Adaptation of actomyosin ATPase in different types of muscle to endurance exercise

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Baldwin, K. M., W. W. Winder, and J. O. Holloszy. Adaptation of actomyosin ATPase in different types of muscle to endurance exercise. Am J Physiol. 229(2): 422-426; 1975—Higher concentrations of actomyosin were found in the red portion of the vastus lateralis and in the white portion of the vastus lateralis muscle than in the soleus or heart in rats. A strenuous program of treadmill running lasting 18 wk or longer did not significantly affect the amount of actomyosin recovered from the different types of muscle. No changes in actomyosin ATPase occurred in fast twitch white (white vastus) or heart muscles in response to the exercise training. In contrast, a decrease of approximately 20% occurred in the specific activity of actomyosin ATPase of fast-twitch red (red vastus) muscle (0.635 ± 0.029 μmol Pi/min per milligram for sedentary vs. 0.529 ± 0.021 μmol Pi/min per milligram for trained), while the actomyosin ATPase activity of slow-twitch red (soleus) muscle increased about 20% (0.209 ± 0.033 vs. 0.257 ± 0.031 μmol Pi/min per milligram). There was a close correlation (r = 0.99, P < 0.001) between actomyosin ATPase activity and phosphofructokinase activity in the three types of skeletal muscle and in heart muscle of exercise-trained and untrained animals, providing further evidence in support of the concept that the glycogenolytic capacity of a muscle and its actomyosin ATPase activity are regulated in parallel.

A fundamental difference between fast and slow skeletal muscles is that the ATPase activity of fast muscle actomyosin is high whereas slow muscle actomyosin is low (7, 8, 9, 12). It seems probable that ATP hydrolysis by actomyosin is the rate-limiting step in the speed of muscle shortening, and that differences in actomyosin ATPase activity reflect differences in the rate of ATP utilization during the contractile process (7, 14, 21, 24). It was believed until quite recently that the capacity of a muscle for aerobic metabolism was inversely related to its speed of shortening and its actomyosin ATPase activity, with slow-twitch muscles having a high content of mitochondria and fast-twitch muscles having few mitochondria. However, it is now well documented that certain fast-twitch muscle fibers can have a high respiratory capacity. For example, in rodents, which have three types of skeletal muscle fibers, the fast-twitch red fibers have both a high respiratory capacity and high actomyosin ATPase activity (9, 26). The other two fiber types are the fast-twitch white, which has a low oxidative capacity and high actomyosin ATPase activity, and the slow-twitch red, which has a moderately high oxidative capacity and low actomyosin ATPase activity (9, 26). On the other hand, there does appear to be a relationship between the glycogenolytic capacity of a muscle and its actomyosin ATPase activity (26).

It was previously found that a prolonged, strenuous program of treadmill running, which induces an approximately twofold increase in the respiratory capacity of rat skeletal muscle, brings about an adaptive increase in the glycogenolytic capacity of slow-twitch red muscle and a decrease in the glycogenolytic capacity of fast-twitch red muscle (6). No changes in glycogenolytic capacity occurred in white muscle (6). If the levels of actomyosin ATPase activity and the levels of the glycogenolytic enzymes are regulated in parallel in muscle, then actomyosin ATPase might be expected to increase in slow-twitch red muscle fibers and decrease in fast-twitch red muscle fibers in response to endurance exercise training. This possibility was examined in the present study in which the adaptive response of actomyosin ATPase in the different types of skeletal muscle fibers was measured in rats subjected to a program of treadmill running. Information was also obtained regarding the response of myocardial actomyosin ATPase.

Methods

Animal care and exercise program. Male rats of a Wistar strain (specific-pathogen-free CFN rats, Carworth) weighing approximately 100 g were placed in individual cages and maintained on a diet of Purina laboratory chow and water. They were assigned to either an exercising or a sedentary group. The exercising group was trained by means of a program of treadmill running as described previously (25). After 12 wk the rats were running continuously for 2 h daily at 1.2 mph up a 15% grade, 5 days/wk. The animals were maintained at this final work load until they were sacrificed between the 18th and 24th wk of training. This exercise program resulted in a large increase in endurance (19); it does not result in muscle hypertrophy (4, 19).

Muscle sampling. Animals were not exercised for 48 h prior to muscle sampling. The left hind limb was removed and the biopsies of the vastus lateralis muscle were taken from the outer half of the muscle. The biopsies were cut into slices for determination of ATPase activity.
to the time they were killed by decapitation. For studies of the fast-twitch red and the fast twitch white types of muscle fiber, the vastus lateralis muscles were dissected out, freed of fat and connective tissue, and separated into a superficial white portion, which consists almost entirely of white fibers, and a deep, red portion, which consists predominantly of fast-red fibers (4, 9). Approximately equal amounts of each type of muscle sample were obtained from the trained and untrained animals. The middle, mixed portion of the vastus lateralis was discarded. The soleus muscle, which consists predominantly of slow-twitch red fibers (1, 4), was used for studies on slow-red muscle. The heart was excised and the great vessels, valves, and atria were trimmed away.

**Tissue preparation and assay methods.** Skeletal muscle samples weighing 400-600 mg and heart muscle samples weighing approximately 900 mg were used for preparation of actomyosin. Muscle samples from the exercise-trained animals and from their sedentary controls were processed at the same time. Soleus muscles from two animals had to be pooled to obtain sufficient actomyosin for analysis. The muscle samples were minced and suspended in 10 vol of the “relaxing buffer” of Zak et al. (35), which contains, in millimoles per liter: KCl, 100; MgCl₂, 5; EGTA, 5; Na pyrophosphate, 5; at pH 6.8; and stirred at 4°C for 20 min. The relaxing buffer was then decanted, and the muscle mince was homogenized in a solution containing (mM): sucrose, 250; KCl, 50; EGTA, 5; and MgCl₂, 5; at pH 6.8; with a glass Potter-Elvehjem homogenizer immersed in ice-water. The homogenates were centrifuged at 1,000 X g for 10 min, and the supernatant was discarded. The crude myofibrillar pellet was resuspended in 100 mM KCl containing 5 mM EGTA, 5 mM MgCl₂, and 0.1% Triton X-100; at pH 6.8; the homogenate was centrifuged at 1,000 X g. This procedure was repeated once. Next, the myofibrils were subjected to two additional washes in 100 mM KCl containing 5 mM EDTA and 10 mM Tris-HCl, at pH 6.8. The washed myofibrils were then homogenized in a solution containing 660 mM KCl, 0.5 mM cysteine, 0.5 M Tris-HCl, at pH 7.4. The homogenate, which contained myofibrils from 1 g of muscle per 20 ml, was stirred for 20 h at 4°C to extract the actomyosin, which was then concentrated by dialysis as described by Bárány and Close (8). The actomyosin precipitate was dissolved in 1.0 M KCl and the protein concentration was measured. The actomyosin solution was then diluted with 1.0 M KCl to give a final concentration of actomyosin of 6 mg/ml.

Mg²⁺-activated ATPase activity of actomyosin was determined in a reaction mixture containing, in millimoles per liter, in a final volume of 4 ml: KCl, 50; MgSO₄, 1; ATP, 1; CaCl₂, 0.01; Na azide, 2; Tris-HCl, 20; and 1.2 mg of actomyosin; at a final pH of 7.4 (cf. 17). Reactions were carried out in 25-ml Erlenmeyer flasks placed in a Dubnoff shaker at 30°C. After preincubation for 10 min, the reaction was initiated by addition of the ATP. After 2 min, the reaction was stopped by the addition of 1 ml of 8% perchloric acid. Control flasks containing the above reaction mixture were treated in the same manner as the experimental flasks with the exception that the perchloric acid was added prior to addition of the ATP.

Ca²⁺-activated ATPase of actomyosin was determined in a reaction mixture containing, in a final volume of 4 ml, 50 mM KCl, 10 mM CaCl₂, 3 mM ATP, 20 mM Tris-HCl, and 1.2 mg of actomyosin (8). Enzyme activity was corrected for any non-Ca²⁺-activated activity measured in control flasks in which 2 mM EGTA replaced the 10 mM CaCl₂ in the reaction mixture. The conditions and time course for the assay were the same as for the Mg²⁺-activated ATPase assay described above. All the enzyme assays were run in duplicate. After centrifugation to remove the precipitated protein, 3-ml aliquots of the actomyosin-ATPase reaction mixtures were assayed for inorganic phosphate (16).

Phosphofructokinase activity was measured as described by Mansour et al. (23). Protein was measured by the biuret method (18).

The statistical significance of the differences between the exercise-trained animals and their sedentary controls was determined with the use of the t test for paired data (29).

### RESULTS

As shown in Table 1, larger quantities of actomyosin were recovered from the fast-twitch red (red vastus lateralis) and fast-twitch white (white vastus lateralis) types of muscle than from the slow-twitch red (soleus) or heart. The exercise training did not have a significant effect on the amount of actomyosin recovered from the different types of muscles (Table 1). That predominantly fast-twitch skeletal muscle has a higher concentration of actomyosin than heart muscle has been reported previously (cf. 21), and our results are in keeping with this finding. There was probably considerable loss of actomyosin as a result of the extensive washing during the purification. Nevertheless, since the different types of muscle were all subjected to the same procedures, our results do raise the possibility that the fast-twitch red and white types of muscle have a higher actomyosin ATPase concentration than the slow-twitch red.

The exercise program did not induce significant changes in Mg²⁺-activated ATPase activity in either fast-twitch white muscle or in the heart (Table 2). In contrast, a decrease of approximately 20% occurred in the actomyosin ATPase activity of red vastus lateralis, while the actomyosin ATPase activity of soleus muscle increased approximately 20% (Table 2). These small but statistically significant changes in actomyosin ATPase activity are similar in magnitude and direction to the changes in glycoenergetic capacity induced in fast-red and slow-red muscle fibers by the running program used in these studies (6). A remark-
ably close parallelism between actomyosin ATPase activity and glycogenolytic capacity is apparent from the correlation (P < 0.001) between Mg²⁺-activated ATPase activity and the level of activity of the rate-limiting enzyme, phosphofructokinase, in the three types of skeletal muscle and in heart muscle of exercise-trained and untrained animals (Fig. 1).

The response of Ca²⁺-activated ATPase was also investigated. No significant change in Ca²⁺-activated ATPase activity was seen in white muscle in response to the running program with a mean value of 0.726 ± 0.096 μmol of P₁ formed per minute per milligram of protein for six sedentary animals compared to an average of 0.700 ± 0.079 μmol/min per milligram protein in six runners. In fast-twitch red muscle a small but statistically significant (P < 0.09) exercise-induced decrease in activity, similar in magnitude to that seen for Mg²⁺-activated actomyosin ATPase, occurred in Ca²⁺-activated actomyosin; the average activity for six sedentary controls was 0.571 ± 0.062 compared to 0.421 ± 0.066 μmol/min per gram for six runners.

DISCUSSION

Slow-muscle myosin and fast-muscle myosin have different biochemical properties (8, 12, 28). When a slow muscle and a fast muscle are cross-innervated, if sufficient time is permitted to elapse, a reversal of contractile properties occurs (8, 12). Concomitantly, the biochemical properties of myosin change so that slow-muscle myosin is "transformed" into fast myosin and vice versa (8, 12). Although this process has commonly been referred to as transformation, it has not actually been shown that one type of myosin is transformed into the other. It seems more likely that cross-innervation of, for example, a slow muscle by a nerve from a fast muscle induces synthesis of fast myosin and represses synthesis of slow myosin, so that fast myosin gradually replaces the slow myosin.

As shown in the present study, less marked, but nevertheless significant, alterations in actomyosin ATPase activity can be induced in the fast-twitch red and slow-twitch red types of muscle fiber in response to strenuous, prolonged exercise. This finding provides evidence that the habitual level of contractile activity can, to some extent, modify the neurally determined myosin isozyme pattern in skeletal muscle. If the fast and slow myosins that have been identified are the only two myosin isozymes in skeletal muscle, the exercise-induced changes in actomyosin ATPase activity could have resulted from either 1) partial derepression of fast myosin synthesis in slow red muscle, and of slow myosin synthesis in fast red muscle, resulting in a mixture of the two isozymes in the fast and in the slow red fibers, or 2) complete replacement of one type of myosin by the other in some of the slow-red and fast-red fibers, with the other fibers being unaffected. The second possibility seems less likely in view of the histochemical evidence that there is no increase in the proportion of slow-twitch red fibers in mixed muscles, such as the gastrocnemius, or in the proportion of fast-twitch red fibers in the soleus in response to exercise training (3, 10, 15).

The absence of a significant alteration in actomyosin ATPase activity in the fast-white muscle fibers in response to the running program is probably due to lack of an adequate adaptive stimulus. The white fibers appear to contract infrequently in leg muscles of rats during prolonged long-distance running, so that most of the work is performed by the fast-red and slow-red fibers (5).

Available evidence indicates that the so called Mg²⁺-activated actomyosin-ATPase reaction, which involves myosin plus actin, Mg²⁺, ATP, and a very low concentration of Ca²⁺, represents the fundamental biochemical process that occurs in the myofibrils during muscle contraction (14). ATP forms a complex with Mg²⁺, which is bound to myosin, and serves as the substrate for the ATPase. In the absence of Ca²⁺, interaction between actin and myosin is inhibited by the modulatory proteins, troponyosin and tropomin. Ca²⁺ at very low concentrations (about 10⁻⁵ M) reverses this inhibition by binding to one component of tropomin. Thus, under physiological conditions, Ca²⁺ does not activate actomyosin ATPase but reverses a preexisting inhibition (14). In the "Ca²⁺-activated" actomyosin-ATPase reaction, the reaction mixture contains 10 mM Ca²⁺ (about 10⁵ times the concentration attained under physiological conditions) and no Mg²⁺. Thus, Ca²⁺-activated ATPase activity of actomyosin, while no doubt of

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TABLE 2. Mg²⁺-activated ATPase activity of actomyosin in different types of skeletal muscle and in heart of exercise-trained and untrained rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Actomyosin ATPase, μmol Pᵢ/min per mg actomyosin</th>
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<tbody>
<tr>
<td></td>
<td>Red vastus</td>
</tr>
<tr>
<td>Untrained</td>
<td>0.635</td>
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<tr>
<td></td>
<td>±0.029</td>
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<tr>
<td></td>
<td>(9)</td>
</tr>
<tr>
<td>Trained</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>±0.021*</td>
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<td></td>
<td>(9)</td>
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</tbody>
</table>

Values are means ± SE. The values in parentheses are the number of animals per group with the exception of soleus, in which muscles from 2 animals were pooled for each determination to give 4 determinations on muscles from 8 animals. *Trained vs. untrained, P < 0.01. † Trained vs. untrained, P < 0.05.
some theoretical interest, has essentially no significance in physiological processes (14). Not surprisingly, Ca++-activated actomyosin ATPase activity does not correlate as well with muscle contractile speed as does Mg++-activated actomyosin ATPase activity (8). Nevertheless, it appeared of interest to investigate the response of Ca++-activated actomyosin ATPase in the fast-twitch red and white types of muscle to our running program, because previous investigators have reported conflicting findings regarding the response to exercise training of the Ca++-activated enzyme activity in the gastrocnemius (3, 31).

Bagby et al. (3) have examined the effects of programs of endurance exercise on actomyosin ATPase activity in the gastrocnemius muscles of rodents and found no change. This lack of a response could have been due to the performance of less strenuous exercise and/or to a shorter duration both of the individual exercise sessions and of the total training program than in the present study. Another, and perhaps more important factor, could relate to the fact that the gastrocnemius is a mixture of the different types of muscle fibers (1). As a result, a small decrease in actomyosin ATPase in the fast-twitch red fibers could have been largely obscured by the absence of an effect in the white fibers and the increase in activity in the slow fibers. A similar situation was seen in previous studies in which we were unable to detect significant changes in various enzymes of glycogenolysis in gastrocnemius (20) and quadriceps (6) muscles, despite a significant decrease in enzyme activity in fast-twitch red and a significant increase in that of slow-twitch red muscle fibers (6).

In contrast to Bagby et al. (3), Wilkerson and Evonuk (31) found a 44% increase in actomyosin ATPase activity in gastrocnemius muscles of rats subjected to an exhausting program of swimming. This difference in response could perhaps be due to a difference in the type of exercise used. However, this is not clear, because Syrovich et al. (30) found no change in actomyosin ATPase activity in the extensor digitorum longus muscle (which is also a mixed, primarily fast, muscle) in rats subjected to a similar swimming program. On the other hand, Syrovich et al. did find a small but significant increase (17%) in actomyosin ATPase activity in soleus muscle, similar to that seen in the present study, in young rats subjected to their swimming program.

Wilkerson and Evonuk (31) and Bhan and Scheuer (11) have shown significant increases in myosin and actomyosin ATPase activities in hearts of rats subjected to programs of swimming that lasted 6–10 wk. Bhan and Scheuer have provided evidence that this increase in activity is associated with alterations in the biochemical characteristics of the myosin molecule and have suggested that the increase in ATPase activity could be responsible for an increase in myocardial contractility (11). The swimming programs used by Bhan and Scheuer involved 90 or 150 min of swimming per day for 8 wk. This is a very mild exercise stress; untrained rats can swim for many hours if water temperature is maintained between 32 and 35°C (13). The present finding that actomyosin ATPase activity was unchanged in the hearts of rats subjected to strenuous, prolonged bouts of treadmill running over a period of 18 wk or longer suggests the possibility that an increase in cardiac actomyosin ATPase activity may be an early adaptation to exercise which disappears as the level of training increases.

Mann and Salafsky (22) found that glycogenolytic enzyme activity increased in fast muscle (tibialis anterior) in cats from birth to 18 wk of age while contraction speed also increased; in contrast, glycogenolytic activity and contractile properties did not change in slow muscle (soleus). In view of the parallel time course of the increases in glycogenolytic activity and speed of contraction (which presumably reflects an increase in actomyosin ATPase activity), these authors speculated that these two parameters might possibly be "... linked through energy demand systems" (22). Our finding of a very close correlation between phosphofructokinase activity and actomyosin ATPase activity in the four types of muscle in trained and untrained animals provides further strong evidence that the glycogenolytic capacity of a muscle and its actomyosin ATPase activity are regulated in parallel. Pettie (27) has shown that certain enzymes in the various pathways involved in the generation of ATP occur in constant proportions to each other in a wide range of tissues. The present results provide evidence for a constant relationship between a major pathway for ATP generation and a major pathway for ATP utilization. Of considerable interest in this context is the finding by Arnold and Pettie (2) that a number of glycolytic enzymes including phosphofructokinase exhibit a strong binding to actin (2).

Since heart muscle contracts continually and has the highest capacity for aerobic metabolism of any mammalian muscle, it seems reasonable that the enzyme patterns for the generation of ATP and for the hydrolysis of ATP during muscle contraction are the optimal ones for continuous and vigorous submaximal contractile activity. Clearly, skeletal muscle has specialized functions, such as the maintenance of posture in the case of the slow-twitch muscles and the performance of short bursts of intense work that exceed the muscle's capacity for aerobic metabolism in the case of the fast-twitch muscles, that preclude an enzyme pattern identical to that in heart. However, in response to endurance exercise training, the fast-twitch red and slow-twitch red types of muscle fiber become at least somewhat more like heart muscle in their enzyme patterns with respect to both the mitochondrial enzymes involved in the generation of ATP via aerobic metabolism and the glycolytic and glycogenolytic enzymes responsible for the generation of ATP anaerobically (4, 6, 32). It is, therefore, of considerable interest that fast-twitch red and slow-twitch red skeletal muscle fibers also become more like heart muscle with respect to their actomyosin ATPase activity, which represents the major pathway for ATP utilization in muscle (Table 2).

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