Physical activity as a factor in the action of dietary restriction on aging: Effects in Fischer 344 rats

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ABSTRACT. Dietary restriction (DR) slows the rate of aging in laboratory rodents but the mechanism of action is unknown. DR is known to induce beneficial effects in a variety of tissues and organ systems. DR also maintains high levels of physical activity over the life span. We tested the hypothesis that lifelong physical activity is an important component of the anti-aging action of DR. Male specific pathogen-free Fischer 344 rats were divided into 4 groups at 6 weeks of age: A: fed ad libitum; AE: fed ad libitum and in cages with running wheels; B: fed 60% ad libitum; BE: fed 60% ad libitum and in cages with running wheels. Running activity and spontaneous cage activity were measured over 24 hours and over the life span. Metabolic rate was measured indirectly by analysis of air entering and leaving cages. AE rats exhibited low levels of running activity and ran very little beyond 6 months of age. In contrast, BE rats sustained high running levels even after all A and AE rats had died. High levels of wheel running did not decrease spontaneous cage activity. Median life span (50% survival) was in the order A = AE < B < BE. Ten percent survival was in the order A = AE < B = BE. BE rats had greatest median life span and also highest specific metabolic rate. Exercise and DR altered pathology: At death BE rats had a high incidence of cardiomyopathy, whereas A and AE rats had high incidence of chronic nephropathy and pituitary tumors. The data indicate that increased physical activity is probably not an important factor in the action of DR on aging.

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INTRODUCTION

Dietary restriction (DR) is the only manipulation known to consistently retard aging processes in mammals (1). Available evidence indicates that it is the reduction in intake of calories without malnutrition which is responsible for this effect, but the mechanism of action is unknown (2). Of the many beneficial effects of DR, one of the most striking is that DR leads to sustained high levels of physical activity. Yu et al. (3) demonstrated that Fischer 344 rats fed a restricted diet (40% less than *ad libitum*) maintained high levels of spontaneous activity over the life span, whereas rats fed *ad libitum* exhibited decreasing activity with age. Similarly, Holloszy and Schechtman (4) found that male Long-Evans rats fed 30% less than *ad libitum* had higher voluntary running activity over the life span than rats fed *ad libitum*.

Sustained physical activity is known to have many effects which counter the effects of aging (5). These results suggest, therefore, that the action of DR on aging may be due, in part, to sustained high levels of physical activity. The early work of Goodrick (6) suggests such a role. This author found that Wistar rats running in exercise wheels from 6 weeks of age had a significant extension of both median and maximum life spans. In contrast, Holloszy et al. (7) found that specific pathogen-free (SPF) Long-Evans rats, running in wheels from 6 months of age, had no extension of maximum life span. These authors suggested the earlier results might have arisen because of the protective effects of exercise against infectious disease in non-SPF rats. This conclusion was strengthened by the work of Holloszy and Schechtman (4). The study combined running wheels and dietary restriction (about 30% less than *ad libitum*) in SPF Long-Evans rats, with running commencing at 3 months of age. The authors found no increase in maximum life span of rats fed the restricted diet and

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running in wheels *vs* sedentary restricted rats. However, the restricted runners exhibited higher initial rates of mortality, i.e., the study suggested possible deleterious consequences of exercise in restricted rats.

The purpose of the present study was to address these effects in the rat strain most often used as a model system for aging studies, i.e., the male Fischer 344 rat. We used the paradigm of DR for which most data are available: restriction by 40% less than *ad libitum*, with both restriction and voluntary running commencing at 6 weeks of age. The possibility that intense wheel running might be compensated by less spontaneous cage activity at other times of the day was examined, as was the effect of DR and exercise on 24-hour energy expenditure. In addition, tissue pathology was determined in all rats at death. Our results demonstrate a major effect of DR on voluntary physical activity in this rat strain, with no significant effect on maximum life span. Pathology present at death was altered by the running activity and median life span was increased. However, the data indicate that physical activity is not a major factor in the action of DR on aging.

MATERIALS AND METHODS

Animal care and dietary procedures

Rat maintenance and dietary manipulations have been extensively reported previously (3,8). In brief, male Fischer 344 rats were purchased from Charles River Laboratories (Kingston, NY) at 4 weeks of age. They were singly housed throughout life in a barrier facility under specific pathogen-free conditions and on a 12:12h light-dark cycle, in accordance with guidelines of the University of Texas Health Science Center. At 6 weeks of age, 40 rats were randomly assigned to each of 4 groups: A: fed *ad libitum* a semisynthetic diet; AE: fed *ad libitum* and housed in cages containing exercise wheels; B: fed 60% of the food consumed by group A rats; BE: fed the same as B rats and housed in cages containing exercise wheels. The semisynthetic diet consisted of (g/100g): 21% casein, 10% corn oil, 15% sucrose, 43.7% dextrin, 5% Ralston-Purina mineral mix and 2% Ralston-Purina vitamin mix. In B and BE rats the vitamin mix was increased to 3.3% of total so that restricted rats received the same vitamin intake as rats eating *ad libitum*. Food intake was measured as described by Yu et al. (3).

Measurements of physical activity

Running activity and spontaneous cage activity were monitored non-invasively. Running wheels (35 cm in diameter) were attached to standard plastic cages with wire mesh floors and these were placed on Hazelton-Enviro Rack systems. Running activity was detected using silent magnetic counters attached to wheels and was monitored 24h/day over the life span. Spontaneous cage activity was measured as before (3): cages were placed in the path of light beams (Digiscan System, Omnitech Corp., Columbus, OH) and movement detected by the interruption of these beams over a 24-hour period. These measurements were carried out using rats randomly selected from each group.

Activity was measured with rats in their home cages in the same room in which they usually lived. Activity monitors were calibrated before and after measurement by determining the number of counts corresponding to movement around the periphery of an area corresponding to the home cage. Cages housing control and exercising rats had floor surface areas of 575 cm^2 and 610 cm^2, respectively, available for voluntary movements. Rats were monitored continuously for 1 week and results averaged to provide counts per 24 hours.

Tissue pathology

All rats were inspected twice daily (from 07.00 to 08.00 and from 15.00 to 16.00h). Dead rats were removed from cages and either necropsied immediately or refrigerated for a brief period. Organs were examined histologically as described by Iwasaki et al. (9). Grades of major diseases present (chronic nephropathy, cardiomyopathy, leukemia/lymphoma, and pituitary tumor) were determined based on the grading criteria that were described previously (8, 10-12). The probable cause of death of each rat was estimated by the severity of diseases found at necropsy. Each rat was diagnosed without reference to experimental group.

Measurements of metabolic rate

Daily energy expenditure was assessed by indirect calorimetry over 24 hours as reported in several previous studies (13). This was done by monitoring the oxygen and carbon dioxide content of air entering and leaving sealed rat cages. In the case of AE and BE rats, this was accomplished by constructing plexiglass housings surrounding the exercise wheels. Monitoring of these cages in the absence of rats and using combustion of known quantities of alcohol confirmed the adequate sealing of these structures. For sedentary A and B rats, cages were sealed with lids containing air outlets and water inlets, as used in previous studies (13). The measurements were carried out using animals selected at random from each of the 4 groups. Rats were acclimated to sealed cages for 48

hours prior to measurement and energy expenditure was then determined over a 24-hour period. Fat-free mass could not be determined in these rats since they were held until death by natural causes and were then necropsied for determination of tissue pathology. Specific metabolic rates were, therefore, determined by normalizing daily caloric expenditure to "metabolic" mass, or body mass in kg raised to the power 0.75 (14).

Statistical analysis

Survival curves were estimated using product limit estimates and curves were compared using the Wilcoxon Test (15). The median and 10th percentile survival times were compared using the quantile test (16). Frequencies of lesions or grades of lesions were analyzed with a χ^2 test or Fisher's exact test for 2×2 tables (17). Interactions between age, nutrition and function were analyzed by analysis of variance (18) with post-hoc identification of significant interactions using the Tukey-Kramer test and assuming significance of differences between mean values at $p<0.05$.

RESULTS

Body weight and food consumption

Variation of body weight with age, diet and exercise is shown in Figure 1. Voluntary running activity had a significant effect on weight. The intense, sustained running of BE rats resulted in significantly lower body weights over most of the life span (3-40 months of age vs B rats). In the case of *ad libitum* fed (AE) rats, low levels of running activity resulted in significantly lower body weight (*vs* A rats) from 12-26 months of age. Daily food consumption of A and AE rats was not significantly different; B and BE rats consumed 40% less food than A rats. The low body weights of BE rats, therefore, resulted in significantly higher specific food consumption (Kcal/g body weight/day) for BE rats than other rats over most of the life span, as shown in Figure 2.

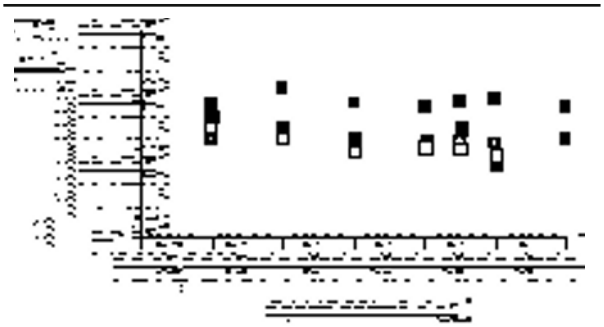

Figure 2 - *Food consumed per gram of body weight with age by rats in each of the four groups. Results are mean ± SE, N=10. □ A: sedentary rats fed ad libitum; ■ AE: exercising rats fed ad libitum; ○ B: sedentary rats fed 40% less than ad libitum; ● BE: exercising rats fed same as B rats.*

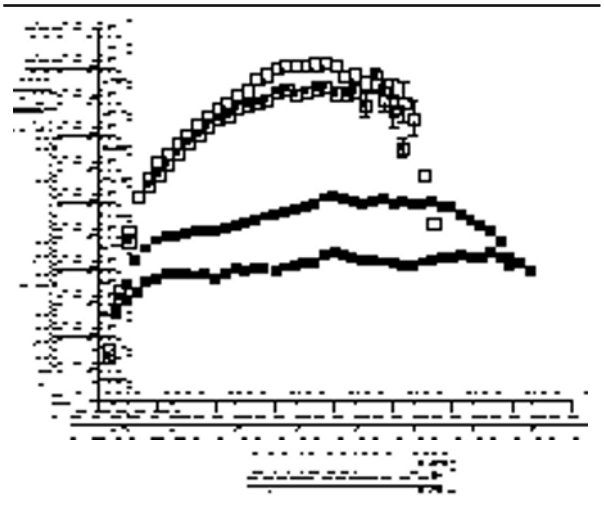

Figure 1 - *Body weights of rats with age. Results are mean ± SE, N=40 in each group. □ A: sedentary rats fed ad libitum; ■ AE: exercising rats fed ad libitum; ○ B: sedentary rats fed 40% less than ad libitum; ● BE: exercising rats fed same as B rats.*

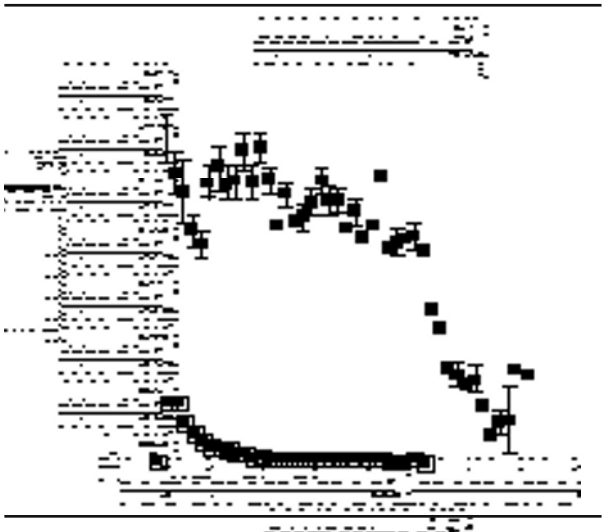

Figure 3 - *Voluntary wheel running per 24 hours of AE and BE rats with age. Running commenced at 6 weeks of age. Results are mean ±SE, N=40. ■ AE: exercising rats fed* ad libitum; *● BE: exercising rats fed 40% less than ad libitum.*

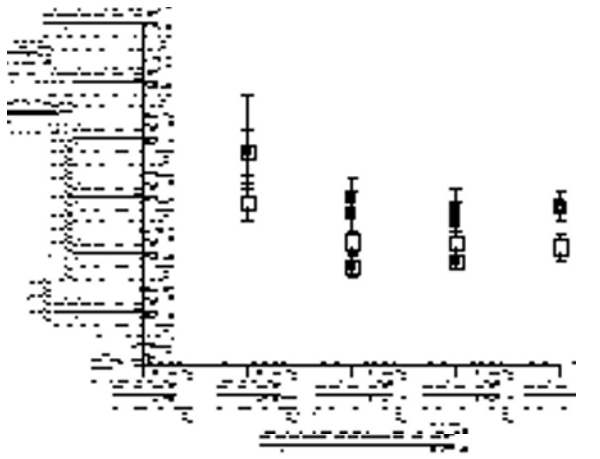

Figure 4 - *Spontaneous cage activity of rats with age. Results are expressed as mean ± SE, N=10. □ A: sedentary rats fed ad libitum; ■ AE: exercising rats fed ad libitum; ○ B: sedentary rats fed 40% less than ad libitum; ● BE: exercising rats fed same as B rats.*

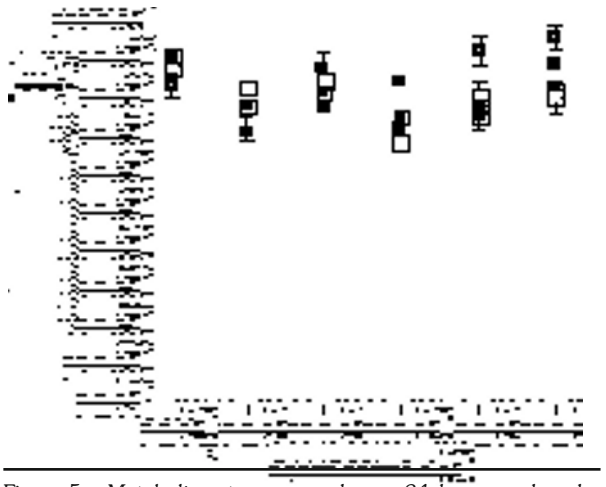

Figure 5 - *Metabolic rate measured over 24 hours and under usual living conditions. Results are mean ± SE, N=10. □ A: sedentary rats fed ad libitum; ■ AE: exercising rats fed ad libitum; ● B: sedentary rats fed 40% less than ad libitum; ○ BE: exercising rats fed same as B rats.*

Physical activity

The very large effect of DR on voluntary running activity is shown in Figure 3. This effect appeared at the onset of the DR regimen and resulted in a large and sustained difference in running activity between DR rats and rats fed *ad libitum*. Fischer 344 rats on the DR regimen continued running at high levels, even at a time when all A and AE rats had died. Running activity of AE rats declined rapidly with age but these rats exhibited low levels of running activity (50-100m/day) over most of their life span. In view of the intense voluntary running of BE rats, measurements of spontaneous cage activity (i.e., movement of rats around their cages unrelated to running-wheel activity) were of interest. These measurements (Fig. 4) show no significant differences between rats over the life span. The results demonstrate that high levels of voluntary wheel running did not result in lower levels of spontaneous cage activity at other times of the day. Rather, the lifelong intense running resulted in high levels of spontaneous cage activity in late life.

Energy expenditure

Measurements of daily energy expenditure are shown in Figure 5. There were no significant differences in specific metabolic rate (SMR) between groups up to age 18 months. From 24 months of age onwards, BE rats exhibited the highest levels of SMR in comparison with rats of all other groups.

Survival

Survival data are shown in Figure 6. Despite the high levels of physical activity, specific food consumption and SMR, BE rats had the greatest longevity. Median life span (50% survival) of BE rats (1123 days) was significantly greater than that of B rats (1024 days), but maximum life span (10% survival) of

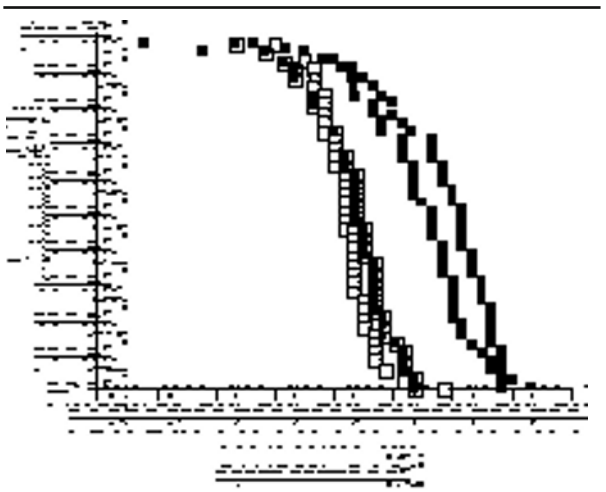

Figure 6 - *Survival of rats in each of the 4 groups. There were 40 rats in each group at the start of the experiment. Dietary restriction and wheel running commenced at 6 weeks of age. □ A: sedentary rats fed ad libitum; ■ AE: exercising rats fed ad libitum; ○ B: sedentary rats fed 40% less than ad libitum; ● BE: exercising rats fed same as B rats.*

Table 1 - *Incidence of severe disease at death (No. of rats exhibiting severe lesions).*

Group	Nephropathy	Cardiomyopathy	Pituitary Tumor	Leukemia/ Lymphoma
A	16	3	7	8
AE	9	3	10	10
B	1	3	1	6
BE	0	10	0	6

A total of 40 rats were necropsied in each group. For nephropathy the numbers represent rats exhibiting lesions of grades 4 and E. For cardiomyopathy, pituitary tumors and leukemia/lymphoma the numbers represent rats exhibiting lesions of grade 3 or greater.

both B and BE rats was similar (1205 days and 1235 days, respectively). Survival of B and BE rats was significantly greater than that of A and AE rats. Exercise did not significantly affect median and maximum length of life of rats fed *ad libitum*. Fifty percent survival of A and AE rats was 768 and 802 days, respectively. Maximum length of life of A and AE rats was 860 and 944 days, respectively, but the difference between these values did not reach statistical significance ($p>0.2$).

Pathology

Effects of diet and exercise on the diseases present at death are given in Table 2. Results are expressed in terms of the incidence of lesions which would be expected to be associated with significant impairment of function. In the case of chronic nephropathy, this corresponded to the presence of lesions of Grades 4 and E; for cardiomyopathy, this corresponded to lesions of Grade 3; for leukemia/lymphoma and pituitary tumor, Grades 3 and 4 (8, 10). In each group all 40 rats were necropsied. Results given in Table 1 represent the number of rats in each group exhibiting such major diseases. A large number of different tissues and lesions were examined (following the procedures of Iwasaki et al., 9). Results are presented only for the most prevalent and serious lesions found. As reported before (9, 10), chronic nephropathy was a major problem for these Fischer 344 rats fed *ad libitum* a diet containing casein as a protein source. Restriction of food intake significantly lessened the incidence of those lesions as did even the mild running activity of AE rats. Incidence of cardiomyopathy was significantly greater in BE rats, presumably a consequence of the intense running activity. The incidence of pituitary tumors was significantly suppressed by dietary restriction, and sustained exercise did not affect this suppression. The incidence of leukemia/lymphoma was not influenced by diet and exercise (Table 1), but it should be noted that B and BE rats exhibited this degree of severity at a much later age since pathology was obtained only following spontaneous death. Both diet and exercise, therefore, influenced tissue pathology presented at death: AE, B and BE rats had significantly lower incidence of chronic nephropathy; B and BE rats had significantly lower incidence of pituitary tumor; and BE rats had the highest incidence of cardiomyopathy at death. The prevalence of neoplastic and non-neoplastic diseases at death in each group are listed in Table 2. BE rats had significantly lower incidence of neoplastic diseases as probable causes of death compared to AE and B rats. This reduction in neoplastic disease is likely a major factor in the extension of median life span of BE *vs* B rats. It should be emphasized that A rats had less incidence of neoplastic diseases because they died earlier, due to severe chronic nephropathy. Interestingly, B and BE rats had high incidence of undetermined causes of death i.e., in some of these rats there was little incidence of severe tissue pathology presented at death.

Table 2 - *Probable cause of death.*

Group	Neoplastic Disease	Non-Neoplastic Disease	Undetermined
A	16	19	2
AE	22	10	3
B	23	6	10
BE	11	17	11

40 rats were necropsied in each of the 4 groups. The text provides references for criteria used in establishing major contributions to death.

DISCUSSION

The major finding of the present study was that lifelong voluntary physical activity did not extend maximum life span of SPF Fischer 344 rats using the 10% survival criterion, both for rats eating *ad libitum* and for rats fed a diet restricted to 40% less than *ad libitum*. Median survival of sedentary rats fed *ad libitum* was similar to that of exercising *ad libitum* fed rats. It should be noted that these rats ran very little over the life span however, and no attempt was made to induce additional running by dietary or other means (such as in reference 4). We have previously shown that even these low levels of running activity reduced lipid peroxidation damage and increased levels of anti-oxidant defenses in cardiac muscle (19), but these beneficial effects did not alter survival. Me-

dian survival of exercising restricted rats was significantly greater than that of sedentary restricted rats. This effect of sustained, high level exercise on survival was associated with reduced incidence of neoplastic disease but higher incidence of cardiomyopathy (Tables 1 and 2). Effects on median survival may be mediated by the intensity of exercise since they were found in restricted but not in *ad libitum*-fed rats.

These results are in general agreement with the previous work of Holloszy et al. (7) and Holloszy and Schechtman (4) in SPF Long-Evans rats. The results are at variance with those of Goodrick (6) for non-barrier maintained Wistar rats. The data suggest that beneficial effects of sustained voluntary exercise do not retard aging processes, as evidenced by a lack of effect of the lifelong running on maximum life span, in contrast to the marked effect of DR in extending both median and maximum life span. There are a number of differences between the present results and those of the earlier studies: exercise appeared to have deleterious consequences for the restricted runners studied by Holloszy and Schechtman (4). This was indicated by increased early mortality (20-30 months of age) in runners *vs* either pair-fed or paired weight sedentary control animals. Goodrick et al. (20) used voluntary wheel running (from 6 weeks of age) to study effects of exercise and every-other-day (EOD) feeding on longevity in male Wistar rats. Voluntary running (about 1.5 miles/day over the life span) increased longevity of *ad libitum* fed rats but decreased longevity of EOD rats: maximum life span (10th percentile survivors) decreased to 145 weeks from 158 weeks in exercising *vs* sedentary EOD rats, respectively. A similar effect appeared in the studies of Skalicky et al. (21), who combined mild DR (10% less than *ad libitum*) with forced treadmill running (5 days/week) from 6 months of age in non-barrier maintained Sprague-Dawley rats. In this study, exercise decreased the beneficial effects of DR as determined by a battery of biochemical and functional indices. No increase in early mortality or decrease in spontaneous physical activity were found in the present work. Rather, the increase in median life span and increased spontaneous cage activity in late life indicate few deleterious consequences of the sustained high levels of physical activity in these SPF Fischer 344 rats.

One adverse consequence of this activity, however, was a significant increase in severe cardiomyopathy, present in 25% of these rats at death (Table 1). The present study also demonstrated that high levels of voluntary wheel running did not result in less spontaneous movement around the cage at other times of the day. Rather, the data of Figure 4 indicate that spontaneous "cage" activity was similar in all groups. BE rats had significantly lower body weights over most of the life span but had the same daily food intake and similar caloric expenditure measured over 24 hours. Consequently, specific food intake and the specific metabolic rate of these rats was greater over most of the life span than those of other rats (Figs. 2 and 5), despite the increased longevity of these rats (Fig. 6). This finding is in contradiction to predictions of shorter life span with increased specific metabolic rate associated with the Rate of Living theory of aging (22). The data thus add to the accumulating literature in disagreement with predictions of that theory (14, 23, 24). It should be noted however, that physiological benefits of exercise, in affording protection from disease, might outweigh the contributions of increased oxidative stress associated with intense lifelong physical activity. Resolution of such paradoxes must await additional study.

A further difference between the present and previous work, is in the level of voluntary running of control rats. There was an immediate and large difference between daily running of restricted *vs ad libitum* fed rats (Fig. 3). In contrast, the earlier study of Holloszy and Schechtman (4) found higher initial running activity in control than in restricted rats. This difference probably reflects strain differences (Fischer 344 vs Long-Evans rats) and differences in age of initiation of running (6 weeks *vs* 12 weeks) but it should also be noted that the latter authors restricted food intake of control rats by a small amount (about 8%) in order to induce sustained running over the life span.

Despite these differences in experimental design and in genetic composition, a consistent finding of the present and previous studies in SPF rats is that maximum life span of restricted rats was not extended by additional physical activity. The present results demonstrate that restricted rats with wheels in cages ran great distances and also sustained high levels of physical activity around their cages over the life span. If physical activity is a significant component of the action of DR on aging, increased activity would be expected to further extend life span in a manner similar to the graded extension of maximum length of life produced by greater levels of DR (25). Since this result has not been found in studies of varying design and using different rodent strains, a reasonable conclusion is that increased physical activity is not a major factor in the action of DR on aging. Rather, the many beneficial effects of voluntary exercise extended life expectancy (50% survival) by providing protection against disease and by altering the pathology present at death.

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