

Muscle triglyceride utilization during exercise: effect of training

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HURLEY, B. F., P. M. NEMETH, W. H. MARTIN III, J. M. HAGBERG, G. P. DALSKY, AND J. O. HOLLOSZY. *Muscle triglyceride utilization during exercise: effect of training.* *J. Appl. Physiol.* 60(2): 562-567, 1986.—The respiratory exchange ratio (RER) is lower during exercise of the same intensity in the trained compared with the untrained state, even though plasma free fatty acids (FFA) and glycerol levels are lower, suggesting reduced availability of plasma FFA. In this context, we evaluated the possibility that lipolysis of muscle triglycerides might be higher in the trained state. Nine adult male subjects performed a prolonged bout of exercise of the same absolute intensity before and after adapting to a strenuous 12-wk program of endurance exercise. The exercise test required 64% of maximum O_2 uptake before training. Plasma FFA and glycerol concentrations and RER during the exercise test were lower in the trained than in the untrained state. The proportion of the caloric expenditure derived from fat, calculated from the RER, during the exercise test increased from 35% before training to 57% after training. Muscle glycogen utilization was 41% lower, whereas the decrease in quadriceps muscle triglyceride concentration was roughly twice as great (12.7 ± 5.5 vs. 26.1 ± 9.3 mmol/kg dry wt, $P < 0.001$) in the trained state. These results suggest that the greater utilization of FFA in the trained state is fueled by increased lipolysis of muscle triglyceride.

muscle glycogen; plasma free fatty acids; plasma glycerol

OXIDATION OF FREE FATTY ACIDS (FFA) can provide much of the energy for prolonged exercise (14, 31). During a long bout of exercise, plasma FFA concentration increases (1, 4, 11, 34). Concomitantly, the respiratory exchange ratio (RER) declines, indicating that a greater proportion of the energy is being supplied by oxidation of FFA (1, 2, 11, 34). It is thought that an important factor in this increase in FFA oxidation is the rise in plasma FFA concentration, because there is a close relationship between plasma FFA concentration and the rate of FFA oxidation (13, 30).

One of the adaptations that characterizes the trained state is a greater reliance on FFA oxidation during submaximal exercise, with a sparing of carbohydrate, as reflected in a lower RER (15, 17, 34). In this context, a puzzling finding is that plasma FFA concentration during submaximal exercise of a given intensity is often lower in the trained than in the untrained state (24, 32, 34). It is conceivable that trained muscle might utilize plasma FFA more rapidly than untrained, resulting in a higher

rate of plasma FFA turnover, despite a lower plasma FFA concentration. However, the finding that blood glycerol concentration during prolonged exercise of the same intensity is also lower in the trained than in the untrained state (32, 34) argues against an increased rate of plasma FFA turnover, because the increase in plasma glycerol concentration is a rough indicator of lipolysis in adipose tissue (18).

Muscle triglyceride concentration can decrease sufficiently during prolonged exercise to account for a considerable portion of the fat oxidized (2, 7). Therefore, in view of the lower plasma FFA levels in the trained state, it seems possible that increased lipolysis of muscle triglycerides might provide the fatty acids that account for the increased oxidation of fat during exercise in the trained state. As a first step in evaluating this possibility, we have examined the effect of adaptation to endurance exercise training on muscle triglyceride depletion during prolonged exercise.

METHODS

Subjects and training program. Nine male subjects age 29 ± 1 yr who had not done endurance exercise training for at least 6 mo participated in this study after giving their written informed consent. This study was approved by the Human Studies Committee of Washington University. After the initial tests, the subjects performed a strenuous training program for 12 wk as previously described in detail (19). On 3 days each week the subjects performed six 5-min bouts of exercise, requiring 90–100% of peak O_2 uptake ($\dot{V}O_2$) on a cycle ergometer, separated by 2-min rest periods. On 3 other days each week they ran continuously for 40 min as rapidly as they could; the running required ~75% of maximum O_2 uptake ($\dot{V}O_{2\text{max}}$). The training program was adjusted on an individual basis each week as necessary to keep training intensity constant relative to $\dot{V}O_{2\text{max}}$.

Measurement of O_2 uptake. $\dot{V}O_{2\text{max}}$ was measured during continuous running on a treadmill as described previously (22). Peak $\dot{V}O_2$ attainable on a cycle ergometer was measured as described by Fox et al. (10). All $\dot{V}O_2$ measurements were made with an open-circuit gas collection system using a Daniels' breathing valve (5) and neoprene meteorological balloons. Gas samples were analyzed with a Perkin-Elmer MGA 1100 respiratory mass

spectrometer, and ventilatory volumes were determined with a Tissot spirometer. The end point for the exercise tests was exhaustion.

Prolonged exercise test protocol. The subjects came to the laboratory in the early afternoon, 5 h after a light breakfast, and in the test done after 12 wk of training, 48 h after their last bout of exercise. Each subject's meals during the 36 h prior to the second study (trained state) were kept the same as those prior to the initial study (untrained state), in terms of both composition and quantity.

A catheter was inserted into an antecubital vein and used for blood sampling. After a muscle biopsy, a baseline blood sample and an expired air sample were obtained, and the subjects began to exercise on a cycle ergometer (Uniwork ergometer, model 845, Quinton Instruments, Seattle, WA). The work rate, which was held constant for each subject during both the pre- and post-training studies, averaged 138 ± 12 W and required $64 \pm 3\%$ of the subjects' peak $\dot{V}O_2$ when they were untrained. We had planned that each subject would exercise for 120 min; however, three of the subjects fatigued more rapidly than expected during the initial test, and their exercise tests were therefore stopped after 90 min both before and after training. Expired air was collected for 2-min periods for determination of $\dot{V}O_2$, $\dot{V}CO_2$ production ($\dot{V}CO_2$), and RER at 10, 30, and 60 min and every 10 min thereafter during the exercise test. The proportions of the energy derived from oxidation of fat and oxidation of carbohydrate were calculated from the RER using the assumption that oxidation of protein made a negligible contribution to the $\dot{V}O_2$. Heart rate was measured for 30 s just prior to each gas collection.

Blood samples. Blood samples were drawn prior to exercise and after 10, 30, 60, 90, and (in 6 subjects) 120 min of exercise. Blood samples were placed 1) in heparinized tubes on ice, after which plasma was separated by centrifugation at 4°C and used for measurement of glucose using a Yellow Springs Instrument model 23A glucose analyzer, FFA (26), insulin (16), and β -hydroxybutyrate (33); or 2) in iced tubes containing 10% perchloric acid, after which the supernatant, separated by centrifugation, was used for measurement of lactate (12) and glycerol (6).

Muscle biopsy. Before and immediately after the prolonged cycle ergometer exercise tests, muscle samples were obtained from the vastus lateralis muscle by needle biopsy as described by Evans et al. (8). The muscle samples were placed as rapidly as possible in liquid N₂ from which dissolved air had been removed under vacuum to lower the temperature of the N₂ below its boiling point and thus speed freezing of the muscle. The frozen samples were divided into portions of ~1–2 mg and lyophilized at -35°C under 10⁻³ Torr for 7 days. The freeze-dried samples were stored under vacuum at -70°C for subsequent biochemical analysis.

β -Hydroxyacyl-CoA dehydrogenase assay. Dry muscle samples (1–2 mg) were weighed at room temperature on a Sartorius analytical balance. They were then homogenized in 1-ml glass homogenizers (Kontes) in 100 or 200 μ l of 20 mM sodium phosphate buffer (pH 7.4) containing

5 mM β -mercaptoethanol, 0.5 mM ethylenediaminetetraacetic acid, 0.02% bovine serum albumin (BSA), and 50% glycerol. β -Hydroxyacyl-CoA dehydrogenase activity was measured at 25°C, as previously described in detail (3).

Muscle glycogen and creatine assays. Dry muscle samples weighing 1–2 mg were extracted with perchloric acid. Each sample was placed in a test tube on top of 100 μ l of 0.3 M perchloric acid and placed in an ethanol bath maintained at -8°C and shaken for 15 min. Next, the extracts were brought to 4°C, mixed intermittently for 10 min, and centrifuged for 10 min at 5,000 g. The supernatant was removed and neutralized with 2 M KOH, 0.4 M imidazole, and 0.4 M KCl.

Glycogen in the perchlorate precipitate was hydrolyzed with 1 ml of 1 M HCl for 2 h in sealed tubes at 95°C (29). Glucose in the hydrolysate was assayed in a medium containing 50 mM tris(hydroxymethyl)aminomethane hydrochloride (pH 8.1), 0.02% BSA, 100 μ M NADP, 0.3 mM dithiothreitol, 500 μ M ATP, 1 mM MgCl₂, and 0.5 μ g/ml of glucose-6-phosphate dehydrogenase. The reaction was started with 5 μ g/ml of hexokinase. NADP reduction was measured fluorometrically (29). Glycogen standards of 3 and 13 μ M were used.

Total creatine was measured in the supernatant as described by Hintz et al. (20).

Muscle triglyceride determination. A 1-mg freeze-dried portion of each muscle sample was used to measure intramuscular triglyceride concentration. Single fibers were teased out and cleaned free of nonmuscle material at room temperature using microdissection techniques. Several hundred dissected fibers were combined to obtain a sample of ~100 μ g. Fiber samples were weighed on a quartz fiber and aluminum pan balance. Lipid was extracted from the pooled fiber samples using a modification of the method of Folch et al. (9); the tissue was homogenized in 500 μ l methanol (HPLC grade, Fischer), with a 1-ml homogenizer (Kontes). Chloroform, 1.0 ml (HPLC grade, Fischer) was added, the tissue further homogenized, and the mixture allowed to stand overnight.

The lipid containing chloroform layer was separated by adding 0.4 ml saline and centrifuging at 1,000 g for 5 min. A 100- μ l aliquot was placed in a test tube and evaporated under N₂; after the sample was dry, 100 μ l of an assay mixture containing 100 mM phosphate buffer (pH 7.4), 4 mM MgCl₂, 0.2 mM ATP, 0.35 mM phosphoenolpyruvate, 0.2 mM NADH, 0.1 μ g/ml sodium dodecyl sulfate, 0.02% BSA, 0.6 U/ml esterase, 0.6 U/ml glycerol kinase, 1 U/ml pyruvate kinase, 6 U/ml lactate dehydrogenase, and 200 U/ml lipase was added to the tube. Duplicate samples were run without lipase. The tubes were then incubated at 25°C for 20 min. The reaction was stopped with 10 μ l of 1 N HCl. A 1.0- μ l aliquot of the mixture was enzymatically cycled using the method of Kato et al. (23) and the product measured fluorometrically.

Expression of data. Glycogen concentration is expressed as millimoles per kilogram muscle dry weight normalized for creatine content, using the highest creatine value obtained on the subjects' biopsies (3). β -Hy-

droxyacyl-CoA dehydrogenase activity is expressed as moles per kilograms of protein per hour. Triglyceride concentration is expressed as millimoles per kilogram of dry muscle fiber weight.

Statistical analysis. Statistical comparisons of responses to the exercise test before and after training were performed with a repeated measures analysis of variance and testing of subhypotheses using appropriate contrasts. Paired *t* tests were used for comparison of muscle triglyceride, glycogen, and enzyme levels. All values are expressed as means \pm SD.

RESULTS

The training program resulted in a 26% increase in $\dot{V}O_{2\text{max}}$ and a 30% increase in peak $\dot{V}O_2$ measured during cycle ergometer exercise (Table 1). There was no significant change in body weight. Further evidence for an adaptation to training was provided by significantly lower heart rate and blood lactate responses during the prolonged exercise test (Fig. 1).

Muscle β -hydroxyacyl-CoA dehydrogenase. β -Hydroxyacyl-CoA dehydrogenase activity, which was used as a marker for the mitochondrial enzymes involved in fatty acid oxidation, increased 90% in quadriceps muscle in response to the training program, averaging 9.2 ± 2.9 mol·kg $^{-1}$ ·h $^{-1}$ before and 17.4 ± 2.6 after training ($P < 0.01$).

Blood metabolite and insulin levels. There was a progressive increase in plasma FFA and blood glycerol concentrations during the prolonged exercise test both before and after training (Fig. 2). However, both FFA and glycerol concentrations were significantly lower toward the end of the exercise in the trained state. There were no significant changes in glucose or β -hydroxybutyrate concentrations during the prolonged exercise either before or after training (Fig. 3). Plasma insulin concentration declined 25–35% during the exercise, but there were no significant differences in response between the untrained and trained states.

Energy utilization and respiratory exchange ratio. There was a gradual, small rise in $\dot{V}O_2$ during the prolonged exercise (Table 2). Training had no effect on the rate of energy utilization during the exercise. RER declined during the exercise both before and after training; however, at all time points, RER was significantly lower in the trained state. As shown in Fig. 4, a significantly smaller proportion of the energy utilized during the exercise came from oxidation of carbohydrate, and a proportionally greater amount came from oxidation of fat in the trained than in the untrained state.

TABLE 1. Weight, maximum O_2 uptake, and peak O_2 uptake

	Before Training	After Training
Weight, kg	73.0 ± 4.8	72.3 ± 5.1
$\dot{V}O_{2\text{max}}, \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	43.2 ± 3.6	$54.4 \pm 5.7^*$
Peak cycle exercise $\dot{V}O_2$, $\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$	38.7 ± 1.3	$50.3 \pm 1.6^*$

Values are means \pm SD for 9 subjects. $\dot{V}O_{2\text{max}}$, maximum O_2 uptake; $\dot{V}O_2$, O_2 uptake. * Before vs. after training, $P < 0.001$.

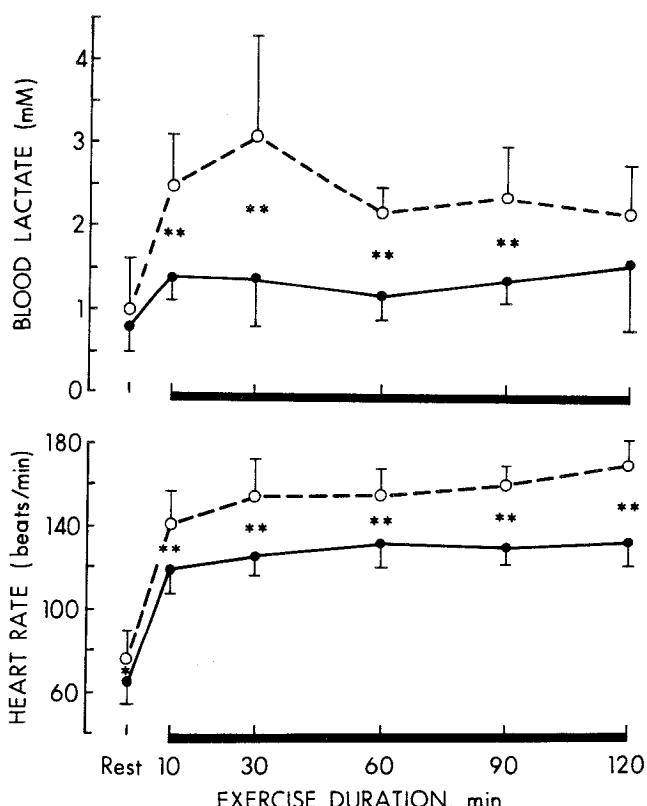


FIG. 1. Blood lactate and heart rate at rest and during the prolonged exercise test before (open circles) and after (filled circles) training. Values are means \pm SD. *Difference between before and after training significant at $P < 0.05$; ** $P < 0.01$.

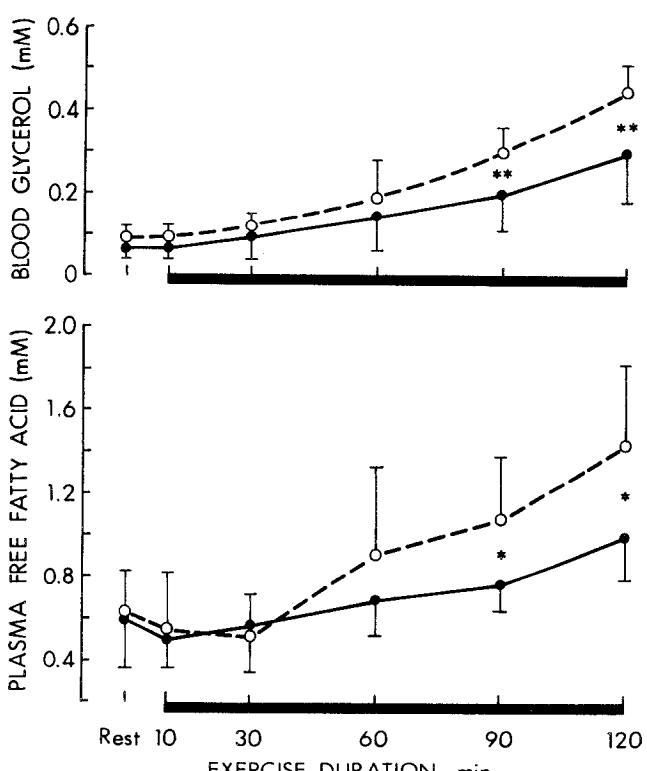


FIG. 2. Plasma free fatty acid and blood glycerol levels at rest and during the prolonged exercise test before (open circles) and after (filled circles) training. Values are means \pm SD. *Difference between before and after training significant at $P < 0.05$; ** $P < 0.01$.

Muscle glycogen and triglycerides. The endurance training resulted in a marked change in the proportions of the quadriceps muscle's glycogen and triglyceride stores depleted during prolonged exercise. The exercise resulted in 41% less muscle glycogen utilization in the trained than in the untrained state (Table 3). The concentration of glycogen remaining in quadriceps muscle after the prolonged exercise was more than twofold higher after than before training. The decrease in quadriceps muscle triglyceride concentration was roughly twice as great in the trained as in the untrained state (Table 3). All nine subjects had a greater decrease in muscle triglyceride concentration after training.

DISCUSSION

Plasma FFA concentration was lower during prolonged

exercise of the same intensity after training in the subjects in this study. A similar finding has been reported in some previous studies (24, 32, 34). The lower plasma levels could be due either to increased utilization or decreased mobilization from adipose tissue. The lower blood glycerol concentration in the trained state favors the interpretation that it is the rate of production that is decreased, because changes in plasma glycerol level usually roughly reflect changes in the rate of lipolysis in adipose tissue (18). The training program used in this study results in a marked blunting of the plasma catecholamine response to submaximal exercise (34). This suggests that decreased sympathoadrenal activity could account for a reduced stimulation of lipolysis in adipose tissue during exercise of the same intensity in the trained, compared with the untrained state.

There is usually a linear relationship between plasma FFA concentration and the rate of plasma FFA oxidation (13, 30). The finding that plasma FFA concentration is lower during submaximal exercise in the trained than in the untrained state suggests that the rate of oxidation of plasma FFA may be reduced in the trained state. Despite the lower plasma FFA concentration, the training program resulted in a 65% increase in the amount of energy derived from fat (calculated from the RER). As a consequence, the proportion of the caloric expenditure derived from fat during the prolonged exercise test increased from 35% before training to 57% after training. These findings provide evidence suggesting that training results in increased utilization of fatty acids derived from a source other than plasma FFA.

The primary purpose of the present study was to determine whether there is a greater depletion of muscle triglyceride stores during the same prolonged exercise test in the trained as compared with the untrained state. Our results clearly show that this is the case, because the decrease in triglyceride concentration was about twice as great in the trained as in the untrained state. In absolute terms, the decrease averaged about 2.7 g/kg wet wt more in the trained than in the untrained state. (An average fatty acid chain length of 17 carbon atoms and a muscle water content of 76% were used in making this calculation.) This quantity of fatty acids released by lipolysis of muscle triglycerides could, if oxidized, account for the greater amount of fat oxidized in the trained state. The increase in the amount of energy derived from fatty acid oxidation calculated from the RER was equivalent to about 22 g of fatty acid. This amount of fatty acid could

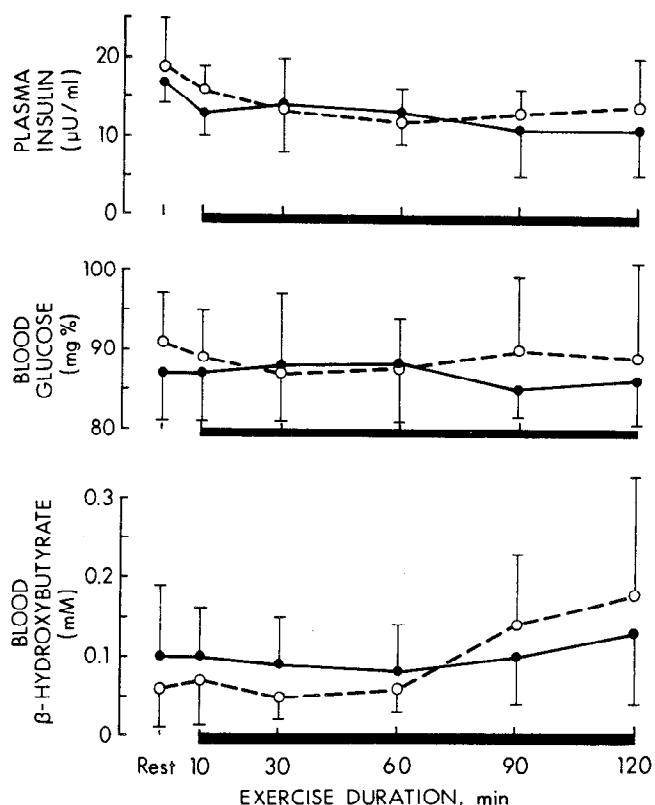


FIG. 3. Plasma insulin and blood glucose and β -hydroxybutyrate levels at rest and during the prolonged exercise test before (open circles) and after (filled circles) training. Values are means \pm SD.

TABLE 2. O_2 uptake and respiratory exchange ratio during prolonged exercise

	Duration of Exercise, min				
	10	30	60	90	120
$\dot{V}O_2$, l/min					
Before training	1.71 \pm 0.33	1.80 \pm 0.33	1.80 \pm 0.36	1.88 \pm 0.30	1.98 \pm 0.39
After training	1.73 \pm 0.51	1.78 \pm 0.45	1.83 \pm 0.36	1.87 \pm 0.36	1.97 \pm 0.44
RER					
Before training	0.94 \pm 0.03	0.93 \pm 0.03	0.88 \pm 0.03	0.87 \pm 0.03	0.90 \pm 0.04
After training	0.88 \pm 0.03*	0.86 \pm 0.06*	0.84 \pm 0.03*	0.83 \pm 0.03*	0.80 \pm 0.04*

Values are means \pm SD for 9 subjects at all time points except for 120-min values, which are the means \pm SD for 6 subjects. $\dot{V}O_2$, O_2 uptake; RER, respiratory exchange ratio. * Before vs. after training, $P < 0.05$.

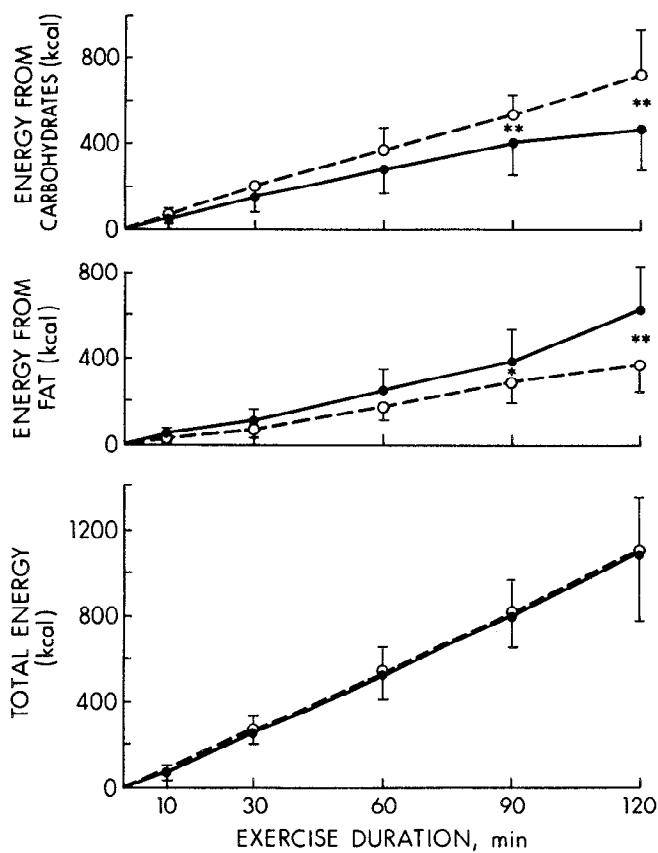


FIG. 4. Cumulative total energy utilized, energy from fat oxidation, and energy from carbohydrate oxidation during the prolonged exercise test before (open circles) and after (filled circles) training. Values are means \pm SD. **Difference between before and after training significant at $P < 0.01$.

TABLE 3. Muscle glycogen and triglyceride concentrations

	Before Training	After Training
Muscle glycogen, mmol/kg dry wt		
Before exercise	285 \pm 111	328 \pm 70
After exercise	82 \pm 51	209 \pm 89†
Decrease	203 \pm 100	120 \pm 55*
Muscle triglyceride, mmol/kg dry wt		
Before exercise	59.2 \pm 7.7	63.3 \pm 17.7
After exercise	46.4 \pm 8.9	37.2 \pm 12.3*
Decrease	12.7 \pm 5.5	26.1 \pm 9.3†

Values are means \pm SD for 9 subjects. * Before vs. after training, $P < 0.05$. † Before vs. after training, $P < 0.001$.

be accounted for by increased lipolysis of muscle triglycerides if a response similar to that seen in our muscle biopsy samples occurred in about 8 kg of muscle.

We have no direct information regarding the muscle mass involved in bicycle exercise requiring ~ 1.8 l O₂/min. However, the value of ~ 8 kg seems a reasonable estimate. In a recent study, Kiens, Christensen, and Saltin (personal communication) estimated, on the basis of a volumetric analysis, that the quadriceps muscle mass in young men with a mean body weight of 73 kg averages 2 kg per lower extremity. It seems reasonable that, in addition to the 4 kg of quadriceps muscle, another 4 kg or so of other muscles are involved in moderate-intensity bicycling.

It seems well documented that the rate of plasma FFA oxidation is a function of concentration (13, 30). However, the rate at which muscle oxidizes FFA is determined not by plasma FFA concentration but by the concentration of FFA in the cytoplasm to which the mitochondria are exposed, as well as by the concentration of mitochondria (i.e., the capacity to oxidize FFA), and perhaps by the availability of other substrate (cf. Refs. 21, 25). The training program used in this study resulted in a large increase in the capacity for FFA oxidation as reflected in the level of β -hydroxyacyl-CoA dehydrogenase, which was used as a marker for the mitochondrial enzymes involved in FFA oxidation. This adaptation might, by itself, have been responsible for the increased FFA oxidation in the trained state if cytoplasmic FFA concentration was similar before and after training. Another possibility that should be considered is that increased lipolysis of muscle triglycerides may result in a higher cytoplasmic FFA concentration in the trained state, contributing to the increased FFA oxidation. This important question will be difficult to answer, because determining the FFA concentration to which the mitochondria are exposed in the cytoplasm involves major technical problems.

It would be of greater interest to know the mechanism responsible for the greater triglyceride lipolysis in trained than in untrained muscle. A possible explanation is suggested by the studies of Oscai and co-workers (28), who found that the level of activity of the type L hormone-sensitive lipase is elevated in muscles of exercise trained rats. This is the enzyme thought to be responsible for triglyceride lipolysis in muscle (27). It will be interesting to see whether a similar adaptation can be demonstrated in humans.

In conclusion, the present study provides evidence that the greater utilization of fat during exercise of the same intensity in the trained compared with the untrained state is fueled by increased lipolysis of muscle triglycerides.

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