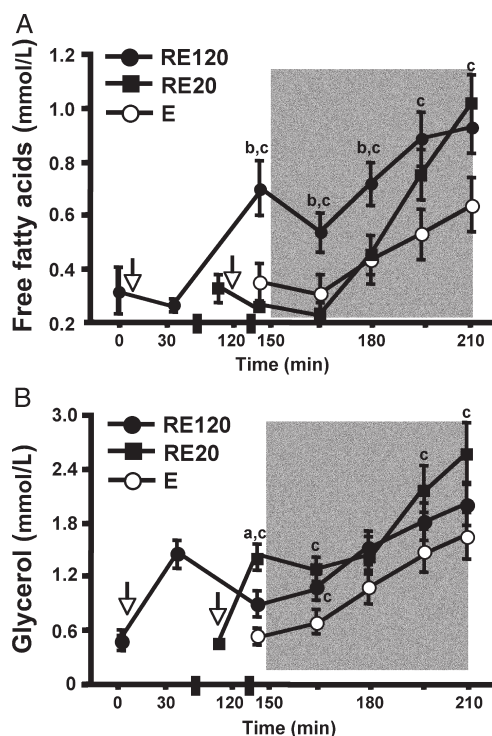


FIGURE 4—Serum free fatty acids (A) and glycerol (B) concentrations during the three exercise regimens. Values are means $\pm$ SE. a, $P < 0.05$ compared with RE120 trial; b, $P < 0.05$ compared with RE20 trial; c, $P < 0.05$ compared with E trial. Arrows in the graph indicate the time points of resistance exercise in the RE120 and RE20 trials. Submaximal exercise is denoted as a shaded area.

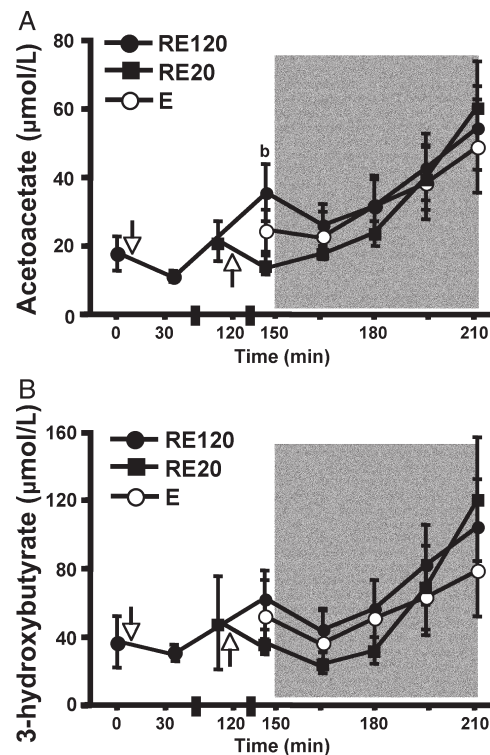


FIGURE 5—Serum acetoacetate (A) and 3-hydroxybutyrate (B) concentrations during the three exercise regimens. Values are means $\pm$ SE. b, $P < 0.05$ compared with RE20 trial. Arrows in the graph indicate the time points of resistance exercise in the RE120 and RE20 trials. Submaximal exercise is denoted as a shaded area.

15 min of exercise. However, no significant difference was observed among three trials when mean values of $\dot{V}\text{O}_2$ throughout 60 min of exercise were compared (RE120 trial: 1.52 ± 0.05 $\text{L}\cdot\text{min}^{-1}$; RE20 trial: 1.57 ± 0.04 $\text{L}\cdot\text{min}^{-1}$; E trial: 1.52 ± 0.04 $\text{L}\cdot\text{min}^{-1}$, NS). Mean values of HR during the submaximal exercise were significantly higher in the RE20 trial (130 ± 4 bpm) and the RE120 (126 ± 3 bpm) than in the E trials (119 ± 4 bpm, $P < 0.01$ for RE20 trial, $P < 0.05$ for RE120 trial). No significant difference was observed in changes in RPE among trials.

Figure 6 shows the relative contributions of fat oxidation to total energy production (% fat) estimated by RER values during submaximal exercise. During the first

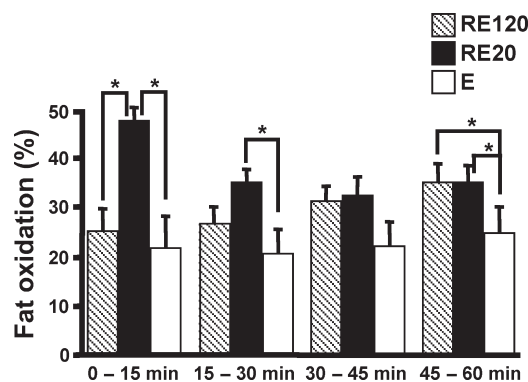


FIGURE 6—Relative contribution of fat oxidation (%) to total energy production during submaximal endurance exercises in the three exercise regimens. Values are means $\pm$ SE. * $P < 0.05$.

30 min of exercise, % fat was higher in the RE20 trial than in the RE120 and E trials ($P < 0.05$), but no significant difference was observed between the RE120 and E trials. During the latter half of exercise, the value of % fat was significantly ($P < 0.05$) greater in the RE20 and RE120 trials than in the E trial during the last 15 min of exercise (45–60 min). In addition, average % fat throughout the latter 30 min of exercise was significantly greater in the RE20 and RE120 trials than in the E trial ($P < 0.05$).

DISCUSSION

The major finding of this study was that fat metabolism during submaximal exercise was strongly affected by prior resistance exercise. In the RE20 and RE120 trials, the blood concentrations of FFA and glycerol during submaximal exercise were higher than in the E trial, suggesting enhancement of fat availability by prior resistance exercise. In addition, in the RE20 trial, the enhanced availability of fatty acids significantly increased the fat oxidation throughout the 60-min exercise. In the RE120 trial, the relative fat oxidation was enhanced during the latter 30 min of exercise. However, marked elevation of FFA concentration before submaximal exercise did not affect the substrate-oxidation pattern during the first half of the 60-min exercise.

The present resistance exercise regimen with moderate intensity (10RM) and a short rest period between sets (1 min) aimed to evoke strong catecholamine and anabolic hormone responses (10). Circulating catecholamine and GH are known to have a potent lipolytic effect (11,23). However, the time course of the lipolytic response to these hormones seems to be quite different: catecholamine causes rapid lipolysis (19,23), whereas GH causes a much-delayed response (11). A GH infusion to healthy individuals increases the serum FFA and glycerol concentrations, with a peak occurring 120–160 min after the infusion (20). As expected, prior resistance exercise caused marked increases in NE and GH, with approximately threefold increases in FFA and acetoacetate during the 120-min rest period in the RE120 trial (Figs. 4 and 5). The interpretation of FFA and ketone body responses demands caution because of limited sampling points during the recovery period, but we demonstrated recently that FFA elevation occurred between 60 and 120 min after a single bout of sprint exercise (data not shown). Moreover, the present delayed elevations of FFA and ketone body were consistent with those reported to occur after the GH administration (11,12). It has also been reported that enhanced fat metabolism during the recovery period after exercise was related directly to GH rather than to NE (22). Therefore, the GH secretion after the resistance exercise might be a major cause for the postexercise elevation of FFA concentration in the RE120 trial.

The FFA and glycerol concentrations during the submaximal exercise were higher in the RE120 trial than in the E trial (Fig. 4), even though no significant differences in $\dot{V}O_2$ and RPE were observed during the submaximal

exercise. Theoretically, the rate of FFA uptake by the working muscles is correlated with blood FFA concentration (8). Therefore, we anticipated that elevation of FFA concentration at the beginning of submaximal exercise in the RE120 trial would facilitate fatty acid use during exercise. However, the present RE120 trial did not increase fat oxidation during the first 30-min period of exercise (Fig. 6). The results are partially consistent with other recent studies (12,17), demonstrating that a marked increase in FFA concentration caused by prior GH administration had no effect on substrate-oxidation pattern during the submaximal exercise. During the initial phase of endurance exercise, carbohydrate oxidation might predominate during exercise, even with an elevated level of blood FFA (12).

The RE20 trial with a much shorter rest period between resistance and submaximal exercises resulted in more rapid increases in FFA, glycerol, and ketone body concentrations during the submaximal exercise than did the E trial (Figs. 4 and 5). In addition, the average fat oxidation throughout 60-min exercise was highest among the three trials. The enhanced lipolytic responses in the RE20 trial might be partially attributable to the preceding elevation of circulating catecholamine level caused by the preceding resistance exercise. The major stimulus for lipolysis seems to be circulating catecholamine in combination with a low insulin concentration (27). In the RE20 trial, catecholamine release already stimulated by the preceding resistance exercise might facilitate lipid mobilization during the subsequent exercise. Moreover, Epi and NE responses during submaximal exercise might be augmented by prior resistance exercise. Adipose tissue lipolysis during aerobic exercise is enhanced by repeated bouts of exercise, partly because of increases in exercise-induced elevations of epinephrine (28) and norepinephrine (25) levels. The higher HR during the submaximal exercise in the RE20 and RE120 trials might reflect enhancements of sympathetic nervous activity and thereby catecholamine response. However, we were unable to determine catecholamine concentrations during the 60 min of exercise, so we have no evidence for the involvement of Epi and NE in the enhanced lipid metabolism in RE20 and RE120 trials. In addition, it is noteworthy that enhanced fat oxidation was found from the early phase of submaximal exercise in the RE20 trial. However, in the RE20 trial, higher lactate concentration caused by prior resistance exercise (Fig. 2) and the resulting increase in hydrogen ions during the submaximal exercise might have affected the validity of the calculation of substrate oxidation because of enhanced carbon dioxide excretion (12).

Insulin also affects lipid mobilization from adipose tissue, and lower concentration of insulin enhances lipolytic response during exercise (13). In the RE20 trial, prior resistance exercise caused a significant increase in insulin concentration, which was kept higher than that in E trial at the start of the subsequent submaximal exercise. However, the insulin concentration decreased rapidly during the first 30 min of the submaximal exercise (Fig. 3). This rapid decrease in insulin concentration might

partially contribute to the enhanced lipolysis during the exercise.

The GH concentration decreased gradually during the submaximal exercise in the RE20 trial (Fig. 3), possibly because of a negative feedback mechanism (7). Conversely, the attenuated GH response shown in the RE120 trial might be caused by elevated blood FFA because increased FFA levels have been shown to suppress GH secretion (5). Therefore, the GH secretion during the submaximal exercise is not likely to be related to the enhancement of lipolysis in the RE20 and RE120 trials. However, the possibility exists that the increased GH after prior resistance exercise affects the fat metabolism. Moreover, indirect effects of GH on lipolysis should be taken into consideration because GH enhances the lipolytic action of adipose tissue by epinephrine through modifications of the β -adrenergic pathway (2).

Unexpectedly, a significant increase in serum glycerol concentration was found immediately after the resistance exercise, even though FFA showed no significant change (Fig. 4). It has been reported that the blood glycerol level is a better indicator for changes in adipose tissue lipolysis than the blood FFA level (1). Although little is known about changes in the circulating glycerol after the resistance exercise, the lipolysis might be stimulated even immediately after the resistance exercise. Hence, for the RE20 trial, we could not exclude the possibility that a rapid increase in lipolysis was already caused by the prior resistance exercise.

The present findings provide practical implications for exercise programs with combined resistance and endurance exercises. We have already demonstrated that GH secretion after resistance exercise was markedly suppressed by 60 min of prior endurance exercise and the resulting elevation of the FFA level (9). On the basis of our previous and present results, an exercise program with preceding

resistance exercise should be desirable for both GH response to resistance exercise and fat metabolism during the submaximal endurance exercise. Further studies should be conducted to determine whether the order of resistance and endurance exercises alters the long-term effects of exercise training.

Several limitations exist in this study. Although we used untrained subjects, different results might be obtained for endurance athletes with greater aerobic capacity. Data at postprandial states might be informative, and the effects of prior resistance exercise on enhancements of fat metabolism might be impaired. In addition, experiments with endurance exercise at higher intensity should be conducted. Furthermore, the possibility remains that any prior exercise might augment fat metabolism during subsequent submaximal exercise. In fact, lipolysis and fat oxidation during aerobic exercise were enhanced significantly by prior exercise of equal duration and intensity, separated by a 60-min recovery period (28). We found recently that responses of glycerol and epinephrine during moderate-intensity exercise (60% $\dot{V}O_{2\max}$) were augmented when the exercise bout was preceded by the same exercise and intervening 20-min rest (data not shown). Although it is not possible to make a clear inference because of different exercise protocols, caution should be given when determining whether prior resistance exercise *per se* is important for augmenting fat metabolism during subsequent submaximal exercise.

In conclusion, this study showed that fat availability during submaximal exercise was enhanced by prior resistance exercise. However, augmentation of fat oxidation during the submaximal exercise was observed only in the trial with a shorter rest period between resistance and submaximal endurance exercises (RE20 trial).

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