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. Schedchman. 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 room temperature was maintained at a temperature between 18 and 22°C. All animals were weaned at 28 days of age and received 1:1 rat chow and water ad libitum. Treatment groups were established from birth and were randomly assigned to one of two treatments at weaning. Each group consisted of 10 rats. In food-restricted groups, the total amount of food was reduced by 15% of the amount consumed by the control group on an equal body weight basis. In exercise groups, the rats were housed in individual cages with running wheels and were allowed to run voluntarily. In food-restricted exercise groups, the rats were housed in individual cages with running wheels and were allowed to run voluntarily. Food was removed at the end of the day, and the amount of food consumed by each rat was recorded. On the following day, rats were allowed to run voluntarily during the 24-h period. At the end of the 24-h period, the amount of food consumed by each rat was recorded. Rats were monitored for weight loss and mortality every day. Mortality data were analyzed by the Kaplan-Meier survival analysis method.

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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 lose interest in wheel running after a few months and markedly reduce their voluntary wheel running quite markedly. It was found that this decrease in running could be prevented or reversed by slightly restricting their food intake, providing evidence for a decreased efficiency.

Table 1. Food intake

<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.

Table 2. Average body weights of rats in four groups

<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>6</td>
<td>380 ± 27</td>
<td>440 ± 34</td>
<td>316 ± 35</td>
<td>320 ± 20</td>
</tr>
<tr>
<td>9</td>
<td>429 ± 37</td>
<td>505 ± 48</td>
<td>340 ± 16</td>
<td>345 ± 20</td>
</tr>
<tr>
<td>12</td>
<td>419 ± 20</td>
<td>565 ± 59</td>
<td>332 ± 14</td>
<td>325 ± 18</td>
</tr>
<tr>
<td>15</td>
<td>424 ± 25</td>
<td>572 ± 61</td>
<td>334 ± 18</td>
<td>337 ± 16</td>
</tr>
<tr>
<td>18</td>
<td>409 ± 25</td>
<td>560 ± 52</td>
<td>323 ± 18</td>
<td>320 ± 27</td>
</tr>
<tr>
<td>21</td>
<td>419 ± 20</td>
<td>586 ± 56</td>
<td>330 ± 14</td>
<td>329 ± 17</td>
</tr>
<tr>
<td>24</td>
<td>420 ± 22</td>
<td>597 ± 73</td>
<td>333 ± 13</td>
<td>330 ± 15</td>
</tr>
<tr>
<td>27</td>
<td>427 ± 21</td>
<td>572 ± 87</td>
<td>326 ± 13</td>
<td>324 ± 21</td>
</tr>
<tr>
<td>30</td>
<td>423 ± 19</td>
<td>570 ± 65</td>
<td>328 ± 22</td>
<td>329 ± 19</td>
</tr>
</tbody>
</table>

Values are means ± SD in grams. At age 3 mo, average weight of all rats was 280 ± 32 g.

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

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 consumptions. After age 9 mo, group C rats ran significantly greater distance/24 h than group A rats (P < 0.04 to P < 0.0001).
nece 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 appar-
cent causes of death, based on the necropsy findings, are
summarized in Table 4. There were no major differ-
ences in cause of death between the two food-restricted
groups, and the necropsy data provide no clues regard-
ing 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
counterbalanced 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 in-
creased mortality over the first ~50% of their mortality

Table 3. Longevity

<table>
<thead>
<tr>
<th></th>
<th>Average Age at Death, days</th>
<th>Range, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (runners)</td>
<td>31 937 ± 171abc</td>
<td>531–1,238</td>
</tr>
<tr>
<td>Group B (sedentary)</td>
<td>65 856 ± 152de</td>
<td>502–1,214</td>
</tr>
<tr>
<td>Group C (food-restricted runners)</td>
<td>31 1,058 ± 166</td>
<td>656–1,328</td>
</tr>
<tr>
<td>Group D (food-restricted sedentary)</td>
<td>65 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: a group B (P < 0.02); b group C (P < 0.01); c group D (P < 0.01); d group C (P < 0.001); e group D (P < 0.001).

Table 4. Apparent cause of death

<table>
<thead>
<tr>
<th></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., abnormal, finding.

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REFERENCES