Effect of exercise and training on mitochondria of rat skeletal muscle

PHILIP D. GOLLNICK AND DOUGLAS W. KING
Exercise Physiology Laboratory, Department of Physical Education for Men, and Electron Microscope Laboratory, Washington State University, Pullman, Washington 99163

The present study investigated the effect of training and exhaustive exercise on the mitochondria of the gastrocnemius muscle of rats.

MATERIALS AND METHODS

Sixteen male rats of the Sprague-Dawley strain, with initial body weights between 180 and 200 g, were used in this investigation. These animals were fed, housed, and maintained as previously described (9). Eight of the animals were trained to run in motor-driven work wheels by progressively increasing the speed and duration of the daily exercise sessions until they were capable of running continuously for 1 hr at 1.0 mph or faster. This initial training period required 4 weeks. The exercise was then continued for an additional 6 weeks with each animal running 40 min at 1.0 mph followed by 20 min at 1.4 mph each day. Mild electrical stimulation was used to encourage the rats to run.

On the final exercise day the eight trained rats were run to exhaustion at speeds between 1.0 and 2.0 mph. Four of these rats were sacrificed immediately at the onset of exhaustion and the remaining four 24 hr later. The eight control animals were allocated into two equal groups and sacrificed either at rest or immediately after completion of an exhaustive swim. Rats swam in groups of four in water at 35°C. This procedure was used to insure vigorous activity and to avoid the effortless floating that occurs when rats swim alone. Swimming was used to exhaust the untrained rats because they would not run in the work wheels long enough to become fatigued. Exhaustion was defined as the point at which the rats were unable to continue running or to regain the water surface. The trained rats ran for an average of 105 min before becoming exhausted, whereas the untrained rats swam for an average of 58 min.

The animals were sacrificed by decapitation, and samples from the belly of the relaxed gastrocnemius muscle (taken from approximately the same location each time) were quickly removed and immediately fixed in cold 1% osmium tetroxide in barbital acetate buffer (pH 7.4) and embedded in Epon (33, 34). Longitudinal...
sections were cut with a glass-knife microtome and mounted on 200-mesh grids. They were stained with uranyl acetate and Reynolds lead citrate (36, 47) and examined with a Philips 100B electron microscope. Micrographs were made at a magnification of 2,600 diam and enlarged photographically to 25,000 and 60,000 for evaluation of number and size of mitochondria. Mitochondrial counts were made on 320 randomly selected micrographs in an area (arbitrary units) and expressed as number of mitochondria per 100 μ² and as mitochondria per 100 sarcomeres of muscle tissue. By the latter technique, possible differences in mitochondrial concentrations due to variation in the tissues in the field of a given micrograph, such as variation in the lengths of the sarcomeres or possible changes as a response to the treatments imposed upon the muscle prior to sacrifice, could be eliminated.

Glycogen concentrations of the biceps and gastrocnemius muscles and of the livers were also determined as an additional means of evaluating the severity of the two types of exercise used to produce exhaustion (39).

RESULTS

The structure of the skeletal muscle from the sedentary control group (Fig. 1A) is similar to that observed for most species including the rat (8). Mitochondria, with a concentration of 26.5/100 μ² or 39.2/100 sarcomeres, were located for the most part in pairs on either side of the Z line. Most of these mitochondria were circular, with a diameter equivalent to approximately half of the I band. Thus, these paired mitochondria were about as long as the I band.

The fine structure of the tissue from the trained animals sacrificed 24 hr after a run to complete exhaustion was similar to that of the control group (Fig. 1G); however, the mitochondria were more numerous and appeared to contain more densely packed cristae. These mitochondria also appeared to be enlarged, with some being as long as a sarcomere. In other instances gross distortions of the shape appeared in the enlarged mitochondria. Because of the uncertainty as to the point on the mitochondria that the sections were made, no attempt has been made to quantitatively compare the mitochondria in the micrographs for differences in size.

Significant alteration was present in the skeletal muscle of the rats that were sacrificed immediately after completion of a run to exhaustion (Fig. 1D). In these micrographs there was evidence of swelling both in the mitochondria and the muscle tissue. Large spaces existed between groups of adjacent myofilaments, and the mitochondria were greatly enlarged. Many of these mitochondria were equal to the length of a sarcomere. The outer compartment of these swollen mitochondria appear to be normal, whereas extensive enlargement of the inner compartment produced large, pale matrices. The cristae of the swollen mitochondria were sparse and disoriented. A curved and circular configuration can be seen in a few cristae, most of which are too short to extend across the increased width of the inner mitochondrial compartment. In contrast, no significant alterations were observed in skeletal muscle structure in the group sacrificed immediately after a swim to exhaustion (Fig. 1B). Tissue structure in these animals was intact, and no change in shape or size of the mitochondria was apparent.

The concentration of mitochondria in the gastrocnemius muscle from the untrained group sacrificed immediately after a swim to exhaustion, expressed either as number of mitochondria per 100 μ² or per 100 sarcomeres, was similar to that of the control group (Table 1). By comparison, skeletal muscle from the trained rats sacrificed 24 hr after a final exercise bout contained significantly (P < 0.01) more mitochondria (per 100 μ² or per 100 sarcomeres) than either of the untrained groups. Muscle from the trained rats sacrificed immediately after a run to exhaustion contained more (P < 0.05) mitochondria per 100 μ² than the untrained rats, but fewer (P < 0.05) than muscle from the trained group sacrificed at rest. However, these mitochondrial concentrations were about the same for both trained groups when expressed on the basis of 100 sarcomeres of tissue.

Glycogen concentration of liver and skeletal muscle are summarized in Table 2. These data show that the glycogen content of the gastrocnemius muscles for the trained rats sacrificed 24 hr after a final exercise bout was significantly (P < 0.01) higher than that of the untrained controls. Liver and biceps muscles were unaltered by the training program. Exercise prior to death significantly reduced the glycogen level in all the tissues assayed, with the greatest depletion occurring in those animals that became exhausted while running.

DISCUSSION

The increased number and the apparent increase in size and cristae concentration of the mitochondria in skeletal muscle of trained rats suggests that metabolic capacity of this tissue can be enhanced by training. In fact, Holloszy (17) has reported that oxygen uptake, enzyme activity, and total protein of the mitochondrial fraction of rat skeletal muscle are increased after a training program of strenuous running. Similar metabolic adaptations, however, have not been found in the skeletal muscle of rats after training programs of swimming 30 min per day for 5-8 weeks (12, 14, 17). The lack of an adaptation in skeletal muscle after training by swimming has been attributed to the fact that it is a relatively mild work load for the rat and one for which the energy requirements can be adequately met with the normal metabolic apparatus. However, single bouts of exhaustive swimming have been shown to increase the mitochondrial mass of dog (25) and rat (24) myocardium. Furthermore, Arcos et al. (1) have reported increases in the mitochondrial mass of rat myocardium after a training program of swimming up to 6 hr per day. Thus, swimming of sufficient duration can produce alterations in mitochondria, at least those in the heart.

The mechanism(s) producing the increase in mito-
FIG. 1. Electron micrographs of longitudinal sections (×11,000) of skeletal muscle from A control animal killed at rest, B untrained animal sacrificed after exhaustive swim, C trained animal sacrificed 24 hr after exhaustive running, and D trained rat sacrificed immediately after running to exhaustion. Tissue in A, B, and C appears to be normal with exception of size and number of mitochondria in C. Skeletal muscle in D shows the existence of mitochondrial swelling, cristae degeneration, and general tissue edema. (See facing page for C and D.)

Mitochondrial concentration of skeletal muscle during training is unknown. However, it is generally believed that mitochondria increase in number by division of pre-existing mitochondria (27, 28). Laguens and Gomez-Dumm (24) have observed invaginations in myocardial mitochondria of rats after single exercise bouts which they have interpreted as a mechanism for replication of internal components of the mitochondrion. Some of the micrographs obtained in this study contained mitochondria with marked evaginations (Fig. 2). One interpretation of these micrographs is that such mitochondria were in the process of dividing. It is known that...
thyroid hormone can stimulate mitochondrial replication (13). However, while it has been suggested that thyroxine production increases during training (20), direct evidence of such an effect is lacking at this time (2, 3). In fact, Tipton and co-workers (45) have found that thyroidectomized rats can be trained at work levels similar to those employed in this study without any apparent impairment of work capacity. Furthermore, mitochondrial swelling and partial uncoupling of oxidative phosphorylation with a partial or complete loss of respiratory control usually accompanies hyperthyroidism (13, 29, 41). It should be pointed out that the mitochondria in the muscles of the trained rats sacrificed at rest in this study were not swollen. In addition, Holloszy (17) did not observe any swelling or loss of respiratory control in the mitochondria isolated from skeletal muscle of trained rats sacrificed at rest.

The swelling of the mitochondria in the skeletal muscle of the trained animals after running to exhaustion is distinctly different from the increased size of myocardial

FIG. 1 C and D
of calcium ion, inorganic phosphate, free fatty acids, sulfhydryl compounds, oxytocin, vasopressin, growth hormone, insulin, and the adenine nucleotides (26). Changes in some of these substances during exhaustive running may also have been involved in producing the mitochondrial swelling seen in this investigation. Of these, the adenine nucleotides may have been particularly important, since it is known that the concentration of ATP in muscle decreases during exercise while ADP, AMP, and inorganic phosphate increase (18, 38). This may be particularly relevant when related to the finding of Vogell and associates (46), that a sharp drop in ATP and a rise in AMP accompanied mitochondrial swelling in rabbit myocardium during asphyxia. In this study, indirect evidence for a reduced ATP level in the skeletal muscle of the rats exhausted by running was a very rapid onset of rigor after death. In addition, micrographs of this tissue showed it to be in various stages of contraction. It is also known that calcium ion is taken up during muscular contraction (4), and that both growth hormone and free fatty acid levels increase in blood during exercise (9, 19, 35).

Swelling was not limited to those mitochondria located in the interior of the muscle fibers but also was observed in mitochondria located immediately under the sarcolemma (Fig. 3). However, in these mitochondria, cristae configuration was not as disrupted as in those located deeper within the muscle. The reason for this difference is not clear but could be related to differences in the availability of oxygen or substrates at the different sites. This may be particularly true if hypoxia is the prime cause of the mitochondrial swelling.

What effect the mitochondrial swelling may have had on the metabolic capacity of the skeletal muscle is unknown. However, it is well known that isolated mitochondria disruption of the basic structural configuration such as occurs with swelling can loosen or completely uncouple oxidative phosphorylation (37, 48). The work capacity of the muscle would probably be reduced if these changes were to occur during exercise. Postexercise oxygen uptake might also be adversely affected if such a loss of respiratory control were to occur.

In addition to the mitochondrial swelling, a general edema existed in the skeletal muscle of the animals run to exhaustion. This edema was characterized by the presence of large clear interfibrillar spaces (Figs. 1D and 2) not seen in normal tissue. Jacobsson and Kjellmer (21, 22) have previously observed swelling in the skeletal muscle of the cat following exercise. They attributed this swelling to an increased accumulation of lymph resulting from an elevated transcapillary pressure. Edema similar to that observed in this study has also been found in heart muscle following asphyxia (46). However, all of the alterations in the skeletal muscle resulting from the exhaustive run appear to have been reversible, since they were not evident in the animals killed after a 24-hr recovery period.

The edema and the fact that the tissue was not in a uniform state of relaxation at the time of fixation produced

### Table 1. Effect of exercise and training on concentration of mitochondria in skeletal muscle of rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Unexercised Control Group</th>
<th>Control Animals Sacrificed at Exhaustion</th>
<th>Trained Animals Sacrificed at Exhaustion</th>
<th>Trained Animals Sacrificed at Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>R</td>
<td>A</td>
<td>R</td>
</tr>
<tr>
<td>Liver</td>
<td>36.85土2.58</td>
<td>51.40土5.45</td>
<td>18.60土2.73</td>
<td>9.58土1.92</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>3.73土0.38</td>
<td>6.31土0.52*</td>
<td>1.31土0.35†</td>
<td>0.62土0.15†</td>
</tr>
<tr>
<td>Biceps muscle</td>
<td>3.95土0.30</td>
<td>2.38土0.50</td>
<td>1.41土0.17†</td>
<td>0.16土0.10†</td>
</tr>
</tbody>
</table>

Values are concentrations in milligrams per gram weight ± SEM. * Trained animals sacrificed at rest vs. control animals sacrificed at rest P < 0.01. † Animals sacrificed at exhaustion vs. animals sacrificed at rest P < 0.01. ‡ Trained animals sacrificed at exhaustion vs. untrained animals sacrificed at exhaustion P < 0.05.

### Table 2. Immediate and chronic effects of exercise on glycogen concentrations of rat liver and skeletal muscles

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Unexercised Control Group</th>
<th>Control Animals Sacrificed at Rest</th>
<th>Trained Animals Sacrificed at Exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>64.4土2.1</td>
<td>72.1土3.1</td>
<td></td>
</tr>
<tr>
<td>Gastrocnemius muscle</td>
<td>47.8土2.2</td>
<td>63.2土4.5</td>
<td></td>
</tr>
<tr>
<td>Biceps muscle</td>
<td>50.1土2.5</td>
<td>74.5土5.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of counts from 20 micrographs. Values are mitochondrial counts per 10 ~2 (column A) and per 100 sarcomers (column B). * Trained groups (sacrificed at exhaustion vs. control groups (sacrificed at rest or exhaustion) P > 0.05. † Trained groups (sacrificed at rest or exhaustion) vs. control groups (sacrificed at rest or exhaustion) P > 0.01. ‡ Trained groups sacrificed at exhaustion vs. trained groups sacrificed at rest P > 0.05.

mitochondria reported by Laguens and Gomez-Dumm (24) after a single exhaustive swim. As can be seen in Figs. 1D and 2, the cristae in the mitochondria of these muscles had separated from each other to the extent that there were spaces of varying size between adjacent cristae, thereby giving these mitochondria a pale appearance. In many instances the cristae appear to have degenerated, with the mitochondrial matrices being filled with substances of varying density. The magnitude and general characteristic of these mitochondrial changes are similar to those seen in the heart and liver of the dog, rabbit, and rat after permanent anoxia, ischemia, and acute and chronic hypoxia (3-7, 15, 16, 23, 30-32, 40, 46). These similarities suggest that hypoxia may have been a factor leading to the mitochondrial swelling that occurred in the skeletal muscle during exhaustive running.

Swelling can be induced in isolated mitochondria by a variety of factors including changes in the concentration...
marked variations in the amount of tissue in the area of the micrographs. Therefore, it seemed appropriate to express mitochondrial concentrations on the basis of the number per 100 sarcomeres in addition to the number per 100 $\mu^2$. The importance of this evaluation is evident in Table 1 where, when expressed as mitochondria per 100 $\mu^2$, it would appear that a decrease in mitochondrial concentration occurred following exhaustive running.

**FIG. 2.** Longitudinal section (X11,000) of muscle tissue from a rat exhausted by running, showing a swollen mitochondrion (upper right) with an evagination. Outer mitochondrial membrane appears to be continuous between the two structures. Evagination may contain developing cristae.

**FIG. 3.** Electron micrograph (X11,000) of a mitochondrial cluster located immediately beneath the sarcolemma in skeletal muscle from a trained rat sacrificed at exhaustion. These mitochondria did not exhibit extensive swelling and cristae degeneration as occurred deeper in the fibrils.
whereas this was not the case on a sarcomere or actual tissue basis.

Failure to find any significant change in the fine structure of rat skeletal muscle following an exhaustive swim may indicate either that this exercise is relatively mild, as suggested by Holloszy (17), or that the gastrocnemius muscle does not contribute much to this activity. An alternate possibility is that skeletal muscle is more resistant to tissue damage of the type seen in the heart after exercise. The differences in the depletion of glycogen after swimming may have been a mild workload; however, the intensity of running used in this study is a strenuous exercise for the rat. This judgment is based on the previous finding that the colonic temperature of the rat increases as a function or work load at these speeds and may exceed 41°C at the point of exhaustion (10). In addition, earlier studies with rats have shown that similar training programs have produced adaptations such as increased work capacity and enhanced metabolic activity (17), increased ligament strength (43, 44), resting bradycardia (42), and a decrease in the cardiac acceleration following atrophic injection (11, 42).

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REFERENCES