Exercise-Induced Increase in the Capacity of Skeletal Muscle to Oxidize Palmitate[1] (34884)

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Oxidation of fatty acids provides a major portion of the energy required for muscle metabolism at rest and during prolonged submaximal exercise (1–3). As the intensity of exercise increases, a progressively greater proportion of the needed energy is derived from carbohydrate (4–6). However, the relative amounts of carbohydrate and fat utilized at different workloads depend on the level of physical training. Fatty acid oxidation is a more important source of energy in physically trained men and animals than in untrained (4–8). To obtain information regarding the basis for the increased utilization of fatty acids in the trained state, we studied the effect of a program of running on the capacity of rat gastrocnemius muscle to oxidize palmitate.

Materials and Methods. Male rats of a Wistar strain (specific pathogen-free CFN rats, Carworth Farms), weighing approximately 100 g were placed in individual cages and maintained on a diet of Purina chow and water. They were divided into one exercising and two sedentary groups. The exercising group was trained for 12 weeks by means of a strenuous program of treadmill running as described previously (9). At the end of 12 weeks, the rats were running continuously for 2 hr daily at 31 m/min. This exercise program results in a large increase in the capacity for prolonged running, but does not result in hypertrophy of the leg muscles (9, 10). Sedentary control rats were divided into a paired-weight group, in which food intake was restricted so as to maintain their body weights the same as those of the exercising animals, and a free-eating group which was provided with food *ad libitum*.

Animals were killed 24 hr after their last exercise session. Gastrocnemius muscle were homogenized (1:10) in 175 m*M* KCl + 0.1 m*M* EDTA as described previously (10). The capacity of gastrocnemius muscle homogenates to oxidize palmitate-1-^{14}C (New England Nuclear) was assessed by measuring the rate of $^{14}CO_2$ production. Reaction mixtures were placed in 25-ml flasks fitted with serum caps and hanging center wells, and incubated in a shaking Dubnoff incubator at 30°. The reaction mixture contained, in a final volume of 2 ml: 5 m*M* $MgCl_2$; 87.5 m*M* KCl; 40 m*M* potassium phosphate buffer, pH 7.4; 10 m*M* Tris buffer, pH 7.4; 2 m*M* EDTA; 0.025 m*M* CoA; 1 m*M* L-carnitine; 2 m*M* ADP; and either 0.5 m*M* (280,000 dpm) or 0.75 m*M* (420,000 dpm) palmitate-1-^{14}C. After 12 min the reaction was stopped with 0.4 ml of 60% citric acid. The $^{14}CO_2$ produced was trapped in Hyamine as described by Jones and Blecher (11). Radioactive Hyamine carbonates were transferred to vials containing 5 ml of Insta-Gel (Packard Instrument Company) scintillator fluid for determination of radioactivity in a Philips liquid scintillation counter.

Succinate oxidase activity was measured manometrically as described by Potter (12).

DPN-specific isocitrate dehydrogenase activity was determined by the method of Plaut and Aogaichi (13), with the modification that Na Amytal was added to the reaction mixture in 2 m*M* concentration. The cyto-

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chrome *c* content of muscle was determined as described by Rosenthal and Drabkin (14).

Results. The rates of palmitate oxidation by gastrocnemius muscle homogenates were the same in the paired-weight sedentary as in the free-eating sedentary animals. Therefore, the results obtained on rats from these two groups have been combined and are referred to jointly under the heading "sedentary animals" or "sedentary group."

Homogenates of gastrocnemius muscles from the exercised rats oxidized palmitate-1-^{14}C at a significantly greater rate than did homogenates of muscles from the sedentary animals (Table I). Increasing the palmitate concentration from 0.5 to 0.75 m*M* resulted in a slight, but not significant, increase in the rate of $^{14}CO_2$ production (Table I). Measurements of palmitate-1-^{14}C oxidation were made under conditions of uncontrolled respiration (*i.e.*, with nonlimiting amounts of P_i and ADP), in the presence of concentrations of carnitine and of coenzyme A that were not limiting. Therefore, the approximately 70% greater rate of palmitate oxidation by trained muscle probably reflects an increase in the levels of activity of mitochrondrial enzymes rather than an increase in the concentration of cofactors.

It has been shown that regularly performed prolonged exercise can result in an increase in muscle mitochondria (10). In the present study, the level of activity of succinate oxidase and the concentration of cytochrome *c* were used as markers for the mitochondrial cristae, and DPN-specific isocitrate dehydrogenase activity was used as a marker for the mitochondrial matrix. The levels of these mitochondrial enzymes in gastrocnemius muscle increased to approximately the same extent in response to the running program as did the capacity to oxidize palmitate (Table II).

TABLE I. Effect of the Exercise Program on the Capacity of Gastrocnemius Muscle to Oxidize Palmitate-1-^{14}C.

Group	Palmitate-1-^{14}C conc (m*M*)	$^{14}CO_2$[a] (dpm/g/min)
Sedentary (10)	0.50	8140 ± 324
	0.75	8952 ± 323
Runners (5)	0.50	13,930 ± 648[b]
	0.75	14,911 ± 478[b]

[a] The rate of $^{14}CO_2$ production is expressed as disintegrations per minute per gram of fresh muscle per minute. Values are means ± SE of mean for 10 sedentary animals and for 5 runners.

[b] Runners versus sedentary controls, $p < 0.001$.

Discussion. In a previous study, it was found that strenuous and prolonged exercise results in a highly significant increase in the enzymes of the respiratory chain involved in the oxidation of succinate and DPNH (10). The concentration of cytochrome *c* and of mitochondrial protein, expressed per gram of muscle, also increases (10), providing evidence that the rise in respiratory enzyme activity is due to a net increase in enzyme protein. This interpretation is supported by the results of subsequent electronmicroscopic studies by Gollnick and King (15) and Kraus *et al.* (16) in which an increase in the number and size of mitochondria occurred in skeletal muscle in response to repeated exercise.

TABLE II. Levels of Activity[a] of DPN-Specific Isocitrate Dehydrogenase and Succinate Oxidase, and Concentration of Cytochrome *c* in Gastrocnemius Muscles from Exercising and Sedentary Animals.

	DPN-specific isocitrate dehydrogenase (μmoles/g/min)	Succinate oxidase (μl of O_2/g/min)	Cytochrome *c* (mμmoles/g)
Sedentary (5)	2.77 ± 0.43	98 ± 2	4.32 ± 0.31
Runners (5)	5.39 ± 0.56[b]	168 ± 8[c]	8.06 ± 0.85[c]

[a] Activity is expressed per gram of fresh muscle per minute. Values are means ± SE of mean for 5 animals.

[b] Runners versus sedentary, $p < 0.01$; [c] $p < 0.001$.

However, the adaptation induced in muscle mitochondria by exercise does not consist simply of an increase in the size and number of mitochondria. A change in mitochondrial composition also occurs. This is evidenced by the finding that the levels of activity, per gram of gastrocnemius muscle, of mitochondrial α-glycerophosphate dehydrogenase (17), creatine phosphokinase[4] and adenylate kinase[4] are unchanged in rats subjected to the same exercise program used in the present study. As a result of the increase in mitochondrial protein, the specific activities of these enzymes, per milligram of mitochondrial protein, are decreased.

The present study was undertaken to determine whether or not the exercise-induced adaptations in skeletal muscle include an increase in the capacity to oxidize fatty acids. An affirmative answer to this question is provided by the significantly greater rate of conversion of palmitate-1-^{14}C to $^{14}CO_2$ exhibited by homogenates of gastrocnemius muscles of the exercised animals as compared to those of the sedentary controls. This finding suggests that the enzymes involved in the oxidation of long chain fatty acids participated in the increase in mitochondrial enzymes, evidenced by the increase in mitochondrial markers shown in Table II, that occurred in the gastrocnemius muscles of the exercised animals in the present study. It also helps to explain the well documented observation that during exercise of light to moderate intensity, physically trained men and animals derive a much greater percentage of their energy from fatty acid oxidation than do untrained individuals at comparable work levels (4–8). In addition to the biochemical adaptations in muscle, physical training appears to produce adaptations in the lipolytic system which result in a greater rate of release of fatty acids from adipose tissue in physically trained than in untrained individuals during exercise (7, 8). It seems likely that the increase in the capacity of skeletal muscle to oxidize fatty acids acts synergistically with this increased rate of fatty acid mobilization to enable physically trained individuals to obtain a greater proportion of the energy required for exercise from fat.

Regularly performed, prolonged exercise can result in large increases in endurance. For example, rats subjected to the training program used in this study can run 6 to 10 times longer, before becoming exhausted, than rats trained at the same treadmill speed for only 10 min daily (10, 18). The maximal duration of vigorous exercise is closely correlated with the time at which muscle glycogen is depleted (19, 20). Although the biochemical basis for this phenomenon is not known, it is well documented that individuals performing vigorous exercise become exhausted and have to stop or slow down markedly when their muscle glycogen stores are used up (19, 20). Thus, a greater utilization of fat for fuel during exercise could, by conserving muscle glycogen, contribute importantly to the trained individual's greater capacity for prolonged exercise.

Summary. Rats were subjected to a 12-week program of treadmill running. Homogenates of gastrocnemius muscle from the exercised rats oxidized palmitate at a significantly greater rate than homogenates of muscles from sedentary controls. This finding indicates that the exercise-induced adaptations in skeletal muscle include an increase in the capacity to oxidize fatty acids. It is suggested that this adaptation plays a role in enabling physically trained individuals, as compared to sedentary, to obtain a greater proportion of the energy needed for submaximal exercise from fat.

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