Interaction between exercise and food restriction: effects on longevity of male rats

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HOLLOSZY, JOHN O., AND KENNETH B. SCHECHTMAN. *Interaction between exercise and food restriction: effects on longevity of male rats.* J. Appl. Physiol. 70(4): 1529–1535, 1991.—Male rats that exercise in running wheels have a longer average survival than freely eating sedentary controls but, in contrast to food-restricted sedentary controls of the same weight, show no extension of maximal life span (*J. Appl. Physiol.* 59: 826–831, 1985). To test the possibility that exercise may counteract a life-extending effect of decreased availability of energy for certain biological processes such as cell proliferation, we examined the combined effects of exercise and food restriction on longevity of male rats. As before, wheel running improved average length of life, 978 ± 172 vs. 875 ± 175 (SD) days, for the sedentary controls ($P < 0.01$) without increasing maximal life span. Paired-weight controls, food restricted (~30% below ad libitum) to weigh the same as the runners, showed increases in both average (1,056 ± 144 days) and maximal life span. Food-restricted runners, with intake restricted to the same extent (~30%), had an increased mortality rate over the first ~50% of their survival curve up to ~900 days of age; their average life span (995 ± 226) was similar to that of the control group of runners and shorter than that of their paired-weight food-restricted sedentary controls (1,088 ± 159 days, $P < 0.05$). However, after ~900 days of age the food-restricted runners' survival became similar to that of the food-restricted sedentary groups, with a comparable increase in maximal life span. Thus the exercise did not counteract the increase in maximal life span induced by food restriction. These findings suggest that the increase in maximal life span induced by food restriction is not mediated by decreased availability of energy for biological processes such as growth, cell proliferation, and fat deposition.

aging; food intake; life span; survival curves; voluntary wheel running

THE RESULTS of a number of studies have provided evidence that rats that exercise regularly live longer than sedentary freely eating controls (6, 7, 9–12). This subject has been reviewed in detail recently (11). In a study of the effect of voluntary wheel running on longevity of male rats, it was found that the runners lived longer than sedentary freely eating animals but not as long as food-restricted sedentary paired-weight controls (12). Although exercise improved survival, it did not increase maximal life span; this was in contrast to the effect of food restriction, which resulted in an extension of life span in the paired-weight sedentary control rats (12).

Food restriction in rodents is the only intervention that has clearly proven effective in prolonging life span in a species of mammal (13, 14, 16, 20, 25–30). Among the mechanisms that have been hypothesized to mediate the life-prolonging effect of food restriction are growth retardation with maintenance of growth potential (16), prevention of excess body fat accumulation (2), a decrease in metabolic rate (21), and a shift in the physiological state of the body from cellular proliferation and reproduction to maintenance/repair pathways (27, 30). Exercise has a number of effects that are similar to those of food restriction and that appear to run counter to some of the changes that occur with aging (3, 5, 8, 17, 18, 23, 24). The adaptation of male rats to chronic exercise is of particular interest in this regard because they generally do not increase their food intake to compensate for the exercise-induced increase in energy expenditure (5, 8, 12, 17, 18). As a result, like food-restricted rats, male rats that exercise regularly show growth retardation and have a decreased body fat content and a reduced availability of calories for cellular proliferation (5, 8, 12, 17, 18).

In this context, there are at least two possible explanations for our previous finding that the relative caloric deficit caused by exercise did not result in extension of life span, whereas a similar caloric deficit produced by food restriction of the sedentary paired-weight rats did prolong maximal life span (12). One is that the life-prolonging effect of food restriction is not mediated by decreased availability of energy for growth, reproduction, fat deposition, and cell proliferation, but by the decreased intake and/or metabolism of food per se (possibly mediated by, for example, a decreased intake and/or formation of toxins and carcinogens and/or an accumulation of waste products). The second is that the life-prolonging effect of food restriction is mediated by decreased availability of energy for one or more biological processes or functions such as cell proliferation but that this effect is nullified by exercise.

If the first possibility were correct, rats subjected to exercise and food restriction in combination should do at least as well as food-restricted sedentary rats in terms of longevity, because both interventions have beneficial effects of survival. On the other hand, if the second possibility were correct, exercise would be expected to counteract the maximal life span-prolonging effect of food restriction. The present study was undertaken to evaluate these possibilities by comparing the separate and combined effects of exercise and food restriction on longevity of male rats.

METHODS

Specific pathogen-free male Long-Evans rats 6 wk of age were obtained from Charles River Laboratories. The animals were housed in temperature- and light-controlled rooms with their own ventilation system, with 15 air changes per hour, 100% intake and 100% exhaust (no recirculation), in a facility in which no other animals are housed. To avoid introducing infections into the rat colony, the people who entered the room to care for the animals did not work with other rats or in areas where they could be exposed to other rats. 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. Three percent of the 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, providing evidence that the rats were pathogen free.

At 3 mo of age the rats were randomly assigned to either exercising or sedentary groups. The exercisers lived in cages with attached running wheels to which they had free access (12). The running wheels were fitted with counters that recorded the number of revolutions. The sedentary rats were housed in stainless steel cages measuring 7 × 14 × 8 in. There were five experimental groups: *group A* were runners that were fed ad libitum initially; *group B* was kept sedentary and pair-fed with *group A; group C* was kept sedentary and food-restricted to keep their (average) body weight the same as that of *group A; group D,* a second group of runners, had their food intake reduced to the same extent as (i.e., were pair-fed with) *group C;* and *group E* was kept sedentary and food-restricted to keep their body weight the same as that of the food-restricted runners in *group D.*

Freely eating rats generally reduce their voluntary wheel running quite markedly after a few months, and mild food restriction reverses this decrease in running (12). The study was therefore designed so that when a runner showed an abrupt decrease in distance run per day, its food intake was decreased by 8% below its ad libitum intake (12). The rats were fed a diet containing, in terms of percent of total weight, 20% casein, 0.15% DL-methionine, 9.65% sucrose, 23% cornstarch, 24% ground wheat, 5% corn oil, 5% lard, 1% brewer's yeast, 2% AIN-76 vitamin mixture, 0.2% choline chloride, 5% AIN-76 mineral mixture, and 5% cellulose. Food intake was measured daily, except on Sunday, by giving the rats premeasured amounts of food and weighing the uneaten food (on Saturdays, the rats were given a 2-day supply of food). In the case of *groups A* and *B* during the period when they were freely eating, ad libitum food intake was determined, and the animals were then given preweighed amounts of food ~10 g above ad libitum intake.

The rats in this longevity study were not subjected to any experimental treatment other than voluntary exercise and/or food restriction. They were permitted to die of natural causes except for 19 rats that appeared to be in pain or acute discomfort and were killed at a late stage of their terminal illness; most of these rats had large invasive and/or metastatic neoplasms that were severely interfering with breathing and/or eating. On 10 of the rats that were killed, cultures were obtained on their respiratory tracts, tympanic bullae, and gastrointestinal contents, and serum was tested for antibodies against pathogenic viruses and mycoplasma. These tests were negative, providing evidence that these rats had remained pathogen free. A detailed necropsy with histological examination was not possible on the rats in this study because of financial constraints. Eight rats, scattered among the five groups, died before the age of 12 mo of various nonaging related causes, including accidents. Because this study concerns aging-related phenomena, these eight rats were eliminated from the study and are not included in the data analysis. Values are presented as means ± SD. The statistical significance of differences in survival between the groups was determined using the generalized Wilcoxon (Breslow) test (4). The significance of differences in average age at death was determined using analysis of variance with testing of subhypothesis with the use of appropriate contrasts (1). The significance of differences between *groups A* and *D* in the distance run per day was evaluated using Student's *t* test.

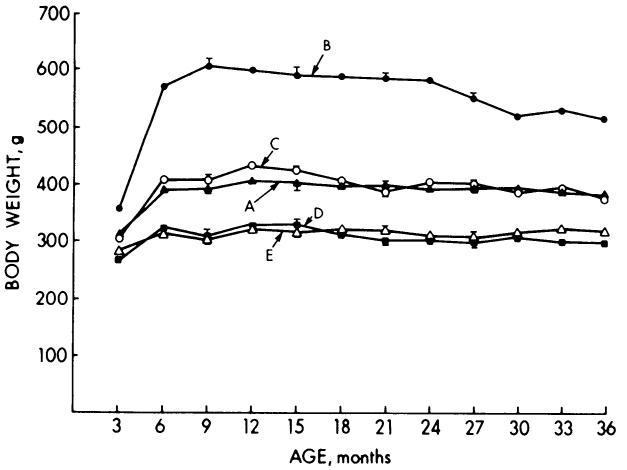

FIG. 1. Average body weights of rats in the 5 groups (*A–E*).

RESULTS

Body weights and food intakes. As shown in Fig. 1, the body weights of the runners in *group A,* and of their sedentary paired-weight controls in *group C,* had stabilized at ~400 g by age 6 mo and remained at this level until the onset of their terminal illness (weights during the last 2 mo of life are not included in the averages in Fig. 1). The body weights of the sedentary rats in *group B* peaked at ~600 g when they were in the 6 to 8-mo age range and were eating ad libitum; thereafter, body weight declined slightly to ~580 g as the result of pair-feeding with *group A.* Between the ages of 24 and 30 mo, the pair-fed rats in *group B* had a further weight loss from ~580 to ~520 g. As found previously (12) and again in the present study, this decrease was due to loss of weight by the surviving animals rather than to longer survival of smaller animals and occurred while the animals still appeared healthy. The body weights of the food-restricted runners in *group D* and of their sedentary paired-weight controls in *group E* had stabilized at ~310 g by age 4 mo and stayed close to this value until the onset of their terminal illness.

TABLE 1. *Food intake*

Age, mo	Group				
	A	*B*	*C*	*D*	*E*
6–9	20.3±1.3	21.0±2.0	14.5±0.2	14.5±0.1	10.5±0.1
9–12	18.0±1.2	18.3±1.4	13.9±0.3	14.0±0.1	10.2±0.1
13–18	17.3±1.0	17.2±0.6	13.4±0.1	13.5±0.2	10.1±0.1
19–24	16.6±1.1	16.8±0.7	12.9±0.2	12.9±0.3	10.0±0.1
25–30	16.8±0.9	16.7±0.6	12.7±0.4	12.8±0.3	10.1±0.1

Values are means ± SD in g/day.

The food intakes of the rats in the five groups are summarized in Table 1. Rats given access to voluntary running wheels generally lose interest in running after a few months when given free access to food and abruptly and markedly decrease their running (12). We have found that slightly restricting food intake reverses this abrupt decrease in running. Therefore, this study was designed so that the food intake of the runners in *group A* was reduced by 8% below their ad libitum intake when they showed a marked decrease in running performance. By the age of 11 mo all the runners in *group A* were on the slightly restricted food intake. As they aged, both the runners in *group A* and the pair-fed sedentary controls in *group B* showed a gradual voluntary reduction in food intake, necessitating further reductions in the amount of food given to them to keep them at ~92% of their ad libitum intake.

The sedentary paired-weight rats in *group C* had their food intake restricted to keep their body weights in the same range as those of the runners in *group A*. During the period from 6 to 30 mo of age, the food intake of the rats in *group C* was ~30% below ad libitum intake. The runners in *group D* were pair-fed with the sedentary food-restricted rats in *group C*. Despite having the same food intake, the runners in *group D* weighed ~90 g less than the sedentary rats in *group C* between 6 and 36 mo of age as the result of their increased energy expenditure. It is interesting that the runners' body weights did not increase after the age of 12 mo, despite a reasonably constant food intake and a progressive decline in the distance run per day (Fig. 2), providing evidence for a decrease in efficiency. The rats in *group E* were food restricted to keep their body weights the same as those of the runners in *group D*. Between 6 and 36 mo of age the food intake of the sedentary rats in *group E* was ~46% below ad libitum intake and ~42% less than that of the sedentary rats in *group B*.

Running performance. Initially, the freely eating runners in *group A* were running a significantly greater distance per day than the food-restricted runners in *group D* (Fig. 2). However, the runners in *group D*, whose food intake was restricted to ~70% of ad libitum, showed a slower decline in running performance initially, and by age 9 mo were running a significantly greater distance per day than the runners in *group A*. By this time, i.e., age 9 mo, the food intake of most of the runners in *group A* had been reduced to 92% of their ad libitum intake. The difference in the amount of running performed by the two groups increased progressively, so that after age 24 mo the rats in *group D* were consistently running more than twice as great a distance as those in *group A*. There was no significant correlation between running performance and longevity.

Survival patterns. The ages at the time of death of the five groups are summarized in Table 2, and their survival curves are shown in Fig. 3. The sedentary rats in *group B* had the shortest survival, the two food-restricted sedentary groups, *C* and *E*, had the longest survival, and the two groups of runners, *A* and *D*, fell in between (Fig. 3).

As expected, food restriction resulted in a significant increase in longevity, with prolongation of both the average and maximal life spans. In the case of *group C*, in which food intake was reduced by ~30% below ad libitum consumption, the increase in average life span was ~6 mo, or 21%, whereas in *group E*, in which food intake was reduced ~46% below ad libitum, average life span was increased by ~7 mo, or 24%, compared with that of the sedentary rats in *group B* (Table 2). As shown in Fig. 4, this increase in longevity was due to a later onset of mortality and to a longer survival of the oldest rats. The oldest three rats in *group C* lived 122 days, or 10% longer, whereas the oldest three rats in *group E* lived 141 days, or 12% longer than the oldest three rats in *group B* (Table 2). An unexpected finding, for which we have no explanation, is that the more severely food-restricted rats in *group E* had only a slight, statistically not significant, improvement in longevity compared with those in *group C*.

The runners in both *group A* and *group D* had a significantly longer average survival than the sedentary rats in *group B* (Table 2, Fig. 5). This increase in the average length of life relative to *group B* was 103 days, or ~12%, for *group A* and 120 days, or ~14%, for *group D*. The sedentary rats in *group B* were on ad libitum food intake initially and then were pair-fed with *group A;* this involved an ~8% reduction in food intake below ad libitum. We have previously found that this minimal degree of food restriction (i.e., ~8%) does not significantly affect longevity (12). The runners in *group D* (food-restricted), in contrast to the runners in *group A*, showed an extension of maximal life span compared with *group B*. Although their average age at death and their overall survival curves were not statistically significantly different, the runners in *group D* had a significantly better survival than those in *group A* after the age of 900 days ($P < 0.01$).

In our first study, the voluntary wheel runners had a significantly shorter survival than the paired-weight sedentary rats that were food restricted to keep their body weights the same as that of the runners (12). In the present study, the difference in average age at death ($P < 0.06$, Table 2) and the difference in survival curves ($P < 0.07$, Fig. 3) between the runners in *group A* and the paired-weight sedentary rats in *group C* did not quite attain statistical significance. However, as before (12), the runners in *group A*, in contrast to the food-restricted rats in *group C*, did not have an increase in maximal life span (Fig. 5) despite a similar retardation of weight gain (Fig. 1).

The runners in *group D*, which were food restricted to the same extent, i.e., pair-fed with, as *group C*, showed an improvement in maximal life span similar to that seen in

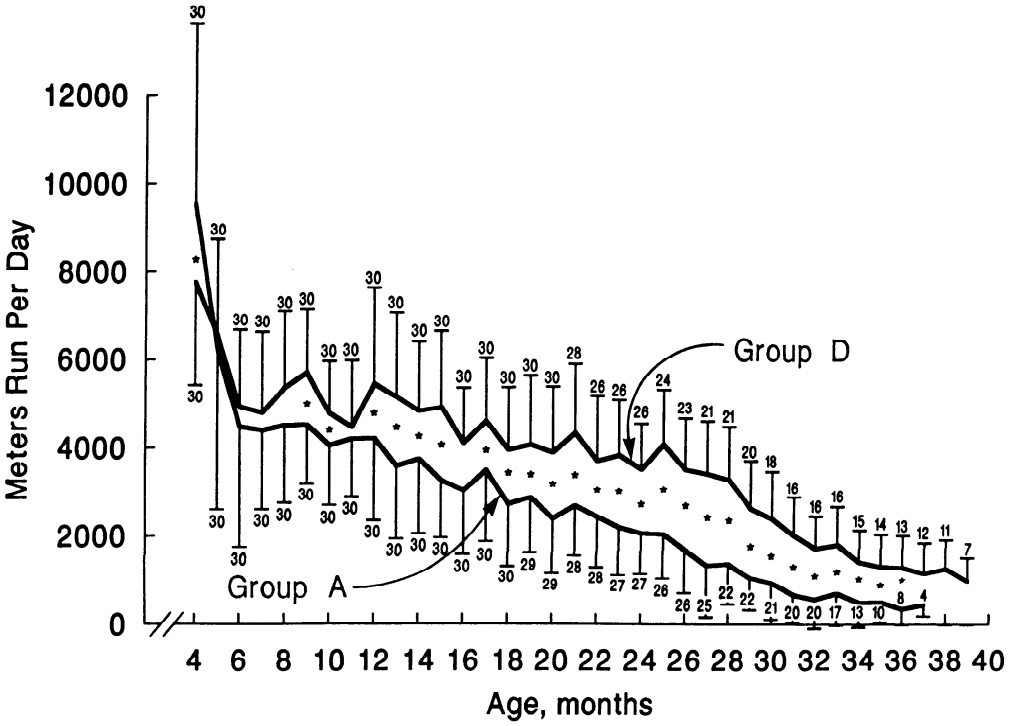

FIG. 2. Decline with age in average distance run per 24 h up to ~4 mo of age; *group A* ran farther than *group D*. After 9 mo, food-restricted rats in *group D* consistently ran farther than runners in *group A*. Number of rats surviving at each time point is shown above or below SD bars. $*P < 0.05$.

TABLE 2. *Longevity*

Group	*n*	Average Age at Death, days	Average Age of 3 Oldest Rats, days
A: runners	30	978±172* (554–1,251)	1,208±58
B: sedentary, pair-fed with *A*	45	875±175† (600–1,246)	1,200±52
C: sedentary, paired-weight with *A*	40	1,056±144‡ (735–1,374)	1,322±69
D: runners, pair-fed with *C*	30	995±226§ (633–1,444)	1,328±111
E: sedentary, paired-weight with *D*	44	1,088±159 (716–1,374)	1,341±42

Values are means ± SD; range in parentheses. * *A* vs. *B*, $P < 0.01$; *A* vs. *C*, $P < 0.06$; *A* vs. *D*, NS; *A* vs. *E*, $P < 0.01$. † *B* vs. *C*, $P < 0.0001$; *B* vs. *D*, $P < 0.01$; *B* vs. *E*, $P < 0.001$. ‡ *C* vs. *D*, NS; *C* vs. *E*, NS. § *D* vs. *E*, $P < 0.05$.

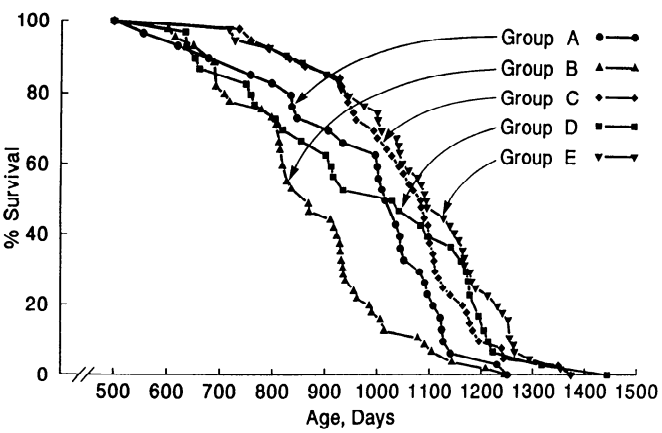

FIG. 3. Survival curves of the 5 groups.

the food-restricted sedentary animals in *groups C* and *E* (Fig. 6, Table 2); in fact, the longest-lived rat in the study (1,444 days) was in *group D*. Thus the exercise did not prevent the prolongation of maximal life span induced by food restriction. However, not only was there a lack of synergism between the beneficial effects of food restriction (Fig. 4) and of exercise (Fig. 5) on survival, but the exercise appeared to be deleterious in the food-restricted runners in *group D* (Fig. 6). This is evidenced by a significantly shorter average age at death in *group D* than in *group E* (Table 2) and by the differences in the shape of the survival curve of the runners in *group D* compared with those of the food-restricted sedentary rats in *groups C* and *E* (Fig. 6). The differences between the overall survival curves do not attain statistical significance (for example, survival curve for *group D* vs. *group E*, $P < 0.085$). However, the rats in *group D* clearly had a higher mortality rate than those in *groups C* and *E* up to ~30 mo of age. When the survival curves up to age 900 days of these groups are compared, *group D* had a significantly poorer survival than either *group C* ($P < 0.02$) or *group E* ($P < 0.02$).

DISCUSSION

As in our previous study (12), exercise, in the form of voluntary wheel running, resulted in a significant improvement in survival without an extension of maximal life span. This was reflected in a significantly older average age at death, compared with sedentary pair-fed controls, with no difference in age between the oldest rats in the two groups (12) (Fig. 5). As previously suggested (11), exercise may bring about this "rectangularization" of the survival curve by counteracting deleterious effects of a

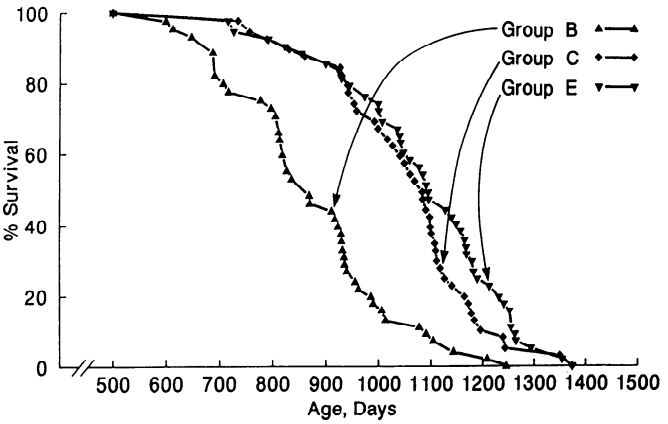

FIG. 4. Effect of food restriction on longevity. For clarity, 3 of the curves from Fig. 3 are shown. Survival curves of both food-restricted sedentary *group C* (intake ~30% below ad libitum) and food-restricted sedentary *group E* (intake ~46% below ad libitum) are significantly different from that of *group B* (sedentary, pair-fed with runners in *group A*; $P < 0.0001$).

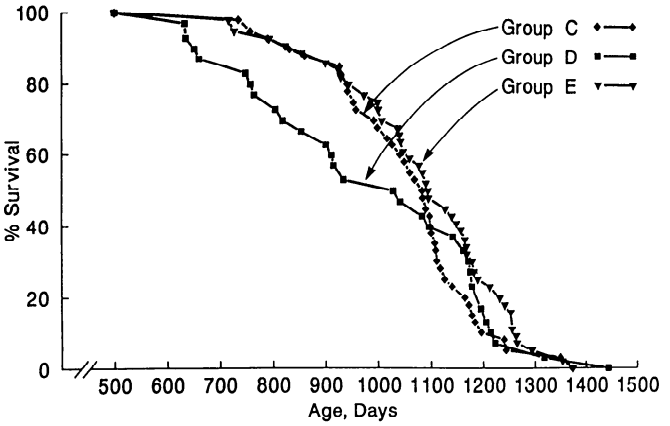

FIG. 6. Effect of exercise plus food restriction compared with food restriction alone. Overall survival curves of food-restricted runners in *group D* are not statistically significantly different from those of their pair-fed controls in *group C* or their paired-weight sedentary controls in *group D*. However, survival of *group D* up to 900 days of age is significantly worse than that of *group C* or *E*, $P < 0.02$.

sedentary life combined with overeating, making it possible for more of the animals to attain old age without slowing primary aging. This is in contrast to food restriction, which has, in numerous studies, been shown to extend maximal life span (13, 14, 16, 20, 25–30).

As before (12), and in keeping with the extensive evidence that food restriction extends longevity, the sedentary paired-weight controls that were food restricted (to ~70% of ad libitum intake) to keep their body weight the same as that of the runners showed a prolongation of maximal life span (Fig. 4). Although the runners in *group A* ate roughly 25% more food than their sedentary paired-weight controls in *group C*, the amount of energy available for growth, cell proliferation, and fat deposition was similar in the two groups, as evidenced by their similar body weights.

It has been hypothesized that the effect of food restriction on longevity is mediated by decreased availability of energy for growth, cell proliferation, reproduction, and fat deposition (2, 16, 29, 30). This hypothesis has been formulated in its current and most sophisticated form by Walford and Weindruch (27, 30). They suggest that decreased availability of energy causes a shift in the physiological state of the body from growth, reproduction, and cell proliferation to maintenance/repair pathways and that increased activity of these pathways slows primary aging (27, 30). This is a reasonable and attractive hypothesis. However, if this hypothesis is correct, one would expect that exercise should prolong life span in our wheel-running male rats, which have a decrease in the amount of energy available for growth, fat deposition, and cell proliferation similar to that of their food-restricted sedentary paired-weight controls.

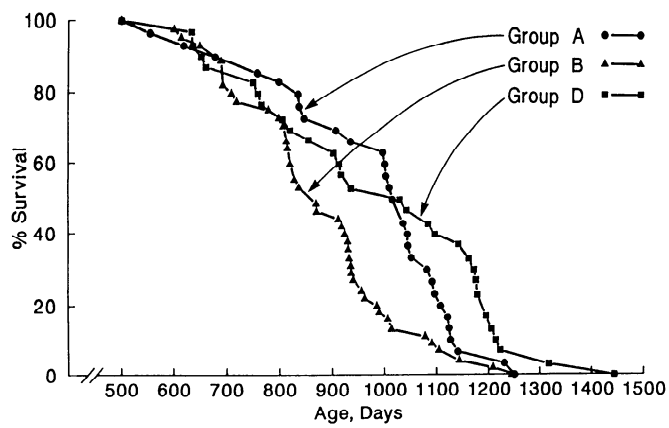

FIG. 5. Survival curves for the 2 groups of runners. Both runners in *group A* and food-restricted runners in *group D* (~30% below ad libitum intake) had a significantly improved survival compared with *group B*. *B* vs. *A*, $P < 0.01$; *B* vs. *D*, $P < 0.05$.

Our finding that the voluntary wheel-running male rats do not have an increase in maximal life span (12) led to the design of the present study in which a second group of runners (*group D*) was food restricted to the same extent as the sedentary paired-weight controls (*group C*) for the first group of runners (*group A*). We expected one of two outcomes. One was that the food-restricted runners would show at least as great an improvement in survival and extension of maximal life span as the food-restricted sedentary animals. This outcome would support the hypothesis that the slowing of primary aging is due to decreased food intake and/or metabolism per se and not to decreased availability of energy for growth, cell proliferation, etc. It would help explain why wheel running does not increase maximal life span in male rats, because it results only in decreased availability of energy for growth and cell proliferation but not in decreased food intake.

The alternative outcome was that the food-restricted runners in *group D* would not show a prolongation of life span, despite their decreased intake and metabolism of food. This outcome would have been compatible with the hypothesis that decreased availability of energy for growth and cell proliferation does cause the slowing of aging by food restriction but that exercise has deleterious consequences that counteract the life-prolonging effect of decreased availability of energy.

The actual outcome of this study was more complex than expected. In keeping with the first of the potential outcomes discussed above, the food-restricted runners in *group D* did show a prolongation of maximal life span. However, the food restriction combined with exercise

clearly also had a deleterious effect. This was manifested by the finding that the runners in *group D* had a significantly poorer survival, i.e., a higher mortality rate, between ~20 and ~30 mo of age than either their pair-fed (*group C*) or paired-weight (*group E*) food-restricted sedentary controls. This evidence of an antagonistic effect of exercise in food-restricted rats is consistent with the findings of Skalicky et al. (22), who reported that an exercise program reduced the beneficial effects of food restriction on a number of biological parameters. We do not yet have any information regarding how this effect is mediated. However, this complicating finding does not prevent the present results from providing insights regarding the mechanisms responsible for the effects of food restriction on longevity.

As found previously (12) and again in this study, exercise in the form of voluntary wheel running did not increase maximal life span in male rats, despite a decrease in the availability of energy for growth, cell proliferation, and other biological processes. In contrast, food restriction of the sedentary paired-weight rats that resulted in a similar decrease in energy availability for growth and cell proliferation did increase maximal life span. As discussed above, this finding provides evidence against the hypothesis (2, 16, 27, 30) that decreased availability of energy for a variety of biological processes including growth, cell proliferation, and fat deposition, mediates the prolongation of the life span by food restriction. The alternative possibility that was tested in this study is that decreased availability of energy for certain biological processes does mediate the increase in maximal life span and that this effect is counteracted by a deleterious consequence of exercise. This explanation now seems unlikely in view of the finding that those food-restricted runners in *group D* that survived beyond ~900 days had as good a survival (Fig. 6) and showed as great an increase in maximal life span as the food-restricted sedentary rats in *groups C* and *E*. Like the food-restricted sedentary rats, the oldest animals in *group D* were ~4 mo older than the oldest rats in *groups A* and *B*.

Thus our results favor the hypothesis that the life-prolonging effect of food restriction is mediated by decreased intake and/or metabolism of food per se. It has been hypothesized that food restriction retards aging by reducing the metabolic rate (20). This hypothesis now seems unlikely in light of the findings by Masoro et al. (14) that rats on a life-prolonging food restriction regimen had greater daily and lifetime intakes of calories per gram body weight than ad libitum-fed rats and by McCarter et al. (15) that metabolic rate per gram lean body mass is the same for food-restricted and ad libitum-fed rats. In the present study, the food-restricted runners in *group D* had a greater food intake per gram body weight than the sedentary controls in *group B*, yet the oldest rats in *group D* lived ~4 mo longer than those in *group B*, further arguing against a decrease in metabolic rate per gram body weight as the mechanism by which food restriction increases life span.

In conclusion, the present results, together with the results of previous studies, provide evidence that the prolongation of life span by food restriction is not mediated by decreased availability of energy for growth, cell proliferation, and fat deposition or by a decreased metabolic rate per gram body weight. Instead, our results favor the interpretation that the increase in maximal life span is due to the decrease in total intake and/or metabolism of food per se. Such an effect could be mediated, for example, by decreased formation of, and exposure of the tissues to, toxins and carcinogens and/or decreased accumulation of waste products. Our results also indicate that exercise can have an antagonistic effect in food-restricted male rats that results in an increased mortality rate over roughly the first 50% of their mortality curve without preventing the extension of maximal life span by the food restriction.

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