Effects of exercise on cardiac weight and mitochondria in male and female rats

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OSCAI, L. B., P. A. MOLÉ, AND J. O. HOLLOSZY. Effects of exercise on cardiac weight and mitochondria in male and female rats. Am. J. Physiol. 220(6): 1944-1948. 1971.—Female rats that swam for 6 hr daily, 6 days/week, gained weight at the same rate as sedentary freely eating controls. Male rats subjected to the same exercise program gained weight significantly more slowly than sedentary freely eating controls. This difference is explained by the finding that the female swimmers are significantly more than their sedentary controls while the male swimmers did not increase their food intake. The hearts of the female swimmers were significantly heavier than those of their sedentary freely eating controls. The hearts of the male swimmers were not heavier than those of their sedentary freely eating controls; however, their hearts were significantly heavier than those of sedentary paired weight animals. The exercise program had no effect on the levels of activity, expressed per gram of ventricular muscle or per milligram of protein, of cytochrome oxidase, succinate oxidase, citrate synthase, and mitochondrial malate dehydrogenase. The concentrations of cytochrome c and of mitochondrial protein, expressed per gram of ventricular muscle were also unchanged.

METHODS

Animals care and exercise program. Rats of a Wistar strain (specific pathogen-free CFN rats) were obtained from Carworth Farms, kept in individual cages, and maintained on a diet of Purina chow and water. In the first experiment, 10-week-old female rats were divided into two groups of equal weight and provided with food and water ad libitum. One group was kept sedentary, the other was exercised using the swimming program described by Arcos et al. (1). The rats swam for 30 min on the 1st day, and the duration of the swim was increased 30 min daily until they were swimming continuously for 6 hr/day, 6 days/week. They were maintained at this work load until they had completed an average of 162 hr of swimming. Water temperature was maintained at 33°C. In the present study, each swimming session was started at approximately 9:00 AM. Exercising animals and their controls were killed over a 6-day period. The second experiment involved both male and female rats. Six-week-old female rats were divided into an exercising and a sedentary group of equal weight and provided with food and water ad libitum. Six-week-old male rats were divided into an exercising and two sedentary groups. A freely eating sedentary group was provided with food and water ad libitum. A paired-weight sedentary group had their food intake restricted so as to maintain their body weights the same as those of the male exercisers. Both the female and male exercising groups were subjected to the same swimming program that was used in the first experiment.

Tissue preparation and assay methods. Animals were anesthetized with ether and decapitated 18–24 hr after their last bout of exercise. The heart was excised, and the great vessels and valves were trimmed away. The ventricles and atria were cut open, rinsed free of blood in ice-cold Ringer solution, blotted, and weighed. The atria were removed, and the ventricles were minced with scissors and used in the procedures described below. Homogenates of ventricular muscle were prepared in 0.175 M KCl containing 0.1 mM EDTA, using a glass Potter-Elvehjem homogenizer at 4°C. The homogenates contained 1 g of ventricle per 20 ml.

Mitochondria were prepared by centrifuging the homogenate for 15 min at 700 X g; the supernatant fluid was decanted and centrifuged again at 700 X g for 15 min. The 700 X g supernatant fluid was then centrifuged for 15 min...
at 14,000 × g. The resulting pellet was suspended in 0.25 M sucrose.

Oxygen uptake was measured in a Gilson differential respirometer, at 30°C, with air as the gas phase. Respiration in the presence of nonlimiting amounts of Pi and ADP, with pyruvate plus malate as substrate, was measured as described previously (4, 9), using whole homogenates instead of isolated mitochondria to avoid possible differences in mitochondrial yield.

Succinate oxidase and cytochrome oxidase activities were measured manometrically as described by Potter (16).

Oxygen uptakes are expressed as microliters of O2 under standard conditions.

Spectrophotometric assays were performed in a Gilford model 240 spectrophotometer with a thermostated cell compartment in 1-ml cuvettes of 1-cm light path at 30°C. Assays were performed under conditions in which the reaction rate was proportional to enzyme concentration.

Mitochondrial malate dehydrogenase activity was measured by following the rate of oxidation of DPNH at 340 μm in a reaction mixture containing 50 mM potassium phosphate buffer, pH 7.4, 0.36 mM oxaloacetate, and 0.2 mM DPNH. Mitochondrial and cytoplasmic malate dehydrogenases were distinguished as described by Shonk and Boxer (20).

Citrate synthase activity was assayed as described by Scree (21), using 5,5-dithiobis (2-nitrobenzoic acid) (DTNB).

Enzyme activities are reported as micromoles of substrate utilized per minute.

The concentration of cytochrome c was determined by the method of Williams and Thorp (25).

Protein was measured by the biuret method (6).

RESULTS

First experiment—response of female rats to 6 hr of daily swimming. The female swimmers gained weight at approximately the same rate as their sedentary controls, which were permitted unrestricted access to food. This was made possible by a voluntary increase in food intake by the swimmers, which took in an average of 75 ± 1 calories/day compared to 61 ± 2 calories/day for the sedentary animals (P < 0.001). As shown in Table 1, the swimming program produced significant cardiac hypertrophy. The concentration of protein in the myocardium was not significantly different in the swimmers and their sedentary controls. There was no change in the levels of activity of succinate oxidase and mitochondrial malate dehydrogenase or in the concentration of mitochondrial protein in the hearts of the swimmers (Table 2).

Second experiment—response of male and female rats to 6 hr of daily swimming. The second group of female swimmers also gained weight at the same rate and had significantly heavier hearts than their sedentary freely eating controls (Table 3). As before, the female swimmers had a significantly greater food consumption than the sedentary female rats.

In contrast, the male swimmers gained weight more slowly and had a significantly lower final body weight than their sedentary freely eating controls (Table 3). The hearts of the male swimmers were lighter than those of the seden-

<table>
<thead>
<tr>
<th>Table 1. Body weights, heart weights, and ratios of heart weight to body weight of female rats in first experiment</th>
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<tr>
<td><strong>Swimmers</strong></td>
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<tr>
<td>Initial body wt, g</td>
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<tr>
<td>Final body wt, g</td>
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<td>Heart wt, mg</td>
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<td>Heart wt/body wt, mg/g</td>
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Values are expressed as means ± SEM. There are 12 animals per group. FE = freely eating. * Swimmers minus sedentary controls.

<table>
<thead>
<tr>
<th>Table 2. Effects of exercise program on succinate oxidase, MMDH, and mitochondrial protein levels in hearts of female rats in first experiment</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>Swimmers</td>
</tr>
<tr>
<td>Male</td>
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</tbody>
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Values are expressed as means ± SEM per gram of fresh muscle. There were 12 animals per group. FE = freely eating. * MMDH = mitochondrial malate dehydrogenase.

<table>
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<th>Table 3. Body weights, heart weights, and ratios of heart weight to body weight of female and male rats in second experiment</th>
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<td><strong>Group</strong></td>
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<tr>
<td>Female</td>
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<td>Male</td>
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Values are means ± SEM. Number of animals per group is given in parentheses. PW = paired weight. FE = freely eating. * Significantly different from female swimmers, P < 0.01. † Significantly different from male swimmers, P < 0.01.
TABLE 4. Cytochrome oxidase and citrate synthase activities and cytochrome c and mitochondrial protein concentrations in hearts of male and female swimmers and sedentary controls in second experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Cytochrome Oxidase, $\mu$O$_2$/min per g</th>
<th>Citrate Synthase, amoles/min per g</th>
<th>Cytochrome c, amoles/g</th>
<th>Mitochondrial Protein, mg/g</th>
</tr>
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<tbody>
<tr>
<td>Female</td>
<td>Swimmers</td>
<td>2367 ± 255 (6)</td>
<td>160 ± 4 (8)</td>
<td>49.5 ± 0.5 (6)</td>
<td>14.8 ± 0.4 (6)</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>2328 ± 117 (6)</td>
<td>156 ± 4 (8)</td>
<td>47.8 ± 0.9 (6)</td>
<td>15.3 ± 0.2 (6)</td>
</tr>
<tr>
<td>Male</td>
<td>Swimmers</td>
<td>2392 ± 142 (6)</td>
<td>150 ± 4 (8)</td>
<td>44.8 ± 1.6 (6)</td>
<td>15.3 ± 0.6 (6)</td>
</tr>
<tr>
<td></td>
<td>Sedentary</td>
<td>2247 ± 115 (6)</td>
<td>146 ± 4 (8)</td>
<td>54.5 ± 1.2 (6)</td>
<td>15.0 ± 0.8 (9)</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SEM per gram of muscle. Number of animals is given in parentheses.

swimmers, compared to 124 ± 7 µl O$_2$/min per gram for six male sedentary rats, 127 ± 4 µl O$_2$/min per gram for six female swimmers, and 123 ± 5 µl O$_2$/min per gram for six female sedentary controls.

The levels of activity per gram of ventricular muscle (or per milligram of protein) of cytochrome oxidase and of citrate synthase were similarly unaffected by the swimming program (Table 4). There were also no significant differences between the exercised and the sedentary animals in the concentrations of cytochrome c or of mitochondrial protein in ventricular muscle (Table 4).

DISCUSSION

In previous studies in this laboratory, male rats that exercised vigorously gained weight more slowly and had significantly lower final body weights than freely eating sedentary controls (2, 9, 15). In addition to increasing caloric expenditure, vigorous exercise was found to have an appetite-suppressing effect in male rats (2). In the present study, male rats which swam for 6 hr daily also had significantly lower body weights than freely eating sedentary controls. However, in contrast to our previous studies, this effect of exercise was mediated solely by increased caloric expenditure, as no appetite suppression occurred. These findings are in keeping with those of Stevenson et al. (23) who found that 30–60 min of running resulted in appetite suppression in male rats, while 4 hr of swimming did not.

It seems likely that this difference is related to the duration and severity of the exercise. Thus, very prolonged light exercise, as in the present study, does not suppress appetite, while a more severe exercise stress of shorter duration does. It is our working hypothesis that appetite suppression induced by exercise is mediated by the increased levels of catecholamines associated with the stress of exercise (17, 24). It seems reasonable that the stress resulting from exercise must vary not only with workload, but also with the condition of the exercising animals. Thus, 2 hr of swimming per day was sufficient to cause appetite suppression in 1-year-old, grossly obese rats that had been kept in individual cages without exercise since the age of 6 weeks (14).

Arcos et al. (1) have reported that female rats that swim for 6 hr daily have body weights similar to those of freely eating sedentary controls. The results of the present study confirm this finding, as both groups of female swimmers gained weight at approximately the same rate as their sedentary controls. This was made possible by a rise in food intake which apparently balanced the increased caloric expenditure associated with the exercise. These findings explain why exercise results in a reduced rate of weight gain in male rats but not in females. The physiological basis for this interesting sex difference in the effect of exercise on appetite in rats remains to be elucidated.

Under normal conditions (i.e., in the absence of pathological states which affect cardiovascular function), two variables appear to affect heart weight. These are the habitual level of physical activity and body weight. Animals that exercise regularly have heavier hearts than sedentary animals of the same weight (1, 7, 22). In sedentary animals, heart weight roughly parallels body weight (7, 19).

The two groups of female swimmers, which gained weight at the same rate as their sedentary controls, demonstrated a significant degree of cardiac hypertrophy (approximately 22% increase in heart weight in the first group, and a 28% increase in the second group). Since their body weights were the same, it seems reasonable to attribute the female swimmers' increased heart weights to the exercise. In contrast to the females, the male swimmers had significantly lower final body weights than their sedentary freely eating controls. The hearts of the male swimmers were lighter than those of the sedentary freely eating animals, but significantly heavier than those of the sedentary pair-fed weight animals. The heart weight-to-body weight ratio of the male swimmers was also significantly increased. Thus, in the male swimmers there were two variables affecting heart weight; these were the lower body weight and the exercise. The effect of the lower body weight is reflected in the finding that the swimmers' hearts were lighter than those of the sedentary freely eating animals. The effect of the exercise is reflected in the swimmers' increased heart weight-to-body weight ratios, and in the finding that their hearts were significantly heavier than those of the sedentary pair-fed weight animals.

Skeletal muscle responds to endurance exercise with an adaptive increase in mitochondria (9, 10) and an associated increase in the capacity to generate ATP aerobically (9). In a previous study (15) we investigated the possibility that a running program which results in an approximately twofold increase in the respiratory capacity of rat hind-limb muscles might also result in an increase in the enzymes of the respiratory chain in heart muscle. No such increase, as reflected in the levels of activity of cytochrome oxidase or succinate oxidase, or in the concentration of cytochrome c, occurred in the heart muscle of the runners (15).

In contrast to these results, Arcos et al. (1) have reported that female rats subjected to 6 hr of swimming daily, 6 days/week for a total of 140–180 hr, have a 52% increase in mitochondrial mass (expressed as milligrams dry weight per gram muscle). Oxygen consumption, expressed per milligram of mitochondrial dry weight, was the same for the swimmers and sedentary animals for a number of
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substrates, indicating that oxidative capacity expressed per gram of heart was also increased 52% (1).

However, Arcos et al. (1) found no increase in the concentration of mitochondria (milligrams dry weight per gram muscle) in the hearts of two other groups of rats subjected to the swimming program for 53–83 hr or for 361–490 hr, despite the fact that both of these groups developed the same degree of cardiac hypertrophy as the 140–180-hr swimmers (1).

Rapid synthesis of new protein occurs during the early stage of cardiac hypertrophy (8, 18). Meerson et al. (11, 12) have reported that mitochondria increase more rapidly than the other components of the myocardial cell, resulting in an “overshoot” with a transient increase in mitochondrial concentration during the first 7 or so days following imposition of a pressure overload. These investigators found that in stable hypertrophy the concentration of cardiac mitochondria is not increased (11, 12). In studies in this laboratory, early cardiac hypertrophy induced by means of a volume overload (A-V fistula) was not associated with an increase in the concentration of mitochondria; rather, all the protein components of the cell increased in parallel (3). However, it does seem reasonable that if a transient increase in mitochondria were to occur, it would take place during the early phase of rapid cardiac hypertrophy when the rate of protein synthesis is elevated, instead of after the hypertrophy has stabilized. The findings of Arcos et al. (1) would indicate that some stimulus appears after work-induced cardiac growth has ceased, transiently induces an increase in the concentration of mitochondria, and then disappears, permitting the concentration of mitochondria to return to normal. Throughout this process, cardiac hypertrophy remains stable. Furthermore, since exercise is the only experimental variable, this new stimulus to mitochondrial synthesis would have to arise from a constant exercise load to which the animals had already been accustomed for weeks.

The results of the present studies provide no support for this surprising sequence of events. The effect of exercise on cardiac mitochondria was investigated, using a number of independent measurements, in two groups of female and one group of male rats subjected to the same swimming program used by Arcos et al. (Average total duration of swimming was 162 hr.)

The levels of activity of succinate oxidase and cytochrome oxidase and the concentration of cytochrome c served as markers for the mitochondrial cristae. In addition, respiratory capacity was evaluated by measuring O2 consumption by whole homogenates of ventricular muscle, under conditions of uncontrolled respiration with pyruvate plus malate as substrate. The levels of activity of citrate synthase and mitochondrial malate dehydrogenase were used as markers for the mitochondrial matrix, while the concentration of mitochondrial protein served as an indicator of the yield of mitochondria per gram of heart.

No differences were found between the swimmers and their sedentary controls in the levels of any of these variables, all of which are indicators of the quantity of mitochondria present per gram of heart. These results, taken together with the previous negative findings on male rats subjected to a running program (15), provide strong evidence that cardiac mitochondria, expressed per gram of ventricular muscle, or per milligram of myocardial protein, do not undergo an adaptive increase in concentration in response to exercise.

These results suggest that the capacity for aerobic metabolism of normal, untrained rat heart muscle is sufficiently large to meet the increased demands for ATP imposed by the exercise program, without the need for an adaptive increase in respiratory capacity. Relative to this point, it is of interest that respiratory enzyme levels, expressed per gram of muscle, are approximately 5 times higher in the heart than in gastrocnemius muscle in the sedentary rat.

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