Biochemical Adaptations in Muscle

EFFECTS OF EXERCISE ON MITOCHONDRIAL OXYGEN UPTAKE AND RESPIRATORY ENZYME ACTIVITY IN SKELETAL MUSCLE*

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JOHN O. HOLLÓSZY

From the Department of Preventive Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

SUMMARY

The capacity of the mitochondrial fraction from gastrocnemius muscle to oxidize pyruvate doubled in rats subjected to a strenuous program of treadmill running. Succinate dehydrogenase, reduced diphosphopyridine nucleotide dehydrogenase, DPNH cytochrome c reductase, succinate oxidase, and cytochrome oxidase activities, expressed per g of muscle, increased approximately 2-fold in hind limb muscles in response to the training. The concentration of cytochrome c was also increased 2-fold, suggesting that the rise in respiratory enzyme activity was due to an increase in enzyme protein. The total protein content of the mitochondrial fraction increased approximately 60%. These changes in the concentration of cytochrome c and total mitochondrial protein are of special interest because they suggest that exercise could serve as a useful tool for studying the biosynthesis of mitochondrial proteins.

Mild exercise, such as that used in previous studies, was found to have no effect on the level of succinate dehydrogenase in muscle, suggesting that the failure of earlier studies to show an increase in respiratory enzyme activity resulted from the use of an insufficient exercise stimulus.

Mitochondria from muscles of the exercised animals exhibited a high level of respiratory control and tightly coupled oxidative phosphorylation. Thus, the increase in electron transport capacity was associated with a concomitant rise in the capacity to produce adenosine triphosphate. This adaptation may partially account for the increase in aerobic work capacity that occurs with regularly performed, prolonged exercise.

Comparative studies have shown that a good correlation exists between the ability of a muscle to perform prolonged exercise and its content of respiratory enzymes (1, 2). Paul and Sperling (1) found the breast muscle of the nonflying chicken to have low α-ketoglutarate and succinate $Q_o$ values and to be poor in mitochondria. In contrast, the breast muscles of mallards and pigeons, which spend long periods in active flight, are rich in mitochondria and have $Q_o$ values which are approximately 10 times as high as those found in the chicken (1). Similarly, Lawrie (2) has reported that the levels of cytochrome oxidase, succinate oxidase, and succinate dehydrogenase activity in psoas muscle of the sedentary laboratory rabbit are approximately one-third to one-half as high as those found in the active wild hare, and only one-fourth to one-sixth as high as the values obtained on horse psoas.

Little is known regarding the factors which control the biosynthesis of mitochondria and the biosynthesis of specific mitochondrial constituents. It does appear clear, however, from studies on the effects of thyroxine feeding, that mitochondria in striated muscle and various other tissues are capable of undergoing adaptive changes in both composition and number (3-6). Lee and Lardy (3) found that feeding desiccated thyroid to rats for 10 days increases the specific activity of mitochondrial α-glycerophosphate dehydrogenase 2- to 3-fold in heart muscle and about 20 fold in liver. The activities of a number of other mitochondrial enzymes, including those of the respiratory chain, also increase significantly in response to thyroxine but to a lesser extent (3-5), while still others appear to be unaffected by hyperthyroidism (3). Electron microscopic studies have shown that thyroxine administration can also increase the number and alter the morphology of mitochondria in skeletal muscle (6).

In view of the evidence, obtained from the above studies with thyroxine, that mitochondria are capable of undergoing adaptive changes, it appeared possible that the differences between the respiratory enzyme levels of active and inactive muscles might be due not only to genetic differences but also to an adaptive process. To test this possibility, controlled studies on the chronic effects of exercise are necessary. Surprisingly few such studies have been reported. Hearn and Wainio (7), in a study on rats subjected to 30 min of swimming daily for 5 to 8 weeks, found no increase in the level of succinate dehydrogenase activity in skeletal muscle. With the use of the same exercise program, Gould and Rawlinson (8) observed no change in the levels of malate dehydrogenase and ATPase activity in rat skeletal muscle. These negative results might reflect the relative mildness of the exercise stress used, since 30 min of swimming is well within the exercise capacity of the untrained rat. It has been observed in

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our laboratory that untrained rats can swim for over 6 hours if the water temperature is kept near 32°.

It appeared of interest, therefore, to investigate the chronic effects of more vigorous and prolonged exercise on skeletal muscle. The data reported in the present paper show that a strenuous program of running increases mitochondrial capacity for oxidative phosphorylation and that this results, at least in part, from an increased ability to carry out terminal electron transport.

EXPERIMENTAL PROCEDURE

Animal Care and Exercise Program—Male rats of a Wistar strain (specific pathogen-free CFN rats), aged 6 weeks were obtained from Carworth Farms, placed in individual cages, and maintained on a diet of Purina laboratory chow and water. They were divided into four groups.

An exercising group was trained to run on a motor-driven treadmill similar to the one described by Kimeldorf (9). It consists of a wide, endless belt riding on metal rollers. A Lucite box, partitioned into individual compartments 30 cm long by 10 cm wide, is suspended over the belt, providing a limited area for each animal to run in. Motivation is provided by a shock grid at the rear of the compartments. Animals learn to avoid being shocked by keeping pace with the belt movement. The treadmill was set at an 8° incline. The animals were exercised 6 days per week. They initially ran for 10 min at 22 m per min, twice daily, 4 hours apart. The work load was progressively increased over 12 weeks. At the end of this period the animals were running continuously for 120 min at 51 m per min, with 12 sprints at 42 m per min, each lasting 30 sec, interspersed at 10-min intervals through the workout. Animals were maintained at the final work level until they were sacrificed; this period varied from 1 to 6 weeks.

The exercising group was provided with food and water ad libitum.

An exercising control group ran on the treadmill for 10 min per day, 5 days per week. Running speed was increased to 51 m per min over 6 weeks and then maintained at this level. The purpose of this program was to maintain running skill, and familiarity with the procedure, while keeping the exercise stimulus relatively minimal. Food intake was adjusted to maintain their body weights in the same range as that for the exercising groups.

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

Preparation of Homogenates and Mitochondria—Animals were not exercised for 24 hours prior to killing. They were killed by decapitation, after an overnight fast, and exsanguinated. Muscles were rapidly dissected out, chilled in ice-cold Ringer's solution, blotted, weighed, chopped into a fine mince with scissors, and homogenized in a glass Potter-Elvehjem homogenizer. Because of the abundant connective tissue, it was found necessary to use loosely fitting tubes, and pestles with cutting teeth on the bottom. The tubes were immersed in ice water during the procedure. After gross breaking up of the tissue, homogenization was completed with 10 complete passes of the tube. The homogenates contained 1 g of muscle per 10 ml. Homogenates for assays of succinate dehydrogenase, succinate oxidase, and cytochrome oxidase activities were prepared in 0.01 M potassium phosphate buffer, pH 7.4.

Preliminary studies confirmed the finding of Ernster, Ikkos, and Luft (10), and of Hedman (11) that sucrose media are unsatisfactory for the isolation of skeletal muscle mitochondria. Mitochondrial yield, in terms of respiratory and enzymatic activity, from muscles homogenized in 0.175 M KCl was approximately twice as great as from paired muscles homogenized in sucrose. Muscle mitochondria were, therefore, prepared from homogenates made in 0.175 M KCl. To isolate mitochondria for spectrophotometric assays of enzyme activity, the homogenates were first centrifuged for 10 min at 700 × g. The supernatant fluid was decanted, and the sediment was resuspended in 0.175 M KCl and centrifuged for 10 min at 700 × g. The 700 × g supernatant suspensions were combined, centrifuged again at 700 × g, decanted, and centrifuged at 14,000 × g for 20 min. The mitochondrial pellet was washed once by resuspension in 0.175 M KCl and recentrifuged at 14,000 × g. The mitochondria from 1 g of muscle were finally suspended in 2 ml of 0.01 M potassium phosphate buffer, pH 7.4, and frozen and thawed twice prior to measurement of DPNH dehydrogenase and DPNH cytochrome c reductase activities.

Mitochondria for O2 uptake studies were isolated from the final 700 × g supernatant fluid by centrifugation at 6000 × g for 20 min. The mitochondria were suspended in 0.25 M sucrose and were used immediately.

Assay Methods—Oxygen uptake was measured in a Gilson differential respirometer, at 30°, with air as the gas phase. The center wells of the Warburg flasks contained 0.2 ml of 15% KOH and a wick of pleated filter paper.

Mitochondrial respiration was measured in triplicate. Each flask contained, in a final volume of 2 ml, 6.5 mM MgCl2, 30 mM KCl, 62.5 mM sucrose, 40 mM potassium phosphate buffer, 10 mM potassium pyruvate, 1.0 mM sodium malate, 25 mM glucose, 2.5 mM ATP, 2 mg of hexokinase, and mitochondria from 0.5 g of muscle. The pH of the mixture was 7.2. After 10 min of thermoequilibration, O2 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. At this point, the first flask was chilled and the reaction was stopped with 5% trichloroacetic acid. O2 uptake was measured for two more 10-min periods in the remaining flasks; the reaction was then terminated by the addition of 5% trichloroacetic acid. The mixtures were filtered, and the Pi present was measured. Phosphate esterified was measured by Pi disappearance during the 20-min period of respiration in the presence of the Pi-trapping system. Respiratory control ratios were calculated by dividing the rate of O2 uptake in the presence of phosphate acceptor by the rate in the absence of phosphate acceptor.

Succinate oxidase and cytochrome oxidase activities were measured manometrically as described by Potter (12). O2 uptakes are expressed as microliters of dry O2 under standard conditions.

Spectrophotometric assays were performed in a Beckman model DU spectrophotometer with a thermococated cell compartment in 1-ml cuvettes of 1-cm light path, at 30°. Readings were taken at 15- or 30-sec intervals. Initial reaction rates were determined from a segment of the linear portion of the change in absorbance and corrected for the rates of any nonenzymatic activity. Enzymatic activities are reported as micromoles of substrate oxidized per min.

DPNH dehydrogenase activity was assayed by the method of Minakami, Ringler, and Singer (13). The reduction of potas-
The methods used for isolating mitochondria and determining O₂ consumption, respiratory control, and P:O ratio are described under “Experimental Procedure.” The substrate used was pyruvate plus malate. There were eight animals in each group. The values shown are means ± the standard errors of the mean. O₂ uptake is expressed as microliters of O₂ utilized per hour by the mitochondria from 1 g of fresh muscle.

<table>
<thead>
<tr>
<th>Group</th>
<th>Oxygen uptake</th>
<th>Respiratory control index</th>
<th>P:O ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>506 ± 53</td>
<td>14.7 ± 2.6</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Exercising</td>
<td>1022 ± 118*</td>
<td>16.1 ± 2.2</td>
<td>2.6 ± 0.1</td>
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</tbody>
</table>

* Exercising versus sedentary, p < 0.01.

Cytochrome oxidase and succinate oxidase activities—Whole homogenates of gastrocnemius and soleus muscles were assayed for cytochrome oxidase and succinate oxidase activities. As shown in Table II, the activity of both of these enzyme systems was significantly greater in the exercising group (p < 0.001). These results, obtained with whole homogenates and, therefore, not subject to errors due to possible differences in the percentage yield of mitochondria, provide further evidence that endurance exercise increases the respiratory capacity of muscle. For the sake of consistency, the O₂ uptakes in Table II are given per g of fresh muscle. However, as the water content of muscle from the two groups was not different, the findings are the same when O₂ uptakes are expressed, in the conventional manner, as microliters of O₂ utilized per hour per mg of dry muscle. The water content of fresh muscles averaged 0.78 ± 0.02 ml per g for the sedentary group and 0.78 ± 0.01 ml per g for the exercising group.

Succinate dehydrogenase—In view of the findings of Hearn and Wainio (7) that 30 min of daily swimming for 6 weeks did not

Despite these differences in weight, no differences were found between the paired weight and free eating sedentary controls in the specific activities of the respiratory enzyme systems measured in muscle. Therefore, in the following sections, the results obtained on animals from these two groups have been combined and are referred to jointly under the headings “sedentary group” or “sedentary controls.”

Respiratory Activity and Oxidative Phosphorylation in Mitochondrial Fraction from Muscle—The respiratory activity of the mitochondrial fraction from gastrocnemius muscles was measured with pyruvate plus malate as the substrate. During uncontrolled respiration (i.e., in the presence of nonlimiting amounts of P₁ and ADP) the mitochondrial fraction of muscles from the exercising group utilized approximately twice as much O₂ as that from the sedentary controls (Table I). As also shown in Table I, respiratory control and P:O ratios were not significantly different for the two groups. It appears from these results that the exercise program brought about a major increase in the ability of the involved muscles to oxidize pyruvate. The high level of respiratory control, together with P:O ratios in the same range as those of the controls, indicates a concomitant increase in the capacity to form ATP.

**Table I**

<table>
<thead>
<tr>
<th>Group</th>
<th>Oxygen uptake</th>
<th>Respiratory control index</th>
<th>P:O ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>305 ± 15</td>
<td>73 ± 5</td>
<td></td>
</tr>
<tr>
<td>Exercising</td>
<td>551 ± 31*</td>
<td>117 ± 8*</td>
<td></td>
</tr>
</tbody>
</table>

* Exercising versus sedentary, p < 0.001.
increase the level of succinate dehydrogenase in the skeletal muscles of rats, it is of interest that, in the present study, a highly significant increase in the activity of this enzyme occurred in the exercising animals. Homogenates of gastrocnemius muscles from nine sedentary controls oxidized an average of 8.3 ± 0.7 μmoles of succinate per min per g of fresh muscle, compared to 15.1 ± 1.4 μmoles per min per g for nine animals in the exercising group (p < 0.001). In contrast, a value of 7.7 ± 0.9 μmoles per min per g was obtained on three rats made to swim 30 min per day, 5 days per week, for 6 weeks; this value is not significantly different from that for the sedentary controls, and confirms the observation of Hearn and Wainio (7).

**TABLE III**

<table>
<thead>
<tr>
<th>1. Group</th>
<th>2. DPNH dehydrogenase</th>
<th>3. Mitochondrial protein</th>
<th>4. DPNH dehydrogenase as units/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary........</td>
<td>5.6 ± 0.6</td>
<td>2.97 ± 0.20</td>
<td>1.96 ± 0.30</td>
</tr>
<tr>
<td>Exercising........</td>
<td>11.8 ± 1.5</td>
<td>4.67 ± 0.30</td>
<td>2.54 ± 0.26</td>
</tr>
</tbody>
</table>

* Exercising *versus* sedentary, p < 0.001.

**Effect of exercise program on DPNH dehydrogenase activity and on mitochondrial protein concentration**

DPNH dehydrogenase activity was measured in the mitochondrial fraction of gastrocnemius muscles as described under "Experimental Procedure." The concentration of ferriyanide was varied, and the results were extrapolated to Vmax with respect to oxidant. Each value represents the mean ± the standard error of the mean for muscles from 10 rats. Enzymatic activity is expressed as micromoles of DPNH oxidized per min by the mitochondria from 1 g of muscle, as compared to 0.25 ± 0.05 μmole per min per g of muscle for seven sedentary controls (p < 0.01).

**Cytochrome c**—In addition to measuring the activity or "functional capacity" of the respiratory chain in the skeletal muscles of rats, it is of interest that, in the present study, a highly significant increase in the activity of this enzyme occurred in the exercising animals. Homogenates of gastrocnemius muscles of the exercising group were significantly higher than that of the sedentary group (p < 0.001). The protein content of the mitochondrial fraction was also significantly higher in the exercising group (p < 0.001), as shown in Table III, Column 3. As a result, the values of DPNH dehydrogenase activity for the two groups are not significantly different when expressed in terms of mitochondrial protein (Table III, Column 4).

The finding that mitochondria obtained from the muscles of the exercised animals exhibited a high level of respiratory control and tightly coupled oxidative phosphorylation indicates that the increase in mitochondrial electron transport capacity is associated with a rise in the capacity to produce ATP. In contrast, mitochondria from tissues of hyperthyroid animals, which also show an increase in respiratory enzyme activity (4, 5) are characterized by partial uncoupling of oxidative phosphorylation and loss of respiratory control (21). Furthermore, on electron microscopic examination, the mitochondria in the skeletal muscles of the exercised animals showed no evidence of swelling or other gross alteration in structure such as have been reported in the tissues of hyperthyroid animals (6, 22).

In contrast to the program of treadmill running, 30 min of daily swimming, which represents a mild work load for the rat, has no effect on the level of succinate dehydrogenase in rat gastrocnemius muscle. This confirms the report of Hearn and Wainio (7), and indicates that a greater exercise stress is needed to bring about an adaptive increase in the enzymes of the mitochondrial electron transport chain. In the present study, the exercise load was progressively increased over a 12-week period to try to achieve a near maximal increase in aerobic work capacity. We now have preliminary data which indicate that similar results can be obtained in as short a time as 3 weeks if the exercise period is sufficiently prolonged.

The intracellular concentrations of numerous substances, including pyruvate, lactate, Pi, ADP, and AMP increase in muscle during exercise (cf. References 23 and 24). Whether or not one of these acts as an inducer of the biosynthesis of the enzymes involved in mitochondrial electron transport is not known. However, it appears clear from the present data that either an increase in the number of mitochondria, an increase in

1 J. R. Williamson and J. O. Holloszy, unpublished observations.
the size of the mitochondria, or an alteration in the composition of the mitochondria must have occurred. Studies in progress on the chronic effects of exercise on the levels of activity of the citric acid cycle enzymes, and on the number and size distribution of mitochondria in muscle may help to determine which of these possibilities is correct. Regardless of which alternative obtains, the finding that exercise can induce an increase in the concentrations of specific mitochondrial enzymes and in total mitochondrial protein in muscle may provide a useful tool for studying the biogenesis of mammalian mitochondria, or the biosynthesis of specific mitochondrial constituents, or both.

The increase in the capacity of muscle to oxidize pyruvate and form ATP has interesting implications relative to the ability of muscle to perform aerobic work. It is well known that physically trained individuals, as compared to untrained, are characterized by the ability to attain a higher maximum rate of \( O_2 \) consumption during strenuous exercise (25, 26) and to maintain lower levels of blood lactate during moderate exercise (27, 28). It has been suggested that cardiovascular adaptations, resulting in the delivery of more blood and \( O_2 \) to the working muscles, are responsible for these effects of training (27, 28). However, it is not possible to explain on this basis the finding that trained individuals have lower blood lactate concentrations than untrained during exercise (27, 28). It appears likely, therefore, that during moderate exercise (27, 28). It has been shown recently that cardiac output and, therefore, also peripheral blood flow, is actually lower at a given level of submaximal exercise in the trained than in the untrained state (30). Trained muscles appear to compensate for this decrease in blood flow and meet their \( O_2 \) requirements during work by increased extracellular \( O_2 \) delivery of more blood and \( O_2 \) to the working muscles, are responsible for these effects of training (27, 28).

REFERENCES

ESSAYS ON APS CLASSIC PAPERS

Nature vs. nurture: can exercise really alter fiber type composition in human skeletal muscle?

Christopher P. Ingalls
Department of Kinesiology and Health, Georgia State University, Atlanta, Georgia 30303

This essay looks at the historical significance of two APS classic papers that are freely available online:


It is well recognized today that mammalian skeletal muscle has a remarkable potential to alter its phenotype. Since the publication of the classic 1960 paper by Buller et al. (2) demonstrating the reversal of contractile characteristics in fast- and slow-twitch muscles after cross-innervation in cat, the plasticity of skeletal muscle has been demonstrated at the molecular and cellular levels using a variety of animal models and experimental treatments. Chronic electrical stimulation, synergistic muscle ablation, hindlimb suspension, and hormone manipulation have all been used to document changes in metabolic enzymes, 

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handling proteins, myosin isoforms and regulatory proteins of skeletal muscle, as well as alterations in muscle fiber type and size. Moreover, John Holloszy’s classic 1967 paper (6) demonstrating the remarkable adaptation of the energy metabolism system in rat skeletal muscle to chronic exercise training indicated that the malleability of muscle could also be observed with a simple physiological stimulus. However, whether a stimulus such as exercise training could produce not only metabolic adaptations, but also transform fiber types in human skeletal muscle, is a question that has been long debated.

The first prospective research study that addressed this question using a fiber type classification system based on histochemical staining of myosin ATPase activity was published in the Journal of Applied Physiology in 1973 by Phil Gollnick (Fig. 1), Bob Armstrong, Bengt Saltin, Carl Saubert, Walt Sembrowich, and Ray Shepherd from the Department of Physical Education for Men at Washington State University (”Effect of training on enzyme activity and fiber composition of human skeletal muscle” Ref. 4). The initial interest in this question arose from the early work of Reggie Edgerton and colleagues at UCLA, whose work was critical for the development of fiber type classification systems. Furthermore, Edgerton’s doctoral dissertation at Michigan State University introduced exercise physiologists to the idea of exercise-induced fiber type transformation in rodent muscle (3). To address the idea of fiber type plasticity in human skeletal muscle, Gollnick needed expertise in the techniques of fiber typing and needle biopsy of muscle. Therefore, Bob Armstrong, a National Science Foundation Predoctoral fellow in Gollnick’s laboratory, spent the summer of 1971 learning fiber type techniques from Reggie Edgerton at UCLA. It was also at this time that Gollnick initiated his life-long research collaboration with Bengt Saltin, an Associate Professor in the Department of Applied Physiology at the Karolinska Institute in Stockholm Sweden, with expertise in the needle muscle biopsy technique and nearly 80 exercise science publications (an impressive publication record considering that he received his PhD in 1964).

Gollnick and coworkers initially applied these techniques in a classic study published in the Journal of Applied Physiology in 1972 (“Enzyme activity and fiber composition in skeletal muscle”)...
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muscle of untrained and trained men” Ref. 5). This may be the first study to address the idea of exercise-induced fiber type plasticity in human skeletal muscle, the 1973 Journal of Applied Physiology article (4) is noteworthy for several reasons. First, the duration and intensity of exercise training are truly remarkable for a human research study. Six subjects exercised on a cycle ergometer for 1 h per day, 4 days per week, for 5 mo. At the completion of the study, most of the subjects were exercising for 1 h at 85–90% of the maximal aerobic power! All of the subjects were either graduate students or the lab director, and five of the six subjects were authors/coauthors on the paper. Considering the commitment of time and intensity of physical effort required by this long-term study, it’s astonishing that this research group was still able to publish nine articles in 1973! Presumably, the reason that Saltin did not participate in the study as a subject, as he was usually accustomed to, was because the long duration of the study kept him from other academic responsibilities and that he was too well trained. Second, the first time that the myosin ATPase-based fiber type system was applied to muscle biopsy samples from humans was in the studies from 1972 (5) and 1973 (4). Although J. B. Peter, R. J. Barnard, and Edgerton had refined the myosin ATPase-based fiber type technique to document three basic fiber types (i.e., type I, slow-twitch, oxidative; type IIa, fast-twitch, oxidative and glycolytic; and type IIb, fast-twitch, glycolytic) in muscles from laboratory animals, the histological approach used by Gollnick and coworkers at the time only allowed them to discriminate between fast- and slow-twitch fibers in human muscle. Third, the conclusion that chronic exercise training does not significantly alter the distribution of fast- and slow-twitch fibers in human skeletal muscle has been a source of debate for years. It should be noted that the magnitude of the mean change in the percent of slow-twitch fibers (32% to 36%) reported by Gollnick et al. has been consistent with numerous subsequent studies that have (e.g., Ref. 7) and have not (e.g., Ref. 1) reported statistically significant alterations in slow-twitch fiber distribution after exercise training. A small sample size (n = 6) and relatively high sampling variance may have precluded Gollnick et al. from observing statistically significant changes. Today, it is generally accepted that exercise training can promote changes within the population of fast-twitch fibers (i.e., type IIb to IIa) and to a lesser extent changes from fast- to slow-twitch fibers. To this end, it is interesting to note that the two subjects (R. B. Armstrong and W. L. Sembrowich) in the Gollnick study with the lowest distribution of slow-twitch fibers before training were the same two subjects that exhibited the greatest absolute increase in slow-twitch fiber percentage (23% to 32%), succinate dehydrogenase (SDH) activity (3.5 to 10.3 μmol·g⁻¹·min⁻¹), and maximal aerobic power (4.1 to 5.0 l/min) after training. Fourth, other observations made in this study have helped fuel exercise science questions over the last 30 years. Gollnick and coworkers reported that endurance training resulted in supercompensation of muscle glycogen storage (153% increase). Furthermore, the disproportionate increase (95%) in muscle SDH activity compared with the small increase (13%) in maximal aerobic power suggested that whole body VO₂ max was not limited by changes in muscle oxidative potential.

It is now generally recognized that skeletal muscle fibers do not exist in three discrete forms at the subcellular level, but rather in a continuum based on the multitude of combinations of myosin heavy and light chain isoforms, polymorphic expression of protein isoforms, metabolic potential, and Ca²⁺ handling characteristics. Moreover, it is clear that all of these cellular characteristics exhibit some degree of plasticity in response to exercise training. The seminal work of scientists such as Edgerton, Holloszy, Ken Baldwin, Frank Booth, Dirk Pette, Saltin, and Gollnick over the years has been critical in shaping our understanding of exercise-induced plasticity of the muscle fiber phenotype. Although all the authors of the 1973 paper have gone on to respectable careers in and out of academia, three deserve special mention. According to the Thomson ISI Web of Knowledge index, Saltin, Gollnick, and Armstrong have published over 600 exercise science articles. Over 90 of these publications have been referenced more than 100 times, and 7 papers have been cited over 400 times. The 1972 and 1973 Journal of Applied Physiology papers have been referenced 554 and 397 times, respectively, making them both true classic papers in exercise physiology.

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www.the-aps.org/publications/classics
Enzyme activity and fiber composition in skeletal muscle of untrained and trained men

P. D. GOLLNICK, R. B. ARMSTRONG, C. W. SAUBERT IV, K. PIEHL, AND B. SALTIN
Department of Physical Education for Men, Washington State University, Pullman, Washington 99163; and Department of Physiology, Gymnastik-och Idrottsföreningen, Stockholm, Sweden

GOLLNICK, P. D., R. B. ARMSTRONG, C. W. SAUBERT IV, K. PIEHL, AND B. SALTIN. Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. J. Appl. Physiol. 33(3): 312-319, 1972.—Succinate dehydrogenase (SDH) and phosphofructokinase (PFK) activities in the skeletal muscle of most species. Several systems have been proposed for the identification of these fiber types (3, 5, 10, 24). In human muscle most investigators have identified only two distinctly different fiber types. These have been classified as type I and type II (7, 16) or red and white (9) fibers. Edström and Nyström (9) have reported differences in the percent distribution of these fibers both between different muscles and within the same muscles comparing different subjects. No consistent pattern for glycogen storage in the two fiber types existed.

Succinate dehydrogenase; glycogen storage; fiber types; phosphofructokinase; human skeletal muscle

AT LEAST THREE DIFFERENT FIBER types have been identified in the skeletal muscle of most species. Several systems have been proposed for the identification of these fiber types (3, 5, 10, 24). In human muscle most investigators have identified only two distinctly different fiber types. These have been classified as type I and type II (7, 16) or red and white (9) fibers. Edström and Nyström (9) have reported differences in the percent distribution of these fibers both between different muscles and within the same muscles comparing different subjects.

The interconvertibility of fiber types has been demonstrated in animals following cross innervation (8). Barnard and co-workers (3) have also reported that physical training resulted in an increase in the percentage of red fibers at the expense of white fibers in guinea pig muscle.

In animals, endurance training can alter the metabolic characteristics of skeletal muscle by increasing the activity of some Krebs cycle enzymes, mitochondrial protein concentration, and the ability to oxidize fat (11, 12, 14). Some of these changes have been observed in human skeletal muscle after training (15, 26). However, the metabolic characteristics and fiber composition in human muscle of trained and untrained subjects and how these relate to the activity of specific muscle groups and to performance characteristics have not been studied. The purpose of this investigation was to approach this problem by studying groups of subjects who had been engaged in various types and intensities of training for prolonged periods of time.

SUBJECTS AND METHODS

Nine groups of men were employed in the study (Table 1). These subjects were selected to represent different age groups and states of physical fitness and to encompass men who were participating in a variety of sport activities that used different muscle groups and training programs. Altogether 74 men between the ages of 17 and 58 years were studied.

Samples of the vastus lateralis and deltoid muscles were obtained with the needle biopsy technique (4) and divided into three parts. One portion of the sample was immediately frozen in liquid nitrogen and stored in Dry Ice for subsequent histochemical analysis. The remaining two parts were weighed and used to determine succinic acid dehydrogenase (SDH) and phosphofructokinase (PFK) activities at 25 C with the methods of Cooperstein et al. (6) and Shonk and Boxer (23), respectively. On some occasions a second biopsy was taken to provide enough tissue for the different measurements. In such cases the biopsy was taken at the same sampling site as the first one.

The samples frozen for histochemical analysis were examined under a dissecting microscope in a cold room at -25 C to determine fiber orientation. They were then cooled to the temperature of liquid nitrogen, placed on specimen holders in OCT embedding medium (Ames Tissue-Tek) at near 0 C, and immediately immersed in liquid nitrogen. Serial sections, 10 µm thick, were cut in a cryostat at -20 C and mounted on cover glasses for staining. Myosin adenosine triphosphatase (ATPase), reduced diphosphopyridine nucleotide-diaphorase (DPNH-diaphorase), and alpha-glycophosphate dehydrogenase activities were estimated with the methods of Padykula and Herman (20), Novikoff and associates (18), and Wattenberg and Leong (27), respectively. The distribution of glycogen in one of the serial sections (16 µm thick) was estimated from the periodic acid-Schiff (PAS) reaction (21). Photographs of the slides were taken and 20 x 25 cm black-and-white
prints made for the purpose of classifying fibers and estimating the distribution of oxidative and glycolytic capacity in the different fibers.

Total glycogen in some samples was determined with the method described by Karlsson et al. (13). Ribonucleic acid (RNA) was determined in the muscle samples of some groups with the modified Schmidt-Thannhauser method described by Munro and Fleck (17). Yeast RNA was used as the standard.

Maximal oxygen uptake (\(\dot{V}O_2\) max) during leg work was determined for all subjects either while running on a treadmill or pedaling a bicycle. In some of the groups whose athletic specialty involved extensive use of the arms, \(\dot{V}O_2\) max was also measured while cranking a specially adapted bicycle with the hands and arms (25). Expired air was collected in Douglas bags and its volume was determined with a wet spirometer. Gas analysis was performed with the Haldane technique.

The area of the muscle fibers from some subjects was determined by planimetry from the photographs of the sections used to determine DPNH-diaphorase activity. A total of 40 randomly selected cells, including 20 of each fiber type, were measured for each subject. Cell diameters were calculated from the area.

### RESULTS

The intent of this investigation was to study subjects of different ages and states of physical training, some of whom were participating in various sport activities. The effectiveness of subject selection in meeting the fitness criteria is illustrated by the \(\dot{V}O_2\) max during leg work (Table 1), which varied from a mean value of 41 (untrained groups and weight lifters) to 75 (young cross-country runners) ml/kg min\(^{-1}\). These values are similar to those previously reported for sedentary individuals and champion athletes (22). It has previously been shown that \(\dot{V}O_2\) during maximal arm work is only about 70\% of that during maximal leg work (1, 25).

### TABLE 1. Physical characteristics, fiber populations, enzyme activities, and \(\dot{V}O_2\) max of groups studied

<table>
<thead>
<tr>
<th>Groups, Age, No.</th>
<th>Height, Weight</th>
<th>Type of Work (Arm/Leg)</th>
<th>% ST Fiber</th>
<th>Enzyme Activities, pmoles/g min(^{-1})</th>
<th>(\dot{V}O_2) max, ml/kg min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untrained, 27 (24-30 yr, n = 12</td>
<td>179 cm (166-187)</td>
<td>A</td>
<td>46.0 ± 6.8</td>
<td>3.6 ± 0.4</td>
<td>21.6 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>76.5 kg (64.6-105.5)</td>
<td>L</td>
<td>36.1 ± 5.0</td>
<td>4.3 ± 0.6</td>
<td>25.3 ± 2.1</td>
</tr>
<tr>
<td>Untrained, 39 (31-52 yr, n = 14</td>
<td>178 cm (168-191)</td>
<td>A</td>
<td>45.2 ± 2.7</td>
<td>3.3 ± 0.4</td>
<td>23.0 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>75.6 kg (63.2-88.6)</td>
<td>L</td>
<td>43.9 ± 4.8</td>
<td>4.4 ± 0.3</td>
<td>23.2 ± 1.9</td>
</tr>
<tr>
<td>Trained, 25 (17-30 yr, n = 12</td>
<td>170 cm (165-189)</td>
<td>A</td>
<td>54.8 ± 3.4</td>
<td>4.0 ± 0.5</td>
<td>19.1 ± 1.8</td>
</tr>
<tr>
<td>Bicyclists, 24 (18-33 yr, n = 4</td>
<td>182 cm (173-189)</td>
<td>A</td>
<td>50.7 ± 4.4</td>
<td>6.0 ± 0.3</td>
<td>19.9 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>74.3 kg (72.0-73.2)</td>
<td>L</td>
<td>50.7 ± 4.8</td>
<td>4.0 ± 0.0</td>
<td>19.4±20.0</td>
</tr>
<tr>
<td>Canoecists, 26 (25-27 yr, n = 4</td>
<td>181 cm (179-186)</td>
<td>A</td>
<td>58.4 ± 3.8</td>
<td>7.9 ± 0.6</td>
<td>25.0 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>74.0 kg (71.0-79.0)</td>
<td>L</td>
<td>61.4 ± 6.2</td>
<td>5.8 ± 0.9</td>
<td>22.2 ± 4.7</td>
</tr>
<tr>
<td>Runners, 23 (19-33 yr, n = 8</td>
<td>177 cm (168-185)</td>
<td>A</td>
<td>4.2</td>
<td>21.0 ± 2.5</td>
<td>72.4 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>69.5 kg (59.1-80.8)</td>
<td>L</td>
<td>58.9 ± 3.7</td>
<td>6.4 ± 0.5</td>
<td>20.1 ± 2.5</td>
</tr>
<tr>
<td>Swimmers, 21 (16-23 yr, n = 5</td>
<td>181 cm (173-189)</td>
<td>A</td>
<td>74.3 ± 5.4</td>
<td>8.0 ± 0.7</td>
<td>22.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>78.3 kg (70.0-78.6)</td>
<td>L</td>
<td>57.7 ± 9.3</td>
<td>7.6 ± 0.5</td>
<td>20.7 ± 2.3</td>
</tr>
<tr>
<td>Weight lifters, 25 (23-29 yr, n = 4</td>
<td>171 cm (159-186)</td>
<td>A</td>
<td>52.6 ± 7.7</td>
<td>3.0 ± 0.5</td>
<td>21.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>61.3 kg (52.0-107.0)</td>
<td>L</td>
<td>46.1 ± 10.5</td>
<td>3.0 ± 0.5</td>
<td>20.8 ± 2.9</td>
</tr>
<tr>
<td>Orienters, 32 (47-50 yr, n = 11</td>
<td>176 cm (161-184)</td>
<td>A</td>
<td>63.1 ± 5.1</td>
<td>4.1 ± 0.5</td>
<td>40.1 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>72.7 kg (59.5-88.0)</td>
<td>L</td>
<td>68.8 ± 5.2</td>
<td>5.7 ± 0.3</td>
<td>50.7 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± se. Values in parentheses are highest and lowest observations. *This group is composed of athletes competing in several different events. †Average of 3 values.
Two fiber types were identified in the muscle samples examined in this study (Fig. 1). One possesses high, and the other low, myosin ATPase activity at alkaline pH (9.4). According to Barnard et al. (2), a high myosin ATPase activity under the conditions of our assay occurs in muscle fibers with fast twitch characteristics and a low activity in muscle fibers with slow twitch characteristics. We have thus designated the two fiber types as fast twitch (FT) and slow twitch (ST). As indicated by alpha-glycerophosphate dehydrogenase activity, FT fibers always have higher glycolytic capacity than ST fibers. The FT fibers generally have a lower oxidative capacity than ST fibers but it is apparent from DPNH-diaphorase activity that a continuum of oxidative capacity exists in both fiber types. In the center of this continuum there is a region where the oxidative capacity of the two fiber types overlaps. As will be shown subsequently, it appears that with training the oxidative capacity of both fiber types is enhanced. Thus, any classification system based either partially or solely on oxidative capacity would be difficult to apply objectively to all muscle samples.

A third fiber type having high myosin ATPase activity at alkaline pH and high oxidative capacity has been identified in skeletal muscle of various species (2). This fiber type has been called red (2), C (24), FT (5), and αβ (10). We have not seen this fiber type in the muscle samples studied. Ogata and Murata (19), however, have identified three fiber types in human intercostal muscle on the basis of SDH and Sudan black staining. These investigators, however, did not consider contractile characteristics as indicated by myosin ATPase activity.

The average fiber distribution in the samples of the deltoid muscle was 46% ST and 45% ST (% FT fibers = 100-ST fibers) for the untrained young and middle-aged groups, respectively (Table 1). These samples contained from 14 to 60% ST fibers in the young subjects and from 34 to 58% ST fibers in the middle-aged group. The average distribution of fibers in the lateral portion of the vastus lateralis muscle was comparable to that of the deltoid with the young subjects having 36% ST and the middle-aged group 44% ST fibers. The range of fiber populations of this muscle was similar to that of the deltoid, 13–73% ST fibers, for both the young and middle-aged groups. Edström and Nyström (9) have reported a similarly wide distribution of fiber types in human skeletal muscle.

The mean percentage of ST fibers in the muscles of the subjects who participated in endurance training was higher than in the sedentary groups. Although there were some exceptions, the highest percent of ST fibers was consistently found in the muscle which was most extensively engaged in the endurance work. The mean value for the leg muscle was about 60% ST fibers. The range of both ST and FT fibers within any specific group of endurance athletes was similar to that of the untrained groups. However, the low and high values were greater than those of the sedentary groups. ST and FT fibers were about equally distributed in both the deltoid and vastus lateralis muscles of the weight lifters.

Fiber diameters and areas and the relative areas occupied by each fiber type in a muscle were determined on 25 samples that included 15 from the leg and 10 from the arm (Table 2). These samples represented all of the groups and included six subjects from which measurements were made on both the arm and leg. The average area of the ST fibers for all samples measured was 5,423.6 and 5,406.5 μ² for the vastus lateralis and deltoid, respectively. FT fiber areas were about 20% larger than ST areas in both vastus lateralis (6,379.8 μ²) and deltoid (6,295.7 μ²). When comparisons were made for subjects where data were available from both leg and arm, ST fiber areas were 5,771.8 and 5,295.7 μ², respectively. In these subjects the ST fibers of
the leg were 9% larger and the FT fibers 8% larger than those of the arm. Marked variations did exist between different muscles from the same subject and between subjects. For several subjects (DM, BL, and DF) ST fibers were larger than FT fibers (Table 2, Fig. 1C). With several exceptions, both fiber types of trained subjects were larger than those of the sedentary individuals. The type of training also seemed to have an influence on the relative size of the two fiber types. In subject MIH (weight lifter) the fibers of the leg were only about 9% larger than those of the arm. However, in both the arm and the leg FT fibers were more than 60% larger than the ST fibers. In contrast, the ST and FT fibers in the arm of canoeist BL were 26% and 64% larger, respectively, than in the leg. In subject SI, a swimmer, the ST fibers of the arm were 24% larger than those of the leg, whereas the FT fibers of the arm were 5% smaller than those of the leg. In this subject both fiber types in the arm were about equal in size, but in the leg FT fibers were 40% larger than ST fibers. These examples illustrate the effects of extensive training of the arm on fiber size. In subject JR, a bicyclist, ST and FT fibers in the arm were 37% and 26% smaller, respectively, than those of the leg. FT fibers in the arm were 34% larger than ST fibers, but in the leg this difference was only 15%. In all of these examples, the area of the fibers in the trained subjects was larger than that of the untrained subjects.

The relative area of a muscle occupied by a given fiber type was quite different in nonendurance as compared to endurance athletes. In the untrained subjects the area comprised of ST fibers ranged from 24.5 to 53.2%. Low values in nonendurance athletes included 17.3% in the leg of a weight lifter and 21.9% in the leg of a swimmer. In contrast, the area occupied by ST fibers was 80.1% in the leg of a distance runner (DM) and 73.7% in the arm and leg of a swimmer (SI), respectively, and 74.5% in the arm of a canoeist (BL).

From the data presented above and in Table 2 it is apparent that for most individuals FT fibers are larger than ST fibers. It also appears that with some types of training a preferential enlargement of either fiber type can occur. Under these conditions the percent distribution of the two fiber types in a muscle may not be indicative of the relative area occupied by a given fiber type. The relationship between the percentage of ST fibers and the relative area

<table>
<thead>
<tr>
<th>TABLE 2. Fiber sizes, populations, and contribution to muscle area of several subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>PC</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>CS</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>MKs</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>GK</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>RP</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>NA</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>DM</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td><strong>DS</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td><strong>DF</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td><strong>RP</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td><strong>BA</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>MH</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td><strong>JR</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>BL</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>SI</strong></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Values are means ± se. Values in parentheses are highest and lowest observations. *See Table 4 for performance data.
they occupy in a muscle sample was determined from all of the data available in the present study. As shown in Fig. 2, a linear relationship existed between ST percent distribution and relative area. This was true even though samples containing vastly different fiber populations and size relationships were included. Data previously published by Edström and Nystrom (9) reporting the percent distribution and relative area of the two fiber types from several different muscles from men and women are also included in Fig. 2. Close agreement exists between the results of the present study and those reported by Edström and Nystrom. All the values from both studies except one fall within the 95% confidence limits of the regression line. Thus the percentage composition of a muscle in terms of fiber types is indicative of the relative area that each fiber type contributes to the total muscle.

SDH activities in the untrained subjects, both young and middle-aged, were about 3.5 and 4.4 μmoles/g min⁻¹ for the arm and leg muscles, respectively (Table 1). The SDH activities of the arm and leg muscles of the weight lifters were 30% less than those of the untrained subjects. SDH activities of the arm and leg muscles of the remaining trained groups were from 20 to 150% higher than the means of the sedentary groups. The highest individual SDH activities were found in the leg muscle of a bicyclist (12.4 μmoles/g min⁻¹) and arm muscle of a swimmer (9.9 μmoles/g min⁻¹).

SDH activities were highest in the most active muscles both for the trained and untrained subjects. In the untrained groups the SDH activity of the leg muscle was 25% greater than that of the arm. In those groups whose training involved leg work the SDH activities of the deltoid muscle average 15–20% more than that of the untrained subjects. In these same subjects, SDH activity in the leg muscles was 55% higher than that of the arms. In the group of middle-aged orienteers the activity of the vastus lateralis was 40% higher than that of the deltoid muscle. Even greater differences existed between the untrained groups and those subjects whose sport event involved extensive use of the leg or arm muscles. Differences between muscles in the same subject also became more pronounced in the well-trained groups. In the case of the bicyclists, the SDH activity of the leg muscle was 2.5-fold greater than that of the sedentary subjects. The activity in the arms of these subjects was 70% higher than the untrained subjects, but only 55% that of the legs. SDH activities of the deltoid muscle of the canoeists and swimmers were 2.2- and 2.4-fold higher, respectively, than those of the untrained groups. In these subjects SDH activities were higher in the arm than leg muscles. These findings suggest a specific localized training effect.

Although PFK activities were not determined for all groups, there was no difference among groups or between the muscles within a given group. Average values were from 19 to 99 μmoles/g min⁻¹, with the lowest mean found in the group of subjects engaged in different sport activities and the highest mean value found in the leg of the swimmers (Table 1).

The importance of variation in the biopsy site on enzyme activity and fiber composition was also investigated by sampling the vastus lateralis at different sites in 18 subjects (including trained and untrained). The standard sampling site used in these experiments was 12–16 cm above the patella at a depth of 4 cm in the muscle. Alternate sites were 4 cm above or below this point. The standard deviation of the differences between these sampling sites was 0.46 and 3.86 μmoles/g min⁻¹ for the SDH and PFK activities, respectively, and 4.6 for the percent distribution of the fibers. Although this represents a small degree of variability, it does illustrate the need for a standard sampling site. Variations in depth of the biopsy might also affect enzyme activities and fiber size and distribution. However, this was not determined in the present investigation.

RNA in the deltoid and vastus lateralis muscles was determined only in the bicyclists, weight lifters, middle-aged orienteers, and swimmers. The mean values for these groups were 1.5 and 1.7 μg/g for the arm and leg, respectively. No significant differences existed among groups or between the two muscles studied. Since these groups included both a wide range of ages (17-58 years) and different sport activities, it appears that only minor differences may be attributed to these factors.

Muscle glycogen concentrations of the trained subjects were generally higher than those of the sedentary individuals (Table 3). It was also apparent that for a single subject the glycogen content was highest in the muscle that was used most extensively in the sport activity. Examples are subject JR (bicyclist), where the highest glycogen content existed in the leg muscle, and subjects SH and BL (swimmer and canoeist), where the pattern was reversed. Based on PAS staining, it appears that no uniform pattern for the storage of glycogen in the two fiber types in skeletal muscle exists. Examples of some of the patterns observed in this study are presented in Fig. 3. In some instances it was impossible to distinguish differences in glycogen concentration between the two fiber types (Fig. 3, A and C). The glycogen content of the tissue in Fig. 3A was low (84 nm

![Fig. 2. Relationship between percentage of ST fibers in a muscle sample and relative area occupied by these fibers. Regression line and 95% confidence limits were determined from data collected in this study. Data from Edström and Nystrom are also plotted.](image)
glucose units/kg), and that in Fig. 3C high (178 mm glucose units/kg). In other examples (Fig. 3, E and G) the pattern varied with either ST or FT fibers staining more intensely. In many muscles the pattern alternated (Fig. 3G). The ability of ST fibers to accumulate glycogen is illustrated by subject DM whose muscle contained 75% ST fibers and a high glycogen content (127 mm glucose units/kg).

**DISCUSSION**

The identification of only two fiber types in human muscle in the present study agrees with several earlier reports. (7, 9, 16). In contrast to these earlier observations we have chosen to identify fibers on the basis of contractile characteristics.

The higher SDH activity in the muscles of the trained groups agrees with the findings reported by Varnauskas and co-workers (26) and Morgan et al. (15). The DPNH-diaphorase activity in the FT and ST fibers of the muscles from the trained subjects was higher than that of the sedentary subjects (Fig. 1). This is evidence of the adaptability of oxidative capacity in both fiber types. In some of the trained groups (Fig. 1), the DPNH-diaphorase activity of the FT fibers appeared to have been as great or greater than that of the ST fibers in the muscles of the untrained groups. The ST fibers in the muscles of the highly trained subjects also appeared to have been more oxidative than the ST fibers from the untrained subjects. This was characterized by a dark subsarcolemmal DPNH-diaphorase stain. In the subjects with the highest SDH activity the DPNH-diaphorase activity of the FT fibers approached that of the ST fibers.

The question arises as to whether the percentage distribution of a specific fiber type in skeletal muscle can be altered by training. Morgan et al. (15) have reported that in man the frequency of red fibers increased following training. Barnard and associates (3) also reported that the percentage of red fibers in guinea pig muscle increased after training. It should be pointed out that the conversion of fibers types as reported by Barnard et al. did not require any change in contractile properties. Furthermore, Morgan and co-workers identified fibers as red or white only on the basis of SDH activity. Since training produces a change in SDH activity (3, 11) and because it has been shown in the present study that the oxidative potential of FT fibers can vary dramatically, it would be easy to identify FT as ST fibers after training from only a histochemical determination of SDH activity. Guth and Yellin (10) have reported a progressive decrease in high myosin ATPase staining fibers in the rat soleus during compensatory hypertrophy. Although the question stated above cannot be answered by the data from this study, we cannot exclude the possibility that such a conversion of fibers can occur in response to training. The wide range of fiber compositions from the trained subjects was higher than that of the sedentary subjects (Fig. 1). This is evidence of the adaptability of oxidative capacity in both fiber types. In some of the trained groups (Fig. 1), the DPNH-diaphorase activity of the FT fibers appeared to have been as great or greater than that of the ST fibers in the muscles of the untrained groups. The ST fibers in the muscles of the highly trained subjects also appeared to have been more oxidative than the ST fibers from the untrained subjects. This was characterized by a dark subsarcolemmal DPNH-diaphorase stain. In the subjects with the highest SDH activity the DPNH-diaphorase activity of the FT fibers approached that of the ST fibers.

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**TABLE 3. Glycogen concentration in arm and leg muscle of untrained subjects and of representative subjects from trained groups**

<table>
<thead>
<tr>
<th>Subj</th>
<th>Event</th>
<th>Glycogen, mm Glucose units/kg</th>
<th>Arm</th>
<th>Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untrained</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JR</td>
<td>Bicyclist</td>
<td>90 (75-120)</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>SH</td>
<td>Swimmer</td>
<td>223</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>Canoeist</td>
<td>105</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>Dist runner</td>
<td>127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DS</td>
<td>Dist runner</td>
<td>127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF</td>
<td>Mid-dist runner</td>
<td>178</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are high and low observations. *Mean of 3 subjects. †Mean of 10 subjects.

**Fig. 3.** Serial sections from vastus lateralis muscle showing PAS stain for glycogen (top) and stain for myosin ATPase (bottom) for four subjects. A and B: untrained middle-aged subject (PC). Both FT and ST fibers stained lightly for glycogen. C and D: middle-distance runner (DF). Both FT and ST fibers show an intense stain for glycogen.
Within the various groups would argue against such a conversion of fibers from one type to another. On the other hand, some of the endurance athletes studied did have a relatively high percentage of ST fibers. This could be due to a selection of these activities by individuals possessing the natural endowment. Additional studies are needed to clarify this point.

Wide variations existed in muscle fiber composition and enzyme activity, and performance characteristics of some of the best athletes studied. Several examples of this are presented in Table 4. A comparison of subjects JR and RP with DM indicates that each had high SDH activity and VO\(_2\)\text{max} but a strikingly different muscle fiber composition. Subject RP, a world champion canoeist, and subject JR, an exceptional bicyclist, both had approximately equal distributions of the two fiber types in the leg and arm muscles, whereas subject DM, a distance runner, had a predominant number of ST fibers (75%) in the vastus lateralis muscle. The high levels of SDH activity in the muscles of subjects JR and RP were the result of a high oxidative capacity in both fiber types as indicated by the DPNH-diaphorase activity. Another difference between these athletes was their ability to perform sprint work. Both subjects RP and JR possessed high capacity for sprint work. Subject RP has performed best in relatively short-distance events, whereas subject DM, a distance runner, has limited sprint capability. Subject JR has been able to perform prolonged endurance work and then to sprint near the end of the competition. Subject JA, a retired middle-distance runner (1:51 mile), has 52% ST fibers in the leg were dominant (76%), whereas SDH activity was only average (4.47 pmoles/g min\(^{-1}\)). These characteristics are indicative of a high capacity for aerobic work. In subject BA, a retired weight lifter who is still somewhat active in other sports, FT fibers in the leg were dominant (76%), whereas SDH activity was only average (1.47 pmoles/g min\(^{-1}\)).

The muscle samples from some of the untrained subjects possessed percentages of ST fibers equal to or higher (70-85%) than the endurance athletes even though VO\(_2\)\text{max} and SDH and DPNH-diaphorase activities were much lower. In every case the highly trained endurance athletes possessed high SDII and DPNH-diaphorase activities. These two characteristics appear to be well related. Since the SDH activity and DPNH-diaphorase activities can vary dramatically in both fiber types, and athletes with high percentages of FT fibers can still possess extremely high oxidative capacities, it would appear that the adaptability of the fibers for aerobic metabolism may be a more important factor in training and performance than the basic composition of the muscle.

One question currently being debated is whether the oxidative capacity of the muscles or the transport capacity of the cardiovascular system is the limiting factor for VO\(_2\)\text{max}. This question can be approached from the standpoint of the oxidative potential of the muscles based on SDH activities. For such a consideration it should be pointed out that SDH activities were measured at 25°C in the present study, and that at a normal body temperature of 37°C there is a 2.2-fold increase in activity (6). If it is assumed that the VO\(_2\) capacity of skeletal muscle is equal to the conversion rate for succinate, 1 kg of skeletal muscle, such as the vastus lateralis from the sedentary groups, could consume 0.6 liter O\(_2\) min\(^{-1}\). For the bicyclists (SDH activity of 11.0 pmoles/g min\(^{-1}\)), this value would be 1.6 liters O\(_2\)/kg min\(^{-1}\). Under these conditions total body oxygen consumption during maximal work could occur in 6-7 kg of muscle for

### Table 4. Fiber population, enzyme activities, VO\(_2\) max, and performance characteristics of several subjects

<table>
<thead>
<tr>
<th>Subj</th>
<th>Age, Ht, Wt</th>
<th>Sample Site (Arm or Leg)</th>
<th>% ST Fibers</th>
<th>Enzyme Activities (pmoles/g min(^{-1}))</th>
<th>VO(_2) max, liter/min</th>
<th>Comments on Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>JR</td>
<td>33 yr, 175 cm, 75 kg</td>
<td>A</td>
<td>51.3</td>
<td>6.73</td>
<td>19.36</td>
<td>Bicyclist; 5 Swedish championships</td>
</tr>
<tr>
<td>CS</td>
<td>26 yr, 189 cm, 74 kg</td>
<td>A</td>
<td>39.3</td>
<td>5.49</td>
<td>12.38</td>
<td>Bicyclist; third in world championship, 1969; 5 Swedish championships</td>
</tr>
<tr>
<td>RP</td>
<td>27 yr, 186 cm, 79 kg</td>
<td>A</td>
<td>72.5</td>
<td>8.21</td>
<td>22.98</td>
<td>Canoeist; Olympic Gold Medal, 1964; Olympic Silver Medal, 1968; world champion 5 times—last 1971</td>
</tr>
<tr>
<td>AB</td>
<td>19 yr, 175 cm, 70 kg</td>
<td>A</td>
<td>71.8</td>
<td>9.60</td>
<td>20.97</td>
<td>Swimmer: 400 m—4:05.8; 1,500 m—16:33.4</td>
</tr>
<tr>
<td>DM</td>
<td>20 yr, 170 cm, 62 kg</td>
<td>A</td>
<td>75.0</td>
<td>8.03</td>
<td>15.07</td>
<td>Distance runner; 4th in NCAA Cross-Country Championships, 1971; 26:15—6 mile</td>
</tr>
<tr>
<td>DF</td>
<td>21 yr, 170 cm, 69 kg</td>
<td>A</td>
<td>75.0</td>
<td>8.03</td>
<td>15.07</td>
<td>Middie-distanc runner, 1:51—880 yd, 4:04 mile</td>
</tr>
<tr>
<td>NP</td>
<td>22 yr, 165 cm, 61 kg</td>
<td>A</td>
<td>55.0</td>
<td>7.20</td>
<td>20.97</td>
<td>Sprinter; 9.3—100 yd</td>
</tr>
<tr>
<td>RA</td>
<td>31 yr, 191 cm, 87 kg</td>
<td>A</td>
<td>24.0</td>
<td>4.67</td>
<td>23.12</td>
<td>Former weight lifter; somewhat active in recreational sports</td>
</tr>
</tbody>
</table>
the untrained group and from 3.35 kg of muscle for the
bicyclist. This would suggest that both in the sedentary and
trained subjects the oxidative capacity of the muscle mass
normally used in leg exercise would exceed reported values
for VO₂ max. These calculations are based on the assumption
that all of the components of the oxidative pathway function
at the rate of the SDH reaction, which is unlikely. However, it has been demonstrated (12) that most com-
ponents of the Krebs cycle and electron transport system increase in proportion to each other during training. Fur-
thermore, SDH activity is representative of total mito-
chondrial protein. Based on these considerations and the
SDH activities of this study, the oxidative capacity of the
leg muscle of the bicyclists is 2.5-fold greater than that of the
sedentary group. VO₂ max of these highly trained ath-
letes, however, was not 2.5-fold greater than that of the
untrained subjects. These considerations seem to point to the
cardiovascular system as imposing an upper limit on
VO₂ max.

What then is the importance of the large aerobic potential
of the skeletal muscle of the trained individuals? It would
seem to relate specifically to the increase in oxidative ca-
pacity of the FT fibers, with the result that these fibers
would be able to better utilize their glycogen stores aero-
bolically and to oxidize fatty acids. In this manner the onset
of fatigue in these fibers would be delayed. This increased
oxidative capacity of the FT fibers may also contribute to
the reduction in lactate production during submaximal
work that occurs after training.

As stated above, it seems unlikely that the capacity of the
body to utilize oxygen during maximal work is limited by
the oxidative capacity of the muscle cell. In spite of this,
profound differences were observed between trained and
untrained muscle groups. The endurance athlete was
characterized by a high percentage of ST fibers in his
trained muscles. The oxidative capacity of these fibers and
the FT fibers was significantly higher than in his less active
muscles or in muscles of untrained persons. The extent to
which the regular training has contributed to this observed
difference cannot be settled by the present study. The ob-
served results suggest that physical conditioning in man
does not influence fiber composition as much as it does the
oxidative capacity of the individual muscle fibers.

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Effect of training on enzyme activity and fiber composition of human skeletal muscle

P. D. GOLLNICK, R. B. ARMSTRONG, B. SALTIN, C. W. SAUBERT IV, W. L. SEMBROWICZ, AND R. E. SHEPHERD

Department of Physical Education for Men, Washington State University, Pullman, Washington 99163

A recent study revealed that the skeletal muscles of highly trained endurance athletes possessed higher oxidative capacities and percentages of slow twitch fibers than those of untrained men (14). The high oxidative potential in the muscles from endurance athletes was consistent with earlier reports on the effect of training on the skeletal muscle of animals and man (1, 3, 16-18, 21, 29). From the previous investigation it was not possible to determine the extent of training on enzyme activity and fiber composition of human skeletal muscle. The purpose of this study was to determine the effects of a 5-month endurance training program on succinate dehydrogenase (SDH) and phosphofructokinase (PFK) activities and fiber composition in skeletal muscle of man. The distribution of aerobic and anaerobic capacity and glycogen concentration in the two fiber types of human muscle before and after training was also estimated.

MATERIALS AND METHODS

Six healthy male subjects were studied (see Table 1 for physical characteristics). All were active in recreational sports but none had engaged in endurance training for at least 2 years prior to this study. Before training maximal oxygen consumption (V_{O2 max}) was determined and muscle samples were taken from two different sites in the lateral portion of the vastus muscle with the needle biopsy technique (4).

The subjects proceeded to train for 5 months by pedaling a cycle ergometer 1 hr/day 4 days a week at a load requiring 75% of their maximal aerobic power. Initially the subjects could not tolerate this load for the full hour and it was reduced to about 65% of V_{O2 max} during a portion of the exercise bout. After about 2 weeks all subjects were able to complete the basic training load for 1 hr. Thereafter each subject attempted to increase the work load to the maximum that could be tolerated for the 1-hr period. Midway through the training program V_{O2 max} tests were repeated to ensure that all subjects were still working at the prescribed load. At the end of the training program most of the subjects were working for 1 hr at 85-90% of their V_{O2 max}.

Three to five days after the final exercise bout multiple biopsy samples were taken from the vastus lateralis muscle at sites near those of the initial sampling. V_{O2 max} was determined at the end of the study.

Maximum oxygen uptake tests were done on the cycle ergometer using the leveling-off criteria. Expired air was collected in a 600-liter gasometer and analyzed with a Beckman E2 oxygen analyzer (8). The accuracy of the analyzer was verified with the Scholander microtechnique.

The muscle samples were divided into three parts. One portion was examined under a dissecting microscope to determine fiber orientation, mounted onto a specimen holder in OCT embedding medium (Ames Tissue-Tek), and frozen in isopentane cooled to the temperature of liquid nitrogen. This sample was used for histochemical analysis. The remaining parts of the sample were weighed and used for the estimation of SDH and PFK activities at 25°C with the methods of Cooperstein and associates (7) and Shonk and Boxer (27), respectively. In some cases a second biopsy sample was taken at the same site to provide enough tissue for all analyses.
TABLE 1. Physical characteristics, enzyme activities, and muscle glycogen concentrations

<table>
<thead>
<tr>
<th>Subj</th>
<th>Age</th>
<th>Ht, cm</th>
<th>Wt, kg</th>
<th>VO&lt;sub&gt;2&lt;/sub&gt;max, liters/min</th>
<th>SDH Activity, μmoles g&lt;sup&gt;-1&lt;/sup&gt; min&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>PFK Activity, μmoles g&lt;sup&gt;-1&lt;/sup&gt; min&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>Muscle Glycogen, mmoles glucose units/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>WLS</td>
<td>29</td>
<td>5'1</td>
<td>183</td>
<td>106.0</td>
<td>3.7</td>
<td>4.4</td>
<td>2.72</td>
</tr>
<tr>
<td>PDG</td>
<td>37</td>
<td>5'9</td>
<td>187</td>
<td>85.5</td>
<td>3.9</td>
<td>4.2</td>
<td>6.23</td>
</tr>
<tr>
<td>CWI</td>
<td>28</td>
<td>5'5</td>
<td>180</td>
<td>79.7</td>
<td>3.7</td>
<td>4.3</td>
<td>5.06</td>
</tr>
<tr>
<td>MRS</td>
<td>40</td>
<td>6'0</td>
<td>160</td>
<td>63.6</td>
<td>2.8</td>
<td>2.9</td>
<td>4.00</td>
</tr>
<tr>
<td>RBA</td>
<td>31</td>
<td>5'8</td>
<td>191</td>
<td>91.0</td>
<td>4.3</td>
<td>5.5</td>
<td>4.29</td>
</tr>
<tr>
<td>RES</td>
<td>30</td>
<td>5'7</td>
<td>187</td>
<td>91.0</td>
<td>5.0</td>
<td>5.4</td>
<td>4.72</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.9</td>
<td>4.5</td>
<td>4.65</td>
</tr>
</tbody>
</table>

B = before training; A = after training. * Before vs. after training P < 0.05. † Before vs. after training P < 0.01.

Serial sections 10 μ thick were cut in a cryostat at −20°C and mounted on cover glasses for histochemical analysis. Myosin adenosine triphosphatase (ATPase), reduced diphosphopyridine nucleotide-diaphorase (DPNH-diaphorase), and alpha-glycosephosphate dehydrogenase activities were estimated with the methods of Padykula and Herman (23), Novikoff et al. (22), and Wattenberg and Leong (30), respectively. The distribution of glycogen in one of the serial sections (16 μ thick) was estimated from the periodic acid-Schiff (PAS) reaction as described by Pearse (24). The tissue remaining after the sections were cut for histochemical analysis was removed from the embedding medium and analyzed for total glycogen with the method described by Karlsson et al. (19).

Muscle fibers were identified as slow twitch (ST) or fast twitch (FT) on the basis of myosin ATPase activity as described previously (14). The area of the two fiber types was determined by direct planimetry on photographs made of the DPNH diaphorase sections.

RESULTS

Individual values are presented in Tables 1 and 2. VO<sub>2</sub>max increased an average of 13% during training (P < 0.05). The largest increase in VO<sub>2</sub>max was 1.1 liters min<sup>-1</sup> (25%) and the smallest, 0.1 liter min<sup>-1</sup> (3.6%).

Mean SDH activity in the vastus lateralis muscle increased 95% (P < 0.01) with training. The smallest pre- and posttraining difference in SDH activity for a single subject was 40%. The largest individual difference, 256%, occurred in the subject with the lowest initial value. Mean PFK activity increased 117% (P < 0.01) during training with a similar change occurring in all subjects.

The percentage of ST and FT fibers in the muscle samples from the vastus lateralis was not significantly altered by the training program. In four of the six subjects the pre- and posttraining fiber compositions of this muscle were nearly identical. In the other two subjects there were about 9% more ST fibers in the posttraining samples.

Oxidative capacity, as indicated histochemically by DPNH-diaphorase activity, appeared to have increased in both fiber types (Fig. 1) following training. In contrast, anaerobic capacity, as indicated histochemically by alpha-glycerophosphate dehydrogenase activity, appeared to have increased only in the FT fibers (Fig. 1).

Muscle samples were available for the determination of total glycogen from only three subjects prior to training. The average glycogen content of these samples was 72 mmoles glucose units kg<sup>-1</sup> wet wt. This value is similar to that of other nontrained subjects (14). Following training the glycogen concentration of the vastus lateralis muscle was 182.0 and 182.3 mmoles glucose units kg<sup>-1</sup> wet wt for the three subjects and total groups, respectively. The difference in glycogen concentration before and after training was also evident from the PAS staining of the muscle sections (Fig 1). There was no clear pattern of a higher glycogen content in one or the other of the two fiber types either before or after training.

The mean ST fiber area prior to training was 5,495 μ<sup>2</sup> as compared to 6,638 μ<sup>2</sup> for the FT fibers (P < 0.01). After training the area of the ST fibers was 6,694 μ<sup>2</sup> (P < 0.05) and that of the FT fibers 6,139 μ<sup>2</sup>. The ratio of the ST to FT fiber areas increased from 0.82 before to 1.11 (P < 0.01) after training. Considerable variation existed in the areas of the two fiber types among subjects. This was less pronounced after training.

DISCUSSION

An increase in VO<sub>2</sub>max was observed in all subjects. These were within the limits of several earlier reports (10). The largest individual change of 25% was only slightly lower than the 37% observed by Saltin and co-workers (25) in sedentary subjects following a 55-day training pro-
Fig. 1. Photomicrographs of serial sections (X130) from one subject before (1) and after (2) training. A, B, C, and D are serial sections stained for myosin ATPase, DPNH-diaphorase, alpha-glycerophosphate dehydrogenase, and glycogen (PAS), respectively.
of running. The difference between the changes in
$V_o_{2\ max}$ of the subjects in this study and that of Saltin et al.
can probably be attributed to the initial fitness of the sub-
jects and the type of training employed.

The magnitude of the increases in muscle SDH activity
following training was similar to the increased oxidative
capacity reported by Holloszy and associates (16-18) for
endurance-trained rats. It was larger than that observed
by Morgan and co-workers (21) and Varnauskas et al. (99)
for men following training. However, the training program
used in this study was longer and more strenuous than
previously used for studying metabolic adaptations in the
skeletal muscle of man. The SDH activities following
training approached those in well-trained bicyclists and
were greater than those seen in endurance runners (14).
These high activities probably reflect a more extensive use
of the vastus lateralis muscle in cycling than during running.
This conclusion was reached from EMG determinations
(J. Hendriksson, personal communications). Bicycle exercise
was chosen for the training in the present study specifically
for this reason. The extensive use of this muscle during
bicycle exercise explains the high enzyme activity even
though the $V_o{2\ max}$ per kilogram for well-trained bicyclists
and the subjects of the present study is lower than that of
well-trained endurance runners.

The increase in PFK activity suggests a greater glycolytic
capacity of the vastus lateralis muscle after training. Eriksson
and co-workers (11) have also observed increases in PFK
activity in the vastus lateralis muscle of 11 to 13-year-old
boys following training with bicycle work. This finding is
in contrast to that of Holloszy et al. (10) in the rat following
endurance training. Recently, however, Baldwin and
associates (2) have reported elevated activities of some
glycolytic enzymes, including PFK, in the soleus but not
quadriceps muscle of the trained rat. The predominant
fiber type of the rat soleus possesses low myosin ATPase and
high oxidative activity (9). In some ways these fibers are
similar to the ST fibers of human muscle. This might suggest
that the ST fibers had increased their glycolytic capacity after
training. However, on the basis of alpha-glycerophos-
phate dehydrogenase activity, it appears that an increase
in glycolytic capacity occurred mainly in the FT fibers.
Since the work load in the present experiment required a
large consumption of muscle glycogen (26), an increase in
PFK activity in either or both fiber types would aid in the
degradation of glycogen to supply energy for muscular
contraction by both the aerobic and anaerobic pathways.

The increases in enzyme activities in this study appear
to be the result of the training and not a response to a single
work bout. This conclusion is based on the relative magni-
tude of the change as compared to other studies with man
and rats (1, 3, 11, 16-18, 21, 29) and from an earlier study
by Eriksson et al. (11), in which SDH and PFK activities
in the skeletal muscle of 10- and 11-year-old boys were
unchanged after 2 weeks but significantly increased after
6 weeks of training.

The elevation in muscle glycogen is consistent with
studies on trained and untrained animals and man (14, 15).
The posttraining glycogen concentrations in this experiment
were similar to those of well-trained distance runners (14).
These changes may have been due to the normal super-
compensation of glycogen seen after a single heavy work
bout and not due to training. This is particularly true since
a time period of 3-5 days elapsed between the final exercise
session and taking the posttraining muscle biopsy. The
glycogen concentrations were, however, higher than those
usually seen after a single work bout (6) but similar to
those produced by exercise and dietary modification (5, 19).
These changes are compatible with the observation that
the glycogen synthetase activity of human skeletal muscle is
increased by training (98).

In five of the six subjects ST fiber size increased following
training, whereas FT fiber area decreased in four subjects.
However, the decrease in FT fiber area was quite small
(about 7%) as compared to the increase in ST fiber area
(23%). The relative area of the muscle composed of ST
fibers increased from 28 to 38% ($P < 0.01$) following
training. This change with training is evidence that the ST
fibers may have been used more extensively during the
training program than the FT fibers. In all cases the relation-
ship between the percent of ST fibers and the relative
area they occupied in the muscle both before and after
training was within the 95% confidence limits of the regres-
sion line published previously (14). The changes with
training resulted in moving all points toward the upper
confidence limit. We have previously observed larger ST
than FT fibers in the muscles of some endurance athletes.

Reports have appeared in the literature in which in-
creases in the percentage of "red" as compared to "white"
fibers occurred in the muscles of animals and man follow-
ing training (3, 12, 13, 21). In all cases these studies have
employed oxidative capacity as measured by SDH or
DPNH-diaphorase activity to classify fibers. On the basis of
the large change in oxidative capacity as identified either
by SDH activity of homogenates or histochemically by
DPNH-diaphorase activity, it is easy to see how such an
interpretation could be made. However, we have concluded
that the apparent ease with which the oxidative capacities
of both fiber types change with training renders any classifi-
cation system based solely on this characteristic tenuous.
Furthermore, Baldwin et al. (1) have shown that the in-
crease in oxidative capacity of all three fiber types of rat
skeletal muscle was approximately twofold following train-
ing. Thus the relative oxidative potential of the fast-twitch
white fiber was unchanged by training. These data suggest
that the relative fatigability of both fiber types in human
muscle, and perhaps also the recruitment pattern during
exercise, would not be significantly altered by training.
In no instance has a change in fiber characteristics as
determined by myosin ATPase been demonstrated.

Based on the criterion of myosin ATPase, the present
data suggest that fiber types are not altered by prolonged
endurance training in adult man. We interpret the approxi
EFFECT OF TRAINING ON HUMAN SKELETAL MUSCLE

indicate that the basic fiber types of skeletal muscle are probably not altered by physical training in adult man. The area of a given fiber type may, however, change in response to training. These findings point to the importance of training in the metabolic characteristic, but not fiber distribution, of human skeletal muscle. They cannot answer the question of whether the changes in enzyme activity (along with increases in mitochondria) produce the metabolic difference between untrained and trained men during exercise.

K. B. Armstrong is a National Science Foundation Predoctoral Fellow.

Present address of B. Saltin, Dept. of Physiology, Gymnasist-och Idrottsförsök, Stockholm, Sweden.

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