Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs. untrained humans

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Turcotte, Lorraine P., Erik A. Richter, and Bente Kiens. Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs. untrained humans. *Am. J. Physiol.* 262 (*Endocrinol. Metab.* 25): E791-E799, 1992.—We studied the effect of local muscle adaptations on free fatty acid (FFA) metabolism during prolonged exercise in trained and untrained subjects. Six trained (T) and six untrained (UT) young human males exercised for 3 h at 60% of their individual maximal dynamic knee extension capacity. The contribution of blood and plasma metabolites as well as intramuscular substrates to oxidative metabolism in the thigh was calculated from arteriovenous differences and femoral-venous blood flow as well as from muscle biopsies in subjects that were continuously infused with [1-^{14}C]palmitate. Arterial plasma FFA concentration increased over time in both T and UT. Fractional uptake of FFA across the thigh remained unchanged over time in T (15%) but decreased in UT (from 15 to 7%), especially during the last hour of exercise. Thus FFA uptake increased linearly over time in T (96 ± 20 to 213 ± 20 $\mu mol \cdot min^{-1} \cdot kg^{-1}$), whereas it leveled off after 2 h in UT (74 ± 16 to 133 ± 46) even though FFA delivery increased similarly in T and UT. Percentage oxidation was similar in T and UT; thus total FFA oxidation was higher in T. Glucose uptake increased in both groups over time and was significantly higher in UT during the last hour of exercise. In conclusion, during prolonged knee extension exercise, FFA uptake increases linearly with FFA delivery in the trained thigh, whereas in the untrained thigh uptake becomes saturated with time. This difference partly explains the increased lipid oxidation in T vs. UT and suggests, furthermore, that local muscle adaptations to training are important for the utilization of FFA during prolonged exercise.

human skeletal muscle; endurance training; free fatty acids; blood glucose

FREE FATTY ACIDS (FFA) are a major fuel source for skeletal muscle metabolism at rest and during low-to-moderate intensity exercise. Endurance training modifies substrate utilization patterns by increasing the contribution of lipids in oxidative metabolism. Hurley et al. (20) found that endurance training increased the utilization of intramuscular triacylglycerols during 2 h of bicycling at the same absolute intensity as before training. Jansson and Kaijser (21) reached the same conclusion based on the fact that the turnover rate of FFA was not different in endurance-trained cyclists bicycling for 1 h at the same relative intensity as untrained subjects. However, during bicycle exercise, Henriksson (19) measured a higher net uptake of FFA across the trained leg compared with the contralateral untrained leg. Similarly, with an equal FFA delivery to the working muscles, Kiens (24) reported a higher net uptake of FFA in the trained thigh performing one-legged dynamic knee extension exercise. Because FFA are both taken up and released from the muscles during exercise, it is sometimes difficult to measure a net uptake across the working muscles. Furthermore, net uptake does not allow conclusions to be made regarding the metabolic fate of the fatty acids taken up.

Studies using bicycling exercise are characterized by a different hormonal response to exercise in trained and untrained subjects. For instance, when work is performed at the same absolute submaximal work load, the increase in plasma catecholamine concentration is less in trained than in untrained subjects (13). Thus differences in metabolism between trained and untrained subjects during exercise may reflect the composite picture of changes in hormone secretion combined with training-induced alterations in muscle.

To evaluate the pure effect of training-induced adaptations in muscle on metabolism during exercise, it is necessary to perform exercise during conditions with a similar internal body milieu. Ideally this would require the use of isolated perfused muscle. In humans, however, an approximation to this technique can be achieved by exercising small muscle groups so that the exercise-induced changes in internal milieu are minimal. Thus, in the present study we utilized the knee extensor model combined with leg catheterization, muscle biopsies, and infusion of [1-^{14}C]palmitate in trained and untrained subjects to focus on the effects of local muscle adaptations on metabolism.

METHODS

Subjects. Six trained and six untrained male subjects, aged 25 yr (range 23-28 yr) and weighing 72.5 kg (range 62-84 kg), gave their informed consent to participate in this study, which was approved by the Copenhagen Ethics Committee. None of the untrained subjects participated in competitive sports or in organized leisure time activities, except for using the bicycle as a form of local transportation. Criteria for selection in the endurance-trained group included regular participation in an established endurance training program for at least 6 mo before the experiment and a maximal oxygen uptake value of at least 60 $ml \cdot kg^{-1} \cdot min^{-1}$. Mean (±SE) maximal oxygen consumption determined by the leveling off criterion of oxygen uptake during incremental work on a Krogh bicycle ergometer was significantly higher in the trained than the untrained subjects (61.7 ± 0.7 vs. 38.6 ± 2.3 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively, $P < 0.05$).

The subjects were then familiarized with the Krogh ergometer modified for one-legged knee extension exercise as previously described (3). With this exercise model, external work is exclusively performed by the quadriceps femoris muscle. The subjects practiced two to three times to ensure that only the knee extensor muscles were used during the exercise. A maximal leg extension capacity test was performed with their dominant leg to determine the maximal work load and peak oxygen uptake of the knee extensors. For 2 days before the experiment, the subjects were instructed to abstain from strenuous physical activity and to eat a mixed diet.

Preparation of infusate. [1-^{14}C]palmitic acid (250 μCi) in ethanol (New England Nuclear, Boston, MA) was put in a sterile round-bottomed flask to which excess NaOH was added. After the mixture was evaporated to dryness, the residue of sodium salts was dissolved in sterile saline and heated to 70°C

while being stirred. When the ethanol could no longer be detected, the mixture was cooled to 55°C and 20% sterile human serum albumin (Statens Serum Institute, Copenhagen, Denmark) was added. The albumin-palmitate solution was sterilized by filtration through a 0.22-μm Millipore filter into sterile containers. The solution was checked for bacterial contamination and kept frozen until use.

Experimental protocol. On the morning of the experiment, subjects reported to the laboratory at 8 A.M. after a 9- to 12-h fast. For blood sampling, Teflon catheters were inserted below the inguinal ligament in the femoral artery and vein of the leg to be exercised and advanced proximally so that the tips of the arterial and venous catheters were located ~2 cm proximal and 2 cm distal to the inguinal ligament, respectively. To allow for the measurement of blood flow by the constant-infusion thermodilution technique (30), a thermistor (Edslab probe 94-030-2.5 F) was inserted through the venous catheter and advanced 6-8 cm proximal to the catheter tip. The thermistor was connected to a cardiac output computer (American Edwards Laboratory, Irvine, CA) and an Elema Mingograph (Siemens, Stockholm, Sweden) to record the changes in temperature. An additional catheter was inserted in the antecubital vein of the left arm for the constant infusion of albumin-bound [1-^{14}C]palmitate (0.2 μCi/min) using a calibrated syringe pump (model 570, NE Medical Instruments, Midway, MA). In three additional subjects, sodium [^{14}C]bicarbonate was infused at a rate of 0.2 μCi/min. The exact infusion rate was determined for each subject by measuring the radioactivity in the infusate.

The infusion of palmitate or sodium bicarbonate was started immediately after the catheterization procedure. After 1 h of supine rest, baseline arterial and venous blood samples were drawn simultaneously and resting thigh blood flow was determined using a modified version of the thermodilution technique, as previously described (30). A needle biopsy of the vastus lateralis muscle to be exercised was taken using the Bergstrom technique (6) including suction. Expiratory air was collected in Douglas bags during the last 20 min of the resting period before obtaining the muscle biopsy. Immediately afterward, the subjects began the exercise protocol that consisted of kicking at 60% of their one-legged maximal knee extension capacity for 3 h. Arterial and venous blood samples were taken at 10, 30, 60, 120, 150, and 180 min of the exercise period, and blood flow determinations were done immediately after each blood sampling. During blood sampling and blood flow determinations, a pneumatic cuff located below the knee was inflated to 220 mmHg. Expiratory air was collected during the last 15 min of every hour. Muscle biopsies were taken after 120 and 180 min of exercise. The second biopsy was obtained through a new incision located 5-7 cm proximal to the first incision, and the third was obtained through the same incision as the second one but with the needle pointed in another direction. Heart rate and blood pressure were continuously monitored and recorded. The volume of expiratory air collected in the Douglas bags was measured with a Tissot spirometer, and the oxygen and carbon dioxide contents were determined with paramagnetic (Servomex) and infrared (Beckman LB-2) systems, respectively.

Blood sample analysis. Blood glucose was determined by the fixed glucose oxidase method using a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). Blood lactate (26), β-hydroxybutyrate (5), and glycerol (5) were determined by specific enzymatic methods adapted to the fluorometer. Plasma FFA were determined fluorometrically as previously described (24). Plasma triacylglycerols minus glycerol were assayed with an enzymatic colorimetric test from Boehringer (Mannheim, FRG). Insulin in plasma was determined using a radioimmunoassay kit kindly donated by Novo-Nordisk (Copenhagen, Denmark). Catecholamines in plasma were determined by a radioenzymatic procedure (8). Blood hemoglobin and oxygen saturation were measured with an OSM II hemoximeter (Radiometer, Copenhagen, Denmark). Hematocrit was determined in triplicate from microcapillary tubes.

To determine plasma palmitate radioactivity, plasma lipids were extracted twice using Dole's extraction mixture (11). The upper phase was removed and evaporated to dryness under nitrogen. The residue was resuspended in chloroform-methanol (2:1 vol/vol) and applied with appropriate lipid standards to a thin-layer chromatography glass plate (silica gel, 250-μm thickness, Sigma, St. Louis, MO) 3 cm from the bottom. Lipid separation was made using a mobile phase of petroleum ether-ether-acetic acid (70:30:1 vol/vol/vol) and allowed to develop to a height of 16 cm (23). Lipids were separated into the following fractions: cholesterol, triacylglycerols, FFA, monoglycerides, and diglycerides. Each lipid fraction was scrapped off the plate, mixed with liquid scintillation fluid (Maxifluor, J. T. Baker, Deventer, Holland), and counted individually in a Tri-Carb Packard liquid scintillation counter, model 2000 CA (Packard Instruments Company, Downers Grove, IL).

The liberation and collection of $^{14}CO_2$ from the blood was performed within 4-5 min of anaerobic collection according to a modified version of the method described by Hagenfeldt (16). In brief, a piece of Whatman paper was glued to the underside of a plastic cap and ethanolamine was spread evenly onto its surface. After the addition of concentrated lactic acid into ordinary screw-cap scintillation vials, a 5-ml blood sample was added to the vial, which was capped immediately and rotated gently until the blood was thoroughly mixed with the acid. Arterial and venous samples were done in duplicate. The vials were left standing at room temperature for 24 h. The cap was then transferred to another counting vial, which contained ethylene glycol monomethyl ether, and left standing upside down to dissolve the glue. After the addition of scintillation fluid, the sample was counted using quench correction by the external standard method.

Muscle sample analysis. The biopsy sample was frozen in liquid nitrogen within 10-15 s. From the resting sample, a section was cut off before freezing and mounted on embedding medium, frozen in isopentane, and cooled to its freezing point in liquid nitrogen, and both parts were stored at −80°C until further analysis. Before biochemical analyses, muscle biopsy samples were freeze dried and dissected free of connective tissue, fat, and blood. Muscle glycogen concentration was determined as glucose residues after hydrolysis of the muscle sample in 1 M HCl at 100°C for 2 h (26). Muscle concentrations of glucose and glucose 6-phosphate were determined in neutralized perchloric acid extracts with standard enzymatic methods (26). Muscle citrate synthase and β-hydroxyacyl-CoA dehydrogenase activities were determined fluorometrically (26). Serial transverse muscle sections were stained for myofibrillar adenosinetriphosphatase (27) to identify fiber types (7) and with the amylase-periodic acid-Schiff method to visualize capillaries (2). Fiber area and capillary density calculations were done as previously described (4).

Calculations. Thigh volume was calculated by using the thigh length, three circumference, and three skinfold measurements (22), and the muscle mass was estimated from a regression equation derived from autopsy studies (24). Fractional uptake was calculated as the difference in radioactivity between the arterial and venous blood samples divided by the radioactivity in the arterial sample (17). FFA delivery was calculated by multiplying plasma flow by the arterial plasma FFA concentration. FFA and triacylglycerol uptake were calculated by multiplying the plasma delivery by the fractional uptake (17). Percentage FFA oxidation was calculated by dividing the total amount of radioactivity recovered as $^{14}CO_2$ by the total amount of radioactivity that was taken up by the muscle (18). Total FFA oxidation was calculated by multiplying the FFA uptake by the

percentage oxidation. In calculating the rate of uptake of FFA into muscle, we have assumed that palmitate is a representative fatty acid for the total plasma FFA concentration. Evidence exists to support this assumption because the fractional uptake of palmitate across the resting and exercising forearm muscle was found to be very similar to the values reported for total plasma FFA (17). Thus we use the term fractional uptake of FFA instead of palmitate.

In calculating the rate of uptake of FFA into muscle, we assumed that the release of radioactive palmitate from the thigh tissue was negligible over time. Our results support this assumption because the specific activity of FFA was always smaller in the vein than in the artery and venous specific activity of FFA did not increase with time in either the trained or the untrained subjects. Regarding the plasma FFA oxidation values, retention of the label via isotopic exchange from the oxaloacetate and α-ketoglutarate pools of the tricarboxylic acid cycle may have led to a slight underestimation of the contribution of plasma FFA to total oxidative metabolism (33). However, our estimates are within the range of previously reported values, which vary from 60 to 100% in exercising humans (17, 18), and there is no reason to believe that such retention would be different between groups.

The equilibrium concentration of unbound palmitate was calculated by the stepwise equilibrium constant method (34), using the dissociation constants for the palmitate-albumin complex reported by Spector et al. (31). Uptake or release of substrates and uptake of oxygen across the thigh were calculated by multiplying the blood or plasma flow by the arteriovenous difference in concentration and were expressed per kilogram of quadriceps muscle mass. The contribution of plasma FFA, blood β-hydroxybutyrate, blood glucose, and muscle glycogen to muscle oxidative metabolism was calculated on the basis of a mean oxygen consumption of 2.03 l O_2/g fat, 0.979 l O_2/g β-hydroxybutyrate, and 0.746 l O_2/g carbohydrate (12).

Statistical analysis. For each physiological variable measured, a two-way analysis of variance with repeated measures for the time factor was performed to test for changes due to time or training status. A two-way Duncan's multiple range test was done to detect differences between time points. Between-group differences in descriptive physical and physiological variables were tested with a Student's t test. In all instances, an α of 0.05 was used to determine significance.

RESULTS

Isotopic steady state. In a subsample of three subjects, we measured plasma FFA radioactivity more frequently during the resting period. Following an initial sharp rise in arterial plasma FFA radioactivity, arterial plasma FFA concentration and radioactivity as well as the arteriovenous difference in radioactivity became stable after 30 min. During the exercise period, arterial plasma FFA radioactivity varied by <15%. To ensure that the carbon dioxide pools were in equilibrium, three separate experiments were performed in which subjects were infused with sodium [^{14}C]bicarbonate at a constant rate. Both arterial and arteriovenous differences in blood carbon dioxide radioactivity levels became relatively stable after 45 min of rest. After a decline in the arterial and venous radioactivity levels during the 1st h of exercise, radioactivity levels varied by <20% during the remaining 2 h, indicating that the carbon dioxide pools were in equilibrium (Fig. 1).

Physical and physiological characteristics. The quadriceps muscle mass and the maximal knee extension capacity of the trained subjects were higher (means ± SE = 2.5 ± 0.2 kg and 57 ± 4 W, respectively) than those of the untrained (1.9 ± 0.2 kg and 38 ± 4 W, respectively, $P < 0.05$). Although the trained subjects worked at a higher absolute work load (34 vs. 23 W, $P < 0.05$), the amount of work done per kilogram of quadriceps muscle mass was similar between the two groups of subjects (pooled mean ± SE = 13.1 ± 1.1 W/kg). Thigh blood flow and leg oxygen uptake (expressed per kg muscle) were not significantly different between the two groups and did not vary during the exercise period. The respective means for these variables were 2.1 ± 0.2 l·min^{-1}·kg^{-1} and 239 ± 20 ml·min^{-1}·kg^{-1} for the trained subjects and 2.1 ± 0.2 and 216 ± 17, respectively, for the untrained subjects. The work load elicited a lower heart rate in the trained than in the untrained subjects (88 ± 2 vs. 97 ± 3 beats/min, respectively, $P < 0.05$) but did not change significantly over time in either group.

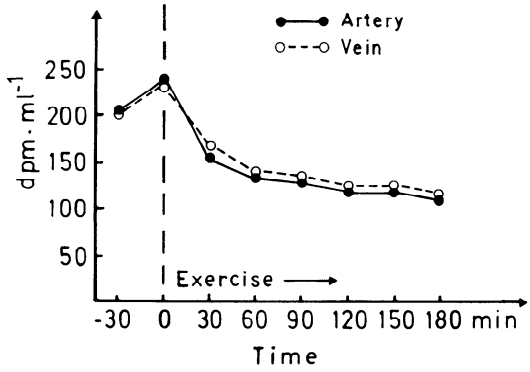

Fig. 1. Mean values of arterial and venous blood CO_2 radioactivity after infusion of sodium [^{14}C]bicarbonate (0.2 μCi/min) during 60 min of rest and 3 h of knee extension exercise in 3 young male subjects exercising at same relative work load as trained and untrained subjects.

FFA metabolism. Arterial plasma FFA concentration increased progressively from values of 543 ± 104 and 373 ± 84 μM after 30 min of exercise to 1,298 ± 186 and 1,378 ± 175 μM after 3 h in the trained and untrained subjects, respectively (Fig. 2*A*). Arterial plasma FFA concentration was not significantly different between the two groups of subjects throughout the exercise period. Because thigh blood flow per kilogram of muscle and arterial plasma FFA concentration were not significantly different between the two groups, FFA delivery to the working muscles was similar for both groups of subjects and displayed the same progressive increase over time as arterial plasma FFA concentration. FFA delivery increased from values of 584 ± 96 and 527 ± 109 μmol·min^{-1}·kg^{-1} after 30 min of exercise to 1,496 ± 131 and 1,720 ± 387 μmol·min^{-1}·kg^{-1} after 3 h in the trained and untrained subjects, respectively.

At rest, the fractional uptake of FFA was similar in the trained (42 ± 8%) and untrained (40 ± 6%) thigh. In both the trained and untrained thigh, FFA fractional uptake was maintained at ~15% for the first 2 h of exercise, at which point in time it decreased sharply in the untrained (Fig. 2*B*). After 3 h of leg kicking, the fractional uptake was down to 7% in the untrained thigh, whereas it was maintained at 15% in the trained ($P < 0.05$). During the first 2 h of exercise, FFA uptake increased progressively

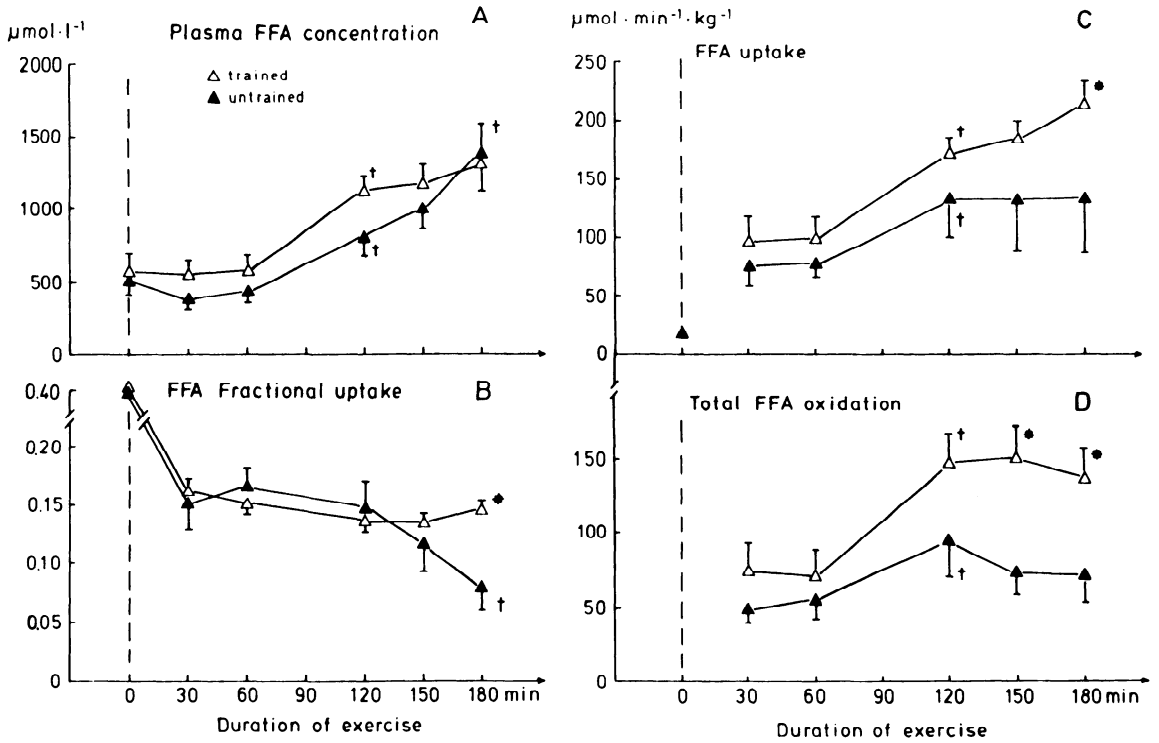

Fig. 2. Arterial plasma concentration (*A*), fractional uptake (*B*), uptake (*C*), and total oxidation (*D*) of free fatty acids (FFA) across thigh during rest and 3 h of knee extension exercise in trained and untrained subjects. Values are means ± SE of 6 subjects in trained and untrained groups. Fractional uptake was calculated as arteriovenous radioactivity difference divided by the arterial radioactivity. Uptake was calculated by multiplying fractional uptake with plasma flow and arterial plasma FFA concentration. Total oxidation was calculated as percentage oxidation times FFA uptake. * $P < 0.05$ compared with untrained; † $P < 0.05$ compared with previous value.

from 96 ± 20 and 74 ± 16 to 171 ± 14 and 132 ± 33 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ in the trained and untrained thigh, respectively (Fig. 2*C*). Only the trained thigh was able to maintain this rate of increase so that, after 3 h of leg kicking, FFA uptake was significantly higher in the trained than the untrained thigh, with values of 213 ± 20 and 133 ± 46 $\mu mol \cdot min^{-1} \cdot kg^{-1}$, respectively. FFA uptake increased with increasing plasma FFA concentration in the trained but reached a plateau in the untrained regardless of whether FFA concentration was expressed as total or unbound concentration (Fig. 3, *A* and *B*).

Throughout the exercise period, the percentage of plasma FFA oxidized did not change and was similar in the trained (76 ± 9%) and untrained (74 ± 12%) thigh. When expressed in absolute terms, total plasma FFA oxidation increased for the first 2 h to reach values of 148.7 ± 20.1 and 95.2 ± 24.3 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ in the trained and untrained thigh, respectively, and remained stable thereafter (Fig. 2*D*).

Substrate concentrations. Resting arterial concentrations for blood glucose, lactate, glycerol, and β-hydroxybutyrate were not different between the trained and

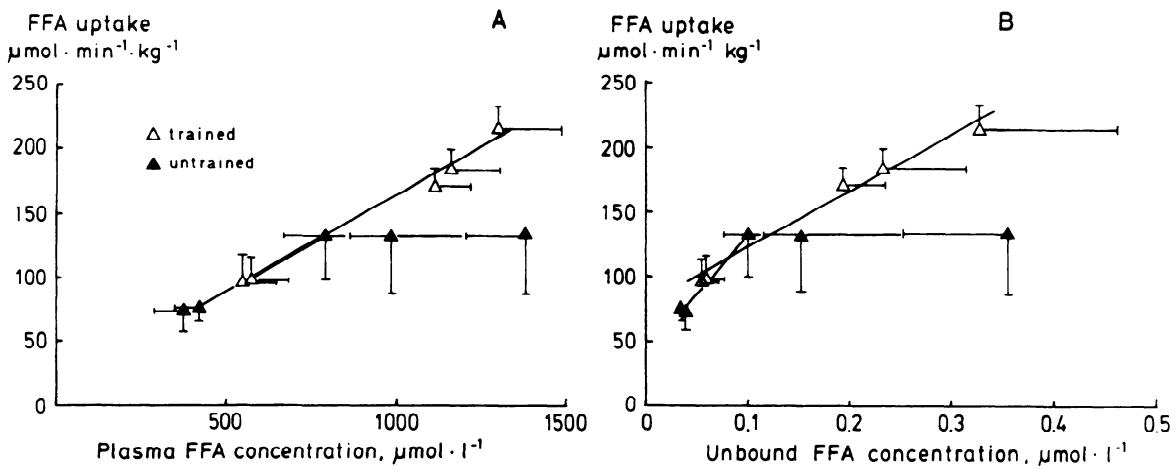

Fig. 3. Relationship between FFA uptake and total plasma FFA concentration (*A*) or calculated unbound FFA concentration (*B*) during exercise in trained and untrained subjects. Each point represents mean ± SE ($n = 6$) total or calculated FFA concentration as plotted against mean FFA uptake at each time point.

untrained subjects (Table 1 and Fig. 4*A*). Resting arterial plasma triacylglycerol concentration was lower in the trained (0.61 ± 0.06 mM) than in the untrained subjects (1.0 ± 0.2 mM; Table 1). In the untrained subjects, arterial blood glucose concentration gradually decreased from 4.8 ± 0.1 mM after 10 min of exercise to 4.0 ± 0.1 mM after 3 h (Fig. 4*A*). In the trained subjects, arterial blood glucose concentration fluctuated only slightly and was not significantly different from the preexercise value of 4.7 ± 0.1 mM after 3 h of exercise. After the 1st h of exercise, arterial blood glucose concentration remained significantly higher in the trained than in the untrained subjects. There were no significant differences in arterial blood lactate, glycerol, and β-hydroxybutyrate concentrations between the trained and untrained subjects throughout the exercise period (Table 1). Arterial plasma triacylglycerol concentration remained lower in the trained subjects throughout the exercise period (Table 1).

Substrate exchange across thigh. In both the trained and untrained thigh, glucose uptake increased progressively from values of 224 ± 41 and 241 ± 38 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ after 10 min of exercise to 637 ± 108 and 882 ± 97 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ after 2 h and remained stable for the last hour of work (Fig. 4*B*). During the last hour of exercise, glucose uptake was higher in the untrained thigh. In both the trained and untrained thigh, lactate and glycerol were released throughout the exercise period, except for the last time point when there was no net release of lactate in the trained thigh (Table 2). There was a similar progressive increase in the uptake of β-hydroxybutyrate in both the trained and untrained thighs during exercise (Table 2).

Arterial hormone levels. Arterial plasma insulin, epinephrine, and norepinephrine concentrations were not significantly different between the trained and untrained subjects both at rest and during exercise (Table 3). Plasma insulin concentration decreased progressively and similarly in untrained and trained subjects. During the first 2 h of exercise, epinephrine concentration increased slowly to values close to double the resting values in both the trained and untrained subjects (0.26 ± 0.05 and 0.14 ± 0.01 ng/ml, respectively). During the last hour of exercise, epinephrine concentration increased further to reach values of 0.37 ± 0.1 and 0.30 ± 0.08 ng/ml, respectively. During the first 30 min of exercise, norepinephrine concentration increased quickly to values of 0.44 ± 0.05 and 0.46 ± 0.06 ng/ml in the trained and untrained subjects, respectively, and did not change significantly during the following 2.5 h of exercise.

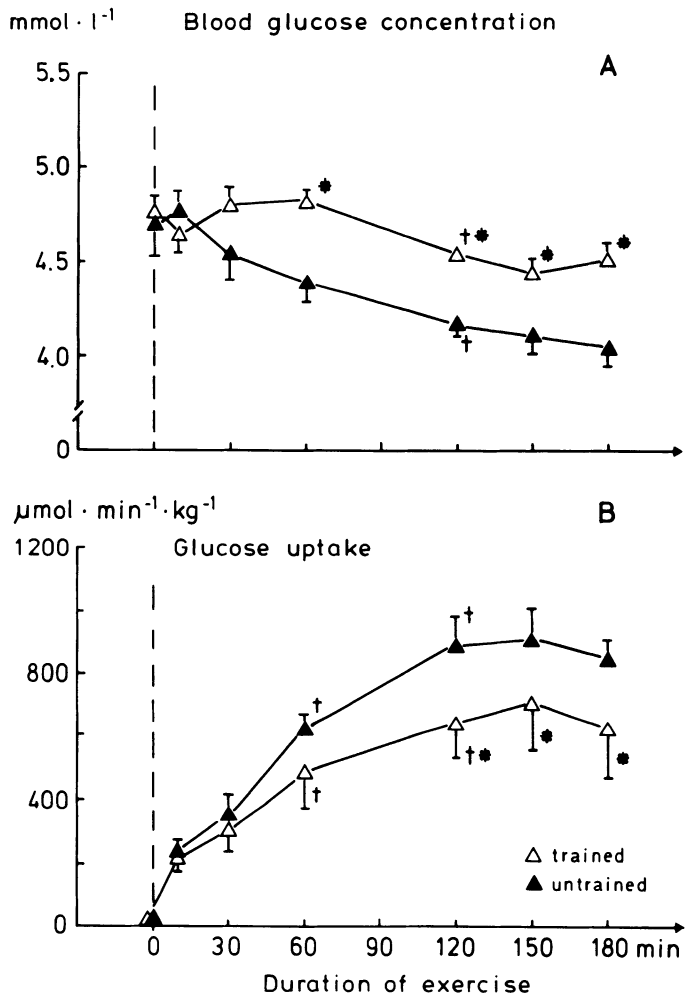

Fig. 4. Blood glucose concentration (*A*) and uptake (*B*) across thigh during rest and 3 h of knee extension exercise in trained and untrained subjects. Glucose uptake was calculated as arteriovenous glucose concentration difference times blood flow. * $P < 0.05$ compared with untrained; † $P < 0.05$ compared with previous value.

Table 1. *Arterial concentrations of substrates during rest and exercise in trained and untrained subjects*

	Rest	Exercise, min				
		30	60	120	150	180
Lactate, mM						
Trained	0.69±0.08	1.04±0.22	0.72±0.12*	0.64±0.10	0.67±0.13	0.67±0.11
Untrained	0.49±0.06	0.81±0.09	0.63±0.06*	0.61±0.05	0.57±0.05	0.64±0.09
Glycerol, μM						
Trained	40.9±5.0	63.9±8.4	68.3±8.6	104.6±7.6*	101.7±9.4	124.6±8.4*
Untrained	43.1±5.9	55.7±6.4	55.9±6.3	87.4±7.4*	101.7±6.8	120.9±8.3*
β-Hydroxybutyrate, μM						
Trained	30.3±9.3	29.2±7.5	33.9±9.4	102.1±21.9*	150.2±33.5*	213.5±46.7*
Untrained	40.3±14.9	36.0±16.3	58.1±25.8	146.3±54.3*	255.0±85.1*	335.6±95.5*
Triacylglycerol, mM						
Trained	0.61±0.06†	0.69±0.08†	0.71±0.08†	0.74±0.09†	0.80±0.09*†	0.82±0.10*†
Untrained	0.99±0.17	1.15±0.14	1.03±0.15	1.06±0.15	1.10±0.15*	1.13±0.14*

Values are means ± SE of 6 subjects in trained and untrained groups. Values were measured in blood, except for triacylglycerol, which was measured in plasma, and obtained immediately before and after 30, 60, 120, 150, and 180 min of knee extension exercise. * $P < 0.05$ compared with previous value. † $P < 0.05$ compared with untrained.

Table 2. *Exchange of substrates across thigh during rest and exercise in trained and untrained subjects*

	Rest	Exercise, min				
		30	60	120	150	180
Lactate release, mmol·min^{-1}·kg^{-1}						
Trained	0.01±0.01	0.18±0.10	0.11±0.08	0.13±0.05	0.10±0.06	−0.04±0.04
Untrained	0.02±0.01	0.26±0.06	0.10±0.07	0.09±0.05	0.12±0.04	0.11±0.03
Glycerol release, μmol·min^{-1}·kg^{-1}						
Trained	2.2±0.6	20.0±3.0	17.9±2.7	35.7±8.9*	49.6±9.7	20.3±5.7
Untrained	4.3±1.1	22.0±3.5	12.5±4.4	24.1±6.4*	18.7±6.7	24.9±8.4
β-Hydroxybutyrate uptake, μmol·min^{-1}·kg^{-1}						
Trained	1.5±0.7	7.3±4.3	4.1±2.2	25.1±5.9*	33.2±9.6	48.9±5.1*
Untrained	3.4±2.4	3.2±6.0	30.7±22.1	40.1±26.4*	47.4±21.8	67.3±22.7*
Triacylglycerol uptake, μmol·min^{-1}·kg^{-1}						
Trained	5.2±4.3	−28.7±29.8	3.1±17.1	12.0±26.9	0.4±15.3	0.3±17.9
Untrained	9.3±3.7	55.9±33.34	15.5±19.6	−27.5±42.4	−39.7±36.5	−54.2±35.6

Values are means ± SE of 6 subjects in trained and untrained groups. Data are expressed per kilogram thigh muscle. Values were obtained immediately before and after 30, 60, 120, 150, 180 min of knee extension exercise. * $P < 0.05$ compared with previous value.

Table 3. *Arterial concentrations of plasma insulin, epinephrine, and norepinephrine during rest and exercise in trained and untrained subjects*

	Rest	Exercise, min				
		30	60	120	150	180
Insulin, μU/ml						
Trained	7.3±1.5	6.5±0.08	6.1±1.1	4.7±1.2	3.9±1.7	4.8±1.2
Untrained	7.6±1.5	7.1±0.08	6.2±1.0	4.6±0.4	3.7±0.4	2.8±0.3
Epinephrine, ng/ml						
Trained	0.13±0.03	0.18±0.02		0.26±0.05*		0.37±0.09*
Untrained	0.08±0.02	0.11±0.01		0.14±0.01*		0.30±0.08*
Norepinephrine, ng/ml						
Trained	0.17±0.02	0.44±0.05		0.49±0.04		0.54±0.04
Untrained	0.25±0.05	0.46±0.06		0.49±0.07		0.72±0.09

Values are means ± SE of 6 subjects in trained and untrained groups. Norepinephrine and epinephrine values were obtained immediately before and after 30, 120, and 180 min of knee extension exercise. Insulin values were obtained at all time points. * $P < 0.05$ compared with previous value.

Muscle substrate and metabolite levels. Resting muscle glycogen levels were higher in the trained than the untrained subjects (Table 4). During the first 2 h of exercise, muscle glycogen concentration decreased at a similar rate in the trained and untrained subjects but the rate was slightly higher in the untrained muscle during the last hour. Muscle glucose and glucose 6-phosphate concentrations were not significantly different between groups at any time point and did not vary significantly during the exercise period.

Table 4. *Muscle concentrations of glycogen, glucose, and glucose 6-phosphate during rest and exercise in trained and untrained subjects*

	Rest	Exercise, min	
		120	180
	mmol/kg dry wt		
Glycogen			
Trained	584±63†	359±67*	309±52*†
Untrained	433±15	247±42*	172±36*
Glucose			
Trained	1.24±0.09	2.17±0.66	1.69±0.32
Untrained	1.92±0.35	1.80±0.42	1.35±0.28
Glucose-6-phosphate			
Trained	0.29±0.08	0.34±0.09	0.43±0.14
Untrained	0.29±0.10	0.31±0.04	0.22±0.05

Values are means ± SE of 6 subjects in trained and untrained groups. Data are expressed per kilogram dry weight. Values were obtained immediately before and after 120 and 180 min of knee extension exercise. * $P < 0.05$ compared with previous value. † $P < 0.05$ compared with untrained value.

Muscle fiber composition and biochemical data. Vastus lateralis muscle enzyme activity for citrate synthase and β-hydroxyacyl-CoA dehydrogenase were higher in the trained (45.7 ± 2.8 and 53.3 ± 1 μmol·g dry wt^{-1}·min^{-1}) than in the untrained muscle (36.2 ± 3.8 and 35.9 ± 2.1 μmol·g dry wt^{-1}·min^{-1}, $P < 0.05$). The number of capillaries per fiber (pooled mean for all 3 fiber types = 4.9 ± 0.3 capillaries/fiber) and the percentage of type I (61 ± 2%), IIa (22 ± 2%), and IIb (17 ± 2%) fibers in the vastus lateralis muscle were not significantly different between the trained and untrained muscle.

DISCUSSION

Our results show that, during the later part of long-term dynamic knee extension exercise at the same relative intensity, thigh uptake and oxidation of plasma FFA are higher in trained than in untrained subjects. With a similar increase in FFA delivery over time, FFA uptake increased in the trained thigh, whereas it reached a plateau after 2 h of exercise in the untrained. Thus, during the 3rd h of exercise, FFA uptake and oxidation were ~60% higher in the trained thigh compared with the untrained. Concomitantly, glucose uptake was decreased in the trained thigh compared with the untrained. Because the internal milieu was largely similar in trained and untrained subjects during exercise, the differences in

FFA metabolism most likely reflect local training-induced differences in muscle.

These results are in line with those of Kiens (24), who found that net uptake of plasma FFA across the exercising thigh muscle was significantly higher in the trained thigh compared with the untrained. Using labeled FFA, we can now confirm these findings and furthermore show that the increased uptake of plasma FFA by the trained thigh is accompanied by a greater rate of plasma FFA oxidation.

The utilization of triacylglycerol was estimated by calculating the total amount of glycerol released over the 3 h of exercise and that amounted to 11.3 and 7.4 mmol in the trained and untrained subjects, respectively. We also estimated that 11.9 (trained) and 9.2 (untrained) mmol of triacylglycerol would need to be combusted to account for the thigh oxygen uptake left over after subtracting the contributions made by plasma FFA, blood β-hydroxybutyrate, blood glucose, and muscle glycogen. If the fact that femoral venous glycerol can originate from the breakdown of intramuscular as well as circulating triacylglycerol and from adipose tissue lipolysis is taken into account, these separate estimates of triacylglycerol utilization are in close agreement with each other and are not different between groups. However, these calculations do not provide us with an exact answer regarding the source of the triacylglycerol utilized. In this study, there was no significant uptake of circulating triacylglycerol by the exercising thigh. However, it is possible that their contribution was not detected because we measured total plasma triacylglycerol. When measuring very low density lipoprotein-triacylglycerol, Kiens and Lithell (25) found a net uptake of circulating triacylglycerol across the exercising thigh during 2 h of leg kicking.

Although different, our results are not necessarily contradictory to other previously published data (20, 21), in which the training-induced increase in fat utilization was covered by intramuscular triacylglycerol, but may be a reflection of the different exercise modes employed. In earlier studies, the subjects exercised on a bicycle ergometer, a type of exercise associated with marked increases in plasma catecholamine concentrations. Our subjects performed one-legged knee extension exercise and the highest catecholamine concentrations measured after 3 h of exercise were only about one-third as high as the values generally reported after prolonged bicycle exercise at a moderate intensity (13). This lower catecholamine response could have affected the use of intramuscular triacylglycerol, since it has been shown in exercising humans that nonselective β-adrenergic blockade prevented muscle lipolysis (9). It thus seems that exercise which leads to small changes in plasma catecholamine levels minimizes the breakdown of intramuscular triacylglycerol. With the exercise mode used in this study, the muscle mainly relies on plasma FFA delivery to increase its utilization of fat and it would appear that the endurance-trained muscle is better able to accomplish this than the untrained muscle.

The training-induced adaptations responsible for the increased utilization of plasma FFA by the muscle could be located at any steps from the mobilization of FFA to skeletal muscle metabolism. If it is taken into consideration that FFA delivery to the muscle increased similarly in the trained and untrained subjects, it is likely that factors inherent to the muscle are responsible for the difference in plasma FFA utilization. It is well accepted that training is associated with an increased capacity to oxidize fats made partly possible by an increase in the activity levels of skeletal muscle oxidative enzymes (14). In our study, β-hydroxyacyl-CoA dehydrogenase and citrate synthase activities were 48 and 26% higher in the trained than the untrained muscle. These results are in agreement with the hypothesis that the training-induced increase in skeletal muscle oxidative capacity leads to an increased utilization of fatty acids during submaximal exercise (15). Significant differences in fiber type, fiber composition, and capillarization between the trained and untrained muscle were not found and could therefore not explain the difference in plasma FFA utilization.

Until recently, transport of FFA across the plasma membrane was believed to be achieved by a simple process of passive diffusion because of the lipid nature of the fatty acid molecule. However, accumulated evidence suggests that transport may partly involve a carrier-mediated process (29, 32). Fatty acid binding proteins have been isolated from the plasma membrane of cultured hepatocytes, adipocytes, and cardiac myocytes (29). These similar 40-kDa proteins possess a high affinity for long-chain fatty acids, and raised antibodies to this class of membrane proteins inhibit both FFA binding to the plasma membrane and cellular influx of FFA in a dose-dependent fashion (29). These fatty acid binding proteins could constitute putative fatty acid transporters and be responsible for at least part of the FFA uptake, and thus FFA uptake could be subject to regulation at the level of the plasma membrane. Our data are compatible with the hypothesis of carrier-mediated FFA transport in skeletal muscle. At high concentrations of FFA, the difference in FFA uptake between the trained and untrained thigh might possibly indicate a training-induced alteration in membrane transport of FFA.

In both the trained and untrained subjects, there was a greater reliance on circulating fuel sources for muscle oxidative metabolism as the exercise continued. The contribution of plasma FFA, blood glucose, and β-hydroxybutyrate increased from 50% after 2 h of exercise to 76 and 78% after 3 h in the trained and untrained subjects, respectively. This shift in fuel sources is in agreement with earlier results showing that the contribution of extracellular substrates to leg muscle metabolism increased from 64% after 40 min of bicycling at 30% of maximal oxygen uptake to 86 and 92% after 3 and 4 h of work, respectively (1).

In the trained subjects, total fat utilization did not change during the exercise period (Fig. 5). However, the preferential use of extracellular fuel sources during the later part of the exercise period led to a 46% increase in the use of both plasma FFA and blood glucose and a concomitant decrease in the use of intramuscular fuel sources. In the untrained subjects, the overall contribution of fats to oxidative metabolism decreased from 46% after 2 h of exercise to 29% after 3 h. Plasma FFA became

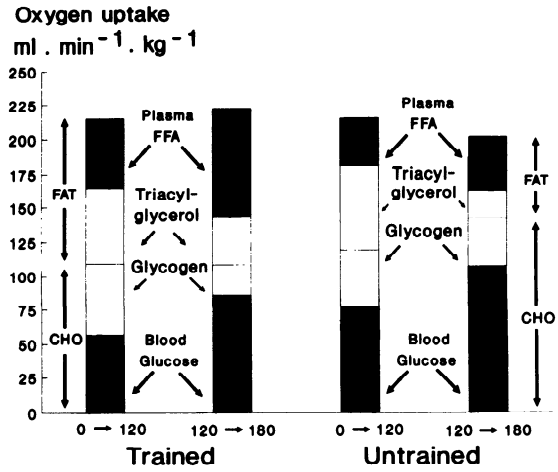

Fig. 5. Calculated contributions of carbohydrate and fat oxidation to total thigh oxygen uptake during first 2 and 3rd h of knee extension exercise in trained and untrained subjects. Values are calculated from plasma FFA oxidation, blood glucose uptake, and muscle glycogen utilization.

the more significant source of fat, but their rate of oxidation did not increase during the last hour of exercise so that their contribution to total oxidative metabolism did not exceed 22%. Conversely, the contribution of carbohydrates increased during the last hour, with a major increase (66%) in the contribution of blood glucose and a maintenance of the rate of muscle glycogen utilization.

Compared with the untrained subjects, there was a decreased reliance on carbohydrates as an energy source in the trained subjects during the last hour of exercise and this was evidenced by a 22% lower glucose uptake and a 32% lower rate of muscle glycogen utilization. Glycogen sparing in muscle has been shown repeatedly in endurance-trained individuals exercising for a prolonged period of time at both the same absolute and relative intensity as untrained subjects (20, 21, 24). Conversely, the maintenance of the rate of muscle glycogen utilization in the untrained subjects was somewhat unexpected, since it has been shown that, during prolonged exercise at a low work intensity, the contribution of fats to total oxidative metabolism increases with time (1). With our exercise model, differences in the rate of muscle glycogen utilization can hardly be ascribed to differences in the concentration of catecholamines but may be related to differences in the muscle fiber recruitment patterns. In accordance with this view, Kiens (24) found that the percentage of type IIa and IIb fibers that were glycogen depleted after 2 h of leg kicking was higher in the untrained than the trained muscle. Thus it may be that, as time progressed, the untrained subjects recruited an increasing number of type IIa and IIb fibers and, because of this, used more muscle glycogen than the trained and, furthermore, increased rather than decreased their reliance on carbohydrates.

Thigh glucose uptake was decreased in the trained compared with the untrained subjects during the later part of the exercise period despite a higher blood glucose concentration in the trained. Our results are in line with findings by Coggan et al. (10) in which 12 wk of endurance training decreased glucose turnover and oxidation in subjects bicycling at the same absolute intensity before and after training. Similarly, Jansson and Kaijser (21) found a lower leg glucose uptake in trained cyclists compared with untrained subjects bicycling at the same relative intensity. The reason for this lower glucose uptake in trained subjects during exercise is not readily apparent. One possibility is that increased fat oxidation in trained subjects would lead to a citrate-mediated inhibition of phosphofructokinase, which might lead to decreased glucose phosphorylation. If this were the case, however, one would expect higher glucose 6-phosphate and glucose concentrations in muscle of trained subjects and such a difference was not found in either the present study (Table 4) or the one by Jansson and Kaijser (21). These observations then directly point to a possible difference in membrane glucose transport between trained and untrained muscle, but such speculations cannot at present be directly supported by data. On the contrary, in rats, 10 wk of endurance swim training increased the number of glucose transporters in fast-twitch red fibers and also increased the rate of glucose transport in perfused muscle during maximal contraction (28). The nature of the mechanism responsible for the decreased glucose utilization in trained compared with untrained subjects during prolonged exercise therefore remains elusive.

In conclusion, with an increased delivery of FFA to the muscle, plasma FFA uptake and oxidation increased in trained subjects during prolonged thigh exercise at the same relative intensity, whereas they reached a plateau in the untrained. Although the contribution of extracellular substrates to thigh oxidative metabolism increased during the 3rd h of exercise in both groups of subjects, only in the trained thigh was there an increased utilization of plasma FFA. The resulting carbohydrate-sparing effect was seen in a decrease of both thigh glucose uptake and muscle glycogen utilization. The findings point to the importance of local muscle adaptations for metabolism of FFA during prolonged exercise.

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