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Inability of Myoglobin to Increase in Dystrophic Skeletal Muscle during Daily Exercise

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Abstract. An exercise program consisting of 80-min daily runs on a treadmill was performed by normal and dystrophic hamsters. Subgroups were sacrificed at various times during the 45-day program. Daily exercise resulted in a significant increase in the myoglobin concentration of gastrocnemius muscles in normal animals but not in dystrophic animals. In the exercise groups of hamsters, there were significant increases in the concentration of cytochrome c, a marker for respiratory capacity, in the gastrocnemius of both normal and dystrophic hamsters.

Key words: Myoglobin – Dystrophy – Exercise – Cytochrome c – Skeletal Muscle.

INTRODUCTION

Myoglobin content is lower in dystrophic than normal skeletal muscle [7, 18, 20]. Since it is known that daily running will increase the myoglobin level in skeletal muscle of normal animals [17, 19], one purpose of the present study was to gain information on whether daily running can increase the level of myoglobin in skeletal muscles of genetically-dystrophic hamsters. Daily treadmill running also increases the respiratory capacity of skeletal muscles in healthy animals. A second purpose of the present study was to determine whether cytochrome *c*, a marker for the respiratory capacity of dystrophic skeletal muscle, increases during daily running-exercise.

METHODS

Animal Care. Male hamsters of a normal, healthy Golden Syrian strain (Engle, Farmersburg, IN) and of a genetically dystrophic (BIO 14.6) strain (Bioresearch, Boston, MA) were obtained at about 30 days of age and were provided with food and water ad libitum. Bioresearch (Boston, MA) guarantees that the genetic constitution of BIO 14.6 hamster is as stated by them.

Exercise Program. All normal and dystrophic animals that were to partake in the exercise program underwent for 4 weeks a 10-min daily period of learning to run on a motor-driven, small-animal treadmill. A sub-group of these animals was sacrificed after this 4-week period of learning and used as baseline controls. The remaining animals were started on a training program consisting of 80 min of daily running at 13.4 m/min up a 15% incline, 7 days per week. Groups of control hamsters were sacrificed at 2, 5, 11, 35 and 45 days of the training program; groups of dystrophic hamsters were sacrificed at 2, 6, 9, 14, 35 and 45 days of exercise training. In each group of animals, both gastrocnemius were dissected out and minced together and used for myoglobin and cytochrome *c* determinations. Visible connective tissue was not included in the mincing.

Group Controls. Since myoglobin content in skeletal muscles increases during growth [15], it was necessary to determine myoglobin concentration in animals not receiving exercise treatments. Subgroups of normal Syrian and of dystrophic BIO 14.6 hamsters were sacrificed at various times during growth. Measurements of myoglobin content in these animals then served as age-control values to determine if exercise treatments enhanced myoglobin.

Tissue Preparation and Assay Methods. After recording body weights, animals were sacrificed by decapitation; the gastrocnemius and quadriceps muscles were quickly dissected, cleaned of fat and connective tissue and then frozen at −80° C prior to weighing, mincing and assaying.

Cytochrome *c* concentration was determined by the method of Williams and Thorp [24], as modified by Booth and Holloszy [2]. Myoglobin concentration was measured with the procedure of Reynafarje [22], as modified by Reiss [21]. Protein concentration was determined with the biuret procedure [10].

RESULTS

Myoglobin. In the gastrocnemius muscles of normal hamsters at 50 days of age, there was 3.6 ± 0.2 mg myoglobin per gram of wet weight (Fig. 1). At 100 days of age, the myoglobin concentration in the gastrocnemius muscle of normal hamsters was significantly higher ($P < 0.0005$) in the group which had undergone daily exercise during the previous 45 days, 5.8 ± 0.1 mg myoglobin per gram muscle, than in the group which had not undergone daily treadmill running, 4.9 ± 0.1 mg myoglobin per gram muscle (Fig. 1).

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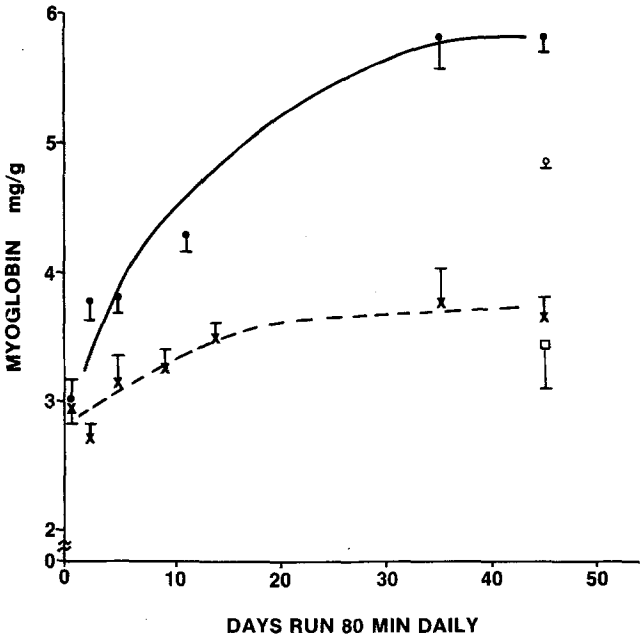

Fig. 1. The effect of the number of days running 80 min daily (x-axis) on the myoglobin concentration (y-axis) in the gastrocnemius muscle of normal (●----●) and dystrophic (×----×) hamsters. Age-matched, non-exercised controls for normal hamsters are symbolized by ○; and age-matched, non-exercised controls for dystrophic hamsters are given by open symbol □. Each point is the mean of 5 animals (except the control 0-day point which is 3 animals and the control 11-day point which is 4 animals). The bar represents the standard error of the mean (SEM)

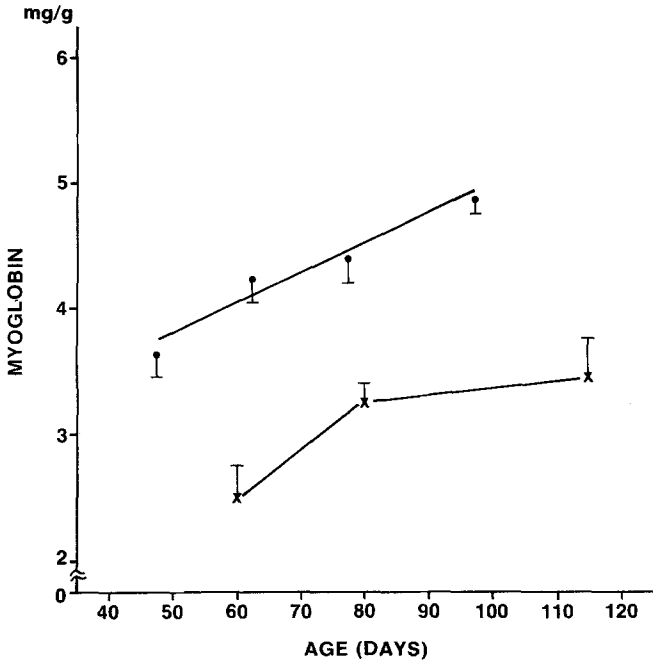

Fig. 2. The effect of age (x-axis) on the myoglobin concentration (y-axis) in the gastrocnemius muscle of non-exercising normal (●----●) and dystrophic (×----×) hamsters. Each point is the mean of 4–5 animals. The bar represents the SEM

In dystrophic hamsters at 70 days of age, there was 3.0 ± 0.2 mg myoglobin per gram of gastrocnemius. After 45 days of treadmill running (at the same work intensity as undergone by the normal hamsters), there was 3.6 ± 0.1 mg myoglobin per gram gastrocnemius in dystrophic hamsters. This level was not significantly different from the mean of 3.4 ± 0.3 mg myoglobin per gram muscle which was measured in age-matched (115 days) dystrophic hamsters that had not taken daily treadmill exercise (Fig. 1). The increase of myoglobin concentration in the gastrocnemius of non-exercising hamsters during their growth is given in Figure 2.

Protein. In the normal hamsters, there were 229 ± 4 mg protein per gram of quadriceps in the group which was 50 days old with no exercise, 241 ± 8 mg/g in the group which was 100 days old with no exercise, and 248 ± 6 mg/g in the group which was 100 days old with daily exercise on the previous 45 days. There was no significant effect of age or exercise on the protein concentration.

In the dystrophic hamsters there were 223 ± 6, 223 ± 4, and 219 ± 3 mg protein per gram of quadriceps in the groups 70 days old with no exercise, 115 days old with no exercise, and 115 days old with exercise during the previous 45 days, respectively. Neither age nor daily exercise significantly affected protein content among these groups of dystrophic hamsters.

Muscle and Body Weights. Between 70 and 115 days, body weights of normal and dystrophic hamsters increased. The gastrocnemius muscle weights were not recorded for the normal and dystrophic hamsters in the exercise study. However, comparison of muscle weights was made in another set of animals for the purpose of gaining information on the progression of the disease process. In 84 day-old animals, the quadriceps weighed 1.11 ± 0.04 g in control and 1.26 ± 0.02 g in dystrophic animals. Body weights were 112 ± 4 g and 95 ± 3 g at 84 days of age in control and dystrophic hamsters, respectively.

Cytochrome c. In a subgroup of normal hamsters sacrificed prior to the start of 80-min daily runs, there was 8.5 ± 0.5 nmoles of cytochrome c per gram of gastrocnemius. After 45 days of daily 80-min runs, there were 16.9 ± 1.0 nmoles of cytochrome c per gram of gastrocnemius in another subgroup of normal hamsters (Fig. 3). Dystrophic hamsters undergoing

an identical program of daily exercise had an increase from 10.3 ± 1.0 nmoles cytochrome *c* per gram of gastrocnemius prior to the start of 80-min runs to 14.9 ± 2.1 nmoles per gram after 45 days of the 80-min runs (Fig. 3).

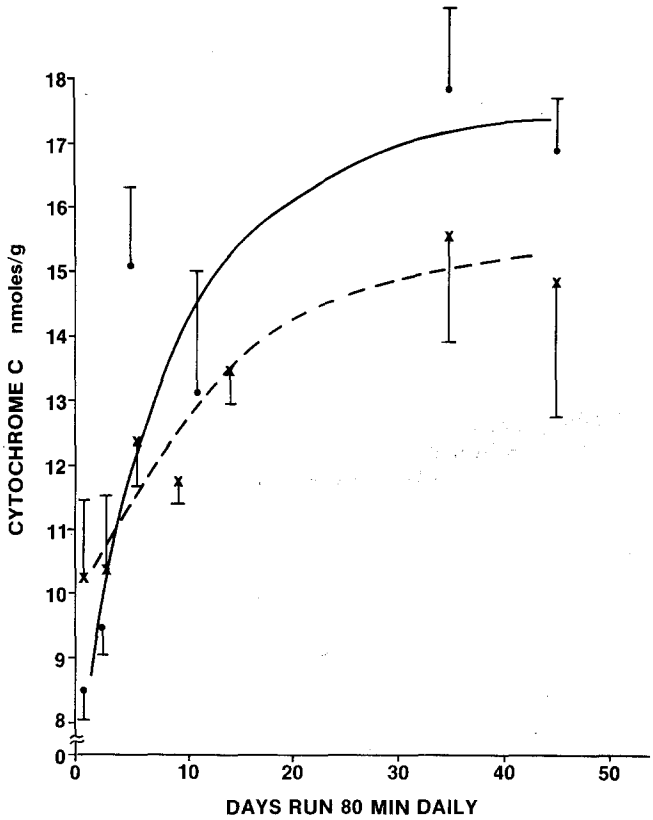

Fig. 3. The effect of the number of days running 80 min daily (*x*-axis) on the cytochrome c concentration (*y*-axis) in the gastrocnemius muscle of normal (●——●) and dystrophic (×——×) hamsters. Each point is the mean of 3 – 5 animals. The bar represents the SEM

DISCUSSION

Many of the published investigations on dystrophy-like myopathies in hamsters have employed the BIO 14.6 strain [12]. Possible reasons for the frequent usage of BIO 14.6 hamsters might include their commercial availability and the guarantee of one supplier that the animals' genetic constitution is BIO 14.6. The disease occurring in the BIO 14.6 animals is due to an autosomal recessive gene [11–13]. The BIO 14.6 strain has been maintained over the years by continued brother × sister mating of homozygous recessive animals [12]. The existence of a dystrophy-like myopathy was confirmed in the animals employed in the present studies by the following criteria. Connective tissue proliferation was usually visible in at least one muscle of the hind limbs of these dystrophic animals. As previously observed in hamsters with a dystrophy-like myopathy [11,13], the microscopic examination of randomly sampled muscles from BIO 14.6 hamsters indicated the presence of centrally located nuclei. The muscle weights of dystrophic animals in the present study were similar to age-matched normal, healthy hamsters. Because the body weights of dystrophic hamsters are smaller, as previously reported [11], than that of age-matched controls, a relative muscular enlargement was present in these dystrophic hamsters. This observation confirms the recent reports of Goldspink [8,9] in which it was stated that the wet weights of diaphragms of BIO 14.6 hamsters were larger than controls of similar ages. In a recent group of BIO 14.6, I have observed that BIO 14.6 swim more poorly than controls (unpublished observation), which confirms earlier statements that muscular weakness in dystrophic hamsters can be demonstrated by swimming [12,13]. All of the above would seem to confirm that the BIO 14.6 hamsters employed in the present study have a dystrophy-like myopathy.

Daily running did not increase the concentrations of myoglobin in the gastrocnemius muscle of dystrophic hamsters over the values observed in age-matched controls. This same exercise intensity was sufficient to cause: (a) a significant increase in gastrocnemius myoglobin concentration in normal hamsters; and (b) significant increases in a marker for respiratory activity, cytochrome *c* concentration, in the gastrocnemius of both normal and dystrophic hamsters. Thus, the level of exercise employed in these studies was sufficient to result in certain adaptations in the muscle. There is insufficient information at present to explain the failure of myoglobin concentration to increase in response to increase levels of contractile activity in dystrophic muscle. It is known, however, that the level of a protein is determined both by its rates of synthesis and degradation [23]. Thus, in the dystrophic muscle, the exercise either did not stimulate myoglobin synthesis or may have increased myoglobin breakdown. Concerning the latter, it is known that exercise can cause loss of sarcoplasmic proteins (such as myoglobin, creatine phosphokinase, and lactate dehydrogenase) into the blood of normal animals and humans [4,6].

The increase in cytochrome *c* concentrations in dystrophic skeletal muscle during daily exercise suggests that the functional work capacity of dystrophic muscle might be enhanced as a result of daily running. Although no measurements of the work of capacity of dystrophic muscle were made in the present study, it is well known that after daily exercise, observed increases in markers for respiratory capacity in skeletal muscle of normal, healthy animals and humans are associated with increased work times until exhaustion

[5], glycogen-sparing during exercise [1,5], and increases in maximal oxygen uptake [3,14,16]. On the other hand, the failure of an exercise-induced increase in myoglobin in dystrophic animals might affect any exercise-induced increase in work capacity. Wittenberg et al. [25] have reported that myoglobin probably transports a significant fraction of oxygen consumed by muscle mitochondria. If this is true, then during muscular work, the oxygen transporting capacity of dystrophic muscle is probably less than that of normal skeletal muscle. At present, there is insufficient information to answer these questions.

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