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The effect of reduced physical activity on longevity of mice

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Abstract

Reduced physical activity leads, in female mice, to a reduction of the average and maximal life span. The average age at death of the inactive experimental group was 497 ± 121 days (mean $\pm$ S.D.) compared to 557 ± 139 days in the active control group, and the six oldest inactive experimental mice died at age 732 ± 50 days, while the six oldest active control mice died at 890 ± 52 days. The restriction of mobility was connected with a higher growth rate and a higher body weight in spite of a significant decrease in food intake. In spite of a reduced food intake leading to a reduced whole body metabolism, the results show that mobility restriction shortens life span in female mice.

Keywords: Female mice; Mobility restriction; Longevity

1. Introduction

Sixty years ago the beneficial influence of food restriction on life expectancy was described for the first time [1]. Since then a lot of studies have reproduced this finding [2–5]. Exercised animals resemble in many ways food restricted animals: exercised animals do not increase their food intake enough to compensate for the increased energy expenditure and food restricted animals show a higher level of spontaneous motor activity [6–8]. Indeed an increasing amount of evidence shows

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that chronically performed exercise like food restriction prevents several characteristic age related changes and increases survival [6,8–13]. Young mice are very active animals, and a forced reduced physical activity should exert a strong effect.

In the present study we compared an active control group of female mice housed in cages with a bottom area of 1820 cm^2 that were covered with a metal grid allowing climbing, and that were provided with running wheels for additional physical activity, with an inactive experimental group of female mice housed in cages with a bottom area of 400 cm^2 that were covered with a plastic grid, preventing climbing and having no running wheels. The aim of our experiment was to investigate the interrelationships between the physical activity, food intake, growth rate and longevity of the animals.

2. Methods

2.1. Animals

Female Swiss-albino mice (Him OF1) were purchased from Forschungsinstitut für Versuchstierzucht und Versuchstierhaltung of the University of Vienna (Himberg, Austria). This mouse stock was originally developed at Carworth Inc. (New City, New York, USA) as CF1 and brought to Iffa-Credo L'Arbresle, France, in 1966. The colony at the University of Vienna was developed from mice obtained from Iffa-Credo in 1980 and is maintained under SPF conditions. Mice are housed in a temperature (22 ± 1°C), humidity ($55 \pm 10\%$) and light controlled (light, 0530 to 1630 hours) room with its own ventilation system. No other animals were present in this facility. At 8 weeks of age weighing 24.0 ± 1.0 g they were randomly assigned to either an active control group of 80 animals or an inactive experimental group of 80 animals and were housed in polycarbonate cages on autoclaved softwood granules. The active control mice were maintained in polycarbonate cages with a bottom area of 1820 cm^2 in groups of five animals each. The cages were covered with metal grids and had three stainless running wheels (circumference 38 cm) attached to them. To get an idea of the amount of exercise performed, five mice were single-housed and were provided with one stainless steel running wheel with a circumference of 38 cm. These wheels were equipped with a permanent magnet, and the revolutions were counted by a hall-sensor which was connected to a personal computer. This counting device allowed a silent monitoring of the wheel revolutions. The inactive experimental animals were housed in polycarbonate cages with about only one fourth of the bottom area of the control group (400 cm^2) also in groups of five animals each. This represents a normal population density, the cages were not overcrowded and no signs of stress were observable. Furthermore the cages were covered with a special plastic grid that prevented climbing, a major form of physical activity. No running wheels were in the cages. The animals were weighed weekly by a veterinarian rodent expert, and on these occasions their health was checked.

None of the animals were subjected to any experimental treatment, and they were permitted to die of natural causes. Three animals in each group died, before the age of 25 weeks, of non-aging related causes; these deaths were considered to be censored observations. All dead animals were dissected, the organs (liver, spleen, kidneys) were weighed and visually inspected for tumors; no histological examinations were carried out. Cages were monitored daily for deaths.

2.2. Diet

All animals had free access to water and food. The diet was a commercial cereal-based preparation supplemented with vitamins and minerals (T 783 Tagger Kraftfutterwerke, Graz, Austria). The diet contained 18.5% crude protein, 3.6% crude fat and 5.4% crude fiber with a metabolizeable energy content of 11.6 MJ/kg. Food consumption was calculated weekly. Spillage was negligible.

2.3. Statistical analysis

Differences between the mean body weights of the two groups of mice were tested for significance by analysis of variance (ANOVA) for repeated measurements. At each time point t ($t = 4$, 8, 12,... weeks) an ANOVA was performed testing retrospectively the difference of the body weight curves, starting at time = 4 weeks up to time t. This type of analysis was performed in order to account for the statistical dependence of repeated measurements as well as for the dynamic evolution of the body weight curves. The same type of statistical analyses was applied to the data on food consumption.

The cumulative survival probabilities for experimental animals versus controls were estimated by the product limit technique [14]. Statistical differences between these survival curves were tested for significance using the generalized Savage test (Mantel-Cox test) which is known to be particulary sensitive to 'late' differences between survival curves. Additionally, the survival data were analyzed by the proportional hazards technique [15] in order to quantitate the effect of mobility restriction on survival.

The statistical analyses were performed using the BMDP software (BMDP Statistical Software, Cork, Ireland). Procedures BMDP2V (ANOVA for repeated measurements), BMDP1L (product–limit method) and BMDP2L (Cox technique) were employed.

To improve the visualization of the data, a logistic growth equation (see Table 1) was fitted to the body weight data and used for graphic representation. The parameters a, b and c were obtained by fitting this curve to the observed data using nonlinear regression analysis (program SigmaPlot, Jandel Scientific Software, Erkrath, Germany).

Table 1
Results of fitting a logistic growth equation[a] to the body weight data of inactive experimental and active control mice[b]

Group		a	b	c
Inactive experimental mice	Coefficient	38.54	0.0752	−0.0466
	S.E.	1.469	0.0080	0.0200
	P-value	<0.0001	<0.0001	0.020
Active control mice	Coefficient	30.46	0.1577	0.0401
	S.E.	0.2731	0.0067	0.0048
	P-value	<0.0001	<0.0001	<0.0001

[a]The following equation was employed:

$$y = \frac{a}{1+e^{-b \cdot t}} + c \cdot t$$

where a, b, c are parameters; y, body weight; and t, time (in weeks).
[b]Weekly measurements of body weights are used, from week 8 to week 80.

3. Results

3.1. Body weight development

Fig. 1 shows the changes in body weight of the inactive experimental and the active control group. Up to the 20th week there were no detectable differences in the body weights between the two groups. From then on the inactive experimental

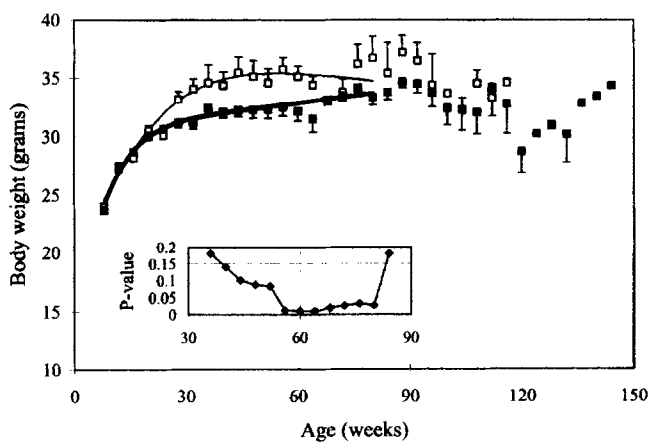

Fig. 1. Average body weights of the inactive experimental (open squares) and the active control mice (full squares). Vertical bars indicate S.E. values. The inset shows the results of statistical analysis of differences between both curves (ANOVA for repeated measurements). The curves show the results of fitting modified logistic growth equations to the data (thin curve, inactive experimental mice; thick curve, active control mice). For mathematical details see Table 1.

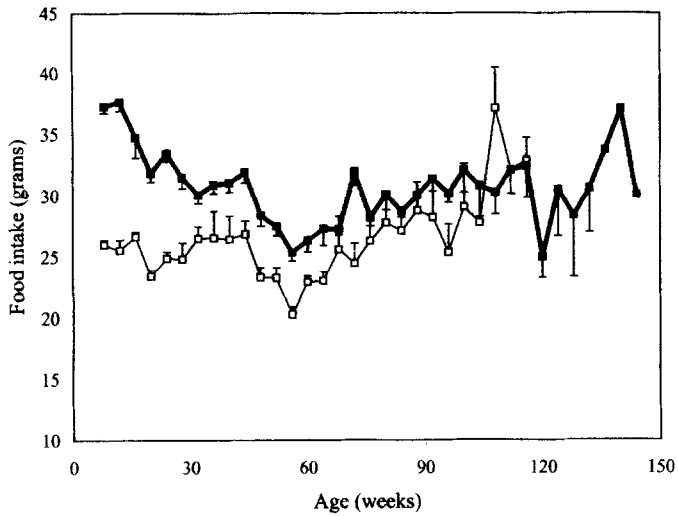

Fig. 2. Average food intake of the inactive experimental (open squares) and the active control mice (full squares). Vertical bars indicate S.E. values.

group gained weight more rapidly and attained a steady state at approximately the 40th week of age with a mean body weight of about 35 g. Thus, at this time they weighed about 10% more than the active control group. In contrast, the active control group increased, more slowly, its body weight up to week 80 when the animals reached an average weight of 34 g. There were significant differences between the body weight curves of the two groups from the 44th week on (ANOVA for repeated measurements). Table 1 shows the results of fitting a modified logistic growth equation to both body weight curves. After the 80th week, the differences between both groups vanished due to the increasing number of deaths rendering statistical analysis increasingly insignificant.

3.2. Food consumption

As shown in Fig. 2, in the first week the mean food consumption of the active control group was, with 37 g, 42% higher than in the inactive experimental group (26 g per week). Right from the start of the experiment, the differences between the food consumption curves of both groups remained highly significant ($P < 0.0001$; ANOVA for repeated measurements). Approximately up to the 90th week the active control group consumed significantly more food. The same curve, but even more pronounced, emerges when the food consumption was calculated per gram body weight (not shown). After the 90th week, the difference between the two curves was no longer significant because, as explained above, statistical analysis became increasingly hampered by the death of the animals.

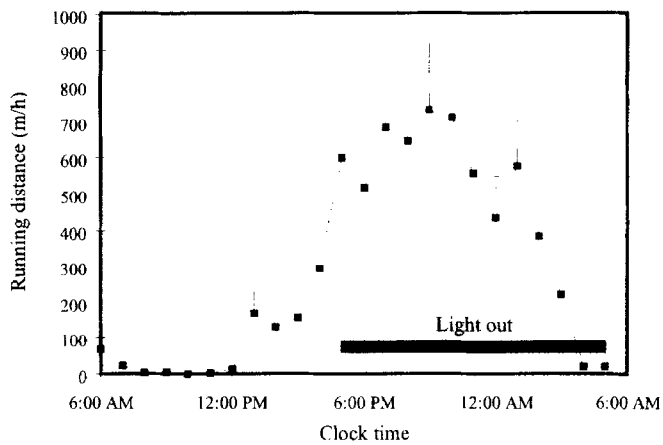

Fig. 3. Running activity per 24 h. Values are the means of five mice at the age of 25 weeks. Vertical bars indicate S.E. values.

3.3. Longevity

Fig. 4 shows the longevity of the two groups. Activity restriction resulted in a significant decrease of the average life span by 60 days or approximately 10%. The average age of death in the inactive experimental group was 497 ± 121 days and in the active control group 557 ± 139 days. The maximal life span was decreased by about 20%. The average age of the six oldest mice of the inactive experimental group was 732 ± 50 days (range 679–805) compared to 890 ± 52 days (range 791–980) in the active control group. All these differences were highly significant

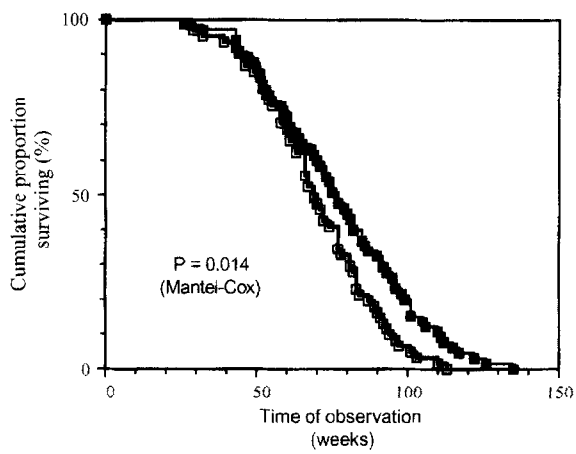

Fig. 4. Cumulative survival curves of the inactive experimental (open squares) and the active control mice (full squares), obtained by the product-limit method.

($P < 0.01$). Analysis of the cumulative survival curves by the product limit approach ($P = 0.014$) as well as a Cox regression analysis ($P = 0.034$) underscored these conclusions. From the Cox regression the effect of reduced physical activity on longevity could be estimated: risk increase of death for an inactive experimental as compared to a active control animal (100% relative risk) is 47%, as was calculated from the regression coefficient of $+0.3834$ (S.E., 0.1846).

3.4. Mortality

The weight of kidneys, spleen and liver was in the normal range, and no differences between the two groups were found. The total incidence of tumors was in, both groups, about 25%, as normally found in this mouse strain [16]. The tumors were preferrably found in the abdominal region of the mamma and on the hind limbs. Again, no statistically significant difference was detected between both groups.

4. Discussion

Mice of the Swiss-albino stock are very active animals. We found from the observation of single-housed mice that in the 25th week each animal ran 6916 ± 1290 (mean $\pm$ S.E.) m/day.The 24 h running rhythm of mice of this age shows a maximum of running between 1700 and 2300 h (Fig. 3). The basis of this 24 h periodicity is not caused by turning the light on or off ('Zeitgeber', [17]) but seems to be an endogenous process [18,19]. The mean values of the amount of running were affected by a remarkably high variance.

There is not much information available on the effect of physical activity and, to our knowledge, none on the effect of physical inactivity on the longevity of mice. Whereas earlier studies showed that physical exercise shortens life span [20,21] more recent studies showed an increase of survival [6,8–13]. It was hypothesized that exercise mediates the extension of life span by the same mechanisms as food restriction: as most exercising animals do not increase their food intake proportionally to their energy expenditure but rather decrease their food intake, hunger and/or appetite [22,23] they are in a state of a decreased availability of energy and thus resemble food restricted animals. But in contrast to food restriction only the average longevity and not the maximal life span seems to be increased [6,8,13]. Also, exercised female rats which in contrast to male rats increase their food intake proportionally to their energy expenditure, only prolong the average length of life [13]. The life prolonging effect of excercise seems so to be a special effect of its own, unrelated to the effect of food restriction.

In our experiment the physical activity of the active control animals did not significantly increase their longevity: their life span was with 557 ± 139 days in the upper range that has been repeatedly observed by us earlier using the same cages as in this study but without wheels (data not shown), and is in a range also reported by others [24]. One reason for this result could be that the amount of exercise with

or without wheels is not significantly different: we observed that animals having the opportunity to run in wheels decrease their activity of running on the floor and climbing on the cover grid. Exercise seems thus to exert only a protective action, keeping the animals in a state of better health without significantly influencing the survival rate. A restriction of the physical activity of this very active mice stock is, with high probability, a dramatic interference in their behavior connected with a series of physiological consequences.

The restraining of physical activity in our inactive experimental group resulted in a series of differences compared to the active control group: the animals gained more weight, had a higher growth rate; thus, they attained earlier a steady state body weight, consumed less food, and finally their average and maximal life spans were significantly reduced.

At the age of 40 weeks the inactive experimental group weighed approximately 35 g, and was about 10% heavier than the active controls. One can assume that due to the low physical activity, this increase in body weight may be attributed to an increase in total body fat; as a matter of fact, the observed increase would result in a duplication of this body compartment. Since a long time increased body fat has been associated with a higher risk of certain chronic diseases as, e.g. diabetes mellitus, hypertension, coronary heart disease and cancer [3,25], all diseases influencing the aging process.

The observed higher growth rate—the inactive experimental group attained the final body weight after 40 weeks compared to 80 weeks in the active control group—led, in all probability, to earlier sexual maturity, a process that has been associated with a reduced life span [26].

Although the inactive experimental animals consumed significantly less food than the active controls and were compared with them 'ad libitum food restricted', they showed a shorter average and maximal life span. The obvious lower metabolic rate as a result of a decreased activity combined with a decreased food intake did not lead to a longer life as could have been expected according to the hypothesis by Sacher [27] who suspected in the decreased metabolic rate the life-prolonging cause of food-restriction. Additionally, the results by Masoro and McCarter [3,28] showed that food restricted rats actually have a higher daily and life time intake of calories per gram body weight. However, this does not rule out that food restriction exerts its life prolonging effect by reducing the metabolic rate in special organs that participate in food absorption and digestion (stomach, intestine, liver, kidney) and increasing in others (muscle tissue) resulting in a constant whole body metabolic rate. Only in experiments with poikilotherms [29] and hibernating animals [30] life span seems to be unequivocably inversely proportional to the rate of whole body metabolism.

Physical inactivity could also exert a less favorable influence on the lipid/lipoprotein profile increasing the risk of cardio-vascular disease [31]. Also a deleterious effect via a reduced function of the immune system is being discussed [32]. It has been shown that the responsiveness of spleen lymphocytes to concanavalin A increases as the level of exercise increases [33]. Also other immune functions like natural killer cell activity and reduced tumor growth have been found to be

influenced by excercise [34]. The most prominent effect of exercise is the enhanced uptake of glucose combined with lower plasma glucose, insulin and triiodothyronine levels [35]. A high glucose level caused by physical inactvity over a long time could be especially responsible for an accelerated aging process caused by glycation of proteins and nucleic acids [36,37].

Our results show that voluntary reduced food consumption caused by reduced physical activity is not accompanied by the effects of forced food restriction like growth retardation, reduced body fat content and probably lower glucose levels and does not lead to a longer life span. On the other hand an increase in metabolism caused by an increase in food intake is not harmful if combined with an increase in energy expenditure.

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