Respiratory capacity of white, red, and intermediate muscle: adaptive response to exercise


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In the present study, the terms white (low oxidative, high myosin ATPase), red (high oxidative, high myosin ATPase), and intermediate (intermediate oxidative capacity, low myosin ATPase), which have been used to classify fiber types in rodent muscle (1, 4, 7), are employed. These correspond to fiber types A, C, and B of Stein and Padykula (21) and Henneman and Olson (8).

A number of histochemical studies have been published in which succinate dehydrogenase, DPNH diaphorase, or malate dehydrogenase staining intensity was used to evaluate the adaptive response of skeletal muscle to endurance exercise (2, 3, 5, 11). With the staining intensity of these enzymes as the criterion for distinction between the fiber types, it was found that the percentage of fibers with the staining characteristics of white muscle decreased while the percentage of red-appearing fibers increased in a mixed muscle in response to endurance exercise (2, 3, 5). This finding was interpreted as indicating that adaptation of skeletal muscle to endurance exercise involves the conversion of white muscle fibers to red fibers with a high respiratory capacity for oxidative metabolism.

The respiratory capacity of white skeletal muscle, which makes up approximately 50% of the mass of gastrocnemius and quadriceps muscles in the rat, is about one-fifth as great as that of red muscle (unpublished observations). Therefore, if the twofold increase in respiratory capacity induced in gastrocnemius and quadriceps muscles by the running program used in our studies was due only to transformation of white fibers into red, then the respiratory capacity of all the white fibers would, on the average, have to increase sevenfold.

This interpretation appeared unlikely to us for at least two reasons. In the first place, we have consistently observed that leg muscles of highly trained animals still have a considerable white-appearing component on gross inspection. Therefore, if the twofold increase in respiratory capacity induced in gastrocnemius and quadriceps muscles by the running program used in our studies was due only to transformation of white fibers into red, then the respiratory capacity of all the white fibers would, on the average, have to increase sevenfold.

A program of prolonged running can induce as much as a twofold increase in the levels of activity of a number of mitochondrial enzymes in skeletal muscle in rats (6, 9, 10, 13). These include palmitoyl-CoA synthetase, palmitoyl-carnitine transferase, palmitoyl-CoA dehydrogenase (13), certain enzymes of the citric acid cycle (10), and the components of the respiratory chain that link the oxidation of succinate and DPNH to oxygen (6, 9). As the above results were obtained on muscles such as the gastrocnemius plus plantaris and quadriceps which are a mixture of fiber types, it seemed of considerable interest to determine which fiber type or types participate in the exercise-induced increase in mitochondrial enzymes.

There is, at present, no standard, generally accepted nomenclature for the different types of skeletal muscle fibers. In the present paper the terms white (low oxidative, high myosin ATPase), red (high oxidative, high myosin ATPase), and intermediate (intermediate oxidative capacity, low myosin ATPase), which have been used to classify fiber types in rodent muscle (1, 4, 7), are employed. These correspond to fiber types A, C, and B of Stein and Padykula (21) and Henneman and Olson (8).

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ing the response of the three types of muscle fibers to exercise.

METHODS

Animal care and exercise program. Male rats of a Wistar strain (specific-pathogen-free CFN rats, Carworth Farms) weighing approximately 110 g were placed in individual cages and maintained on a diet of Purina chow and water.

They were divided into an exercising group and a sedentary group. The exercising group was trained for 12 weeks by means of a program of treadmill running described previously (17). At the end of the 12 weeks, the rats were running continuously for 2 hr daily, 5 days/week (17). 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, 17). The animals were maintained at the final work level until they were killed; this period varied from 1 to 10 weeks. The exercising group was provided with food and water ad libitum. The sedentary group had their food intake restricted so as to maintain their body weights approximately the same as those of the exercised animals. It has been shown that this degree of food restriction has no effect on the levels, expressed per gram of muscle, of the variables measured in this study (9, 13, 15).

Muscle sampling for biochemical studies. The runners were not exercised for 72 hr prior to sacrifice. The animals were killed by decapitation and exsanguinated. The soleus muscles were used for studies on the effect of the exercise program on intermediate muscle fibers. For comparative studies of red and white muscle, the quadriceps muscles were dissected out, freed of fat and connective tissue, and, on the basis of gross visual appearance separated into a superficial white portion, a deep red portion, and a mixed middle portion.

Tissue preparation and assay methods. Muscle homogenates were prepared in a glass Potter-Elvehjem homogenizer, with the use of a pestle with cutting teeth on the bottom.

For studies of palmitate-U-14C and of pyruvate-2-14C oxidation, the muscle homogenates were prepared in 175 mM KCl containing 0.1 mM EDTA. The capacity of whole homogenates of muscle to oxidize palmitate-U-14C was assessed by measuring the rate of 14CO2 production as described previously (13).

The capacity of whole homogenates of muscle to oxidize pyruvate-2-14C was evaluated by measuring the rate of 14CO2 production at 30 C. The reaction mixture contained, in a final volume of 2 ml, 5 mM MgCl2, 87.5 mM KCl, 40 mM potassium phosphate buffer, 2 mM EDTA, 2 mM ADP, 10 mM Tris-Cl, 0.070 mM cytochrome c, and 10 mM pyruvate-2-14C. The pH of the mixture was 7.2. 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 14CO2 produced was trapped and radioactivity was determined as described previously (13).

Oxygen uptake was measured in a Gilford differential respirometer, at 30 C, with air as the gas phase.

Succinate oxidase activity was measured manometrically as described by Potter (19). Oxygen uptakes are expressed as microliters of O2 under standard conditions.

Spectrophotometric assays were performed in a Gilford model 240 spectrophotometer with a thermostated cell compartment in 1-ml cuvettes of 1-cm light path at 30 C.

Citrate synthase and carnitine palmityltransferase activities were measured on muscle homogenates prepared in 100 mM potassium phosphate buffer, pH 7.4, containing 10 mM glutathione. The mitochondria in the homogenates were disrupted by exposure to sonic oscillation with a Blackstone ultrasonic probe, set at maximum intensity, for three 15 sec periods spaced 45 sec apart at 0 C.

Citrate synthase activity was assayed as described by Sorensen (20), with the use of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB).

Carnitine palmityltransferase activity was determined as described previously (13). Assays were performed under conditions in which the reaction rate was proportional to enzyme concentration. Enzyme activities are reported as micromoles of substrate utilized per minute.

The concentration of cytochrome c was determined by the method of Williams and Thorp (23).

Histochemistry. Gastrocnemius plantaris, quadriceps, and soleus muscles were rapidly removed from the animal, mounted on a piece of cardboard, and frozen in isopentane cooled in liquid nitrogen. Serial sections (10 μ) were cut from each muscle and processed for hematoxylin-eosin and Gomori trichrome staining as well as histochemical reactions for DPNH-diaphorase (14), myosin ATPase (16), and phosphorylase (22). Photographs were taken from slides to permit classification of each fiber. Red fibers were characterized by their abundance of coarse diformazan granules which are most prevalent in the periphery of the fiber. White fibers were characterized by the presence of a few ditormazan granules which are also most prevalent in the periphery of the fiber. Intermediate, red fibers were characterized by their light staining with myosin ATPase and their pattern of more uniformly dispersed diformazan granules (1). One experimental and two control gastrocnemius-plantaris-soleus groups were serially sectioned with representative samples taken every 50 sections (500 μ) to construct a replica of the muscle architecture so that sections from control and experimental animals could be matched on subsequent more limited samples.

RESULTS

Muscle weights and distribution of fiber types. As in previous studies, the running program did not result in muscle hypertrophy (Table 1). The muscles of the trained animals appeared redder than those of the sedentary ones. However, it was still possible to distinguish the superficial, white and
WHITE, RED, AND INTERMEDIATE MUSCLE RESPONSE TO EXERCISE

white, red, and intermediate muscle response to exercise

Figs. 1-5 are photographic representatives of tissue from each area. S is a similar area from soleus for comparison. Stained for DPNH-diaphorase. All photographs ca. X115.

Fields I-V are photographic representatives of tissue from each area. S is a similar area from soleus for comparison. Stained for DPNH-diaphorase. All photographs ca. X115.

depth, red portions of the quadriceps muscles. Similar amounts of tissue were obtained from the runners and their sedentary controls on separation of the superficial, white, deep, red, and the middle, mixed portions of the quadriceps on the basis of their gross appearance (Table 1).

Histochemical findings. The quadriceps muscle has marked regional variation in proportions of red, intermediate, and white fiber content. The variation is well ordered, however, and constant within control and experimental groups. Histochemical examination corroborated the impression obtained on gross visual inspection. As shown in Fig. 1, the most superficial portion of the quadriceps contained 100% white fibers, while the deepest area consisted of red and intermediate fibers. When the muscle is arbitrarily subdivided into five layers (Fig. 1), the mean percentage of fibers in each layer (500 adjacent fibers in each of two control animals) are: layer I: 100% white; layer II: 85% white, 9% intermediate, 6% red; layer III: 60% white, 28% intermediate, 12% red; layer IV: 10% white, 40% intermediate, 50% red; layer V: 30% intermediate, 70% red. In soleus, 96% of fibers were intermediate and 4% were red (Fig. 15).

A similar but less well-ordered lamination is noted in the gastrocnemius-plantaris group. However, in this muscle the consistent separation of the plantaris by fibrous tissue septa allows for identification of matched experimental and control samples throughout the length of the muscle independent of the proportion of the various fibers. Figure 2 depicts sections of plantaris muscle obtained from paired trained and sedentary weight matched animals and stained for DPNH diaphorase activity. It can be seen that the percentage of red-appearing fibers is increased and the percentage of white-appearing fibers is decreased in the muscle from the runner as compared to the sedentary control. No such increase in staining for the respiratory enzyme was evident in the soleus muscle.

Oxidation of pyruvate-2-14C and palmitate-U-14C. As shown in Table 2, the capacity to oxidize pyruvate-2-14C and palmitate-U-14C increased significantly in the superficial, white and deep, red portions of the quadriceps, and also in the soleus muscle, in response to the exercise program. This finding provides evidence that endurance exercise induces an increase in the respiratory capacity of all three muscle fiber types. The use of whole homogenates instead of the mitochondrial fraction in these studies avoids possible differences in the percentage yield of mitochondria from the
different muscle types in the two groups of animals. In evaluating these results one should remember that, under ideal conditions, oxidation of one molecule of pyruvate yields 15 molecules of ATP, while oxidation of 1 molecule of palmitate yields 130 molecules of ATP.

TABLE 2. Oxidation of pyruvate-2-14C and palmitate-U-14C by homogenates of different types of muscles from exercised and sedentary animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Quadriceps</th>
<th>Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superficial, white</td>
<td>Deep, red</td>
</tr>
<tr>
<td>Sedentary</td>
<td>96±22 (6)</td>
<td>324±56 (6)</td>
</tr>
<tr>
<td>Runners</td>
<td>207±42 (6)</td>
<td>332±149 (6)*</td>
</tr>
<tr>
<td></td>
<td>5.5±2.5 (6)</td>
<td>40.5±5.6 (6)</td>
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<tr>
<td></td>
<td>15.6±2.3 (6)*</td>
<td>88.0±16.0 (6)*</td>
</tr>
</tbody>
</table>

Values are means ± se. The number of animals per group is given in parentheses. The concentration of pyruvate-2-14C was 10 mM, while that of palmitate-U-14C was 0.75 mM. The pyruvate-2-14C contained approximately 70,000 dpm per pmole; the palmitate-U-14C contained approximately 400,000 dpm per pmole. The flasks contained homogenate equivalent to either 100 mg of white, 40 mg of red, or 40 mg of soleus muscle. *Runners vs. sedentary, P < 0.05.

TABLE 3. Levels of activity of citrate synthase and carnitine palmitoyltransferase in muscles from exercised and sedentary animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Quadriceps</th>
<th>Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superficial, white</td>
<td>Deep, red</td>
</tr>
<tr>
<td>Sedentary</td>
<td>10.3± (9)</td>
<td>35.5± (9)</td>
</tr>
<tr>
<td>Runners</td>
<td>18.5± (9)</td>
<td>69.9± (9)</td>
</tr>
<tr>
<td></td>
<td>0.11± (6)</td>
<td>0.10 (8)</td>
</tr>
<tr>
<td></td>
<td>0.20± (6)</td>
<td>1.0 (8)</td>
</tr>
<tr>
<td></td>
<td>0.02 (6)*</td>
<td>0.09 (8)*</td>
</tr>
</tbody>
</table>

Values are means ± se. The number of animals per group is given in parentheses. *Runners vs. sedentary, P < 0.01.

Carnitine palmitoyltransferase, μmoles/min per g

Carnitine palmitoyltransferase, μmoles/min per g

Values are means ± se. The number of animals per group is given in parentheses. *Runners vs. sedentary, P < 0.01.
and the components of the respiratory chain that link the oxidation of succinate and DPNH to O$_2$ (6, 9, 10, 15).

chain fatty acids (13), enzymes of the citric-acid cycle (10), involved in the activation, transport, and oxidation of long-number of mitochondrial enzymes. These include enzymes capacity for oxidative metabolism is a rise in the levels of a mitochondrial fraction of muscle to oxidize pyruvate and long-

metabolism (6, 9, 10, 13, 15). This is evidenced by an in-

in these studies is an increase in the capacity for aerobic metabolism, citrate synthase, and carnitine palmi-

tytransferase, which also increase in mixed muscle in the respiratory chain. Two other enzymes involved in aerobic metabolism, citrate synthase, and carnitine palmi-

tytransferase, which also increase in mixed muscle in response to exercise (10, 13), served as additional mito-

DISCUSSION

The major feature of the adaptation that occurs in the limb muscles of rats subjected to the running program used in these studies is an increase in the capacity for aerobic metabolism (6, 9, 10, 13, 15). This is evidenced by an in-

creased capacity of whole homogenates and of the mitochondria. This does not seem compatible with the concept that white muscle fibers are converted to red, and that this change in fiber type is responsible for the increase in respiratory capacity of skeletal muscle that occurs with endurance exercise.

The results of the biochemical portions of the present study show that this is not the case, but that the capacity of all three fiber types for aerobic metabolism increases proportionally to the same extent. In these studies the capacity to oxidize pyruvate and long-chain fatty acids was assessed by measuring the rate of $^{14}$CO$_2$ production from pyruvate-$2^{14}$C and palmitate-$U^{14}$C by whole homogenates of muscle. The concentration of cytochrome $c$ and the level of activity of cytochrome oxidase were used as markers for the respiratory chain. Two other enzymes involved in aerobic metabolism, citrate synthase, and carnitine palmit-

tytransferase, which also increase in mixed muscle in response to exercise (10, 13), served as additional mito-

chondrial markers.

From the response of these mitochondrial marker en-

dymes and the measurements of oxidative capacity, it is clear that the mitochondria in soleus muscle and in the superficial, white and deep, red portions of the quadriceps (Tables 3 and 4). The concentration of the respiratory chain con-

stituent, cytochrome $c$, was also found to be significantly increased in the white and red portions of the quadriceps of the runners (Table 5). There was insufficient tissue available to measure cytochrome $c$ levels in the soleus.

In addition to the superficial white and deep red portions of the quadriceps, a number of assays were performed on the remaining middle portion of the quadriceps, which is a mixture of the fiber types (Fig. 1). The levels of activity of citrate synthase and carnitine palmitoyltransferase (Table 3), and the concentration of cytochrome $c$ (Table 5), were significantly increased in the middle portion of the quadriceps of the runners. The magnitude of the exercise induced increase in the mitochondrial enzymes was similar in the three portions of the quadriceps muscle.

A number of groups of investigators, employing histo-

tical techniques, have demonstrated an exercise-in-

duced increase in the percentage of fibers in mixed muscles which appear to be red and a decrease in those that appear white on the basis of staining intensity of a number of mitochondrial enzymes (2, 3, 5). No changes were noted in the soleus (3). This finding has been interpreted as indicating that white muscle fibers are converted to red, and

the unavoidable conclusion that the approximately twofold increase in respiratory capacity of the soleus and of the superficial, white and deep, red portions of the quadriceps is due to a twofold rise in the respiratory capacities of the intermediate, the white, and the red muscle fibers.

Since exercise induced an approximately twofold increase in the capacity for aerobic metabolism of all three muscle fiber types, their relationship to each other with respect to respiratory capacity was unchanged. In other words, white fibers still had only approximately one-fifth as great a capacity for oxidative metabolism as red fibers in the muscles of the trained animals. This does not seem compatible with the concept that white muscle fibers are con-

verted to red.

The present histochemical observations confirm the findings of others (2, 3, 5) that, by the criterion of staining intensity for respiratory enzymes, the percentage of red appearing fibers in a mixed muscle increases, and the per-

centage of white appearing fibers decreases, while no changes are evident in the soleus, in response to endurance exercise. Since the biochemical data are not compatible

**TABLE 4. Cytochrome oxidase activity in muscles from exercised and sedentary animals**

<table>
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<th>Soleus</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Superficial, white</td>
<td>Deep, red</td>
</tr>
<tr>
<td>Sedentary</td>
<td>167 ± 8 (9)</td>
<td>830 ± 64 (5)</td>
</tr>
<tr>
<td>Runners</td>
<td>339 ± 21 (9)*</td>
<td>2041 ± 60 (5)*</td>
</tr>
</tbody>
</table>

Values are means ± se. The number of animals is given in parentheses. *Runners vs. sedentary, $P < 0.001$

**TABLE 5. Concentration of cytochrome $c$ in muscles of exercised and sedentary animals**

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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superficial, white</td>
</tr>
<tr>
<td>Sedentary</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Runners</td>
<td>6.3 ± 0.7*</td>
</tr>
</tbody>
</table>

Values are means ± se, each value is the mean for 5 animals. *Runners vs. sedentary, $P < 0.01$.
with the interpretation that the exercise-induced increase in respiratory capacity of mixed muscle is due to transformation of white muscle fibers into red, another explanation is needed for these histochemical findings.

The most likely explanation, in our opinion, relates to the relative insensitivity of the histochemical staining techniques, which are qualitative in nature and not appropriate for quantitation of enzymatic activity. In other words, the stains for the respiratory enzymes, as they are generally used, serve to distinguish fibers with an oxidative capacity above some critical level, which makes them appear red, from white fibers whose oxidative capacity is below this level. However, the staining methods do not appear to be sufficiently sensitive or reproducible to permit grading of intensities of redness or quantitation of enzyme levels in studies involving comparisons of muscles from different animals.

It seems likely that the population of white muscle fibers in a mixed muscle is not homogeneous, but consists of a spectrum of fibers of varying respiratory capacity. The exercise apparently increased respiratory enzyme levels sufficiently in certain white fibers, probably those with the highest initial respiratory capacity, to reach the critical level required to give a red appearance, thus decreasing the percentage of white appearing fibers. On the other hand, the initial staining intensity of the respiratory enzymes in red and intermediate fibers is already so dark that any further darkening of the stain due to the exercise-induced increase in respiratory enzyme levels does not appear to be readily detectable.

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