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


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Baldwin, K. M., G. H. Klinkerfuss, R. L. Terjung, P. A. Mole, and J. O. Holloszy. Respiratory capacity of white, red, and intermediate muscle: adaptive response to exercise. Am. J. Physiol. 222(2): 373-378. 1972.—A program of running can result in a twofold increase in the respiratory capacity of mixed muscles such as the gastrocnemius and quadriceps in the rat. This study was undertaken to determine which fiber type or types participate in the increase in oxidative capacity. The soleus muscle, and the superficial, white and deep, red portions of the quadriceps were used to determine the responses of intermediate, white, and red muscle to endurance exercise. The capacity to oxidize pyruvate-2,14C and palmitate-U-14C, the levels of activity of cytochrome oxidase, carnitine palmitoyltransferase, and citrate synthase, and the concentration of cytochrome c increased to approximately the same extent in all three muscle types in rats subjected to a program of running. These results are not compatible with the interpretation that the exercise-induced increase in oxidative capacity of muscle is due to transformation of white into red fibers as appears to be the case by histochemical appearance. Since the respiratory capacity of all three muscle types increased approximately twofold, the approximately fivefold difference in capacity for oxidative metabolism between red and white muscle was maintained in the trained animals.

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, palmityl-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).

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 with a low respiratory capacity to red fibers with a high 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. A sevenfold increase in respiratory capacity of the white fibers might be expected to result in a uniformly red-appearing muscle, since myoglobin content appears to parallel respiratory capacity (12). In the second place, it was previously found that soleus muscle, which is a mixture of intermediate and red fibers (4), undergoes an adaptive increase in the levels of activity of cytochrome oxidase and succinate oxidase in response to exercise (9). This finding seems incompatible with the interpretation that the increase in oxidative capacity induced by exercise is due to a conversion of white to red muscle fibers. The present study was, therefore, undertaken to obtain further information regard-
ic samples in l-ml cuvettes of l-cm light path at 30 C. The 14C02 produced was trapped and radioactiv-
ity was determined as described previously (13). Oxygen uptakes are expressed 
as microliters of O2 under standard conditions. 

Enzyme activities are reported as micromoles of substrate 

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 Srere 
(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 phos-
phorylase (22). Photographs were taken from slides to per-
at a representative sampling 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 diformazan 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 experi-
mental 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 

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 seden-
tary group. The exercising group was trained for 12 weeks 
by means of a program of treadmill running described pre-
viously (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 pro-
vided 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 restric-
tion 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 
obidation, 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 radioactiv-
ity was determined as described previously (13). 

Oxygen uptake was measured in a Gilson 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 
compartiment 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 
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<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Superficial, white</th>
<th>Deep, red</th>
<th>Middle, mixed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>399±6</td>
<td>1146±37</td>
<td>391±20</td>
<td>1061±45</td>
<td>2601±56</td>
</tr>
<tr>
<td>Runners</td>
<td>393±6</td>
<td>1131±35</td>
<td>408±18</td>
<td>1088±30</td>
<td>2628±53</td>
</tr>
</tbody>
</table>

Values are means ± se. There were 10 animals in each group. None of the above values were significantly different for the 2 groups.
WHITE, RED, AND INTERMEDIATE MUSCLE RESPONSE TO EXERCISE 375

FIG. 1. Diagram represents a midsection of quadriceps. Fiber counts were obtained from area demarcated by dotted lines. Top is superficial; bottom is deep. Artificial division of tissue into 5 subareas allowed comparison of proportion of fiber types from superficial to deep.

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, the 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
Fig. 2. Plantaris muscle from weight-matched control (A) and exercise-trained (B) rats. When sections were orientated by matching to a serially sectioned replica of gastrocnemius-plantaris-soleus, nearly exact levels could be compared. Increase in numbers of fibers rich in oxidative enzyme in B was a constant finding at all levels in exercised animals. Stained for DPNH-diaphorase. Magnification ca. X20.

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>Pyruvate-2-14C oxidation, nmol/min per g</td>
<td>96±22 (6)</td>
</tr>
<tr>
<td>Runners</td>
<td>Palmitate-U-14C oxidation, nmol/min per g</td>
<td>207±42 (6)*</td>
</tr>
<tr>
<td>Sedentary</td>
<td>Palmitate-U-14C oxidation, nmol/min per g</td>
<td>5.5±2.5 (6)</td>
</tr>
<tr>
<td>Runners</td>
<td>Carnitine palmityltransferase, pmol/min per g</td>
<td>15.6±2.3 (6)*</td>
</tr>
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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 palmityltransferase 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>Citrate synthase, µmol/min per g</td>
<td>10.3± (9)</td>
</tr>
<tr>
<td>Runners</td>
<td>Carnitine palmityltransferase, µmol/min per g</td>
<td>1.8 (9)*</td>
</tr>
</tbody>
</table>

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

Citrate synthase, carnitine palmityltransferase, cytochrome oxidase, and cytochrome c levels. Further information regarding the effects of the exercise program on the different fiber types was obtained by measuring the response of a number of mitochondrial marker enzymes. The levels of activity of the citric acid cycle enzyme, citrate synthase, the long-chain
and the components of the respiratory chain that link the oxidation of succinate and DPNH to 02 (6, 9, 10, 15). Chain fatty acids (13), enzymes of the citric-acid cycle (10), involved in the activation, transport, and oxidation of long-chain fatty acids (6, 9, 13). Underlying this increased capacity for oxidative metabolism is a rise in the levels of a number of mitochondrial enzymes. These include enzymes and the respiratory chain enzyme, cytochrome oxidase, all measured to oxidize pyruvate and long-chain fatty acids was assessed by measuring the rate of 14CO2 production from pyruvate-2-14C and palmitate-1-14C 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 palmitoyltransferase, which also increase in mixed muscle in response to exercise (10, 13), served as additional mitochondrial markers.

In 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 percentage 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>
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<th>Table 4. Cytochrome oxidase activity in muscles from exercised and sedentary animals</th>
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<tr>
<td>Group</td>
</tr>
<tr>
<td>Superficial, white</td>
</tr>
<tr>
<td>Sedentary</td>
</tr>
<tr>
<td>Runners</td>
</tr>
<tr>
<td>339 ± 21 (9)*</td>
</tr>
<tr>
<td>Values are means ± se. The number of animals is given in parentheses. * Runners vs. sedentary, P &lt; 0.001</td>
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<th>Table 5. Concentration of cytochrome c in muscles of exercised and sedentary animals</th>
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<tr>
<td>Group</td>
</tr>
<tr>
<td>Sedentary</td>
</tr>
<tr>
<td>Runners</td>
</tr>
<tr>
<td>6.3 ± 0.7*</td>
</tr>
<tr>
<td>Values are means ± se, each value is the mean for 5 animals. * Runners vs. sedentary, P &lt; 0.01</td>
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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 increased capacity of whole homogenates and of the mitochondrial fraction of muscle to oxidize pyruvate and long-chain fatty acids (6, 9, 13). Underlying this increased capacity for oxidative metabolism is a rise in the levels of a number of mitochondrial enzymes. These include enzymes involved in the activation, transport, and oxidation of long-chain fatty acids (13), enzymes of the citric-acid cycle (10), and the components of the respiratory chain that link the oxidation of succinate and DPNH to O2 (6, 9, 10, 15).
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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