Skeletal muscle mitochondria and myoglobin, endurance, and intensity of training

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HARMS, SANDRA J., AND ROBERT C. HICKSON. *Skeletal muscle mitochondria and myoglobin, endurance, and intensity of training.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 54(3): 798–802, 1983.—Female rats were trained by treadmill running 40 min/day, 6 days/wk up to 11, 22, or 44 m/min for 14 wk. Low-intensity exercise (11 m/min) increased the activities of the mitochondrial markers, citrate synthase and succinate dehydrogenase, by 50 and 58% in fast-twitch red vastus lateralis (FTR) muscles and by 32 and 15%, respectively, in slow-twitch red soleus (STR) muscles. Running up to fourfold faster did not significantly elevate the activities of these enzymes beyond those obtained after running at 11 m/min. In contrast, there was a small but direct trend of training intensity with citrate synthase activity in fast-twitch white vastus lateralis (FTW) muscles. Myoglobin concentration tended to increase as a linear function of training intensity in FTR muscles. In STR muscle, the concentration of myoglobin increased 15% in the 11 m/min group but was essentially the same as control values in the 22 and 44 m/min groups. The concentration of myoglobin was not changed with training intensity in FTW muscle. Exercise time to exhaustion increased in proportion to training intensity. We conclude that *1*) low-intensity running [approx 50% maximal O_2 uptake or less] is a sufficient stimulus to induce most of the total possible mitochondrial increase in the red fiber types, whereas extremely fast running speeds are needed to stimulate significant increases in white muscle mitochondria; and *2*) the mitochondrial differences in skeletal muscle between intensity groups cannot account for the differences in performance.

exercise; fast-twitch white muscle; fast-twitch red muscle; slow-twitch red muscle; citrate synthase; succinate dehydrogenase; female rats

TWO OF THE PRINCIPAL cellular constituents that can influence the aerobic potential of muscle are the mitochondria and myoglobin. The mitochondria are organelles that are involved in the utilization of substrates (mainly carbohydrates and fats during exercise) with O_2 for ATP production. Myoglobin, a hemoprotein, functions in the storage of O_2 (2, 14, 22) and in the transport of O_2 within the muscle cell to the mitochondria (10, 16, 17, 21). Recent studies have shown that cytochrome *c*, a mitochondrial marker, and myoglobin have different degradation rate constants in the red types of skeletal muscle when exercise training is stopped (12). In addition, cytochrome *c* and myoglobin have a different pattern of response to training frequency in slow-twitch red and in fast-twitch white fiber types (11). Based on these differences in response to detraining and frequency of exercise, one purpose of this investigation was to gain insight into whether the mitochondria or myoglobin, or both, play a significant and coordinated role in the adaptation to exercise intensity.

At this time, there is only minimal information available regarding the effects of training intensity in skeletal muscle, and the results of one report are at odds with that found with frequency and duration of training. Dohm et al. (6) observed essentially the same increases in succinate dehydrogenase activity in gastrocnemius (mixed) muscles of rats trained to run at three intensities between 20 and 35 m/min. In contrast, mitochondrial content and performance have been shown to be related to both frequency and duration of training paticularly in the red types of muscle (9, 11). Therefore, another purpose of this work was to attempt to resolve these apparent discrepancies and thereby gain a clearer understanding of the impact of all three components on the training process. Specifically, these goals were *1*) to study the influence of training intensity on the mitochondrial markers, citrate synthase and succinate dehydrogenase, and myoglobin in the three types of skeletal muscle; *2*) to evaluate the influence of training intensity on performance; and *3*) to examine the relationships of the mitochondrial and myoglobin adaptations with performance.

METHODS

Animal care and training program. Female Wistar rats (Charles River Laboratories, Wilmington, MA) were obtained for this study. At the commencement of the experiment, all animals were 95 days of age with an average body weight of 223 g. The animals were housed individually and were provided an ad libitum diet of Purina rat chow and water. Rats were randomly assigned to one of four groups: a sedentary control group and three groups that were trained on a motor-driven treadmill (Quinton 42-15). The intensity of exercise was varied by adjusting running speeds among the groups. Speed was progressively increased over a 10-wk period. For each exercise session, the lowest-intensity group always ran at one-half the speed of the medium-intensity group, which ran at one-half the speed of the highest-intensity group. The training groups were exercised 6 days/wk for a total of 12–14 wk, with duration increased to the same extent in all groups. During the last 3–5 wk of training, the animals were running either 11 (S_{11}), 22 (S_{22}), or 44 (S_{44}) m/min up a 5° incline for 40 min/day, which was preceded by a 5-min warm-up of slower running. According to literature values (4, 5), the final running speeds

are estimated to represented 50% or less (11 m/min), 65–75% (22 m/min), and 100% or more (44 m/min) of maximum O_2 uptake ($\dot{V}O_{2\,max}$) depending on the training state of the animals.

Evaluation of endurance performance. An exercise test was administered on the 68th day of training, after 11 wk of exercise. Six animals were randomly selected from each of the three training groups. Body weights of these animals were recorded before the run and statistically analyzed to determine whether there were group differences. The 18 animals were run at 28 m/min up a 5° incline until exhaustion at a room temperature of approximately 15.5°C. The point of exhaustion was determined by the animal's repeated failure to avoid the shock grid (located at the rear of the treadmill) and failure to upright itself when placed on its back.

Muscle sampling. After at least 12 wk of training, rats were killed by decapitation and exsanguinated; the trained animals were killed approximately 24 h after their last bout of exercise. Prior to killing, the animal's body weights were determined. The muscles selected for study were dissected out from both hindlimbs, trimmed of fat and connective tissue, blotted, weighed, and stored at −82°C until analyses. The whole muscles chosen for analyses were the soleus, which consists mainly of slow-twitch red fibers, and the plantaris, a mixed muscle, which consists of approximately 40% fast-twitch white, 50% fast-twitch red, and 10% slow-twitch red fibers (1). The gastrocnemius and quadriceps femoris were two additional skeletal muscles dissected out from the hindlimbs. The quadriceps, which consist of four distinct muscles, was removed as a unit from its insertion to its origin from the right hindlimb. The superficial portion of the vastus lateralis, one of the quadriceps muscles, and the deep portion of the vastus lateralis were used in subsequent analyses. The superficial portion, commonly termed the "white vastus," consists of fast-twitch white fibers. The deep portion is commonly termed the "red vastus" and is made up of fast-twitch red fibers. (In the case where two muscles of the same type were excised, the average weight of the two muscles was used in calculating the group statistics.)

Assay methods. Citrate (*si*)synthase (EC 4.1.3.7) activity was determined according to the method described by Srere (18) with the use of 5,5′-dithiobis(2-nitrobenzoic acid); muscle homogenates were frozen and thawed three times prior to assay. Succinate dehydrogenase (EC 1.3.99.1) activity was determined as described by King (13). Myoglobin content was measured according to the procedures of Reynafarje (15).

Statistical procedures. The data were analyzed using analysis of variance and Student's *t* tests. Tukey test post hoc procedures were used to evaluate means following significant analysis of variance results (8). Statistical significance was set at the 0.05 level.

RESULTS

Body weights and muscle weights. The body weights of the sedentary control and the three training intensity groups were not different from each other (Table 1). Gastrocnemius muscle weights of the 44 m/min group were significantly greater than those in the sedentary control group. In the other muscles, the weights of the 44 m/min group tended to be greater than the weights in other groups, but these differences did not reach statistical significance. The issue of whether or not high-intensity, rapid, repetitive, contractile activity, such as sprinting, can increase muscle size still remains unresolved; nevertheless, these muscle weight data support this contingency.

TABLE 1. *Effect of endurance training at three intensities on body weight and selected skeletal muscle weights*

Group	*n*	Body Wt, g	Muscle Wt, mg			
			Soleus	Plantaris	Gastrocnemius	Right quadriceps
SC	14	280 ±5	133 ±4	289 ±9	1,381 ±29	2,280 ±53
S_{11}	17	296 ±5	138 ±4	314 ±7	1,490 ±33	2,380 ±54
S_{22}	16	279 ±5	135 ±4	302 ±6	1,435 ±28	2,350 ±59
S_{44}	16	285 ±5	141 ±5	315 ±7	1,504* ±31	2,445 ±54

Values are means ± SE; *n*, no. of animals per group. S_{11}, S_{22}, and S_{44}, no. of m/min rats ran up a 5° incline for 40 min/day. * Significantly different from sedentary control (SC) ($P < 0.05$).

Citrate synthase and succinate dehydrogenase. The red portion of the quadriceps muscle had the greatest increases of these enzymes with training (Figs. 1 and 2). In the fast-twitch red vastus lateralis (FTR) muscle, citrate synthase activities increased between 50–58% ($P < 0.01$) above controls in the three training groups, and succinate dehydrogenase (SDH) activities increased 58–88% ($P < 0.01$) in the three intensity groups. In the slow-twitch soleus (STR) muscle, the levels of activity of citrate synthase increased to the same extent (30–35%) ($P < 0.05$) above sedentary control values in all training groups. The small increases in the activities of SDH were not statistically significant. Citrate synthase activity in the plantaris, a mixed muscle, increased 33% in the 11 m/min, 49% in the 22 m/min, and 72% ($P < 0.01$) in the 44 m/min groups. Similarly, SDH activities increased 17%, 36%, and 68% ($P < 0.05$) in these respective groups. This linear effect in mixed muscle was probably due to the greater range of running speeds than employed previously (6). In fast-twitch white vastus lateralis (FTW) muscle, citrate synthase activity increased 13% in the 11 m/min, 29% in the 22 m/min, and 48% in the 44 m/min groups, thereby demonstrating a small but direct effect of training intensity. However, only the 44 m/min runners' citrate synthase activity was significantly different from that of the sedentary control levels in FTW muscle fiber type. The increases in SDH activity were not statistically significant in white vastus muscle, but there was a trend similar to that seen for citrate synthase in this muscle type.

Myoglobin. In the FTR portion of the vastus lateralis muscle, there was a nonsignificant trend for myoglobin concentration to increase in proportion to training intensity (Fig. 3). The increases were 7, 11, and 17%, respec-

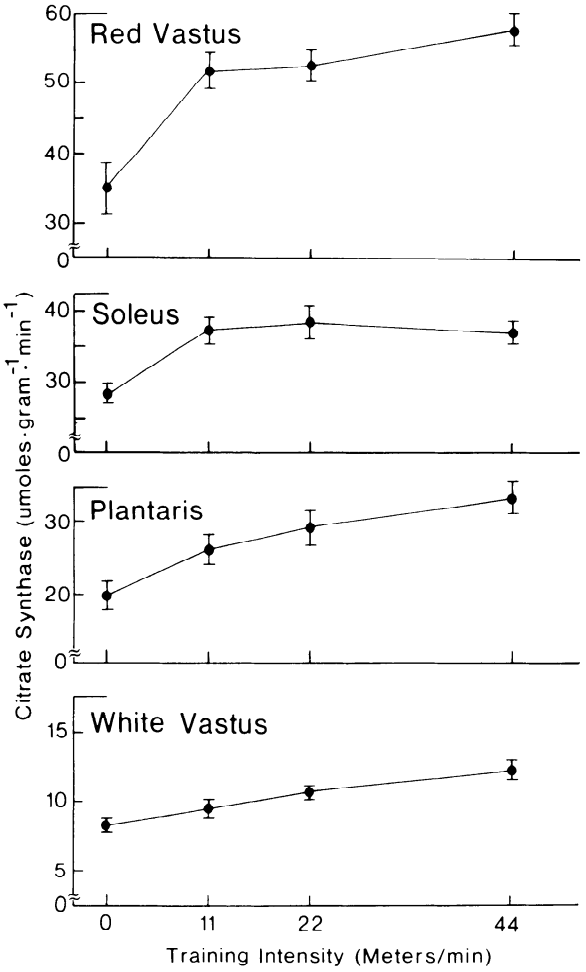

FIG. 1. Effects of training intensity on citrate synthase activity in plantaris muscle and in 3 types of skeletal muscle. Zero intensity represents sedentary control values. There is a minimum of 5 animals/*point* in sedentary control group and a minimum of 10 animals/*point* in training groups.

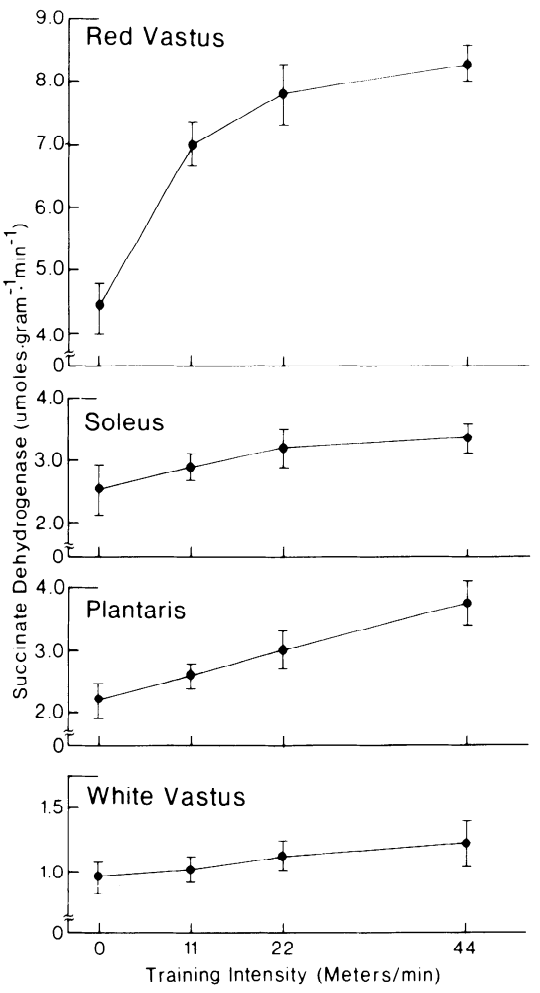

FIG. 2. Effects of training intensity on succinate dehydrogenase activity in plantaris muscle and in 3 types of skeletal muscle. Zero intensity represents sedentary control values. There is a minimum of 6 animals/*point* in sedentary control group and a minimum of 10 animals/*point* in training groups.

tively. Myoglobin levels increased 15% in soleus muscles of the 11 m/min runners; however, the myoglobin values in the 22 and 44 m/min groups were essentially the same as those in the sedentary control group. In the plantaris muscle, myoglobin concentration increased between 24 and 29% ($P < 0.01$) in all training groups. The concentration of myoglobin remained unchanged by training intensity in FTW muscle.

Endurance performance. The six animals randomly selected from each of the training groups for the endurance test showed no significant difference in body weights on the testing day. Total run time to exhaustion averaged 127 ± 9 min in the 11 m/min group, 314 ± 32 min in the 22 m/min group, and 569 ± 75 min in the 44 m/min group. All values were significantly different from each other.

DISCUSSION

In the red types of muscle, low-intensity training (11 m/min) resulted in near-peak adaptive increases in the activities of citrate synthase and succinate dehydrogenase. At the faster running speeds, there were relatively small or no further increases in the activities of these enzymes. Therefore, at a given duration and frequency of training, these results imply that low levels of physical activity (approx 50% $\dot{V}O_{2\,max}$ or less) are sufficient to induce most of the total possible increases in mitochondria of fast-twitch red and slow-twitch red muscles.

The finding that mitochondrial levels are not entirely related to intensity of training in the red types of muscle suggests significant roles for the other training components, namely duration and frequency. For example, if the duration of exercise is extended to 2 h/day, a number of mitochondrial marker proteins, including citrate synthase, increase twofold in the red types of muscle (3). The increases in citrate synthase activity in the present investigation are 40–70% less than that found when exercise duration is 2 h/day (3). Thus exercise duration appears to have a more direct and stronger influence on mitochondrial adaptations than intensity of training. In addition, when frequency of training is varied (2, 4, or 6 days/wk) a direct relationship of mitochondrial content in the red types of muscle is observed (11). Hence, an argument can be made that if frequency is greater than 4 days/wk, duration of training can be a significant factor in inducing red muscle mitochondrial adaptations. Con-

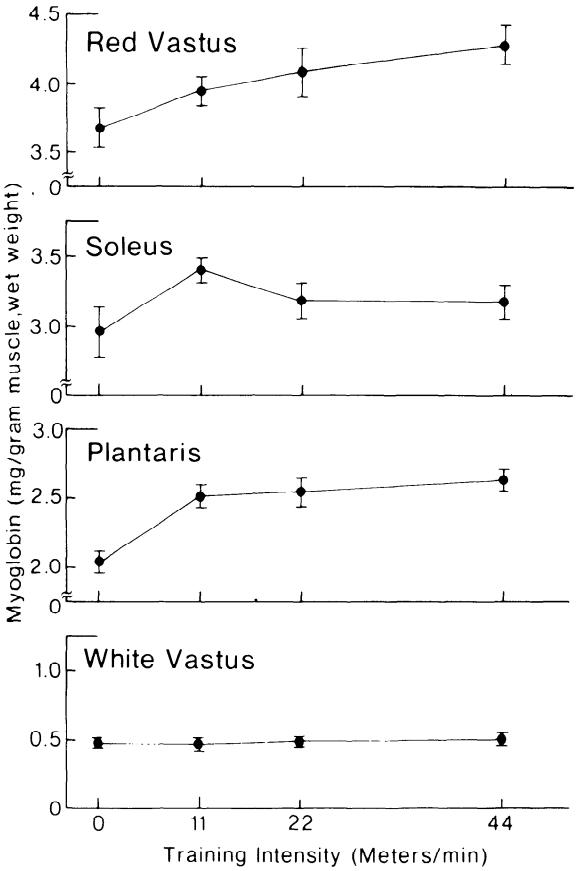

FIG. 3. Effects of training intensity on myoglobin concentration in plantaris muscle and in 3 types of skeletal muscle. Zero intensity represents sedentary control values. There is a minimum of 6 animals/*point* in sedentary control group and a minimum of 10 animals/*point* in training groups.

versely, an argument can be made for frequency of training as an important factor in inducing mitochondrial adaptations in the red muscle types if the exercise is performed at some optimal duration.

In white vastus muscles, the pattern of increase in the mitochondrial enzymes appeared to be linear. Yet, the absolute changes in citrate synthase and succinate dehydrogenase activities were 50 and 400% greater, respectively, in red vastus than in white vastus muscles between 11 and 44 m/min. Faster running speeds are needed to elicit further responses by white fibers; but this would be possible only at the expense of dramatically reducing training duration and consequently reducing the red fibers mitochondrial adaptations. Two studies (7, 20) have also observed that as exercise becomes more strenuous there is a greater recruitment of fast-twitch white muscle as evidenced by increased levels of the mitochondrial markers, citrate synthase and cytochrome *c*; however, it is difficult to compare these observations with the present data, because both intensity and duration were varied in these studies.

The pattern of changes in myoglobin content with exercise intensity was specific to each of the fiber types. In fast-twitch red muscle, there was a linear trend of myoglobin concentration with intensity of training, but the amount of increase in the highest intensity runners was still only one-half of that seen when animals are trained up to 2 h/day at slower running speeds (11, 12). The concentration of myoglobin in fast-twitch red muscle has also been shown to be related to frequency of exercise (11). Cumulatively then, in fast-twitch red muscle the increases in myoglobin appear to be related to all three training mechanisms.

In the soleus, myoglobin increased above control levels after running at a low intensity (11 m/min). Surprisingly, myoglobin concentration was not further increased in this predominantly slow-twitch red muscle as intensity was increased. Rather, myoglobin concentration declined back to control levels at the higher intensities. For some unknown reason, it appears that the stimulus to elevate myoglobin in this muscle may be "shut off" as exercise speeds increase.

White muscle myoglobin levels were not elevated even in the highest intensity runners. Other studies have been unable to demonstrate any training effect in this fiber with greater durations of running or with different frequencies of exercise (11, 12). It is possible white muscle myoglobin is unresponsive to all three components of training. Using glycogen depletion as an index of fiber activity, Sullivan and Armstrong (19) did not observe significant recruitment of white fibers during a 200-m run in rats until running speeds exceeded 60 m/min. Therefore, another possibility is that much higher intensities than running at 44 m/min would be required to stimulate increased myoglobin content in the white fibers.

When expressed per gram of muscle, the absolute increases (0.5–0.6 mg/g) in the concentration of myoglobin in plantaris muscle with training were similar to the absolute increases in myoglobin in red vastus muscles. But 1 g of plantaris muscle contains approximately 40% fast-twitch white muscle fibers (1). These results suggest there may have been a greater magnitude of change by one or more of the fiber types within the plantaris than was found in the individual fiber type sections that were examined.

Recent unpublished data from this laboratory indicate that the 3- to 5-wk steady-state running period was adequate time for soleus myoglobin to increase 90–100% of the total response. However, a longer time is needed for complete red vastus changes. These differences in myoglobin half-life between fast and slow red muscle and an insufficient training time to reach steady state limits direct comparisons between fiber types.

Previous studies have found long-term endurance to be highly correlated with frequency (11) and duration (9) of training. Furthermore, these earlier studies also demonstrated that endurance was related to the mitochondrial content of the red muscle types and mixed muscle. Although endurance performance was directly affected by training speed in the present study, there was no relationship between performance and the mitochondrial content of the red fiber types. However, the sampling of the red muscle was limited and may not reflect all of the responses of these fiber types within the active musculature. For instance, the fast-twitch red fibers in gastrocnemius muscle may have responded quite differently to the training than those in red vastus muscle. In fact, the observation that gastrocnemius muscle showed the greatest relative change in mass at the highest training inten-

sity suggests it may have undergone the greatest training response. Nevertheless, these findings distinguish intensity as a unique component of the training process. To our knowledge, this is the first data to show that differences in endurance cannot be explained by differences in mitochondria in the trained state. While a correlation can be made between endurance performance and the white fibers' mitochondrial content, it is highly unlikely that the large differences in running times between groups could be explained by the small increases in the white fibers. The mechanisms accounting for the performance by each group are unknown but are probably interrelated with carbohydrate and lipid utilization and work intensity.

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