Skeletal muscle respiratory capacity, endurance, and glycogen utilization

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Fitts, R. H., F. W. Booth, W. W. Winder, and J. O. Holloszy. Skeletal muscle respiratory capacity, endurance, and glycogen utilization. Am. J. Physiol. 228(4): 1029-1033. 1975.—This study was undertaken to evaluate the relationship between physical performance capacity and the mitochondrial content of skeletal muscle. Four groups of rats were trained by means of treadmill running 5 days/wk for 13 wk. One group ran 10 min/day, a second group ran 30 min/day, a third group ran 60 min/day, and a fourth group ran 120 min/day. The magnitude of the exercise-induced adaptive increase in gastrocnemius muscle respiratory capacity varied over a twofold range in the four groups. There were significant correlations between the levels of three mitochondrial markers (cytochrome c, citrate synthase, respiratory capacity) in the animals' gastrocnemius muscles and the duration of a run to exhaustion. There was also a significant correlation between the amounts of glycogen remaining in liver and skeletal muscle after a 30-min-long exercise test and the respiratory capacity of the animal's leg muscles. These findings are compatible with the interpretation that a close relationship exists between skeletal muscle mitochondrial content and the capacity to perform endurance exercise.

Methods

Animal care and exercise programs. Six-week-old male rats of a Wistar strain (specific-pathogen-free CFN rats, Carworth) were housed in individual cages and maintained on a diet of Purina chow and water. They were separated into six groups. Four of the groups were trained for 5 days/wk on a motor-driven treadmill set at a 15% grade and a speed of 1.2 mph (14). The length of the training sessions was progressively increased, but to different final durations, for the four groups. The final duration of the exercise session was 10 min for the group designated T10, 30 min for the group designated T30, 60 min for the group designated T60, and 120 min for the group designated T120. The hypothesis on which this experimental design was based was that the magnitude of the exercise induced adaptive increase in muscle mitochondrial could be varied by varying the duration of the training sessions. One sedentary group, designated free eaters, was allowed to eat ad libitum. A second sedentary group, designated fed, was fed ad libitum.

Exercise tests. An endurance exercise test, in which the animals run to exhaustion, was administered at the end of the 12th wk of training. The work load for the initial 10 min of the run was 1.2 mph up a 15% grade, after which it was increased to 1.5 mph up a 15% grade for the remainder of the run. In this study, exhaustion was defined as the point when animals seemed unable to keep pace with the treadmill and avoid the shock grid at the rear of the treadmill. To avoid bias, a person who was not aware of the degree of training of the individual rats was assigned the task of determining the point of exhaustion. Blood was obtained from the rats' tails at the end of the run.

After 13 wk of training, a 30-min-long exercise test was administered for the purpose of determining the response of skeletal muscle and liver glycogen stores to a standardized bout of exercise. This test was administered between 9 and 11 A.M., 48 h following the last training session. Rats were not fasted prior to the test. Animals from each of the four trained groups were separated into a resting group, which
was sacrificed without running on the day of the test, and an exercised group, which was sacrificed after running a total of 30 min, with the first 15 min at 1 mph up a 20% grade and the second 15 min at 1.2 mph up a 25% grade. The rats were anesthetized with 6 mg/100 g body wt of Na pentobarbital given intraperitoneally immediately following completion of the exercise test.

Tissue preparation and assay methods. Approximately 5 min following administration of the anesthetic, the right gastrocnemius muscle was dissected free and quick-frozen with Wollenberger tongs cooled in liquid nitrogen (32). One lobe of the liver was then frozen. Next, the rats were exsanguinated via the abdominal aorta. Next, the left gastrocnemius muscle was excised, trimmed free of connective tissue, and used for preparation of a homogenate in 175 mM KCl, 10 mM glutathione, 2 mM EDTA, pH 7.4. After removal of an aliquot of this homogenate for measurement of respiratory capacity, the remainder was frozen for determination of citrate synthase activity.

Respiratory capacity of the fresh whole homogenate was determined manometrically in a Gilson differential respirometer at 30°C, in the presence of nonlimiting amounts of P_i and ADP, with pyruvate plus malate as substrates, as described previously (14).

Citrate synthase activity was determined by the method of Srere (27) with the use of 5,5'-dithiobis (2-nitrobenzoic acid). Gastrocnemius muscle homogenates were frozen and thawed 3 times prior to the assay.

Cytochrome c concentration of gastrocnemius muscle was determined by the method of Williams and Thorp (30).

Glycogen concentrations were determined on the quick-frozen muscle and liver samples with the use of anthrone reagent as described by Ihasid and Abraham (11).

Blood glucose levels were determined on Nelson-Somogyi filtrates of whole blood by the enzymatic method of Stein (26). Blood lactate levels were determined on perchloric acid extracts of whole blood by the enzymatic method of Hohorst (13).

Analyses of variance were conducted, and significant differences among group means were determined at the P < 0.05 level, with Duncan's procedure (28). The Student t test was used to compare two group means independent of other groups. The correlation between two variables was determined by means of a linear regression analyses (28).

RESULTS

No significant differences with respect to any of the biochemical variables measured were observed between the paired weight and the freely eating sedentary animals. We have, therefore, combined the biochemical data from these two groups.

Mitochondrial adaptations. The respiratory capacity of gastrocnemius muscle, as reflected in the rate of O_2 uptake by whole-muscle homogenates, in the presence of nonlimiting amounts of ADP and P_i, with pyruvate plus malate as substrates, increased in proportion to the duration of the daily sessions of running (Table 1). The magnitude of the increases in citrate synthase activity and in the concentration of cytochrome c also increased with the duration of the daily training sessions (Table 1).

Endurance run to exhaustion. The average durations of the run to exhaustion for the four groups of trained animals are shown in Table 1. Blood glucose concentrations after the run to exhaustion were 76 mg/100 ml for the T_10, 60 mg/100 ml for the T_30, 57 mg/100 ml for the T_60, and 61 mg/100 ml for the T_120 group. Blood lactate levels were not significantly elevated above control in any of the groups after the run to exhaustion.

Correlation between endurance and muscle respiratory capacity. Endurance, as reflected in the duration of the run to exhaustion, was significantly correlated (P < 0.001) with gastrocnemius muscle cytochrome c concentration and citrate synthase activity and with the O_2 uptake capacity of gastrocnemius muscle homogenates. Figure 1 shows the relationship between endurance and the cytochrome c concentration (A) in gastrocnemius muscle (correlation coefficient of 0.79) and the O_2 uptake capacity (B) of gastrocnemius (correlation coefficient of 0.69).

Thirty-minute-long exercise test. The running programs resulted in significant glycogen supercompensation in the gastrocnemius muscle, but not in the liver, in all four trained groups (Table 2). No significant differences were present between any of the resting exercise-trained groups with respect to muscle or liver glycogen concentration (Table 2). There was a trend for the amount of glycogen depleted from gastrocnemius muscle to vary inversely with the duration of training, but, because of the limited range of the changes in glycogen concentration, the intergroup differences are small. A striking effect of the level of training on the rate of glycogen depletion was seen in the liver, with, at the extreme, no depletion of liver glycogen stores in the T_120 group, and, at the other extreme, an approximately 70% decrease in liver glycogen in the T_10 group.

Blood glucose values were not low in any of the rats following the exercise test, averaging 123 ± 8 mg/100 ml in the T_10, 120 ± 18 mg/100 ml in the T_30, 141 ± 16 mg/100 ml in the T_60, and 161 ± 18 mg/100 ml in the T_120 group compared to an average resting value of 160 mg/100 ml. Post-exercise blood lactate levels were not significantly different from resting levels except in the T_10 group in which the

<table>
<thead>
<tr>
<th>Groups</th>
<th>Malate-Pyruvate Oxidation, pmol/g per min</th>
<th>Citrate Synthase, nmol/g per min</th>
<th>Cytochrome c, nmol/g</th>
<th>Run Time to Exhaustion, min</th>
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</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>36.6 ± 0.8* (16)</td>
<td>20.0 ± 0.7* (16)</td>
<td>10.0 ± 0.5* (13)</td>
<td>1.39 (16)</td>
</tr>
<tr>
<td>T_10</td>
<td>39.6 ± 1.3 (12)</td>
<td>22.9 ± 1.0 (12)</td>
<td>11.6 ± 0.7 (7)</td>
<td>1.22 (12)</td>
</tr>
<tr>
<td>T_30</td>
<td>44.5 ± 3.2 (6)</td>
<td>31.4 ± 2.7 (6)</td>
<td>13.1 ± 1.1 (5)</td>
<td>1.11 (6)</td>
</tr>
<tr>
<td>T_60</td>
<td>57.1 ± 2.0† (8)</td>
<td>37.3 ± 2.4† (8)</td>
<td>19.2 ± 0.7 (5)</td>
<td>1.11 (8)</td>
</tr>
<tr>
<td>T_120</td>
<td>75.6 ± 2.3† (12)</td>
<td>45.5 ± 2.3† (12)</td>
<td>10.6† (8)</td>
<td>1.16† (12)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Number of animals per group is given in parentheses. * Sedentary significantly lower than T_10, T_30, and T_60, P < 0.05. † Significantly different from all other exercised groups, P < 0.05.
FIG. 1. Correlations between endurance and (A) concentration of cytochrome c in gastrocnemius muscle, and (B) respiratory capacity of gastrocnemius muscle as reflected in rate of O2 uptake by whole homogenates in presence of nonlimiting amounts of ADP, Pi, and pyruvate.

TABLE 2. Glycogen depletion in gastrocnemius muscle and liver in four trained groups during 30-min-long exercise test

<table>
<thead>
<tr>
<th>Group</th>
<th>Glycogen, mg/g wet wt</th>
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<tbody>
<tr>
<td></td>
<td>Gastrocnemius</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
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<tr>
<td>Sedentary</td>
<td>8.0 ± 0.3*</td>
</tr>
<tr>
<td>T10</td>
<td>10.4 ± 0.3</td>
</tr>
<tr>
<td>T30</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>T60</td>
<td>10.4 ± 0.8</td>
</tr>
<tr>
<td>T120</td>
<td>10.0 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE for four animals except for T15 in which n = 3. * Significantly different from all other groups, P < 0.05.

blood lactate concentration averaged 7.49 ± 1.00 μmol/ml compared to a resting value of 2.16 ± 0.66 μmol/ml.

Relationship between glycogen utilization and muscle respiratory capacity. As shown in Fig. 2A, there was a significant correlation (P < 0.02) between the respiratory capacity of gastrocnemius and the concentration of glycogen remaining in the gastrocnemius after the standardized exercise test. There was also a significant correlation (P < 0.001) between the respiratory capacity of the gastrocnemius and the concentration of glycogen remaining in the liver after the exercise test (Fig. 2B). Since the concentrations of glycogen in muscle and liver were not significantly different in resting animals in the four trained groups, we interpret the relationships shown in Fig. 2, A and B, to indicate that there is an inverse relationship between the amount of glycogen depleted from liver and muscle during the exercise test and the respiratory capacity of the animal's leg muscles. On examination of the raw data, it appeared that, in groups T10, T30, and T60, those rats that had the greatest degree of muscle glycogen depletion had the least hepatic glycogen depletion and vice versa, suggesting that depletion of total-body carbohydrate stores might be a more meaningful measurement, in the present context, than either muscle or liver glycogen concentration. We, therefore, estimated the total amount of glycogen remaining in muscle plus liver in each of the rats that had been subjected to the exercise test. As shown in Fig. 2C, there is an excellent correlation (P < 0.001) between the respiratory capacity of gastrocnemius muscle and the total amount of glycogen remaining in the liver plus the muscle after the exercise test. There was a similar close relationship (r = 0.91, P < 0.001) between gastrocnemius cytochrome c concentration and total liver plus muscle glycogen remaining after the exercise test. The estimation of total muscle glycogen was based on the assumption that the weight of the muscles involved in the exercise was 60 g and that the response to the exercise test of the glycogen in this mass of muscle paralleled that seen in the gastrocnemius. The value of 60 g was obtained by weighing the muscles of the fore- and hindlimbs, as well as those muscles that cross the hip or the shoulder joints, in rats weighing approximately 350 g.

DISCUSSION

It was possible, in the present study, to vary the magnitude of the increase in skeletal muscle mitochondria induced by exercise over a twofold range in rats by varying the duration of their exercise sessions between 10 and 120 min per day. This wide range in adaptive response made it possible to correlate the levels of a number of mitochondrial markers, which were used as indicators of the concentration of mitochondria in skeletal muscle, with endurance and with
glycogen depletion during exercise. In contrast to the report by Barnard and Peter (4), a highly significant positive correlation was found between how long the animals could run before they became exhausted and the levels of each of the mitochondrial markers measured in the present study.

In the study by Barnard and Peter (4), guinea pigs were exercised on a treadmill, and the intensity of the training was increased each week until the 9th wk. This program resulted in a 39% increase in cytochrome c concentration. Groups of three to five animals were tested and killed after 0, 3, 6, 9, and 12 wk. An increase in the duration of the run to exhaustion from 9 to 33 min occurred during the first 3 wk of training, while cytochrome c in gastrocnemius muscle did not increase significantly. One factor that may have played an important role in this increase in running time probably relates to the fact that naive rodents will not exercise to exhaustion on a treadmill but have to first be thoroughly familiarized with treadmill running. Since the pre-training group was exercised on the treadmill at a slow pace for 5–10 min only 3 times prior to the exercise test, we think it likely that these animals stopped running after 9 min for reasons other than exhaustion. Additional factors such as an improvement in cardiac function and adaptive changes in the neuroendocrine system may also have contributed to the 3-wk trained group's longer run time. Between 3 and 6 wk of training, significant increases occurred in both run time to exhaustion and gastrocnemius cytochrome c. However, no changes occurred thereafter; there were no significant differences in cytochrome c concentration or run time to exhaustion between the groups tested after 6, 9, and 12 wk. Clearly, in order to demonstrate a correlation between cytochrome c concentration and endurance on a treadmill, a wide range of cytochrome c concentrations is needed, and all the animals tested must be thoroughly familiar with treadmill running. Barnard and Peter's (4) study did not meet these criteria. This may explain why they did not find a significant correlation between these variables.

The wide variation in the duration of the run to exhaustion of animals in the same groups in the present study, as reflected in the large standard errors of the means (Table 1), points to a problem in the use of a run to exhaustion to evaluate the exercise capacity of rodents. Since determination of the end point is largely a subjective judgment made by the experimenter, it is often difficult to be sure that an animal has quit because of exhaustion rather than for some other reason. It, therefore, seems useful to also employ standardized exercise tests with fixed end points, in which the objective measurement of differences in metabolic response can be used to evaluate the level of training.

In the present study, glycogen depletion was the response used to evaluate the relative metabolic stress of the standardized, 30-min-long exercise test. The concentrations both of liver and of gastrocnemius muscle glycogen remaining after the test were significantly correlated with the respiratory capacity of skeletal muscle. However, the highest correlation was between total glycogen remaining in the liver plus the working muscles after exercise and leg muscle respiratory capacity. Since there were no significant differences between the four trained groups in the concentrations of liver and of muscle glycogen at rest, it seems clear that carbohydrate utilization during exercise was inversely correlated with the concentration of mitochondria in the animal's leg muscles. Since all the animals performed the same amount of work and, therefore, expended similar amounts of energy, it seems reasonable that the animals that utilized less carbohydrate must have utilized proportionally more fat, and vice versa. We have, so far, been unable to surmount the technical difficulties involved in measuring O2 consumption and respiratory quotient in our rats during exercise, and cannot, therefore, calculate the actual amounts of fat oxidized. However, it seems inescapable from our results that there must be a positive correlation between the percentage of the total energy expenditure derived from fatty acid oxidation and the concentration of mitochondria in the working skeletal muscles in rats performing the 30-min-long exercise test used in this study.

There is considerable evidence that the oxidation of fatty acids inhibits the utilization of carbohydrates (21–23). It is also well documented that trained individuals oxidize more fat and less carbohydrate than untrained when performing submaximal work of the same absolute intensity (7, 12, 18). In a recent study in this laboratory (unpublished data) it was found that rats trained by means of a program of swimming had a slower depletion of glycogen in the different types of skeletal muscle and in liver during a standardized treadmill test than did untrained controls. The results of the present study show that the magnitude of this glycogen sparing effect of training is correlated with the increases in muscle respiratory capacity. The most highly trained animals in the present study (T120) demonstrate an extreme degree of this glycogen-conserving effect of exercise training. It can be estimated that the T120 group, which showed minimal muscle glycogen depletion, no liver glycogen depletion, and the highest postexercise blood glucose levels of any of the groups, utilized approximately 120 mg of glucose during the 30-min exercise test. At the other extreme, the least-trained (T16) group utilized more than 380 mg of glucose while performing the same amount of work.

While it seems clear that animals with the highest levels of mitochondria in their skeletal muscles utilize the least carbohydrate during submaximal work, the mechanism by which hepatic glycogenolysis and glucose release are closely matched to glucose utilization by the muscles are not known. Achou and Hetenyi (1) concluded from cross-circulation experiments that the overall rate at which glucose is utilized by the tissues regulates the rate of hepatic glucose release, but provided no information regarding the mechanisms by which this control is mediated. However, in light of what is known regarding the regulation of hepatic glycogenolysis, it seems likely that the final steps in the regulatory pathway involve hormonally induced alterations in the levels of cyclic AMP in the liver (25). It is of interest in this context that plasma catecholamine levels increase less (10) and that insulin levels decrease less (10) during exercise at the same absolute work level in the physically trained as compared to the untrained state.

In conclusion, our finding of a significant correlation between skeletal muscle respiratory capacity and endurance does not, of course, prove a cause-and-effect relationship. However, as discussed in detail previously (15, 16), a strong case can be made in support of the view that the exercise-induced increase in skeletal muscle mitochondria is re-
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REFERENCES


