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# Exercise Increases Average Longevity of Female Rats Despite Increased Food Intake and No Growth Retardation

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*In previous studies, male rats given access to voluntary running wheels showed improved survival. Because the male runners did not increase food intake, it was not clear whether their improvement in average longevity was due to decreased availability of energy for cell proliferation and growth or to another effect of exercise. In this study, female rats, which increase their food intake in response to wheel running, were used to determine whether exercise can increase longevity when availability of energy for cell proliferation and growth is not decreased. At age 5 mo, the female voluntary wheel runners were running 9173 ± 3640 m/day (mean ± SD); running distance declined to 965 ± 483 m/d by age 34 mo. From 5 mo to 10 mo of age, the runners ate ~37% more than the sedentary rats. Thereafter, the runners ate ~20% more. The runners and sedentary rats attained similar peak body weights. However, the runners gained weight more rapidly, attaining steady state by 11 mo; the sedentary rats' weights did not plateau until ~15 mo. The runners had a significant prolongation of average longevity without an increase in maximal life span. The sedentary rats' average age at death was 924 ± 155 days (mean ± SD; range, 619–1263 d) compared to 1009 ± 132 days (range, 693–1259 d) for the runners, $p < .001$. These results show that exercise improves average longevity of rats independent of decreased availability of energy for cell proliferation and growth. They also provide evidence that an increase in food intake is not harmful when balanced by an increase in energy expenditure.*

RESEARCH has shown that food restriction increases maximal life span in rats and mice (McCay et al., 1935; Ross, 1972; Weindruch et al., 1979; Masoro et al., 1982; Weindruch and Walford, 1982, 1988; Yu et al., 1982, 1985; Masoro, 1985; Walford et al., 1987). It has variously been hypothesized that this increased longevity is mediated by decreased availability of energy for cell proliferation (Walford et al., 1987; Weindruch and Walford, 1988) with growth retardation (McCay et al., 1935), and reduced fat deposition (Berg and Simms, 1960), with a shift in physiological state from cell proliferation and reproduction to maintenance/repair pathways (Walford et al., 1987; Weindruch and Walford, 1988). Another mechanism that was thought to be involved is a decrease in metabolic rate (Sacher, 1977); however, this now seems unlikely in light of the results of a study by McCarter et al. (1985), who found that metabolic rate per gram lean body mass was the same in rats fed ad libitum and rats maintained on a life-prolonging food-restriction regimen.

Regularly performed exercise, which induces a number of adaptations that run counter to some of the changes that occur with aging (Oscai et al., 1972, 1974; Spurgeon et al., 1983; Starnes et al., 1983; Beyer et al., 1984), has also been shown to improve survival (Edington et al., 1972; Drori and Folman, 1976; Goodrick, 1980, 1983; Holloszy et al., 1985; Holloszy and Schechtman, 1991). However, in our studies of the effects of voluntary wheel running in male rats, exercise resulted in an improvement only in average length of life, without extension of maximal life span (Holloszy et al., 1985; Holloszy and Schechtman, 1991). This finding is of interest relative to the mechanism by which food restriction acts because, unlike most mammals, male rats do not increase their food intake to compensate for the increased energy expenditure caused by exercise. As a result, exercised male rats resemble food-restricted rats in that they have a decreased availability of energy for cell proliferation, with growth retardation and reduced fat cell size and number (Holloszy et al., 1985; Craig et al., 1987; Holloszy and Schechtman, 1991). Sedentary rats food-restricted to keep their body weights the same as those of the runners, i.e., similar decrease in energy availability for cell proliferation and growth, showed the expected extension of maximal longevity (Holloszy et al., 1985; Holloszy and Schechtman, 1991).

It is not clear from these findings whether the improved survival of the wheel runners was due to the exercise per se, or to decreased availability of energy for cell proliferation and growth. The finding that the wheel runners' maximal life span was not increased despite decreased availability of energy for cell proliferation and growth further raises the possibility that the life-extending effect of food restriction is mediated by a mechanism other than decreased availability of energy.

Female rats generally increase their food intake sufficiently in response to exercise to compensate for the increased energy expenditure (Oscai et al., 1971, 1973; Ivy et al., 1983). This makes it possible to examine the effects of exercise without the complication of a decreased availability of energy for cell proliferation and growth. In the present study we used female rats that exercised by means of

voluntary wheel running to determine whether exercise can increase longevity despite a large increase in energy intake.

## METHODS

Female, pathogen-free Long-Evans rats aged 3 mo were obtained from Charles River Laboratories and housed in temperature- and light-controlled rooms with their own ventilation system, with 15 air changes per hour, 100% intake and 100% exhaust with no recirculation. No other animals were housed in the facility, and the animal caretakers did not work with other rats. The animal rooms were lighted between 0600 and 1800 hr, and maintained between 18° and 22 °C. Three rats, selected at random, were killed and necropsied. Plasma was tested for antibodies against pathogenic viruses and mycoplasma, and cultures were obtained on the respiratory tracts, tympanic bullae, and gastrointestinal contents. The results of these tests provided evidence that the rats were pathogen free. At 4 mo of age, the animals were randomly assigned to either an exercising group of 62 rats (the size of which was determined by the number of running wheels available), or a sedentary group of 65 rats. The sedentary rats were housed in stainless steel cages measuring 7 × 14 × 8 in. The exercisers lived in cages of the same dimensions that had running wheels attached to them (Holloszy et al., 1985). The rats had free access to the running wheels, which were fitted with counters that recorded the number of revolutions. The rats were fed ad libitum a diet containing, by weight, casein 20%, DL-methionine 0.3%, ground whole wheat 20%, corn starch 20%, sucrose 18%, corn oil 5%, lard 5%, brewer's yeast 1%, AIN-76 vitamin mixture 1.5%, choline bitartrate 0.3%, AIN-76 mineral mix 3.5%, calcium carbonate 0.4%, cellulose 5%. Food intake was measured 6 days/week on 20 rats per group. The animals, whose food intake was measured, were rotated every 2 wk; i.e, the first 20 rats in a group the first 2 wk, the next 20 rats the next 2 wk, and so on. The animals were given preweighed amounts of food ~5 g above ad libitum intake, and the uneaten food was weighed. On Saturdays, the rats were given a 2-day supply of food.

The rats in this longevity study were not subjected to any experimental procedures. It was not possible to perform necropsies with histological examination because of financial constraints. Two runners and one sedentary rat died before the age of 15 mo of nonaging-related causes and are not included in the data analysis. Values are presented as means ± *SD*. The significance of the difference in survival between the two groups was determined with the Generalized Wilcoxon (Breslow) test (Breslow, 1970). The significance of differences in average age at death, in body weight, and in food intake was determined using Student's *t*-test. The Bonferroni procedure was used to adjust for bias associated with multiple comparisons. The acceptable level for statistical significance was set at .05. Least square linear regression analysis was used to evaluate the relationship between distance run and longevity in the voluntary wheel runners.

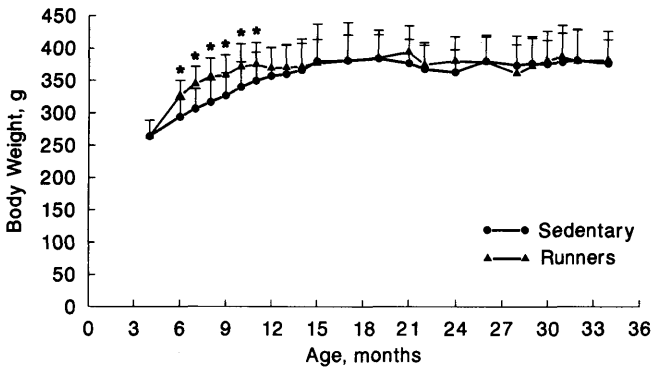


Figure 1. Average body weights of the runners and sedentary rats. *$p < .01$.

## RESULTS

*Body weights and food intake.* — As shown in Figure 1, the runners gained weight more rapidly than the sedentary controls. The runners' body weights had attained steady state by ~11 mo of age, while the sedentary rats' weights increased to age ~15 mo. Thereafter, the two groups had similar body weights. The difference in body weights between the runners and sedentary rats was statistically significant ($p < .01$) at all time points from 6 mo through 11 mo. The runners' food intake was significantly greater than that of the sedentary animals. From 5 to 10 mo of age the runners ate ~37% more than the sedentary rats; thereafter, the difference averaged ~20% (Table 1).

*Running activity.* — The amount of voluntary running done by the rats declined rapidly from 9173 ± 3640 meters/day at 5 mo to 4077 ± 2092 m/day at 11 mo of age, and then remained fairly constant for the next 8 months (see Figure 2). Then, after a sharp decline during the 20th month, for which there was no obvious explanation, average running distance decreased gradually from 2575 ± 1287 m/day at age 20 mo to 965 ± 483 m/day at age 34 mo. (The distance that rats ran during the last 2 mo of life was not included in the averages.)

*Longevity.* — Figure 3 shows that the voluntary wheel running resulted in a shift to the right, i.e., rectangularization, of the survival curve, with a significant prolongation of average life span (Table 2). This increase in average length of life of 85 days or ~9% was due to a later onset of mortality without an increase in maximal life span; the oldest runner died at age 1259 days while the oldest control died at

Table 1. Food Intake of Sedentary and Exercising (Runners) Rats

| Age Period (months) | Sedentary | Runners |
|---|---|---|
| 5–9 | 14.2 ± 1.5[a] | 19.5 ± 2.2* |
| 10–14 | 16.0 ± 1.4 | 19.4 ± 2.1* |
| 15–19 | 15.3 ± 1.2 | 18.5 ± 2.4* |
| 20–24 | 14.6 ± 0.7 | 17.8 ± 1.5* |
| 25–29 | 14.7 ± 0.9 | 17.5 ± 1.1* |

Values are means ± *SD*.
[a]Food intake, grams.
*Runner vs Sedentary, $p < .01$, after the Bonferroni adjustment.

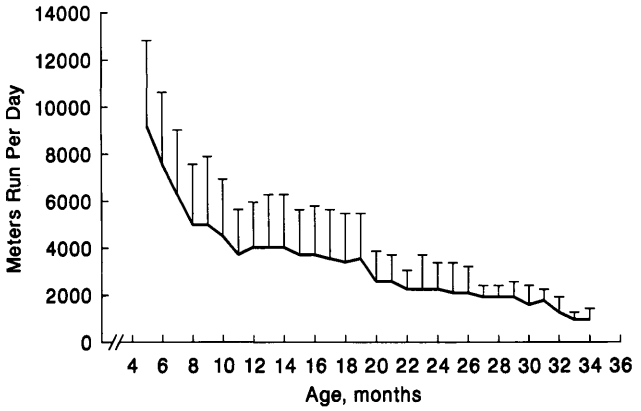


Figure 2. Decrease with age in average distance run per day.

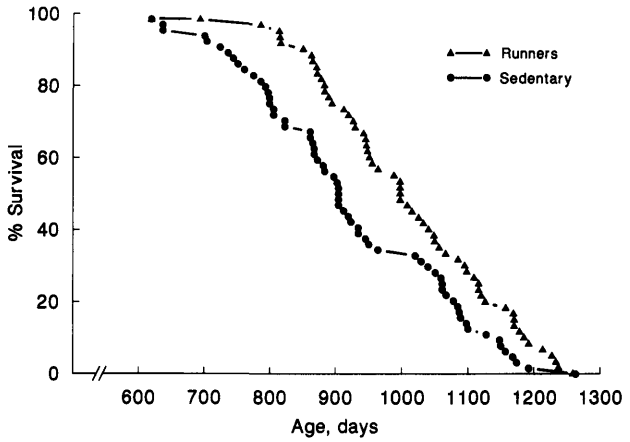


Figure 3. Survival curves of the two groups.

Table 2. Longevity of Sedentary and Exercising (Runners) Rats

| Group | *n* | Average Age at Death (days) | Age of 4 Oldest Rats (days) |
|---|---|---|---|
| Sedentary | 64 | 924 ± 155 (619–1263) | 1199 ± 44 |
| Runners | 60 | 1009 ± 132* (693–1259) | 1239 ± 14 |

Age at death and age of 4 oldest rats are means ± *SD*. The range of ages at death is shown in parentheses. *Runners vs sedentary, $p < .001$.

age 1263 days. There was no significant correlation between longevity and average daily running distance for the wheel runners. For example, the correlation coefficient was 0.043 ($p = .37$) for longevity and average daily running distance during months 10–12.

## Discussion

In our studies on male rats, voluntary wheel running did not result in an increase in food intake (Holloszy et al., 1985; Holloszy and Schechtman, 1991). As a consequence, the male runners had a relative caloric deficiency that resulted in growth retardation with attainment of a peak body weight that was approximately two-thirds as great as that of sedentary freely eating rats. The runners also had smaller and fewer fat cells than the sedentary animals (Craig et al., 1987). It was, therefore, not clear whether the improvement in average longevity of the male runners was due to decreased availability of energy for cell proliferation and growth (Walford et al., 1987; Weindruch and Walford, 1988) or to other effects of exercise unrelated to the relative energy deficit.

It seems evident from the present findings that regularly performed exercise improves longevity of rats by mechanisms that are independent of decreased availability of energy for cell proliferation and growth. The female wheel runners' average length of life was increased to a similar extent (~9%) as that of male runners in two previous studies (11.8%, Holloszy et al., 1985; and 9.6%, Holloszy and Schechtman, 1991), despite a large increase in energy intake that prevented growth retardation.

In our earlier studies, wheel running did not increase maximal life span of male rats despite a decreased availability of energy for cell proliferation and growth (Holloszy et al., 1985; Holloszy and Schechtman, 1991). In contrast, sedentary rats that were food restricted to keep their body weights in the same range as the runners showed a significant increase in maximal longevity (Holloszy et al., 1985; Holloszy and Schechtman, 1991). This finding suggests the possibility that food restriction mediates the extension of life span by some effect of decreased intake or metabolism of food other than a reduced availability of energy for cell proliferation and growth. Possibilities could, for example, include a decreased intake of toxins or a decreased accumulation of cellular waste products. This line of reasoning raised the further possibility that an increase in food intake might shorten survival.

Because of their smaller size, male rats that exercise regularly have a higher average energy expenditure per gram body weight than sedentary control rats eating the same amount of food. They also have intermittent increases in energy expenditure as a result of the running. However, total daily energy intake (Holloszy et al., 1985; Holloszy and Schechtman, 1991) and, therefore, after attainment of steady state body weight, also total daily energy expenditure, are the same in the male runners and sedentary controls. Female rats are different in that they have large increases in total food intake and energy expenditure, with no growth retardation, in response to wheel running. In this context, the finding that the female runners showed as great an improvement in average longevity as the male runners provides evidence that a large increase in food intake is not harmful when it is balanced by a proportional increase in energy expenditure that prevents development of obesity. An earlier study on the effects of intermittent cold exposure led to the same conclusion (Holloszy and Smith, 1986). In contrast to exercise, however, the cold exposure did not result in improved survival (Holloszy and Smith, 1986).

The finding that the female runners gained weight more rapidly and attained steady state body weight earlier than the sedentary animals is in sharp contrast to the reduced growth rate and peak body weight seen in male wheel-running rats (Holloszy et al., 1985; Holloszy and Schechtman, 1991).

The weights of a number of muscles of the forelimbs and hindlimbs are significantly increased in female wheel runners (Brown et al., 1992, and unpublished results), and this finding, together with the observation in previous studies on female rats exercised by swimming that (a) lean body mass is significantly increased, and (b) total body fat content and fat cell size are decreased in the swimmers (Oscai et al., 1973; Craig et al., 1983), makes it likely that the runners' more rapid weight gain is due to an increase in lean body mass.

In conclusion, this study shows that voluntary wheel running increases average length of life in female rats despite a large increase in food intake. The magnitude of this increase in longevity was similar to that seen previously in male rats that do not increase their food intake and, therefore, have a decreased availability of energy for cell proliferation and growth. This finding provides evidence that (a) the increase in average longevity induced in rats by voluntary wheel running is not due to decreased availability of energy for cell proliferation and growth, and (b) a large increase in food intake is not harmful if it is balanced by an increase in energy expenditure.

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