Mortality rate and longevity of food-restricted exercising male rats: a reevaluation

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Holloszy, John O. Mortality rate and longevity of food-restricted exercising male rats: a reevaluation. J. Appl. Physiol. 82(2): 399–403, 1997.—Food restriction increases the maximal longevity of rats. Male rats do not increase their food intake to compensate for the increase in energy expenditure in response to exercise. However, a decrease in the availability of energy for growth and cell proliferation that induces an increase in maximal longevity in sedentary rats only results in an improvement in average survival, with no extension of maximal life span, when caused by exercise. In a previous study (J. O. Holloszy and K. B. Schedtman, J. Appl. Physiol. 70: 1529–1535, 1991), to test the possibility that exercise prevents the extension of life span by food restriction, wheel running and food restriction were combined. The food-restricted runners showed the same increase in maximal life span as food-restricted sedentary rats but had an increased mortality rate during the first one-half of their mortality curve. The purpose of the present study was to determine the pathological cause of this increased early mortality. However, in contrast to our previous results, the food-restricted wheel-running rats in this study showed no increase in early mortality, and their survival curves were virtually identical to those of sedentary animals that were food restricted so as to keep their body weights the same as those of the runners. Thus it is possible that the rats in the previous study had a health problem that had no effect on longevity except when both food restriction and exercise were superimposed on it. Possibly of interest in this regard, the rats in this study did considerably more voluntary running than those in the previous study. It is concluded that 1) moderate caloric restriction combined with exercise does not normally increase the early mortality rate in male rats; 2) exercise does not interfere with the extension of maximal life span by food restriction, and 3) the beneficial effects of food restriction and exercise on survival are not additive or synergistic.

FOOD RESTRICTION has been shown to increase maximal longevity in a number of species, including rats (see Ref. 15 for review). It has been hypothesized that the life-prolonging effect of food restriction is mediated by a shift in biological state from cellular proliferation and reproduction to one in which cellular maintenance and repair mechanisms are maximized (14, 15). In contrast to food restriction, exercise appears to improve average survival time in rats without increasing their maximal longevity (7–9). Male rats are unusual in their response to exercise in that they do not increase their food intake to compensate for the increase in energy expenditure (8, 9). As a consequence, like food restriction, exercise results in a reduced availability of energy for cell proliferation and growth in male rats; it does not, however, increase their maximal life span (7, 9). It seemed possible that the failure of exercise to increase maximal longevity in male rats despite a reduced availability of energy for growth and cell proliferation might be due to some effect of exercise that counteracts or prevents a life-prolonging effect of a decreased availability of energy.

To test this possibility, a study was conducted on male rats in which exercise and food restriction were combined (8). In that study, exercise did not prevent the life-extending effect of food restriction, and the oldest food-restricted runners lived as long as the oldest food-restricted sedentary rats. However, the food-restricted exercisers had an increased mortality rate between the ages of 600 and 900 days (8). Because of a cut in research funding that made it impossible to obtain necropsies, we were unable to determine the cause of this unexpected increase in the death rate of the food-restricted exercisers during the first one-half of their survival curve.

Our research on rats is done with the assumption that it has relevance to humans. It is not unusual for people who participate in sports in which leanness is an advantage to combine exercise training with decreased caloric intake, either to provide a competitive edge or for psychological reasons (5, 10, 13, 16). It, therefore, seemed important to determine the cause of the increased early mortality observed in our food-restricted exercising rats. In this context, the present study was performed with the intention of determining the pathological basis for the increased early mortality in food-restricted exercising male rats.

METHODS

Male specific-pathogen-free Long-Evans rats (6 wk of age) were obtained from Charles River Laboratories. The rats were housed in temperature- and light-controlled animal rooms with their own ventilation system, 100% intake and 100% exhaust, i.e., no recirculation, with 15 air changes per hour, in a building in which no other animals were housed. The animal rooms were lighted between 6:00 A.M. and 6:00 P.M., and maintained at a temperature between 18 and 22°C. To protect against introduction of infections into the rat colony, the animal care technicians involved in this study did not work with other rats or in areas where they could be exposed to other rats. Six rats, selected at random, were killed and necropsied. Cultures were obtained on their respiratory tracts, tympanic bullae, and gastrointestinal contents. Serum was tested for antibodies against pathogenic viruses and mycoplasma. These tests were negative for pathogens, providing confirmatory evidence that the animals were pathogen free. Over the next 3 yr, serum was tested for antibodies against pathogenic viruses and mycoplasma on 11 rats from this aging colony. These tests were negative, providing evidence that the rats had remained pathogen free.

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At age 3 mo, the rats were randomly assigned to four groups. Group A rats were runners that had their food intake restricted to 92% of ad libitum intake. Group B rats were kept sedentary and pair fed with group A (as voluntary wheel running does not affect the ad libitum food intake of male rats, the degree of food restriction was also ~8% below ad libitum in group B). Group C rats, a second group of runners, had their food intake restricted to ~70% of ad libitum. Group D rats were kept sedentary and food restricted to keep their body weight the same as that of the food-restricted runners in group C. The reason for the 8% below ad libitum food intake restriction for the runners in group A is that freely eating rats generally reduce their voluntary wheel running quite markedly after a few months, and mild food restriction reverses this decrease in running (8, 9); therefore, to prevent this decline in voluntary running, the runners in control group A were restricted to 92% of voluntary intake from the beginning of the study. This mild degree of food restriction, which was also used in the preceding studies, does not affect longevity of sedentary rats (9). The rats were fed a pellet diet obtained from Teklad (Madison, WI), containing in terms of grams per kilogram, 200 g casein, 3 g methionine, 315.984 g sucrose, 275 g cornstarch, 80 g corn oil, 70 g cellulose, 35 g American Institute of Nutrition (AIN)-76 mineral mix, 3 g calcium carbonate, 15 g AIN-76A vitamin mix, 3 g choline bitartrate, and 0.016 g ethoxyquin. Food intake was measured daily, except on Sunday, by giving the rats premeasured amounts of food and weighing any uneaten food. On Saturdays, the rats were given a 2-day supply of food. Any wasted food was collected on aluminum foil sheets placed under the cages and weighed. The exercised rats lived in cages with attached running wheels to which they had free access (9). The running wheels were fitted with counters that recorded the number of revolutions. The sedentary rats were housed in stainless steel cages that measured 7 × 14 × 8 in.

A necropsy was performed on all the rats except for those in which autolysis was too advanced by the time their death was discovered. Four rats died of non-aging related causes before the age of 12 mo and are not included in the data analysis. Values are given as means ± SD. The statistical significance of differences in survival between groups was determined by using the generalized Wilcoxon (Breslow) test (3). The significance of differences in average age at death was determined by using analysis of variance with testing of subhypotheses with the use of appropriate contrasts (1). The significance of the differences in distance run per day between groups A and C was evaluated by using Student’s t-test.

RESULTS

Food intake and body weights. The food intakes of the rats in the four groups are summarized in Table 1. Male rats generally lose interest in wheel running after a few months and markedly reduce their running. It was previously found that this decrease in running could be prevented or reversed by slightly restricting their food intake, and this strategy was used in previous studies to keep the animals running. Therefore, in the present study, the runners in group A and the sedentary rats in group B had their food intake restricted by ~8% below ad libitum. This degree of food restriction has no significant effect on longevity (9). The runners in group C had their food intake restricted to the same extent, i.e., 30% below ad libitum, as in our previous study in which the food-restricted wheel runners had an increased mortality rate between ~600 and 900 days of age (8). The sedentary rats in group D were food restricted so as to keep their body weights in the same range as those of the food-restricted runners.

The average body weights of the four groups are summarized in Table 2. The body weights of the runners in group A, the food-restricted runners in group C, and the food-restricted sedentary rats in group D were all similar to those of the comparable groups in our previous study (8). As before, the body weights of the runners in groups A and C did not increase after age 12 mo despite a progressive decline in running distance (Fig. 1) and an essentially constant food intake, providing evidence for a decreased efficiency.

Running performance. The average distances run per day for groups A and C are shown in Fig. 1. From the age of 9 mo on, the runners in group C, for which food intake was restricted to ~70% of ad libitum, ran a

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>7–9</td>
<td>18.4 ± 1.2</td>
<td>18.6 ± 1.2</td>
<td>14.4 ± 0.9</td>
<td>10.3 ± 0.5</td>
</tr>
<tr>
<td>10–12</td>
<td>16.9 ± 1.3</td>
<td>16.9 ± 1.5</td>
<td>13.2 ± 0.2</td>
<td>9.6 ± 0.1</td>
</tr>
<tr>
<td>13–18</td>
<td>17.1 ± 1.2</td>
<td>17.3 ± 1.2</td>
<td>13.3 ± 0.3</td>
<td>9.8 ± 0.1</td>
</tr>
<tr>
<td>19–24</td>
<td>17.2 ± 1.6</td>
<td>17.3 ± 1.0</td>
<td>13.4 ± 0.3</td>
<td>10.0 ± 0.1</td>
</tr>
<tr>
<td>25–30</td>
<td>17.1 ± 1.7</td>
<td>17.6 ± 1.6</td>
<td>13.0 ± 0.9</td>
<td>10.0 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SD in g/day.

Fig. 1. Decrease with aging in average distance run per day by rats in groups A and C. Food intake of rats in group C was restricted to ~70% of ad libitum food consumption. After age 9 mo, group C rats ran significantly greater distance/24 h than group A rats (P < 0.04 to P < 0.0001).
and food-restricted sedentary rats in group D, food-restricted runners in group C showed a significant increase in maximal longevity. In marked contrast to the four groups at the times of death are summarized in Table 3, and the survival curves are shown in Fig. 2. As in previous studies, the food restriction increased both the average and maximal life spans of sedentary rats. As before, wheel running alone improved the average survival of group A rats but did not result in an extension of maximal life span, whereas food restriction of the runners in group C caused a significant increase in maximal longevity. In marked contrast to the previous study, in which the food-restricted runners had an increased mortality rate between the ages of 600 and 900 days (8), the food-restricted runners in group C showed an increase in average survival time as well as in maximal life span (Fig. 2) and had a survival curve that was virtually identical to that of the food-restricted sedentary control animals. Also in contrast to our previous results, the food-restricted runners (group C) had a significantly longer average survival than the runners in group A, with an average age at death similar to that of the food-restricted sedentary rats in group D (Table 3).

Necropsy findings. In the previous study, in which the food-restricted runners had an increased mortality rate between ~600 and 900 days of age (8), it was not possible to obtain necropsies. The goal of the present study was, therefore, to determine the pathological cause for the excess mortality in the food-restricted runners. Because there was no increase in mortality rate in the food-restricted runners in the present study, it was not possible to answer this question. The apparent causes of death, based on the necropsy findings, are summarized in Table 4. There were no major differences in cause of death between the two food-restricted groups, and the necropsy data provide no clues regarding the cause of the excess mortality in the food-restricted runners in our previous study.

**DISCUSSION**

Male rats generally do not increase their food intake to compensate for the increase in energy expenditure in response to chronic exercise (8, 9). As a consequence, male rats that exercise regularly are similar to food-restricted rats in that they show growth retardation and have a decreased body fat content and reduced availability of calories for cellular proliferation (4, 6, 8, 9, 12). It has been variously hypothesized that the life-prolonging effect of food restriction is due to growth retardation with maintenance of growth potential (11), prevention of excess body fat accumulation (2), and/or a shift in biological state from cell proliferation and reproduction to maintenance/repair pathways (14, 15). However, in a previous study in this laboratory, the relative caloric deficit induced by exercise did not result in extension of maximal life span in male rats, whereas a similar caloric deficit produced by food restriction did (9). This finding raised the possibility that the life-prolonging effect of a reduced availability of calories is countered by some harmful effect of exercise. In a study designed to test this possibility, it was found that exercise did not prevent the increase in maximal life span induced by food restriction (8); this finding was confirmed in the present study.

However, the earlier study (8) was complicated by the finding that the food-restricted runners had an increased mortality over the first ~50% of their mortality

### Table 3. Longevity

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Average Age at Death, days</th>
<th>Range, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (runners)</td>
<td>31</td>
<td>937 ± 171^abc</td>
<td>531–1,238</td>
</tr>
<tr>
<td>Group B (sedentary)</td>
<td>65</td>
<td>858 ± 152^de</td>
<td>502–1,214</td>
</tr>
<tr>
<td>Group C (food-restricted runners)</td>
<td>31</td>
<td>1,058 ± 166</td>
<td>656–1,328</td>
</tr>
<tr>
<td>Group D (food-restricted sedentary)</td>
<td>65</td>
<td>1,051 ± 157</td>
<td>672–1,390</td>
</tr>
</tbody>
</table>

Values for average age at death are means ± SD; n = no. of rats. Significantly different compared with: ^group B (P < 0.02); ^group C (P < 0.01); ^group D (P < 0.01); ^group C (P < 0.001); ^group D (P < 0.001).

### Table 4. Apparent cause of death

<table>
<thead>
<tr>
<th>Causes of Death, %</th>
<th>Number Necropsied</th>
<th>Renal Disease</th>
<th>Neoplasia</th>
<th>Cardiovascular</th>
<th>Pulmonary</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (runners)</td>
<td>29</td>
<td>38</td>
<td>35</td>
<td>17</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Group B (sedentary)</td>
<td>59</td>
<td>24</td>
<td>63</td>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Group C (food-restricted runners)</td>
<td>29</td>
<td>21</td>
<td>44</td>
<td>7</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Group D (food-restricted sedentary)</td>
<td>57</td>
<td>14</td>
<td>52</td>
<td>9</td>
<td>9</td>
<td>16</td>
</tr>
</tbody>
</table>
curve. This increase in mortality, which seemed to indicate that exercise has a harmful effect when combined with caloric restriction, was disturbing because of the possibility that such an interaction might not be limited to rats. The combination of caloric restriction and exercise is common in athletes who participate in sports that involve weight divisions, such as wrestling and boxing, or in which being light is an advantage, such as riding race horses or long-distance running (16). In addition, eating disorders that result in reduced caloric intake are fairly common in compulsive exercisers (5, 10, 13). In this context, the purpose of the present study was to determine the cause of the increased early mortality in food-restricted male rats given access to running wheels (8).

The results of the present study shed no light on this question because, in contrast to the previous study, the same degree of food restriction did not result in any increase in mortality. The present findings are in keeping with those of two other studies in which food restriction also did not increase the mortality rate in wheel-running rats (R. J. M. McCarter and B. P. Yu, personal communication). In view of these findings, it seems possible that the rats in our previous study may have had a subclinical health problem that had no effect on longevity except when both food restriction and exercise were superimposed on it. Perhaps of relevance in this regard is that the rats in the present study did considerably more voluntary running than those in our previous study at the same ages. For example, at age 17 mo, the food-restricted runners in the present study were running 5,833 ± 1,333 m/24 h compared with 4,445 ± 1,588 m/24 h for the previous group; at 23 mo the values were 4,333 ± 1,667 m/24 h for the present group and 3,502 ± 1,212 m/24 h for the previous group; and at 29 mo the values were 3,307 ± 1,632 m/24 h for the present group and 2,421 ± 1,068 m/24 h for the previous group. Clearly, the lower early mortality rate in the food-restricted runners in the present study compared with our previous study (8) was not the result of lower exercise stress.

Some studies on mice and rats have provided evidence suggesting that the more severe the degree of dietary restriction the greater is the increase in longevity (15). It is therefore of interest that the 30% food-restricted runners in group C and the paired-weight sedentary rats in group D, which had to be food-restricted by ~50% to keep their body weights the same as the runners’, had similar survival curves despite the difference in degree of restriction. Interpretation of this finding is complicated because, although food intake was different, the relative caloric deficit and the degree of growth retardation were similar in the food-restricted runners and the paired-weight sedentary rats. However, our previous study included both ~30% and ~50% food-restricted sedentary groups, and their survival times were also not statistically significantly different (8). This suggests that in the Long Evans strain of rat, a maximal or near-maximal effect of dietary restriction on longevity occurs with an ~30% reduction in food intake. In conclusion, exercise improves average survival but, despite resulting in a relative caloric deficit, does not extend maximal life span in male rats. The present results confirm that the failure of an exercise-induced reduction in availability of energy for cell proliferation and growth to increase maximal longevity is not due to a harmful effect of exercise that counters the life prolonging of a caloric deficit. This is evidenced by the finding that food-restricted wheel running rats live as long as food-restricted sedentary animals. As discussed in detail previously (8), this finding provides evidence that the life-prolonging effect of food restriction is not due to the energy deficit per se but is mediated by some other consequence of decreased intake and metabolism of food. The present results show that exercise does not interfere with the extension of maximal life span induced by food restriction. The beneficial effects of food restriction and exercise on survival are not additive or synergistic in male rats. It appears that a moderate, i.e., ~30%, caloric restriction does not normally result in an increase in the early mortality rate of wheel-running male rats and that our previous finding of an increase in early mortality was an unusual, i.e., abnormally, finding.

The excellent technical assistance of Marjie Kennedy is gratefully acknowledged.

This research was supported by National Institute on Aging Research Grant AG 00425.

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Received 10 July 1996; accepted in final form 22 October 1996.

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