Augmentation of skeletal muscle myoglobin by a program of treadmill running

PAUL K. PATTENGALE2 AND JOHN O. HOLLOSZY3

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

Augmentation of skeletal muscle myoglobin by a program of treadmill running. Am. J. Physiol. 213(3) : 783-785. 1967.—The purpose of these experiments was to obtain information, under controlled conditions, regarding the effect of regularly performed exercise on the concentration of myoglobin in skeletal muscle. Rats were subjected to a vigorous program of treadmill running of progressively increasing intensity lasting 15 weeks. This program resulted in a highly significant increase in the capacity for prolonged running. The weight and water content of the leg muscles was unaffected. The myoglobin concentration in the quadriceps and hamstring muscles increased approximately 80%, while that of the abdominal muscles was unaffected. This suggests that a local factor, limited to the exercising muscles, was responsible for the increase. It appears from these results that the level of habitual physical activity may play an important role in determining the concentration of myoglobin in skeletal muscle.

METHODS

Six-week-old male rats of Carworth's "specific pathogen-free" CFN strain were divided into three groups, placed in individual cages, and maintained on a diet of Purina laboratory chow and water.

An exercising group was trained to run on a motor-driven treadmill similar to the one described by Kimeldorf (5). It consists of a wide, endless belt riding on metal rollers. One roller is driven at a selected rate by a variable-speed control system. A Lucite box, partitioned into individual compartments 30 cm long by 10 cm wide, is suspended over the belt, providing a separate running area for each animal. A shock grid is located at the rear of the compartments; the 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 5 days per week, and the program was made progressively more vigorous. They initially ran for 10 min at 22 m/min, twice daily, 4 hr apart; after 3 weeks they were running 40 min at 28 m/min, twice, 4 hr apart; after 6 weeks, 60 min at 31 m/min, twice, 4 hr apart; after 9 weeks, 120 min continuously at 31 m/min; after 12 weeks, 120 min at 31 m/min, with 12 sprints at 42 m/min, each lasting 30

Received for publication 21 November 1966.

1 This work was supported by National Institute of Child Health and Human Development Research Grant HD 01618.
2 Student Summer Research Fellow supported by Public Health Service Grant 5-T5-GM-8-10.
3 Inquiries regarding this paper should be directed to JOH.

Whipple appears to have been the first to suggest that exercise might increase the concentration of myoglobin in muscle (13). This suggestion was prompted by his finding, during the course of a study on myoglobin in dogs, that the muscles of an active hunting dog had a higher concentration of myoglobin than the muscles of the other, more sedentary, animals. In support of this concept, Lawrie found, in an investigation of the well-known phenomenon that active muscles generally have a darker red color than inactive muscles, that there is a good correlation between the activity of a muscle and its myoglobin content (6). Further supportive evidence comes from a study by Shenk (12) who found that cattle grazing in a pasture had a higher concentration of myoglobin in their muscles than cattle kept in an enclosure and provided with food.

Taken together, the foregoing findings strongly suggest that exercise may increase the concentration of myoglobin in muscle. Nevertheless, the possibility that the reported differences were on a genetically or nutritionally determined basis, rather than the result of variations in physical activity, cannot be ruled out, since these factors were not all controlled.

It appeared of interest, therefore, to investigate the effect of physical activity on muscle myoglobin content under controlled conditions of exercise, diet, and heredity. The present paper describes the effect of a program of treadmill running of progressively increasing intensity on the concentration of myoglobin in three different muscle groups.
sec, interspersed at 10 min-intervals through the workout. Animals were maintained at the final work level for 3 weeks and then were sacrificed. The exercising group was provided with food and water ad lib.

An exercising control group ran on the treadmill for 10 min/day, 5 days per week. Running speed was increased to 31 m/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 those of the exercising group.

Sedentary control rats were confined to individual cages measuring 17 x 34 cm, but no attempt was made to restrict their spontaneous activity. Their food intake was adjusted so as to maintain their body weights approximately the same as those of the exercising group.

Animals were not exercised for 48 hr prior to sacrifice. After an overnight fast, they were killed by decapitation and exsanguinated. Muscles were dissected out, trimmed of fat, rinsed in Ringer solution, and blotted with moist filter paper. The procedure for myoglobin extraction, reduction, and conversion to carboxymyoglobin was based on DeDuve's method (3) as modified by Anthony et al. (1). The optical densities of the carbon monoxide compounds were measured in a Beckman DU spectrophotometer. The concentrations of myoglobin and hemoglobin were calculated using the equations and extinction coefficients given by Poel (8). These were found, in preliminary studies, to give more consistent results than the equations of DeDuve (3). This is in keeping with the conclusions of Rosenmann and Morrison (10). These investigators have empirically validated Poel's equations using mixtures of different concentrations of myoglobin and hemoglobin (10).

RESULTS

Following 12 weeks of training, the animals were subjected to an "all-out" exercise test in which they ran to exhaustion at a treadmill speed of 31 m/min. It was found that the training program had resulted in a large increase in exercise capacity. The average run time for the exercising group was 192 ± 3 min, compared to 29 ± 3 min for the exercising controls (P < 0.001).

A comparison of sedentary controls with the exercising animals showed that the quadriceps muscles did not increase in weight in response to the endurance exercise (Table 1). The weights of gastrocnemius, soleus, gluteus, and hamstring muscles were similarly unaffected by the training program. No chronic changes in the water content of the quadriceps muscles occurred in the exercised animals (Table 1).

The myoglobin content of the quadriceps muscles was found to be approximately 75% higher in the exercising group than in the sedentary controls (Table 1). This increase in myoglobin was apparently limited to those skeletal muscles which were directly involved in the activity of running. As shown in Table 2, the concentration of myoglobin was essentially the same in the abdominal muscles of the exercising and sedentary groups. In contrast, the concentration of myoglobin was approximately 80% higher in the hamstring and quadriceps muscles of the exercising group (Table 2).

TABLE 1. Effects of a 15-week program of running on the weight, water content, and myoglobin content of quadriceps muscle, and on total body weight in the rat

<table>
<thead>
<tr>
<th></th>
<th>Body</th>
<th>Quadriceps Muscle</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wi, g</td>
<td>Weight, g</td>
<td>Water content, %</td>
</tr>
<tr>
<td>Exercised group</td>
<td>369±20</td>
<td>3.06±0.13</td>
<td>73.8±2.7</td>
</tr>
<tr>
<td>Sedentary group</td>
<td>374±27</td>
<td>3.14±0.19</td>
<td>76.8±2.4</td>
</tr>
<tr>
<td>Difference</td>
<td>5</td>
<td>0.08</td>
<td>0.8</td>
</tr>
<tr>
<td>P</td>
<td>NSD</td>
<td>NSD</td>
<td>NSD</td>
</tr>
</tbody>
</table>

Values are expressed as means ± the se of the mean for six rats. NSD: no significant difference between the means.

DISCUSSION

The present study demonstrates that prolonged exercise, regularly performed, can significantly increase the concentration of myoglobin in skeletal muscle confirming the conclusions of Whipple (13) and Shenk et al. (12). The concentration of myoglobin almost doubled in muscles of the hindlimb in response to the program of running, but remained unchanged in the abdominal muscles. It appears, therefore, that a local factor, limited to the exercising muscles, was responsible for the increase.

In individuals, or within a species, the level of habitual physical activity may, in the absence of iron deficiency, be the most important factor in determining the concentration of myoglobin in a skeletal muscle. Both the

TABLE 2. Effect of a 15-week program of running on the concentration of myoglobin in various rat skeletal muscles

<table>
<thead>
<tr>
<th>Myoglobin Content</th>
<th>Hamstrings</th>
<th>Quadriceps</th>
<th>Rectus abdominus</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/g Wet weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercised group</td>
<td>1.90±0.05</td>
<td>2.17±0.12</td>
<td>0.73±0.6</td>
</tr>
<tr>
<td>Sedentary group</td>
<td>1.05±0.10</td>
<td>1.20±0.07</td>
<td>0.70±0.4</td>
</tr>
<tr>
<td>Difference</td>
<td>0.85</td>
<td>0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NSD</td>
</tr>
</tbody>
</table>

Values are expressed as means ± the se of the mean for muscles from six rats. NSD: no significant difference between the means.
magnitude of the exercise-induced increase and the absence of an overlap between the myoglobin values obtained on the hindlimb muscles of the exercising and sedentary rats favor this possibility. However, the concentration of muscle myoglobin attained by the rats, despite a program of prolonged exercise which pushed them close to the limits of their capacity, was only approximately one-fourth that reported in the skeletal muscles of horses and various other animals (6). (Attempts to increase the exercise load above the level attained after 12 weeks of training resulted in weight loss.) This suggests that major interspecies differences in muscle myoglobin may, to a considerable extent, be genetically determined.

It has been postulated, on the basis of direct measurements on contracting muscles (7) and indirect evidence obtained on humans (2), that myoglobin acts as an $O_2$ store and helps to support aerobic metabolism by releasing $O_2$ to cytochrome oxidase when $O_2$ becomes limiting during muscle contraction. The observation that myoglobin can, in vitro, increase $O_2$ transport across a fluid layer (4, 11) suggests that myoglobin might also facilitate $O_2$ utilization by enhancing $O_2$ transport through the myoplasm.

In this context, it appears possible that the increase in muscle myoglobin could play a role in the adaptive improvement of the capacity for prolonged, aerobic exercise which, as in the present study, can occur with physical training (9).

The authors express their appreciation to Dr. Hugh Chaplin and Dr. Robert E. Shank for helpful discussions, and to Miss Judith Leahey for skillful technical assistance.

REFERENCES