Effect of Endurance Training on Glycerol Kinetics During Strenuous Exercise in Humans

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Glycerol kinetics were evaluated during high-intensity exercise in five untrained and five endurance-trained subjects. Glycerol rate of appearance (Ra) in plasma was determined by infusing [$^2H_5$]glycerol during rest and 60 minutes of cycle ergometer exercise performed at 70% $\dot{V}O_2$ peak. Mean plasma glycerol concentration was greater in trained than untrained subjects throughout exercise ($P < .05$). The average glycerol Ra during exercise and the integrated lipolytic response to exercise, expressed as total glycerol Ra above baseline, were both greater in trained (7.85 ± 0.72 μmol · kg^{-1} · min^{-1} and 289 ± 50 μmol · kg^{-1} · h^{-1}, respectively) than in untrained (5.68 ± 0.90 μmol · kg^{-1} · min^{-1}, and 198 ± 31 μmol · kg^{-1} · h^{-1}, respectively) subjects ($P < .05$). We conclude that whole-body lipolytic rates are greater in endurance-trained athletes than in sedentary controls during high-intensity exercise performed at the same relative intensity.

ENDOGENOUS TRIGLYCERIDES provide an important fuel for working muscles during endurance exercise. The use of fat is influenced by previous exercise training and many studies have consistently documented increased fat oxidation during exercise in trained compared with untrained subjects.[1-4] In contrast, assessment of fat mobilization, by measuring the rate of appearance (Ra) of free fatty acids (FFA) in plasma, has yielded conflicting results. FFA Ra has been found to be higher,[5] the same,[6,7] or lower[8] in trained compared with untrained subjects.

One possible reason for the differences observed between studies is that FFA Ra in plasma may not be an accurate measure of lipolytic activity during exercise. Glycerol Ra provides a better index of whole-body lipolytic rate than does FFA Ra because glycerol released during lipolysis cannot be metabolized by adipose tissue and must enter the bloodstream.[9] In contrast, a portion of fatty acids released during lipolysis are reesterified and thereby retained within adipose tissue. During high-intensity exercise, FFA Ra is a particularly poor marker of lipolysis and alterations in FFA kinetics may not even parallel alterations in glycerol kinetics. Romijn et al found that FFA Ra was 35% lower while glycerol Ra was 25% higher during high-intensity than during low-intensity exercise.[10] Several mechanisms may be responsible for the discrepancy between FFA Ra and glycerol Ra. A portion of released fatty acids may become trapped in adipose tissue because adipose tissue blood flow is not sufficient to remove the large amount of released fatty acids.[10,11] In addition, fatty acids released from intramuscular lipolysis may be oxidized locally, preventing their entry into the bloodstream. Therefore, it is possible that differences in FFA Ra observed between trained and untrained subjects may not represent true differences in lipolytic activity.

We recently found that glycerol kinetics were the same in trained and untrained subjects during low-intensity exercise performed at the same absolute intensity.[6] Exercise performed at higher intensities should provide a greater lipolytic stimulus because of the increase in sympathoadrenal activity. However, assessment of glycerol Ra during moderate- or high-intensity exercise in trained compared with untrained subjects has never been reported.

The purpose of the present study was to evaluate the effect of endurance training on glycerol kinetics during high-intensity exercise. Glycerol Ra, measured by infusing [$^2H_5$]glycerol, was determined during 30 to 60 minutes of cycle ergometer exercise performed at 70% of peak oxygen uptake in endurance-trained male athletes and in untrained healthy young adult men. The relative intensity of the exercise bout was the same in both groups, but the absolute intensity was higher in trained subjects because of their higher capacity for oxygen consumption.

METHODS

Subjects

Five untrained subjects and five endurance-trained athletes participated in this study (Table 1). The trained subjects were competitive distance runners and were actively involved in a vigorous running program for more than 3 years. The untrained subjects had not been involved in a regular exercise program for at least 1 year. All subjects were considered to be in good health after a careful medical evaluation that included a history, physical examination, and blood tests (complete blood cell count, electrolyte panel, blood urea nitrogen, creatinine, and liver chemistries). Informed consent was obtained from each subject. This study was approved by the Institutional Review Board and the General Clinical Research Center of The University of Texas Medical Branch at Galveston, TX.

Exercise Capacity

Measurement of $\dot{V}O_2$ peak was determined during an incremental cycle ergometer exercise protocol. The workload increased every minute until volitional exhaustion was reached. Oxygen consumption and carbon dioxide production were measured using a metabolic measurement cart (Sensormedics, Anaheim, CA). The

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Table 1 Characteristics of the Study Subjects

Characteristic	Untrained Subjects (n = 5)	Trained Subjects (n = 5)
Age (yr)	27.6 ± 2.1	28.6 ± 3.6
Gender (male/female)	5/0	4/1
Height (cm)	176.7 ± 2.5	174.9 ± 4.3
Weight (kg)	70.7 ± 3.4	67.1 ± 4.1
$\dot{V}O_2$max (mL · kg^{-1} · min^{-1})	44.6 ± 2.1	66.4 ± 2.8*

NOTE. Values are means ± SE.

*Significantly different from untrained subjects, $P < .05$.

following criteria were used to establish that maximal oxygen uptake was attained: (1) respiratory exchange ratio greater than 1.15; (2) a leveling off of $\dot{V}O_2$ and heart rate despite increases in workload; and (3) attainment of predicted maximal heart rate.

Experimental Protocol

Subjects were admitted to the General Clinical Research Center 1 day before the isotope infusion study was performed. The untrained subjects completed $\dot{V}O_2$ peak testing in the afternoon after admission. All trained subjects had known $\dot{V}O_2$ peak values from recent testing and completed their usual training run in the afternoon after admission.

The isotope infusion study was completed on the second day after the subjects had fasted overnight (12 hours). One catheter was placed in the antecubital vein of one arm for the infusion of labeled glycerol and another catheter was placed in the contralateral dorsal hand vein, which was heated, for arterialized venous sampling.[12] After collecting baseline blood samples, a primed (0.9 μmol/kg), constant (0.06 μmol · kg^{-1} · min^{-1}) infusion of [$^2H_5$]glycerol (Cambridge isotopes,) was started using a calibrated syringe pump (Harvard Apparatus, Natick, MA). The precise infusion rate was determined by measuring the infusate glycerol concentration. After 60 minutes of isotope infusion at rest, the subjects began cycling on an electronically braked ergometer at 70% $\dot{V}O_2$ peak. They were instructed to cycle for 60 minutes if possible. Oxygen consumption was determined continuously beginning 15 minutes before and throughout exercise using a metabolic measurement cart (Sensormedics). The ergometer work load was adjusted during the first 10 minutes of exercise to elicit the desired $\dot{V}O_2$. The rate of isotope infusion was increased threefold at the onset of exercise to minimize changes in plasma substrate isotopic enrichment.[11] Blood samples were obtained at 45, 50, 55, and 60 minutes to determine glycerol kinetics at rest and every 10 minutes during exercise to determine the lipolytic response to exercise. Blood was placed in chilled heparinized tubes and the plasma was promptly separated by centrifugation.

Sample Analysis

Plasma glycerol concentration was determined enzymatically with an automated analyzer using a glycerol oxidase method (Technicon, Tarrytown, NY). Deuterium enrichment of plasma glycerol was determined by gas chromatography-mass spectrometry (GC-MS) as described previously.[13] Plasma proteins were precipitated with barium hydroxide and zinc sulfate and charged molecules removed by a mixed cation and anion exchange column. A trimethylsilyl derivative of glycerol was formed and injected into an MSD 5971 GC-MS (Hewlett-Packard, Palo Alto, CA) with an HP-1 12 m × 0.2 mm fused silica capillary column (Hewlett-Packard). Ions were produced by electron impact ionization, and glycerol enrichment was determined by selectively monitoring ions at mass-to-charge ratios of 205.1, 206.1, 207.1, and 208.1.

Calculations

Glycerol Ra in plasma was used as a measure of whole-body lipolytic activity. Steele's equation[14] for steady-state conditions was used to calculate glycerol Ra during the resting period when a physiologic and isotopic steady-state was present. During exercise non–steady-state conditions were present and Steele's equation[14] for non–steady-state conditions was used to calculate glycerol Ra.

Statistical Analysis

The significance of differences between the trained and untrained subjects was evaluated by using Student's t test for independent samples and analysis of variance (ANOVA) with repeated measures. A P value $\leq .05$ was considered to be statistically significant. All data are expressed as means ± SE.

RESULTS

Exercise Bout

Each subject attempted to exercise at 70% of their individual $\dot{V}O_2$ peak for 60 minutes. All trained, but only four untrained subjects, were able to complete the entire exercise protocol. One untrained subject stopped cycling after 30 minutes because of exhaustion. Therefore, the data in the untrained group represent the mean of five subjects during the first 30 minutes of exercise and four subjects during the second 30 minutes of exercise.

Indirect Calorimetry

The target workload was reached within the first 10 minutes of exercise and was maintained throughout the remainder of the exercise period. Mean oxygen consumption during exercise in the trained subjects (3,120 ± 36 ml · kg^{-1} · min^{-1}) was significantly higher than in the untrained group (2,197 ± 27 ml · kg^{-1} · min^{-1}) ($P < .001$) because of the considerable difference in $\dot{V}O_2$ peak between the two groups. Respiratory exchange ratios were lower in the trained than in the untrained subjects throughout exercise (data not shown).

Glycerol Concentrations

Basal plasma glycerol concentrations were similar in the trained and untrained subjects. However, during exercise plasma glycerol increased more in the trained than in the untrained subjects, with differences becoming statistically significant by 10 minutes of exercise (Fig 1A). At the end of exercise, mean plasma glycerol concentration in the trained group was almost double that of the untrained group.

Glycerol Kinetics

Glycerol Ra at rest and during 60 minutes of cycle ergometer exercise at 70% $\dot{V}O_2$ peak in trained and untrained subjects are shown in Fig 1B. Glycerol Ra increased progressively throughout exercise in both groups. The lipolytic response to exercise, expressed as either average glycerol Ra (Fig 2A) or additional glycerol released above basal (Fig 2B), was greater in trained (7.85 ± 0.72 μmol · kg^{-1} · min^{-1} and 289 ± 50 μmol · kg^{-1} · h^{-1}, respectively) than in untrained (5.68 ± 0.90 μmol · kg^{-1} · min^{-1} and 198 ± 31 μmol · kg^{-1} · h^{-1}, respectively) subjects ($P < .05$).

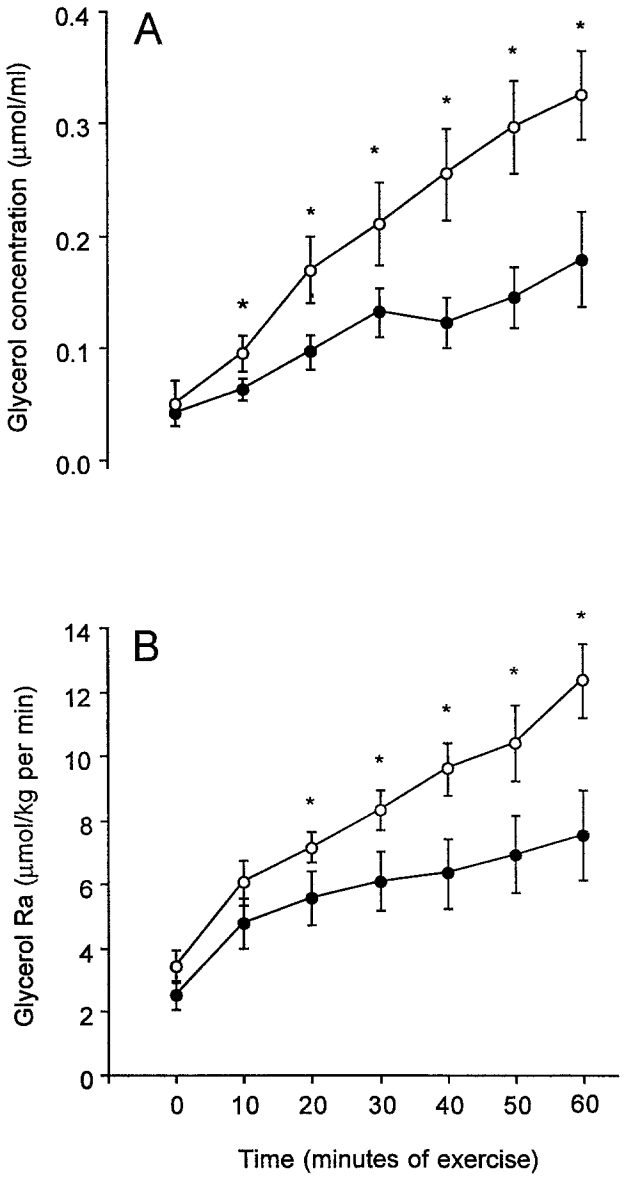

Fig 1. Plasma glycerol concentration (A) and glycerol Ra (B) in trained (○) and untrained (●) subjects at rest (time 0) and during 60 minutes of cycle ergometer exercise performed at 70% peak $\dot{V}O_2$. Values are means ± SE. *Value for trained subjects significantly different from corresponding value for untrained subjects, $P < .05$.

DISCUSSION

Endurance training increases the oxidation of fat by working muscles during exercise.[15,16] The results of the present study demonstrate that glycerol Ra, an index of whole-body lipolysis, is greater in endurance-trained athletes than in untrained subjects during strenuous exercise performed at the same relative intensity (% peak $\dot{V}O_2$). Therefore, an increase in lipolytic capacity is an important adaptative mechanism for facilitating fat oxidation during high-intensity exercise in trained subjects.

In this study, we used glycerol Ra in plasma to assess the lipolytic response to exercise. Glycerol can be released into plasma from lipolysis of several sources of endogenous triglycerides, including adipose tissue, muscle, and circulating lipoproteins. The method used in our study can only quantify the total rate of glycerol appearance in the systemic circulation, but is unable to separate the proportional contribution from each source. Therefore, we cannot determine whether enhanced lipolysis in one or more tissues was responsible for the higher rate of glycerol release observed in our trained subjects. Data from in vitro studies suggest that endurance training increases lipolysis of adipose tissue triglycerides because of an increase in β-adrenergic receptor sensitivity. Both longitudinal training studies[17] and cross-sectional studies of endurance-trained athletes compared with sedentary controls[18-20] have found that training increases epinephrine-stimulated lipolysis in adipocytes obtained from subcutaneous abdominal adipose

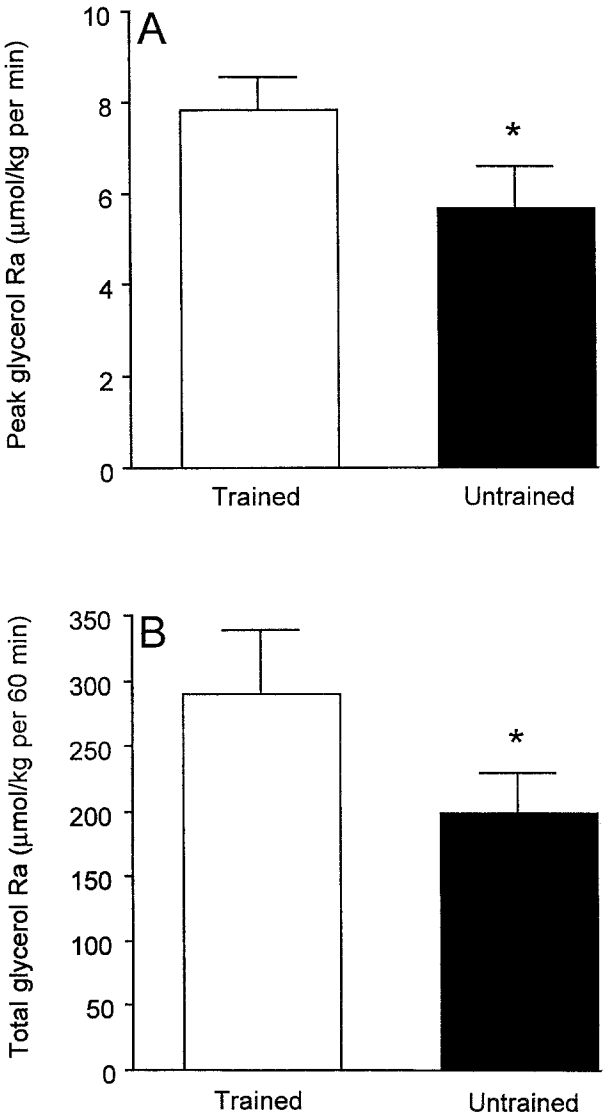

Fig 2. Average glycerol Ra (A) and the increase in glycerol Ra above baseline (B) in trained and untrained subjects during 60 minutes of cycle ergometer exercise performed at 70% peak $\dot{V}O_2$. Values are means ± SE. *Value for untrained subjects is significantly different from corresponding value for trained subjects, $P < .05$.

tissue. Data from both human and animal studies suggest that endurance training may also increase the amount of glycerol released into plasma by increasing the mobilization of intramuscular triglycerides. Muscle biopsies obtained from human volunteers before and after exercise demonstrate greater depletion of intramuscular lipids in trained than in untrained subjects.[16] Furthermore, training in humans increases triglyceride content in skeletal muscle.[21] Comparative morphometric studies of muscles from mammalian species with widely different aerobic capacities have found that animals with a high $\dot{V}O_2$ max have larger intramuscular triglycerides stores than do those with a low $\dot{V}O_2$ max.[22] More importantly, muscle lipid droplets were always found in direct contact with mitochondria, and the size of the contact area between lipid droplets and the outer mitochondrial membranes was strongly correlated with aerobic capacity.

Endogenous triglycerides are the body's largest fuel reserve. The mobilization and oxidation of fat during exercise spares the use of more limited carbohydrate stores. In the present study, lipolytic rates (glycerol Ra) increased progressively during exercise in both trained and untrained subjects and did not reach a plateau after 1 hour of intense exercise. However, glycerol Ra was greater throughout exercise in the trained than in the untrained group. In fact, glycerol Ra at the end of exercise in the trained subjects (~ 12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was double that found in the untrained group (~ 6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). The increased mobilization of endogenous triglycerides is consistent with the finding that endurance training attenuates muscle glycogen depletion during exercise.[16]

Our results demonstrate that exercise is the most potent physiological stimulus for lipolysis. Peak glycerol Ra during exercise in the trained subjects was threefold higher than that reported during critical illness[23] or after 84 hours of starvation.[24] The maximal capacity for lipolysis during exercise in humans is not known. Data from interspecies studies suggest that the ability to mobilize fat may be related to the level of aerobic fitness. The relationship between lipolysis during exercise and peak $\dot{V}O_2$ is relatively constant in mammalian species, despite a wide range of aerobic capacities.[25] The results of the present study taken in conjunction with data from animal studies suggest that training itself can influence lipolytic capacity.

In contrast to our findings, two studies have reported that lipolytic activity, measured by FFA Ra, was the same or lower during exercise in trained than in untrained subjects.[7,8] Jansson and Kaijser[7] found that FFA Ra was the same in both trained and untrained groups. However, the apparent discrepancy between our study and that of Jansson and Kaijser[7] may be related to factors that affect glycerol and FFA appearance in plasma rather than true differences in lipolytic activity. Fatty acids released during lipolysis of adipose tissue triglycerides can be reesterified back to triglycerides within the adipocyte, preventing FFA entry into the systemic circulation. However, glycerol released during lipolysis must enter the bloodstream because glycerol kinase, the enzyme needed to metabolize glycerol, is virtually absent in adipose tissue.[9] Therefore, increased fatty acid reesterification in trained subjects would decrease measured FFA Ra, but would not affect glycerol Ra. Differences in fatty acid reesterification could explain the similarity in FFA kinetics in the trained and untrained groups reported by Jansson and Kaijser,[7] in the face of higher rates of glycerol appearance (lipolysis) found in trained subjects in the present study. In addition, an increase in the mobilization and oxidation of intramuscular triglycerides in trained subjects could also cause an increase in glycerol Ra without a concomitant increase in FFA Ra. Local oxidation of fatty acids released during lipolysis of intramuscular triglycerides would prevent fatty acid entry into plasma, whereas released glycerol would likely enter the plasma compartment because of the low or absent levels of glycerol kinase activity in muscle.[26,27] Martin et al[8] found that 12 weeks of endurance training blunted the exercise-induced increase in FFA Ra during exercise. However, the posttraining exercise bout was performed at the same absolute intensity and therefore at a lower relative intensity than before training. Furthermore, differences in recent exercise activity and exercise training between studies may have also contributed to differences in results.

In summary, the present study provides evidence that endurance training increases the lipolytic response to high-intensity exercise. Glycerol Ra was greater in trained than in untrained subjects during 1 hour of strenuous cycle ergometer exercise performed at the same relative intensity. The mechanism responsible for this adaptive response is not known, but other studies performed in humans and animals suggest that training may upregulate lipolysis of both adipose tissue and intramuscular triglycerides.

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