Adaptation of Muscle to Exercise

INCREASE IN LEVELS OF PALMITYL CoA SYNTHETASE, CARNITINE PALMITYLTRANSFERASE, AND PALMITYL CoA DEHYDROGENASE, AND IN THE CAPACITY TO OXIDIZE FATTY ACIDS

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ABSTRACT The capacity of gastrocnemius and quadriceps muscles to oxidize palmitate, oleate, linoleate, palmityl CoA, and palmityl carnitine doubled in rats subjected to a program of treadmill running. The rate of palmitate oxdation by whole homogenates of, or the mitochondrial fraction from, leg muscles was twice as great per gram wet weight of muscle in the trained as in the sedentary animals over a wide range (0.125–1.5 mM) of palmitate concentrations.

The levels of activity of carnitine palmityltransferase, palmityl CoA dehydrogenase, and mitochondrial ATP-dependent palmityl CoA synthetase expressed per gram of muscle doubled in gastrocnemius and quadriceps muscles in response to the running program. The protein content of the mitochondrial fraction from these muscles was increased approximately 60%.

INTRODUCTION

Long-chain fatty acids can serve as the major energy source for skeletal muscle metabolism during prolonged exercise (1–5). The relative amounts of fat and of carbohydratae utilized during a bout of exercise depend in part on the individual's level of physical training. Men and animals that have adapted to regularly performed endurance exercise, such as long-distance running, derive more of their energy from fatty acid oxidation and less from carbohydrate catabolism than do their untrained counterparts during exercise of submaximal intensity (1, 6–8). This difference viewed in the context of studies demonstrating that muscle mitochondria can undergo major adaptive changes in response to exercise (9–12) suggested the possibility that physical training might induce an increase in the capacity of skeletal muscle to oxidize fatty acids. Evidence that such an adaptation does occur was obtained in a preliminary study (13) in which it was found that homogenates of leg muscles from chronically exercised rats released $^{14}CO_2$ from palmitate-1-^{14}C at a significantly increased rate. This observation was confirmed in the present detailed study in which it was further found that the levels of activity per gram wet weight of muscle of carnitine palmityltransferase (palmityl CoA-carnitine palmityltransferase, EC 2.3.1.-), palmityl CoA dehydrogenase (fatty acyl CoA : FAD oxidoreductase, EC 1.3.2.2) and mitochondrial ATP-dependent palmityl CoA synthetase (palmitic acid : CoA ligase [AMP], EC 6.2.1.3) doubled in leg muscles of rats subjected to a program of running. The capacity of gastrocnemius and quadriceps muscles to oxidize oleate, linoleate, palmityl CoA, and palmityl carnitine also increased twofold in response to the exercise.

METHODS

Animal care and exercise program. Male rats of a Wistar strain (specific pathogen-free CFN rats) weighing 100 g were obtained from Carworth Laboratory Animals, New City, N. Y., kept in individual cages, and fed Purina chow and water. They were divided into an exercising and two sedentary groups. The exercising animals were trained by means of a program of treadmill running as previously

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described (9, 10). After 12 wk, the exercisers were running continuously for 2 hr day, 5 days/wk, up an 8° incline at 31 m/min, with twelve 1 min intervals of running at 42 m/min interspersed through the work period. They were maintained at this work level until they were sacrificed. This exercise program has been found to result in a large increase in capacity for prolonged running and does not result in muscle hypertrophy (9, 14).

Sedentary control rats were divided into a paired weight group in which food intake was adjusted so as to maintain their body weights the same as those of the exercising animals, and a freely eating group which was provided with food ad lib. These animals were not subjected to treadmill running.

Tissue preparation. Animals were killed approximately 66 hr after their last exercise session. They were anesthetized with ether, decapitated, and thoroughly exsanguinated. Gastrocnemius and quadriceps muscles were dissected out and chopped into a fine mince which was thoroughly mixed. A portion of the mixed muscle mince was homogenized in 0.175 M KCl, containing 0.1 mM EDTA, using a glass Potter-Elvehjem homogenizer immersed in ice water. After gross breaking up of the muscle, homogenization was completed with two complete passes of the tube. The homogenate contained 1 g of muscle per 10 ml. The remainder of the muscle mince was used for measurement of cytochrome c concentration.

Mitochondria were prepared by first centrifuging a portion of the homogenate for 15 min at 700 *g*; the 700 *g* supernatant fluid was decanted and centrifuged again at 700 *g* for 15 min. The second 700 *g* supernatant fluid was then centrifuged for 15 min at 8000 *g*. The resulting mitochondrial pellet was suspended in 250 mM sucrose, containing 2 *m*M EDTA, pH 7.4.

Reagents. Palmitic-1-^{14}C acid, oleic-1-^{14}C acid, uniformly labeled palmitic-^{14}C acid, and palmityl-1-^{14}C-CoA were obtained from New England Nuclear Corp., Boston, Mass.

Bovine serum albumin, Fraction V, "essentially fatty acid-free," was obtained from Sigma Chemical Co., St. Louis, Mo., and extracted according to the method of Goodman (15) to further decrease fatty acid content. This preparation is subsequently referred to as "fatty acid-free" albumin. The molecular weight of albumin was taken to be 66,000 (16).

CoA, cytochrome c (type II), hexokinase, ADP, ATP, and palmitic acid were obtained from Sigma Chemical Co. L-Carnitine, palmityl CoA, and oleic acid were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

L-Palmityl carnitine was a generous gift from Dr. Rubin Bressler, Duke University Medical Center.

The fatty acids were dissolved in ethanol (17). After addition of the alcoholic solutions of the free fatty acids, the final concentration of ethanol was 1% in the reaction media used for studies of fatty acid oxidation (17).

Assay methods. Mitochondrial O_2 uptake was measured in a Gilson differential respirometer (Gilson Medical Electronics, Inc., Middleton, Wis.) at 30°C with air as the gas phase. In those experiments in which P : O ratios were not determined, each flask contained, in a final volume of 2 ml: 5 mM $MgCl_2$, 20 mM KCl, 125 mM sucrose, 30 mM potassium phosphate buffer, 2 mM EDTA, 10 mM Tris-HCl, 1 mM Na malate, 0.15 mM fatty acid–free albumin, 0.078 mM cytochrome c, 10 mM ADP, 1 mM ATP, and mitochondria from $\frac{1}{3}$ or $\frac{1}{2}$ g of muscle. The concentrations of substrate and of CoA and L-carnitine (when present), are indicated for each experiment. The pH of the mixture was 7.2. After 10 min of thermoequilibration, O_2 uptake was measured for a 10 min period; the ADP was then tipped in from the side arm, and O_2 uptake was measured for two more 10-min periods.

For determination of P : O ratios during the oxidation of palmityl CoA or palmityl carnitine, the reaction medium contained, in a final volume of 2 ml: 5 mM $MgCl_2$, 20 mM KCl, 100 mM sucrose, 25 mM glucose, 30 mM potassium phosphate buffer, 2 mM EDTA, 10 mM Tris-HCl, 1 mM Na malate, 0.15 mM fatty acid–free albumin, 0.078 mM cytochrome c, 2.5 mM ATP, 2 mg of hexokinase, and either 0.3 mM palmityl CoA and 1 mM carnitine, or 0.3 mM palmityl carnitine and 0.025 mM CoA. The pH of the mixture was 7.2. After 10 min of thermoequilibration, O_2 uptake was measured for 10 min in the absence of phosphate acceptor. The ATP, glucose, and hexokinase were then tipped in from the side arm, and O_2 consumption and P_i disappearance during the following 20 min period were measured (9).

The capacity of whole homogenates of muscle to oxidize ^{14}C-labeled fatty acids was assessed by measuring the rate of $^{14}CO_2$ production. The reaction mixture contained, in a final volume of 2 ml: 5 mM $MgCl_2$, 87.5 mM KCl, 40 mM potassium phosphate buffer, 2 mM EDTA, 2 mM ADP, 10 mM Tris-Cl, 0.078 mM cytochrome c, 0.15 mM fatty acid–free albumin, and homogenate equivalent to 100 mg of muscle. The concentrations of ^{14}C-labeled substrate and of CoA and carnitine are indicated for each experiment. Reaction mixtures were placed in 25-ml flasks fitted with serum caps and hanging center wells, in a shaking Dubnoff incubator at 30°C. The $^{14}CO_2$ produced was trapped in 0.4 ml of Hyamine hydroxide, placed in the center well, as described by Jones and Blecher (18). Radioactive Hyamine carbonates were transferred to vials containing 5 ml of Insta-Gel (Packard Instrument Co., Inc., Downers Grove, Ill.) scintillator fluid for determination of radioactivity in a Philips liquid scintillation counter. Efficiency was monitored by the external standard channel ratio method with ^{133}Ba as the external source of radiation.

Mitochondrial suspensions were frozen and thawed three times, followed by vigorous homogenization before measurement of enzymatic activity. Assays were conducted at 30°C.

ATP-linked palmityl CoA synthetase activity was measured as described by Pande and Mead (19) using the hydroxamate trapping method of Kornberg and Pricer (20). Concentrations of substrates in the reaction mixture were 4 mM palmitate and 1.2 mM CoA.

Palmityl CoA-carnitine palmityltransferase activity was determined by measuring the palmityl CoA formed from L-palmityl carnitine and CoA (cf. reference 21). The reaction mixture (cf reference 22) contained, in a final volume of 1 ml: 500 mM hydroxylamine, pH 7.4; 0.6 mM CoA; 5 mM glutathione; 0.6 mM palmityl carnitine; and 75 mM Tris-Cl, pH 7.4. A control lacking CoA was included for each experimental sample. After a 5 min period of thermoequilibration, the reaction was started by addition of enzyme. After 30 min of incubation with shaking, the reaction was terminated by the addition of 2 ml of 6% perchloric acid. The tubes were centrifuged, and the supernatant fluid was decanted. The residue was extracted with 3 ml of reagent A of Hill that had been freshly diluted 1 : 10 in absolute ethanol (23). The absorbance of the iron-palmitylhydroxamate complex was measured at 520 mμ in a Gilford model 240 spectrophotometer.

Palmityl CoA dehydrogenase activity was measured by following the rate of 2,6-dichlorophenolindophenol reduction at 600 mμ (cf. reference 24). Assays were performed in a Gilford model 240 spectrophotometer, in 1 ml cuvettes of 1 cm light path. Phenazine methosulfate (PMS) was used as the intermediate electron carrier. Reaction rates were measured at several different PMS levels. Results were extrapolatd to infinite PMS concentration (V_{max}, PMS) by plotting the reciprocal of the velocities against the reciprocal of PMS concentrations. The reaction mixture contained 100 mM potassium phosphate buffer, pH 7.0; 0.034 mM 2,6-dichlorophenolindophenol; 0.12 mM FAD; 0.2 mM palmityl CoA; and 0.26 mM, 0.33 mM, 0.65 mM, or 1.3 mM PMS. After a variable period, when change in absorbance due to oxidation of endogenous substrates in the mitochondrial preparations had stopped, the reaction was started by addition of palmityl CoA.

The concentration of cytochrome c in muscle was determined by the method of Williams and Thorp (25). Protein was determined by the biuret method (26). Homogenates and mitochondria were prepared for protein measurement by the procedure of Cleland and Slater (27).

RESULTS

L-Carnitine and CoA requirements for oxidation of long-chain fatty acids by homogenates and mitochondria of skeletal muscle. L-Carnitine and CoA were necessary cofactors for the oxidation of palmitic and oleic acids by skeletal muscle mitochondria. The mitochondrial fraction from 1 g of muscle consumed approximately 50 μl of O_2 per hr when incubated in the absence of any substrate other than malate. Addition of 0.75 mM palmitate to the medium without L-carnitine or CoA did not result in an increase in O_2 consumption above the values seen with malate alone. The presence of either CoA or L-carnitine alone resulted in a negligible increase in palmitate oxidation (Table I). However, when L-carnitine and CoA were both added in what appeared to be optimal concentrations, mitochondrial O_2 uptake with palmitate as substrate increased approximately 10-fold (Table I). A similar response was seen with oleic acid as substrate.

In contrast to the response of the mitochondrial preparation, the rate of palmitate-1-^{14}C oxidation by whole homogenates of muscle increased approximately two- to threefold on addition of either CoA or carnitine alone (Table I). A possible explanation for this difference in response may be that whole homogenates have higher endogenous levels of these cofactors than does the mitochondrial fraction. Addition of both cofactors together in optimal concentrations resulted in an approximately sevenfold increase in the rate of oxidation of palmitic-1-^{14}C acid. A similar response was seen when oleic-1-^{14}C acid was used as substrate.

The concentrations of these cofactors that resulted in the highest rates of fatty acid oxidation with palmitic (or oleic) acid as substrate were 0.025 mM for CoA, and above 0.5 mM for L-carnitine (Table I). Higher concentrations of CoA had a slight inhibitory effect (Table I).

TABLE I

Effects of CoA and of L-Carnitine on Palmitic Acid Oxidation

CoA concentration	L-Carnitine concentration	Mitochondrial O_2 uptake	Homogenate $^{14}CO_2$ production
μM	*mM*	*μl O_2/hr per g**	*dpm/min per g‡*
0	0	52	1134
25	0	69	3483
0	1.0	64	2597
5	1.0	390	—
10	1.0	483	6544
25	1.0	543	7966
50	1.0	424	6535
100	1.0	377	6687
25	0.1	367	—
25	0.25	475	6621
25	0.5	535	7002
25	2.0	524	8011

The concentration of palmitic acid was 0.75 mM. In experiments involving measurement of $^{14}CO_2$ production the palmitic acid contained 300,000 dpm of palmitic-1-^{14}C acid per μmole. Values are averages for two representative experiments.

* O_2 uptake is expressed as microliters of O_2 utilized per hour by the mitochondrial fraction from 1 g of muscle.

‡ $^{14}CO_2$ production is expressed as disintegrations per minute released per minute by homogenate of 1 g of muscle.

Oxidation of palmitate, oleate, linoleate, palmityl CoA, and palmityl carnitine by the mitochondrial fraction of muscle. The paired weight sedentary and the freely eating sedentary animals were not significantly different in any of the biochemical variables measured in the present study. Therefore, the results obtained on these two groups have been combined and are referred to jointly under the headings Sedentary Group or Sedentary Controls.

The mitochondrial fraction of (combined) gastrocnemius and quadriceps muscles from the exercised animals utilized approximately twice as much O_2 as that of the sedentary group under conditions of uncontrolled respiration with palmitate as substrate (Table II, Fig. 1). This difference in the capacity to oxidize palmitic acid was demonstrable over a wide physiologic range of concentrations (Fig. 1). As shown in Table II, the rate of O_2 consumption by the mitochondrial fraction from muscle of the runners was also approximately double that of the sedentary controls with oleic and linoleic acids, palmityl CoA, or palmityl carnitine as substrate. It is of interest that with the incubation medium used in these studies (containing CoA, L-carnitine, ADP, ATP, malate, albumin, and EDTA), the rates of O_2 uptake with palmitic, oleic, or linoleic acids as substrate were similar to those seen with palmityl carnitine (Table II). Respiratory rates in both the exercised and sedentary groups were lower with palmityl CoA than with the other substrates. The concentration of palmityl CoA that gave the highest rate of O_2 consumption was

TABLE II

Oxidation of Palmitate, Oleate, Linoleate, Palmityl CoA, and Palmityl Carnitine by Mitochondrial Fraction of Muscles from Exercised and Sedentary Rats

Group	O_2 consumption				
	Palmitate	Oleate	Linoleate	Palmityl CoA	Palmityl carnitine
			μl O_2/hr per g		
Runners	1069 ±37*	1084 ±50*	962 ±60*	776 ±42*	1120 ±55*
	(12)	(5)	(3)	(5)	(8)
Sedentary	509 ±22	498 ±20	523 ±36	440 ±39	516 ±25
	(14)	(8)	(5)	(8)	(12)

Substrate concentrations were 0.75 mM for palmitic, linoleic, and oleic acids, and 0.3 mM for palmityl CoA and palmityl carnitine. Cofactor concentrations were 0.025 mM for CoA and 1 mM for L-carnitine. Carnitine was omitted from the flasks containing palmityl carnitine. The flasks contained mitochondria from either ⅓ g (runners) or ½ g (sedentary) of muscle, to give approximately 1.8–2 mg of mitochondrial protein per flask. Values are means ±SEM. The number of animals per group is given in parentheses. O_2 uptake is expressed as microliters of O_2 utilized per hour by the mitochondrial fraction from 1 g of fresh muscle.

* Runners vs. sedentary, $P < 0.001$.

0.3 mM; higher levels resulted in a reduction of respiratory rate.

Respiratory control indexes and P:O ratios with palmityl CoA or palmityl carnitine as substrates were not significantly different in the mitochondrial fractions from muscle of the exercised and sedentary animals.

The average P:O ratio was 2.4 ±0.4 with palmityl CoA and 2.4 ±0.4 with palmityl carnitine for six runners compared with 2.3 ±0.2 with palmityl CoA, and 2.4 ±0.3 with palmityl carnitine for eight sedentary controls. The respiratory control index averaged 6.4 ±0.7 with palmityl CoA and 6.1 ±0.5 with palmityl carnitine in the exercisers and 6.2 ±1.2, and 6.8 ±1.1 for the sedentary controls.

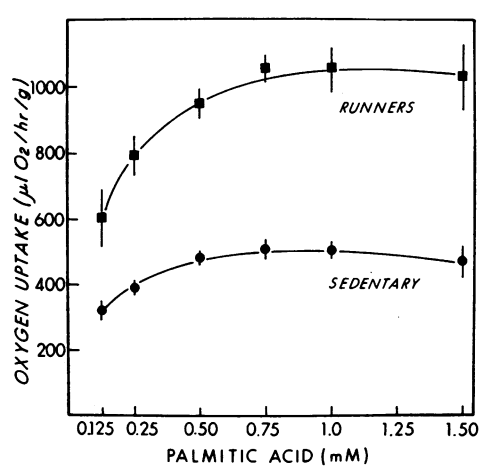

FIGURE 1 Relationship between palmitic acid concentration and rate of O_2 utilization by the mitochondrial fraction from muscles of exercised and sedentary animals. The reaction mixture and the methods used are described under Experimental Procedure. Each flask contained 1 mM L-carnitine and 0.025 mM CoA. Each point is the mean for four to six animals. The vertical bars represent twice the SE. O_2 uptake is expressed as microliters of O_2 utilized per hour by the mitochondrial fraction from 1 g of fresh muscle.

The protein content of the mitochondrial fraction from gastrocnemius and quadriceps was increased approximately 60% in the exercised animals. The average mitochondrial protein concentrations was 6.0 ±0.2 mg/g of fresh muscle for 12 runners, compared with 3.7 ±0.1 mg/g for 14 sedentary controls ($P < 0.001$). As a result, the increase in the capacity of the exercised animals' leg muscles to oxidize fatty acids is partly obscured when O_2 consumption is expressed per milligram of mitochondrial protein. For example, O_2 consumption with palmitate as substrate was 182 ±13 μl of O_2/hr per mg of mitochondrial protein for 12 runners, compared with 140 ±8 μl/hr per mg for 14 sedentary controls. This difference is still statistically significant ($P < 0.01$).

Oxidation of palmitate-1-^{14}C, palmitate-U-^{14}C, oleate-1-^{14}C, and palmityl-1-^{14}C CoA by whole muscle homogenates. The rate of $^{14}CO_2$ production by whole homogenates of muscle, under conditions of uncontrolled respiration with palmitic-1-^{14}C acid as substrate, was significantly greater in the exercised than in the sedentary group (Table III). This difference in the rate of palmitate oxidation by homogenates of muscle from the trained and sedentary animals was demonstrable over a wide physiologic range of palmitate concentrations (0.125–1.5 mM). The capacity of muscle homogenates to oxidize palmitic-U-^{14}C acid, oleic-1-^{14}C acid, and palmityl-1-^{14}C CoA was also examined in a small number of exercised

TABLE III

Oxidation of Palmitate-1-^{14}C, Palmitate-U-^{14}C, Oleate-1-^{14}C, and Palmityl-1-^{14}C CoA by Homogenates of Muscles from Exercised and Sedentary Animals

Group	$^{14}CO_2$ released			
	Palmitate-1-^{14}C	Palmitate-U-^{14}C	Oleate-1-^{14}C	Palmityl-1-^{14}C CoA
	dpm/min per g			
Runners	16161 ±1270*	18708 ±1180*	18617 ±1845*	14506 ±3280‡
	(10)	(3)	(3)	(3)
Sedentary	8905 ±130	8897 ±720	9547 ±816	5220 ±1750
	(14)	(3)	(3)	(3)

The concentration of palmitate-1-^{14}C, palmitate-U-^{14}C, and oleate-1-^{14}C was 0.75 mM, while that of palmityl-1-^{14}C CoA was 0.3 mM. Each substrate contained approximately 300,000 dpm of ^{14}C per μmole. The reaction medium contained 1 mM L-carnitine and 0.025 mM CoA. Values are means ±SEM. The number of animals per group is given in parentheses. $^{14}CO_2$ production is expressed as disintegrations per minute of $^{14}CO_2$ formed per minute by homogenates of 1 g of muscle.

* Runners vs. sedentary, $P < 0.01$.

‡ $P < 0.02$.

and sedentary animals. As shown in Table III, the running program resulted in a similar approximately twofold, increase in the capacity of gastrocnemius and quadriceps muscles to oxidize these substrates. The similar rates of oxidation of palmitate-1-^{14}C and of palmitate-U-^{14}C provide evidence that once β-oxidation of palmityl CoA was initiated, it went to completion.

ATP-dependent palmityl CoA synthetase, carnitine palmityltransferase, and palmityl CoA dehydrogenase activities. The levels of activity of these three enzymes involved in the activation, transport, and catabolism of long-chain fatty acids were measured in the mitochondrial fraction of gastrocnemius and quadriceps muscles. As shown in Table IV, the values obtained for all three enzymes were approximately twice as high in the exercised as in the sedentary group. Enzymatic activity is expressed per gram wet weight of muscle as this seems the most useful reference when relating enzyme content to functional capacity. As mentioned above, the protein content of the mitochondrial fraction was approximately 60% higher in the exercised groups. As a result, the adaptive increase in the levels of activity of these enzymes is largely obscured when activity is expressed per milligram of mitochondrial protein.

The magnitude of the increase in the levels of activity of these enzymes was similar to that of the concentration of cytochrome c, which was used as a marker for the respiratory chain. The concentration of cytochrome c in (combined) gastrocnemius and quadriceps muscles was 18.7 ±0.5 nmoles/g of muscle for 14 runners, compared with 9.4 ±0.5 nmoles/g for 14 sedentary animals ($P < 0.001$).

DISCUSSION

Earlier reports that palmitate is oxidized more slowly by mitochondria from heart (28, 29) and skeletal muscle (29) than is palmityl carnitine suggested that mitochondrial palmityl CoA synthetase and/or formation of palmityl carnitine might be rate limiting. The results of the present study in which muscle mitochondria oxidized palmitate and palmityl carnitine at approximately the same rate when incubated in the presence of optimal levels of substrate and cofactors, show that this is not the case. The very low rates of palmitate oxidation by muscle mitochondria noted in the previous studies were probably due to the absence of CoA from the incubation media (28, 29). As reported by Fritz and Yue for heart mitochondria (21) and demonstrated in the present study with skeletal muscle mitochondria,

TABLE IV

ATP-Dependent Palmityl CoA Synthetase, Carnitine Palmityltransferase, and Palmityl CoA Dehydrogenase Activities in Hind Limb Muscles of Exercised and Sedentary Rats

Group	ATP-dependent palmityl CoA synthetase	Carnitine palmityltransferase	Palmityl CoA dehydrogenase
		nmoles/min per g	
Runners	75 ±6* (9)	132 ±8* (6)	970 ±106* (6)
Sedentary	33 ±1 (9)	70 ±4 (8)	416 ± 57 (6)

Values are means ±SEM. Number of animals in each group is given in parentheses. Enzymatic activity is expressed as nanomoles of substrate utilized per minute by the mitochondrial fraction from 1 g of muscle, wet weight.

* Runners vs. sedentary, $P < 0.001$.

both CoA and carnitine are necessary cofactors for the oxidation of long-chain fatty acids by intact muscle mitochondria.

It was previously found that the program of exercise used in these studies results in an approximately twofold increase in the levels of activity of the mitochondrial respiratory chain enzymes linking the oxidation of NADH and succinate to oxygen (9). This rise in enzyme activity appears to be due to an increase in enzyme protein, as evidenced by a doubling of the concentration of cytochrome c, and a 60% increase in the protein content of the mitochondrial fraction of skeletal muscle (9, 10). A study by Gollnick and King (11) has provided electron microscopic evidence that the adaptation to exercise involves an increase in both the size and number of muscle mitochondria. However, although mitochondria are the site of fatty acid oxidation, it could not be assumed a priori that adaptation to exercise results in an increase in the capacity of muscle to oxidize fatty acids, because muscle mitochondria do not increase as a unit but undergo adaptive changes in composition.

For example, the capacity of muscle to oxidize α-glycerophosphate is not increased in physically trained rats (10). Mitochondrial α-glycerophosphate dehydrogenase, although also a component of the mitochondrial cristae, does not participate in the adaptive increase in the constituents of the respiratory chain that occurs in response to exercise (10). Thus, because of the increase in mitochondrial protein, α-glycerophosphate dehydrogenase activity is significantly decreased in leg muscles of physically trained rats when expressed per milligram of mitochondrial protein (10). Similarly, mitochondrial adenylate kinase and creatine kinase do not participate in the adaption to exercise and show a significant decrease in specific activity per milligram of mitochondrial protein.[1] The finding in the present study that exercise significantly increased the capacity to oxidize palmitate not only per gram of muscle but also per milligram of mitochondrial protein provides further evidence of a change in mitochondrial composition.

It was previously found that the levels of activity of citrate synthase and NAD-specific isocitrate dehydrogenase and of the respiratory chain enzymes increased to the same extent, approximately twofold, in leg muscles of rats subjected to the exercise program used in these studies (12). In contrast, the level of activity of the citric acid cycle–related enzyme, glutamate dehydrogenase, increased by only one-third (12). Pette and his coworkers (30, 31) have shown that certain mitochondrial citric acid cycle and citric acid cycle–related enzymes occur in constant proportions to each other and to the flavoproteins and cytochromes of the respiratory chain. Viewed in this context, the present finding that the levels of mitochondrial palmityl CoA synthetase, carnitine palmityl transferase, and palmityl CoA dehydrogenase all increased to about the same extent as did the concentration of cytochrome c (approximately twofold), raises the possibility that these enzymes of fatty acid catabolism may also be "constant proportion" enzymes which are regulated in a coordinated, quantitatively related manner with the respiratory chain enzymes.

The rate of oxidation of free fatty acids by muscle increases with plasma concentration in resting and exercising animals (32, 33). A similar effect of fatty acid concentration was seen in vitro in the present study in which the rate of palmitate oxidation by the mitochondrial fraction (and whole homogenate) of leg muscles from both the exercised and sedentary animals increased with palmitate concentration in the range from 0.125 to 0.75 mM. However, at all concentrations studied, the rate of palmitate oxidation was approximately twice as great in the muscle preparations from the trained as from the sedentary animals.

Physically trained individuals derive a much greater percentage of their energy from fatty acid oxidation than do untrained during exercise of the same intensity (1, 6–8). The present results, showing that regularly performed endurance exercise results in an adaptive increase in the capacity of muscle to oxidize fatty acids, helps to explain this difference. In addition to its effect on muscle, exercise appears to produce adaptations, which result in a greater rate of release of fatty acids from adipose tissue and higher levels of free fatty acids in blood, in trained than in untrained subjects during exercise (5, 34). It seems likely that the increase in the capacity of muscle to oxidize fat and the greater mobilization of fatty acids act synergistically to account for the physically trained individual's greater utilization of fat as an energy source during exercise.

Men and animals that have adapted to regularly performed endurance exercise, such as long-distance running, demonstrate a marked increase in their capacity to perform aerobic work (9, 35–37). One manifestation of this adaptation is an increase in endurance, measured as an increase in the period of time for which an individual can maintain a given submaximal level of exercise (9, 14, 38). The shift in the carbon source for the citric acid cycle in skeletal muscle that occurs in response to physical training with increased oxidation of fat and a reduction in carbohydrate utilization (1, 6–8), may play an important role in this increase in endurance.

One factor which has been implicated in the develop-

[1] Oscai, L. B., and J. O. Holloszy. Manuscript in preparation.

ment of the muscle fatigue, which forces an exercising individual performing prolonged exercise to stop or markedly slow his pace, is a depletion of muscle glycogen (39, 40). Why glycogen depletion should have this effect, when large amounts of substrate in the form of fatty acids are still available, remains to be clarified; nevertheless, the fatigue and reduction in work capacity associated with glycogen depletion are well documented (39, 40). Clearly, the glycogen-sparing effect of increased utilization of fat could postpone depletion of muscle glycogen and the fatigue associated with it in the exercising muscle of physically trained individuals.

Another factor which may limit endurance in individuals performing heavy work of several hours duration is hypoglycemia with associated central nervous system symptoms (41, 42). An increase in the oxidation of fatty acids decreases carbohydrate utilization (43). This appears to be mediated, in part, by decreased uptake of glucose by muscle (44). A greater oxidation of fatty acids by skeletal muscle could, through this mechanism, protect physically trained individuals against the development of hypoglycemia during prolonged exercise.

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