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Longevity of exercising male rats: effect of an antioxidant supplemented diet

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Abstract

Food restriction increases maximal life span in rodents. Male rats that exercise in voluntary running wheels do not have an increase in maximal longevity despite a relative caloric deficit. In contrast, sedentary rats that are food restricted so as to cause the same caloric deficit have an extension of maximal longevity. It seemed possible that exercise-induced oxidative stress might prevent a maximum life span-extending effect of a caloric deficit to manifest itself. This study was done to determine if antioxidants would allow a maximal longevity-extending effect of exercise to manifest itself in male rats. The antioxidant diet had no effect on longevity of the runners (Antiox., 951 ± 158 days versus control 937 ± 171 days), or of the sedentary controls (875 ± 127 versus 858 ± 152 days). As in previous studies, wheel running modestly increased average longevity ($\approx 9\%$), but had no effect on maximal life span. The finding that antioxidants had no effect on longevity of the wheel runners supports the interpretation that the caloric deficit induced by exercise in male rats does not have a life-extending effect that is countered by oxidative tissue damage.

Keywords: Body weight; Food intake; Maximal lifespan; Wheel running

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1. Introduction

Studies on rats have shown that exercise has a beneficial effect on survival (Goodrick, 1980; Holloszy et al., 1985; Holloszy, 1993). In contrast to food restriction, which results in an increase in maximal life span in rats (Yu et al., 1985; Walford et al., 1987; Weindruch and Walford, 1988; Masoro and Austad, 1996), our studies on the effects of voluntary wheel running have shown that exercise improves average survival time without increasing maximal longevity (Holloszy et al., 1985; Holloszy, 1993, 1997). It has been hypothesised that food restriction increases maximal life span by decreasing the energy available for growth and reproduction and shifting the biological state from cellular proliferation to one in which maintenance and repair mechanisms are enhanced (Yu et al., 1985; Walford et al., 1987; Weindruch and Walford, 1988; Masoro and Austad, 1996). Male rats are atypical in that they do not increase their food intake in response to exercise. As a result, male rats that exercise regularly also have growth retardation and a reduced availability of energy for cell proliferation (Holloszy et al., 1985; Holloszy, 1988, 1997). Sedentary rats that are food restricted to keep their body weights the same as those of the runners, i.e. have a similar relative caloric deficit, show a significant extension of maximal life span, yet the runners do not (Holloszy et al., 1985; Holloszy, 1988). This finding led to the hypothesis that, in addition to its beneficial effect on average survival time, exercise might also have a deleterious effect that counters a longevity-prolonging action of a decreased availability of energy for cell proliferation.

Evidence has accumulated that the increase in oxygen consumption during exercise results in increased free radical production and oxidative tissue damage (Davies et al., 1982; Alessio et al., 1988; Ji, 1995; O'Neill et al., 1996) and that supplementation with antioxidants and free radical scavengers may protect against free radical mediated tissue injury during exercise (Sumida et al., 1989; Meydani et al., 1993; Goldfarb et al., 1994; Rokitzki et al., 1994; Kanter, 1995). In this context, the present study was conducted to test the possibility that a diet supplemented with antioxidants and free radical scavengers would, by protecting against free radical damage, allow a maximal life span-extending effect of decreased energy availability to manifest itself in exercising male rats.

2. Materials and methods

Specific-pathogen-free male Long Evans rats were obtained from Charles River Laboratories at the age of 6 weeks. They were housed in temperature- and light-controlled rooms with their own non-recirculating ventilation system, with 15 air exchanges per hour, in a facility in which no other animals were housed. The technicians who cared for the animals did not work with other rats or in areas where they could be exposed to other rats. The animal rooms were maintained at a temperature between 18 and 22°C and lighted between 06:00 and 18:00. A total of six rats selected at random were killed and necropsied. Cultures were obtained

on their gastrointestinal contents, respiratory tracts and tympanic bullae and serum was tested for antibodies against pathogenic viruses and mycoplasma. These tests were negative for pathogens, providing confirmatory evidence that the rats were pathogen-free. During the next 3 years, serum was tested for antibodies against pathogenic viruses and mycoplasma on eleven animals from this ageing rat colony and were also found to be negative.

When the rats were 3 months old, they were randomly assigned to four groups. There were two groups of voluntary wheel runners with 31 rats per group and two groups of sedentary rats with 65 rats per group, that were pair fed with the runners. One group of runners and one group of sedentary animals were fed a diet supplemented with antioxidants and the other two groups were fed the same diet without the antioxidant supplements. The two control groups that did not receive the antioxidant supplements also served as controls for another study, performed concomitantly, of the interaction between exercise and food restriction (Holloszy, 1997). It was found in previous studies that rats markedly reduce their voluntary wheel running after a few months unless their food intake is slightly restricted (Holloszy et al., 1985; Holloszy and Schechtman, 1991; Holloszy, 1997); therefore, as in these previous studies, the runners' food intake was restricted by 8% below ad libitum intake. This mild food restriction does not affect longevity of sedentary rats (Holloszy et al., 1985). The animals were fed pellet diets obtained from Teklad (Madison, WI); the control diet contained, in terms of grams per kilogram: 200 g casein, 3.0 g DL-methionine, 315.984 g sucrose, 275 g corn starch, 80 g corn oil, 70 g cellulose, 35 g AIN-76 mineral mix, 3 g calcium carbonate, 15 g AIN-76A vitamin mix, 3 g choline bitartrate, 0.016 g ethoxyquin. The antioxidant and free radical scavenger supplemented groups were fed a diet containing 200 g casein, 3.0 g DL-methionine, 310.364 g sucrose, 275 g corn starch, 80 g corn oil, 70 g cellulose, 35 g AIN-76 mineral mix, 3 g calcium carbonate, 15 g AIN-76A vitamin mix, 3 g choline bitartrate, 0.016 ethoxyquin, 2.5 g coated ascorbic acid, 2 g DL-alpha tocopherol (1100 U/g), 1 g BHT, 0.1 g beta carotene, 0.02 g menadione sodium bisulfite complex. Food intake was measured daily, except on Sundays; the rats were given premeasured amounts of food and any uneaten food was weighed. On Saturdays the animals were given a two day supply of food.

The runners lived in cages that had running wheels attached to them; the rats had free access to the running wheels (Holloszy et al., 1985). The running wheels were equipped with counters that recorded the number of revolutions. Both the runners and the sedentary rats were housed in cages that measured $7 \times 14 \times 8$ inches.

2.1. Assays

Alpha-tocopherol in serum of 4 runners on the antioxidant diet and 4 runners on the control diet was determined by a modification of the reverse-phase high performance liquid chromatography method of Stacewicz-Sapuntakis, et al.

(1987). Ascorbic acid was assayed by the 2,4-nitrophenylhydrazine method of Roe and Kuether (Roe and Kuether, 1943). These assays were kindly performed by Dr Ed Norkus (Our Lady of Mercy Medical Centre, Bronx, NY)

2.2. Statistical analysis

The statistical significance of differences in survival between groups was determined using the generalised Wilcoxon (Breslow) test (Breslow, 1970). The significance of differences in average age at death was determined using two-way analysis of variance (Barr et al., 1979). The significance of the differences in serum ascorbic acid and alpha-tocopherol levels was evaluated using Student's t test. Significance was set at $P < 0.05$.

3. Results

3.1. Body weights and food intake

The body weights of the runners and the sedentary rats are shown in Fig. 1; there were no differences in the rates of weight gain, or in the peak body weights attained, between the rats on the antioxidant supplemented diet and those on the control diet. As shown in Table 1, there were no significant differences in food intake between the four groups.

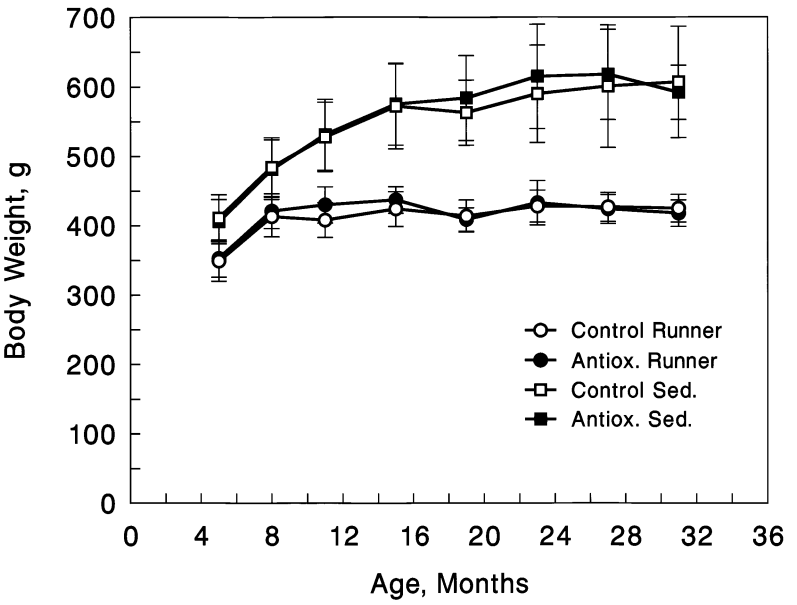

Fig. 1. Average body weights of the runners and sedentary controls. Values are means ± S.D.

Table 1
Food intake

Age (months)	Group (food intake, g/day)			
	Sedentary		Runners	
	Control	Antioxidant	Control	Antioxidant
5–7	18.2 ± 1.1	18.6 ± 1.3	18.9 ± 1.7	18.8 ± 0.8
8–13	17.6 ± 1.3	17.3 ± 1.5	17.3 ± 1.5	17.2 ± 1.3
14–19	17.3 ± 1.2	17.2 ± 1.1	17.2 ± 1.2	17.1 ± 1.0
20–25	17.3 ± 1.1	17.3 ± 1.2	17.1 ± 1.6	16.8 ± 1.2
26–31	17.2 ± 1.5	17.3 ± 1.1	17.0 ± 1.7	16.9 ± 1.1

Values are means ± S.D.

3.2. Running activity

As in previous studies, there was a progressive decline in the distance run by the rats with advancing age (Fig. 2). There were no significant differences in the distances run between the antioxidant diet and control diet groups.

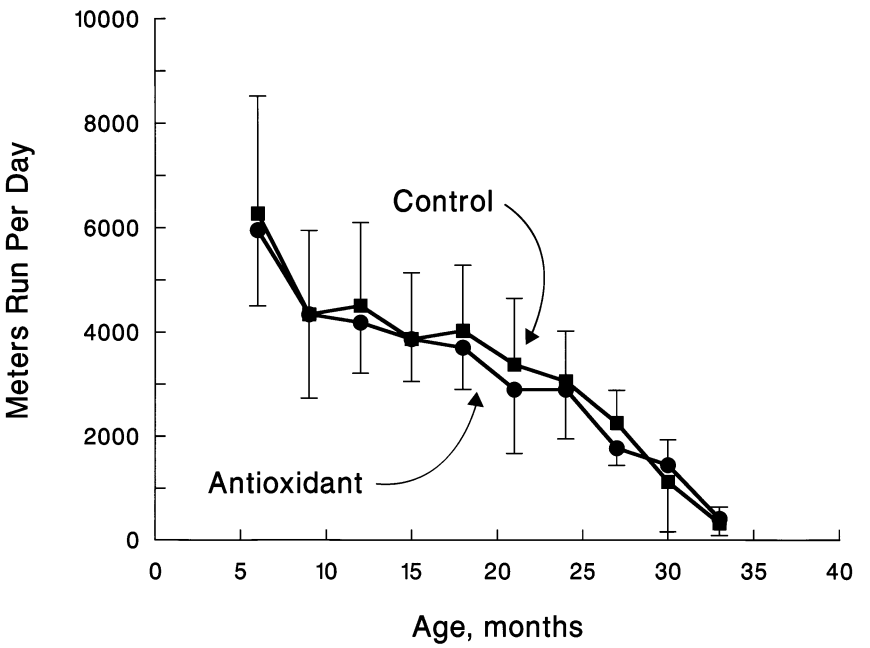

Fig. 2. Average distance run per day by the voluntary wheel runners on the control and antioxidant diets. Values are means ± S.D.

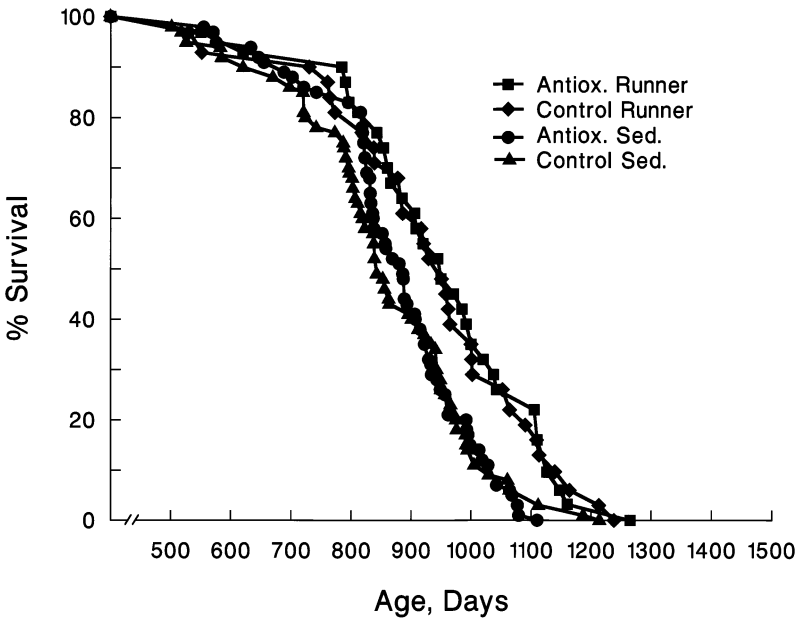

Fig. 3. Survival curves for the four groups. The survival curves for the runners are significantly different from those of the sedentary rats in both diet groups ($P < 0.02$).

3.3. Survival patterns and longevity

The survival curves of the four groups are shown in Fig. 3 and the average lengths of life are summarised in Table 2. As in our previous studies, the voluntary wheel running resulted in a modest, $\approx 9\%$, but statistically significant increase in average survival time. The antioxidant supplemented diet had no significant effect on longevity of the runners. The antioxidant diet also did not increase either average or maximal survival time of the sedentary animals. Two-way analysis of variance revealed no significant interactions between the effects of diet and exercise ($P = 0.95$). There was a significant main effect of exercise on longevity ($P < 0.01$). There was no significant main effect of diet on longevity ($P = 0.49$).

Table 2
Age at time of death

Group	No.	Average age at death days	Range days
Control sedentary	65	858 ± 152	502–1214
Antiox. sedentary	65	875 ± 127	555–1132
Control runners	31	937 ± 171	531–1238
Antiox. runners	31	951 ± 158	550–1263

Average age at death values are mean $\pm$ S.D.
Antiox., rats on the antioxidant supplemented diet.
Runners versus sedentary, $P < 0.01$.

3.4. Serum ascorbic acid and alpha-tocopherol levels

After the animals had been on the antioxidant or control diets for 12 months, serum alpha-tocopherol concentration averaged 0.92 ± 0.35 mg/dl in the controls and 2.22 ± 0.67 mg/dl in the antioxidant group (values are means $\pm$ S.D. for four rats/group; $P < 0.01$). Serum ascorbate levels in the same animals averaged 0.61 ± 0.08 mg/dl in the controls and 1.05 ± 0.11 mg/dl in the antioxidant diet group ($P < 0.01$).

4. Discussion

Male rats do not increase their food intake to compensate for the increase in energy expenditure caused by exercise (Holloszy et al., 1985; Holloszy, 1988, 1997). Despite the relative caloric deficit induced by the exercise, male rats exercised by means of wheel running do not show an extension of maximal longevity. In contrast, the food restriction required to keep the body weight of sedentary rats the same as that of the runners ($\approx 30\%$ below ad libitum intake) results in a significant increase in maximal life span in sedentary animals (Holloszy et al., 1985; Holloszy and Schechtman, 1991; Holloszy, 1997). The oxidative stress associated with exercise results in free radical-mediated tissue damage (Davies et al., 1982; Alessio et al., 1988; Witt et al., 1992; Goldfarb et al., 1994; Rokitzki et al., 1994; Ji, 1995; O'Neill et al., 1996). It seemed possible that supplementation with antioxidants and free radicals scavengers, by protecting against the damaging effects of free radicals (Dillard et al., 1978; Machlin and Bendich, 1987; Sumida et al., 1989; Kanter et al., 1993; Meydani et al., 1993; Goldfarb et al., 1994; Rokitzki et al., 1994; Kanter, 1995; O'Neill et al., 1996) might improve the survival of exercising animals and perhaps, unmask a maximal life span-extending effect of the relative caloric deficit induced by exercise in male rats.

In the present study, the antioxidant and free radical scavenger-supplemented diet had no effect on the longevity of either wheel running or sedentary male rats. The finding that the antioxidants did not have a life-extending effect in the sedentary rats is in keeping with the results of previous studies see (Yu, 1995) for review. The ineffectiveness of the antioxidant diet in prolonging the longevity of the wheel runners argues against the hypothesis that the oxidative stress associated with exercise prevents a life-extending effect of a reduced energy availability to manifest itself in exercising male rats. There is considerable evidence that antioxidants, particularly alpha-tocopherol, can protect against the free radical tissue damage induced by exercise (Sumida et al., 1989; Meydani et al., 1993; Goldfarb et al., 1994; Rokitzki et al., 1994; Kanter, 1995).

The conclusion that the oxidative stress associated with exercise does not have a deleterious effect that counters a life-prolonging effect of a reduced availability of energy for cell proliferation and growth is supported by a study conducted concomitantly with the present one. In that study exercise and food restriction were combined (Holloszy, 1997). Even though the food restricted runners ($\approx 30\%$ below

ad lib) did considerable more running than the wheel running controls, they showed the same extension of maximal life span as a food restricted sedentary control group (Holloszy, 1997). If the oxidative stress induced by exercise had a deleterious effect that counters a life-extending effect of a caloric deficit, the wheel-running should have reduced the effect of food restriction on longevity. As discussed in detail previously (Holloszy and Schechtman, 1991; Holloszy and Kohrt, 1995; Holloszy, 1997) these findings raise the possibility that it is not a caloric deficit per se that extends maximal life span, but some other consequence of a decreased intake and metabolism of food that does not occur in exercising male rats, because they have a normal food intake.

In conclusion, the present results show that a diet supplemented with high concentrations of antioxidants and free radical scavengers has no effect on longevity of male rats exercised by means of wheel running. Another study, conducted concomitantly, showed that exercise does not decrease the life-extending effect of food restriction (Holloszy, 1997). Taken together, these findings provide evidence that the relative caloric deficit induced by exercise in male rats does not have a maximal lifespan-extending effect that is prevented from manifesting itself because of increased free radical-mediated tissue damage.

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