ELSEVIER

Mechanisms of Ageing and Development
105 (1998) 61–73

mechanisms of ageing and development

The life-shortening effect of reduced physical activity is abolished by a fat rich diet

Walter Mlekusch [a,*], Manfred Tillian [b], Manfred Lamprecht [a], Karl Oettl [a], Hannes Krainz [a], Gilbert Reibnegger [a]

[a] *Institute of Medical Chemistry and Pregl Laboratory, Karl-Franzens-University Graz, Harrachgasse 21/II, Graz, A-8010, Austria*
[b] *Institute of Biochemistry, Karl-Franzens-University Graz, Halbärthgasse 5, Graz, A-8010, Austria*

Received 22 March 1998; received in revised form 6 July 1998; accepted 13 July 1998

Abstract

In female mice on a control diet (3.6% fat) reduced physical activity leads to a reduction of the average life span. So the average age at death of an inactive group is 500 ± 166 compared to 565 ± 175 days in an active control group. If the animals are kept on a fat rich diet (12.4% fat) this effect of physical activity restriction is no longer observable and the average age at death is 570 ± 142 days, within the range of the control animals. The increased fat intake seems to reduce the stress or to increase the resistance to stress in the activity restricted animals. So stress is a crucial determinant of life span.

Keywords: Mice; Mobility restriction; Fat diet; Longevity; Stress

1. Introduction

Acute restraint and immobilisation stress have been reported to suppress food intake and cause weight loss (Kennett et al., 1986; Krahn et al., 1990) and have

* Corresponding author. Tel.: +43 316 3804162; fax: +43 316 3809610; e-mail: walter.mlekusch@kfunigraz.ac.at

0047-6374/98/$ - see front matter
*PII S*0047-6374(98)00080-3

been proposed as an animal model of anorexia nervosa. In contrast, stressors such as a brief 20-min restraint exposure or tail pinch have been shown to promote feeding and weight gain in ad libitum fed rats (Morley and Levine, 1980; Badiani et al., 1996). Also young, very active mice stressed by a chronic reduction of physical activity do not adjust their food intake proportional to their reduced energy expenditure and show an increased weight gain. Besides, a restriction of the physical activity of female mice kept on a control diet significantly shortens their life-span as compared to a group that is provided with running wheels (Mlekusch et al., 1996b). In the present study we compared active with inactive mice kept on a control diet and on a fat-rich diet, respectively. The life shortening effect of physical activity restriction demonstrated in animals on a control diet was no longer observable under the fat diet regimen. The possible underlying mechanism could be an attenuation of stress.

2. Materials and 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). 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 ± 10%) and light controlled (light, 05:30–16:30 h) room with its own ventilation system in polycarbonate cages on autoclaved softwood granules. No other animals were present in this facility. At 8 weeks of age, weighing 24.0 ± 1.0 g, each animal was tested for its spontaneous activity by means of a running wheel. 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 very active animals running more than 10 km in 24 h and the very inactive animals running less than 1 km in 24 h were not used in the study. The animals were randomly assigned to four groups: two active and two inactive with 70 animals in each group. The active groups 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, allowing climbing, and had three stainless running wheels (circumference 38 cm) attached to them. The two inactive groups 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. Furthermore the cages were covered with a special plastic grid that prevented climbing, a major form of physical activity for mice. No running wheels were in the cages. The animals were weighed weekly by a veterinary rodent expert and on these occasions their health was checked.

None of the animals was subjected to any experimental treatment, and they were permitted to die of natural causes. The animals that died before the age of 25 weeks of non-aging related causes were considered to be censored observations. All dead animals were weighed, dissected, the organs (liver, spleen, kidneys) removed and 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. One active and one inactive group received a commercial cereal-based diet (control diet groups) supplemented with vitamins and minerals (T 783 Tagger Kraftfutterwerke, Graz, Austria). The pellets contained 3.6% crude fat, 18.5% crude protein, and 5.4% crude fiber. The metabolizable energy content amounted to 11.0 MJ/kg. A further active and inactive group got the same pellets that had been soaked in refined corn oil under reduced pressure (fat diet groups). One-hundred grams of pellets ≈ 10 g corn oil. This changed the composition of the pellets to 12.4% fat, 16.8% protein, and 4.9% fiber. The metabolizable energy content amounted to 14.0 MJ/kg. Corn oil is a highly unsaturated fat containing more than 50% as linoleic acid. The ratio of polyunsaturated to saturated fatty acids is ≈ 4.5 and the vitamin E activity is ≈ 220 mg/100 g. Food consumption was calculated weekly. Spillage was negligible.

2.3. Statistical analysis

Differences between mean body weights of the groups were tested for significance by analysis of variance (ANOVA) for repeated measurements. At each time point t ($t = 8,12,16...$ weeks) an ANOVA was performed testing retrospectively the difference of the body weight curves. After week 100 the increasing number of death rendered the statistical analysis insignificant. 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 analysis was applied to the data on food consumption.

The cumulative survival probabilities were estimated by the product limit technique (Kaplan and Meier, 1958). Statistical differences between these survival curves were tested for significance using the generalized Savage test (Mantel–Cox test) which is known to be particularly sensitive to 'late' differences between survival curves. To test for 'early' differences, we also employed the generalized Wilcoxon test. Additionally, the survival data were analyzed by the proportional hazards technique (Cox, 1972) in order to quantitate the effect of diet and 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. All significant test statistics are reported.

3. Results

3.1. Food consumption

During the 1st year (8–52th weeks) the active fat diet animals consumed ≈8% more energy than the active control diet group. This value was significant ($P = 0.0003$, ANOVA for repeated measurements). This significance was no longer observable during the 2nd year ($P = 0.4141$). Both curves declined continuously from ≈430 kJ in the 8th week and reached a plateau at ≈370 kJ at the end of the 1st year. This surplus of energy consumption by the fat diet animals was more pronounced when comparing the two inactive groups. During the 1st year the inactive fat diet animals consumed ≈20% more than the inactive control diet group. This value was significant ($P < 0.0001$, ANOVA for repeated measurements). This pattern was also observable during the 2nd year ($P < 0.0001$ and $P = 0.0153$). The energy intake of both inactive animal groups was remarkably constant over the whole life-time. Comparing the active with the inactive groups a pronounced difference was especially observable at the beginning of experiments when the two active groups consumed ≈430 kJ or 43% more than the inactive groups consuming ≈300 kJ. Whereas in the control diet groups (Fig. 1) this difference was significant over the 1st ($P < 0.0001$, ANOVA for repeated measure-

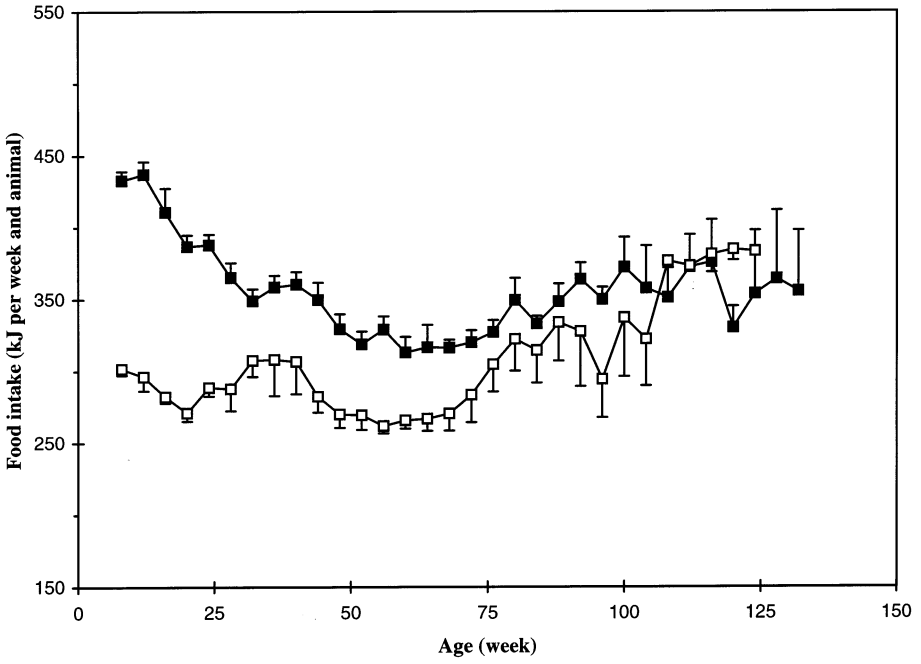

Fig. 1. Average food intake of the active control diet mice (filled squares) and of the inactive control diet mice (open squares). Vertical bars indicate S.E. values.

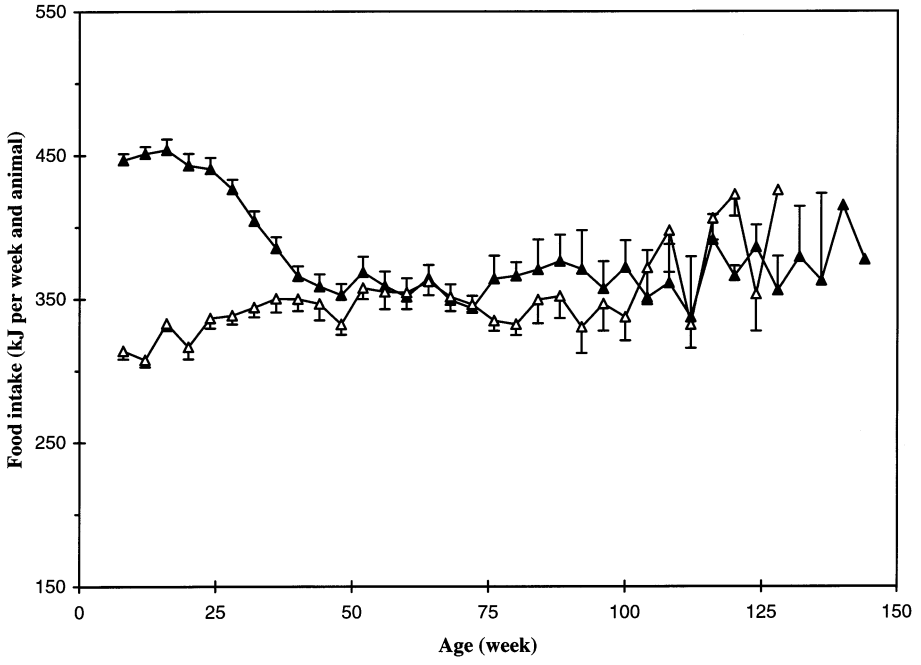

Fig. 2. Average food intake of the active fat diet mice (filled triangles) and of the inactive fat diet mice (open triangles). Vertical bars indicate S.E. values.

ments) and the 2nd year ($P = 0.0017$), it disappeared between the fat diet groups ($P < 0.0001$, after the 1st year and $P = 0.2162$ after the 2nd year; Fig. 2).

3.2. Body weight development

There was no significant difference detectable in body weights between the two active groups over the whole life span ($P = 0.3887$, ANOVA for repeated measurements). After a rapid rise during the first 24 weeks body weights slowly increased up to approximately the 80th week when the animals reached an average weight of 34 g. Both inactive groups gained weight more rapidly; the control diet group attained a steady state at approximately the 44th week of age with a mean body weight of ≈ 35 g and the fat diet group continued to increase its body weight up to the 56th week reaching ≈ 40 g. Compared to the active groups the inactive groups had significantly higher body weights over most of their life span (Figs. 3 and 4). So the P-value for repeated measurements between the two control groups in the time period from the 24th to the 92th week was 0.0416, and for the fat diet groups was 0.0042. From there on the differences between the groups vanished due to the increasing number of deaths rendering statistical analysis increasingly insignificant.

3.3. Longevity

There was no significant difference in longevity between the active control diet group and the active fat diet group. Analysis of the cumulative survival curves by the product limit approach produced P-values of 0.48 (generalized Savage test) and 0.20 (generalized Wilcoxon test). The average age of death in the active control diet group was 565 ± 175 days and in the active fat diet group 528 ± 186 days and showed no significant difference ($P = 0.2378$). Also the maximal life spans (10% survival) of 855 ± 56 days (range 798–931) in the active control diet group and 878 ± 96 days (range 770–1029) in the active fat diet group were not significantly different ($P = 0.6230$). The restriction of physical activity in the fat diet group (Fig. 5) did not lead to a significant change in the cumulative survival statistics ($P = 0.92$, generalized Savage test; $P = 0.28$, generalized Wilcoxon test). Neither the average age of death (570 ± 142 days, $P = 0.1434$) nor the maximal life span (10% survival, 786 ± 75 days, range 735–917, $P = 0.0903$) were significantly different. In contrast, the physical activity restriction in the control diet group (Fig. 6) resulted in a significant decrease in survival (cumulative survival data: $P = 0.02$, generalized Savage test and $P = 0.04$, generalized Wilcoxon). The average life span was 500 ± 166 days and the maximal life span (10% survival) was 765 ± 66 days (range 700–889). Both values were significantly different from the active control diet

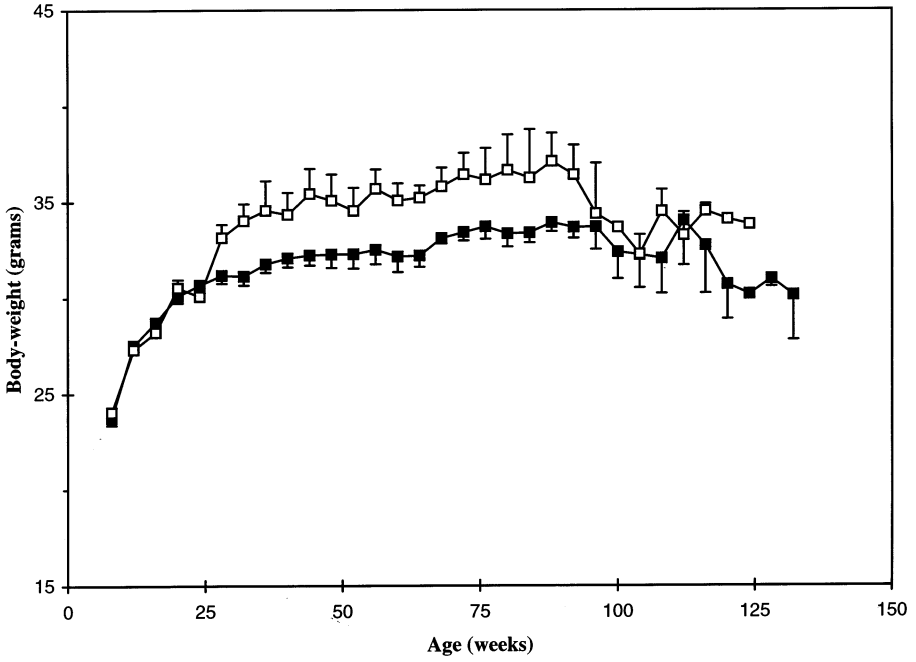

Fig. 3. Average body weights of the active control diet mice (filled squares) and of the inactive control diet mice (open squares). Vertical bars indicate S.E. values.

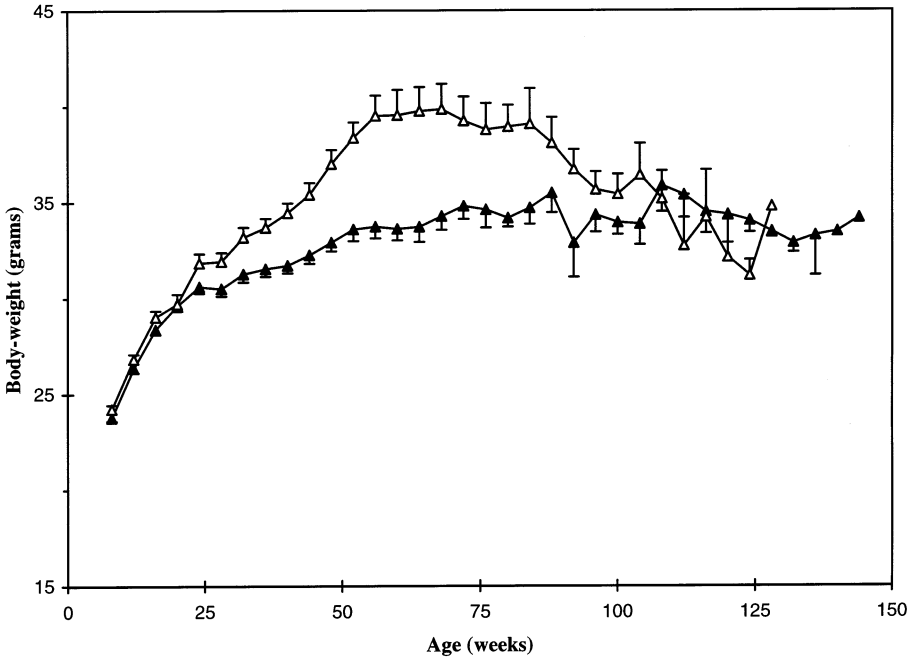

Fig. 4. Average body weights of the active fat diet mice (filled triangles) and of the inactive fat diet mice (open triangles). Vertical bars indicate S.E. values.

group ($P = 0.0304$; $P = 0.0257$). From the above results it is clear that significant differences were also observable between the inactive control diet group and the inactive fat diet group (generalized Savage test: $P = 0.05$; generalized Wilcoxon test: $P = 0.01$; Fig. 7). From the Cox regression the effect of reduced physical activity on longevity in the control diet group could be estimated: the risk of death is reduced by physical activity to 66.5% compared with inactive animals (100% relative risk). This was calculated from the regression coefficient $= -0.4076$ (S.E. $= 0.1783$, $P = 0.021$). In the same way the effect of fat diet on longevity in the inactive animals could be estimated: the risk of death is reduced in inactive fat diet animals to 71% compared with inactive control diet animals (100% relative risk). Again, this was calculated from the regression coefficient $= -0.3389$ (S.E. $= 0.1731$, $P = 0.049$).

3.4. Organ weights and tumor incidence

The body weights, and the weights of kidneys, spleens and livers were in the normal range, and no differences between the groups were found. The total incidence of tumors in both groups was $\approx 25\%$, as normally found in this mouse strain (Prejean et al., 1973). The tumors were preferably 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

In our previous publication (Mlekusch et al., 1996b) and in the present work we compared voluntary wheel running mice with mice restricted in their activity by a special cage, kept on a control diet, and observed a significant reduction in life span in the inactive group. This result stands in accord with investigations that have shown positive effects of physical activity to varying degrees on longevity of rodents; Goodrick (1980) found that voluntary wheel running Wistar rats lived 3–4 months longer than sedentary controls. A similar result was found by Holloszy et al. (1985) using Long–Evans rats: though the maximum life span was not changed by exercise, the average life span was increased. McCarter (McCarter et al., 1997) found in a study that rats with life-long food restriction and access to running wheels significantly increase their mean survival. He arrives at the conclusion that the beneficial effect of voluntary exercise lies in its protection against disease. It was also hypothesized that exercise mediates the extension of life by the same mechanisms as food restriction since most exercising animals do not increase their food intake proportional to their energy expenditure (Stevenson et al., 1966; Dohn et al., 1977) and so reach a state of voluntary food restriction. On the other hand dietary restriction leads to sustained high levels of physical activity (Yu et al., 1985; McCarter et al., 1997). So life-long physical activity could be an important component of the antiaging action of dietary restriction.

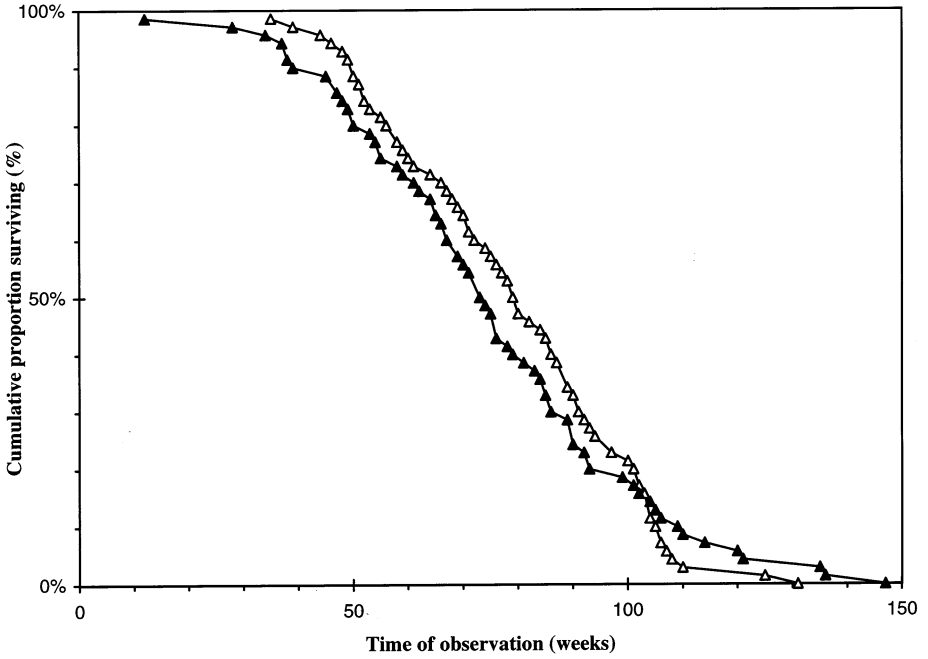

Fig. 5. Cumulative survival curves of the active fat diet mice (filled triangles) and of the inactive fat diet mice (open triangles), obtained by the product-limit method. The curves are not significantly different ($P = 0.92$).

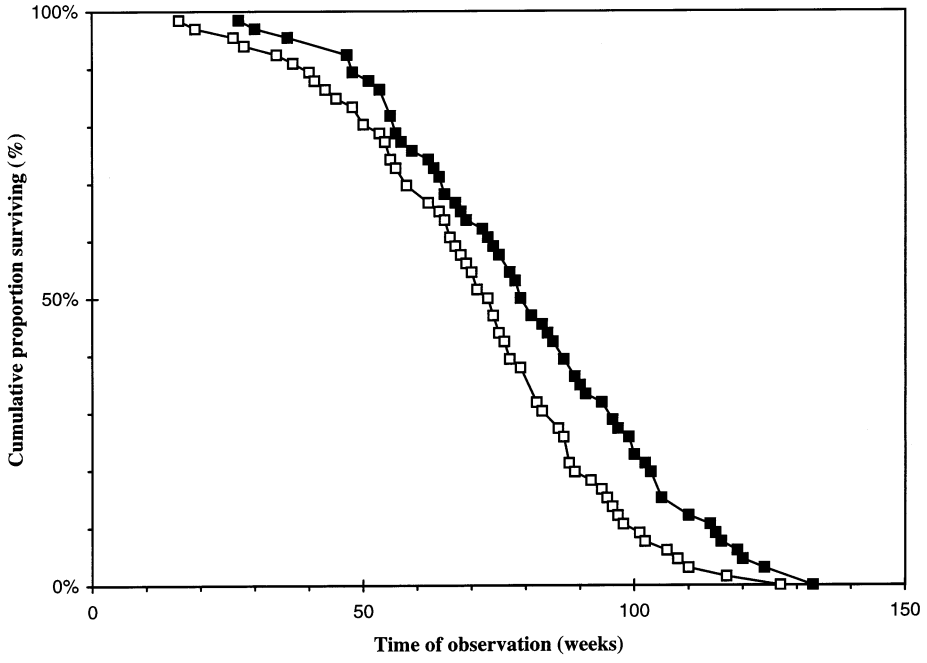

Fig. 6. Cumulative survival curves of the active control diet mice (filled squares) and of the inactive control diet mice (open squares), obtained by the product-limit method. The curves are significantly different ($P = 0.02$).

It is known that exercise and immune function interact in a complex network including central and peripheral nervous and endocrine systems (Nehlsen-Cannarella et al., 1997). Physical activity restriction could reduce survival by exerting a suppressive effect on the immune function (Hoffman-Goetz and Watson, 1994) leading to an increased susceptibility of the host to infection. Another possible contributing factor could be sensorimotor disturbances as described by Marshall (1982). The most prominent metabolic effect of exercise is the enhanced uptake of glucose combined with lower plasma glucose and insulin (Ivy and Holloszy, 1981). A high glucose level caused by physical inactivity over a long time could be especially responsible for an accelerated aging process caused by glycation of proteins and nucleic acids (Monnier, 1990; Sohal and Allen, 1990; Kristal and Yu, 1992; Mlekusch et al., 1996a).

But to what extent these factors contribute to the life prolonging effect of exercise, and vice versa the life shortening effect of physical inactivity, remains to be elucidated. The hypothesis that the life span is associated with the ability to respond to environmental stress becomes more and more significant.

The chronic restriction of physical activity creates a high probability of a stressful situation disturbing the homeostatic equilibrium especially in mice as active as the Swiss-albino stock where we found from the observation of single-housed mice that each animal in the 25th week of life ran on an average 7000 m/day (Mlekusch et al.,

1996b). A relationship between stress and aging has been observed for a long time; a stress theory of aging was proposed in the 1950s (Pare, 1965) and the glucocorticoid hypothesis of brain aging was proposed in the late 1970s (Landfield and Eldridge, 1994). There have been many reports showing that immobilisation stress induces oxidative damage through the increased production of free radicals and that this oxidative damage could contribute to the degenerative diseases of aging (Hidalgo et al., 1991; Kovacheva-Ivanova et al., 1992; Sosnovsky and Kozlov, 1992; Sosnovsky et al., 1992; Shvets and Davydov, 1993; Liu et al., 1994, 1996). Life extension seems to be associated with increased ability to respond to environmental stress and decreased susceptibility to stress-induced damage. Many studies show that energy restricted rodents have elevated ability to respond to stress such as reactive oxidant species and heat stress (Weindruch and Walford, 1988; Aly et al., 1993; Heydari et al., 1993) and an altered response to stress has long been thought to be part of the problem in aged animals (Pacifici and Davies, 1991).

In the present work we examined the influence of reduced physical activity on longevity of female mice kept on fat rich diet compared to a group kept on a control diet. For the control diet fed mice the results were the same as in our previous study: the physical activity restricted animals as compared with the active group gained more weight, had a higher growth rate and their average life span was

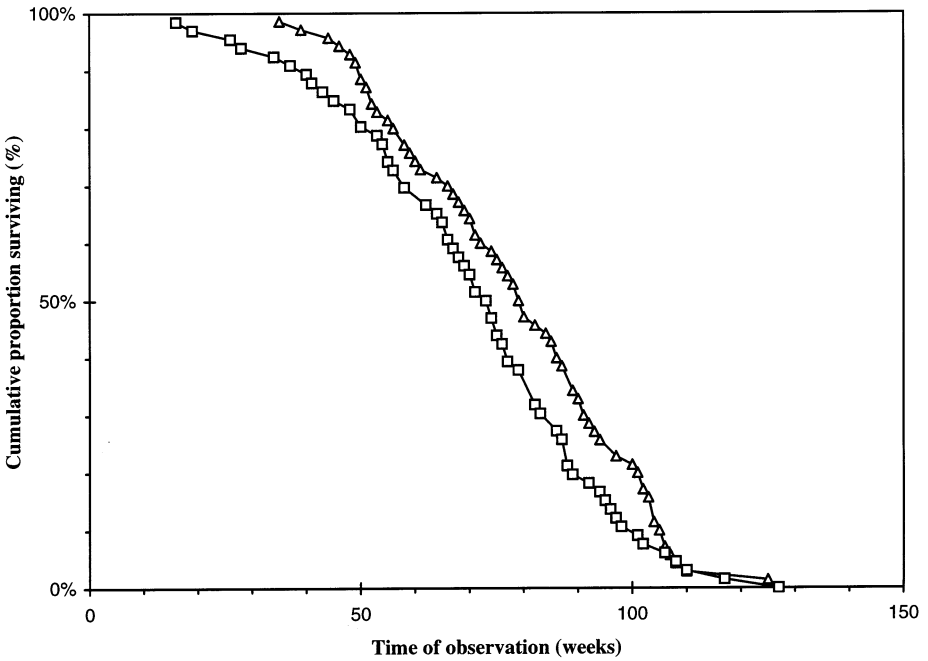

Fig. 7. Cumulative survival curves of the inactive control diet mice (open squares) and of the inactive fat diet mice (open triangles), obtained by the product-limit method. The curves are significantly different ($P = 0.05$).

significantly reduced. The oil-enriched diet showed no great influence on the active mice group: body weight development was identical with the control diet group. The animals showed a pronounced ability to adjust their food intake according to the density of their diets: they reduced the amount of weight of consumed pellets and only slightly exceeded the energy intake compared to a normal diet fed group. The small increase of energy intake was obviously compensated by increased exercise as the body weight curves of the two groups were nearly identical. As also observed in humans physical activity seems to play a pivotal role in energy regulation (Saltzman and Roberts, 1995). The average and maximal life spans of the two active groups were not statistically different. A quite different picture was observable when physical activity was restricted: the fat diet group ate nearly the same amount in weight as the control diet group and so consumed more than 20% extra energy and gained significantly more weight.

The most remarkable result of our investigation was, however, that the life reducing effect of physical inactivity was not observable under the fat feeding regimen. The survival data of the activity restricted fat diet animals were not different from the wheel runners. It looks as if the increased intake of fat helped the animals to cope with the stress caused by the restriction of physical activity. A connection between fat intake and stress was also observed by Dess et al. (1998): inescapable shock increased high fat food selection and attenuated stress-induced weight lost and anorexia. The mechanism behind this effect remains to be elucidated. Several lines of evidence suggest a connection between stress hormones, especially glucocorticoids, and fat intake. It was observed that fat intake is dramatically reduced following adrenalectomy, and further it was demonstrated that corticosterone replacement in adrenalectomized rats is linked with an enhanced fat appetite in a dose-dependent fashion (Wurdeman et al., 1978). Corticosterone is also a necessary condition for the expression of obesity promoted by highly palatable diets (Langley and York, 1990). These results were confirmed by Bligh et al. (1990). They showed that rats stressed by food deprivation, exhibiting elevated corticosterone levels, in a self-selected diet enhanced their fat consumption. Further results indicating a connection between fat intake and stress hormones was published by Yamaguchi and Matsuoka (1982). They found that in rats the increase in the activity of adenylate cyclase by electric stress, the main enzyme translating the fast acting stress hormones like catecholamines in their second messenger cyclic AMP, was reduced by high fat diet. A similar result was observed for glucocorticoid (Yamaguchi and Matsuoka, 1981). An association between stress and an increased fat intake was also observed in humans (Hellerstedt and Jeffery, 1997). Chronic exposure to high strain jobs is positively associated with a high fat intake.

From our results it could be concluded that stress in female mice evoked by physical activity restriction may be attenuated by an increased intake of fat. The reduction of stress or the increase of stress resistance with increased fat intake seems to be a more crucial determinant of life extension than the overweight produced by the high fat intake. So two factors, fat feeding and inactivity, each one considered as unfavorable for longevity, seem to cancel each other out if applied together.

Further studies will be directed at determining the connection between immobilisation stress, corticosterone levels and fat intake.

Acknowledgements

This research was supported by the Austria Nutrition Society, Vienna, Austria, by C.H. Knorr Nahrungsmittelfabrik Ges. m.b.H., Wels, Austria and Franz Lanyar Stiftung der Karl Franzens Universität, Graz, Austria. The assistance of Heinz Plank is gratefully appreciated.

References

Aly, K.B., Pipkin, J.L., Hinson, W.G., Feuers, R.J., Duffy, P.H., Lyncook, L., Hart, R.W., 1993. Chronic caloric restriction induces stress proteins in the hypothalamus of rats. Mutat. Res. 295, 191–200.

Badiani, A., Jakob, A., Rodaros, D., Stewart, J., 1996. Sensitization of stress-induced feeding in rats repeatedly exposed to brief restraint: the role of corticosterone. Brain Res. 710, 35–44.

Bligh, M.E., DeStefano, M.B., Kramlik, S.K., Douglass, L.W., Dubuz, P., Castonguay, T.W., 1990. Adrenal modulation of the enhanced fat intake subsequent to fasting. Physiol. Behav. 48, 373–381.

Cox, D.R., 1972. Regression models and life tables (with discussion). J. R. Stat. Soc. 34, 187–220.

Dess, N.K., Choe, S., Minor, T.R., 1998. The interaction of diet and stress in rats: high-energy food and sucrose treatment. J. Exp. Psychol. Anim. Behav. Proc. 24, 60–71.

Dohn, G.L., Bechner, G.R., Stephenson, T.P., Womack, M., 1977. Adaptations to endurance training at three intensities of exercise. J. Appl. Physiol. 42, 753–757.

Goodrick, C.L., 1980. Effects of long-term voluntary wheel exercise on male and female Wistar rats. 1. Longevity, body weight and metabolic rate. Gerontology 26, 22–33.

Hellerstedt, W.L., Jeffery, R.W., 1997. The association of job strain and health behaviours in men and women. Int. J. Epidemiol. 26, 575–583.

Heydari, A.R., Wu, B., Takahashi, R., Strong, R., Richardson, A., 1993. Expression of heat shock protein 70 is altered by age and diet at the level of transcription. Mol. Cell. Biol. 13, 2909–2918.

Hidalgo, J., Gasull, T., Garcia, A., Blanquez, A., Armario, A., 1991. Role of glucocorticoids and catecholamines on hepatic thiobarbituric acid reactants in basal and stress conditions in the rat. Horm. Metab. Res. 23, 104–109.

Hoffman-Goetz, L., Watson, R.R., 1994. Immune function in exercise, sport and inactivity. In: Hickson, J.F., Wolinsky, I. (Eds.), Nutrition in Exercise and Sport. CRC Press, Boca Raton, FL, pp. 475–493.

Holloszy, J.O., Smith, E.K., Vining, M., Adams, S.A., 1985. Effects of voluntary exercise on longevity of rats. J. Appl. Physiol. 59, 826–831.

Ivy, J.L., Holloszy, J.O., 1981. Persistent increase in glucose uptake by rat skeletal muscle following exercise. Am. J. Physiol. 241, C200–203.

Kaplan, E.L., Meier, P., 1958. Non-parametric estimation from incomplete observations. J. Am. Stat. Assoc. 53, 457–481.

Kennett, G.A., Chaouloff, F., Marcou, M., Curzon, G., 1986. Female rats are more vulnerable than males in an animal model of depression: the possible role of serotonin. Brain Res. 382, 416–421.

Kovacheva-Ivanova, S., Bakalova, R., Kagan, V., Georgriev, G., 1992. Activation of lipid peroxidation and changes in vitamin E contents in the lungs under oxidative stress. Bull. Exp. Biol. Med. 113, 132–134.

Krahn, D.D., Gosnell, B.A., Majchrzak, M.J., 1990. The anorectic effects of CRH and restraint stress decrease with repeated exposures. Biol. Psychiatry 27, 1094–1102.

Kristal, B.S., Yu, B.P., 1992. An emerging hypothesis: synergistic induction of free radicals and Maillard reaction. J. Gerontol. Biol. Sci. 47, B107–114.

Landfield, P.W., Eldridge, J.C., 1994. Evolving aspects of the glucocorticoid hypothesis of brain aging: hormonal modulation of neuronal calcium homeostasis. Neurobiol. Aging 15, 579–588.

Langley, S.C., York, D.A., 1990. The effects of the antiglucocorticoid RU-486 on the development of obesity in the obese 'fafa' Zucker rat. Am. J. Physiol. 259, R539–544.

Liu, J., Wang, X., Mori, A., 1994. Immobilization stress-induced antioxidant defense changes in rat plasma: effect of treatment with reduced glutathione. Int. J. Biochem. 26, 511–517.

Liu, J., Wang, X., Shigenaga, M.K., Yeo, H.C., Mori, A., Ames, B.N., 1996. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. FASEB J. 10, 1532–1538.

Marshall, J.F., 1982. Sensorimotor disturbances in the aging rodent. J. Gerontol. 37, 548–554.

McCarter, R.J.M., Shimokawa, I., Ikeno, Y., Higami, Y., Hubbard, G.B., Yu, B.P., McMahan, C.A., 1997. Physical activity as a factor in the action of dietary restriction on aging: effects in Fischer 344 rats. Aging Clin. Exp. Res. 9, 73–79.

Mlekusch, W., Lamprecht, M., Oettl, K., Tillian, M., Reibnegger, G., 1996a. A glucose-rich diet shortens longevity of mice. Mech. Ageing Dev. 92, 43–51.

Mlekusch, W., Tillian, H., Lamprecht, M., Trutnovsky, H., Horejsi, R., Reibnegger, G., 1996b. The effect of reduced physical activity on longevity of mice. Mech. Ageing Dev. 88, 159–168.

Monnier, V.M., 1990. Non-enzymatic glycosylation, the Maillard reaction and the aging process. J. Gerontol. Biol. Sci. 45, B105–111.

Morley, J.E., Levine, A.S., 1980. Stressed-induced eating is mediated through endogenous opiates. Science 209, 1259–1261.

Nehlsen-Cannarella, S., Fagoaga, O., Folz, J., Grinde, S., Hisey, C., Thorpe, R., 1997. Fighting, fleeing, and having fun: the immunology of physical activity. Int. J. Sports Med. 18 (Suppl. 1), 8–21.

Pacifici, R.E., Davies, K.J.A., 1991. Protein, lipid, and DNA repair systems in oxidative stress: the free radical theory of aging revisited. Gerontology 37, 166–180.

Pare, W.S., 1965. The effect of chronic environmental stress on premature aging in the rat. J. Gerontol. 20, 78–84.

Prejean, J.D., Peckham, J.C., Casey, A.E., Griswold, D.P., Weisburger, E.K., Weisburger, J.H., 1973. Spontaneous tumors in Sprague–Dawley rats and Swiss mice. Cancer Res. 33, 2768–2773.

Saltzman, E., Roberts, S.B., 1995. The role of energy expenditure in energy regulation: findings from a decade of research. Nutr. Rev. 53, 209–220.

Shvets, V.N., Davydov, V.V., 1993. State of induced lipid peroxidation in the heart of mature and aged rats under stress. Ukr. Biokhim. Zh. 65, 91–96.

Sohal, R., Allen, R., 1990. Oxidative stress as a causal factor in differentiation and aging: a unifying hypothesis. Exp. Gerontol. 25, 499–522.

Sosnovsky, A.S., Kozlov, A.V., 1992. Increased concentration of thiobarbituric acid-reactive material in the hypothalamus of rats subjected to a one hour emotional stress. Bull. Exp. Biol. Med. 113, 486–488.

Sosnovsky, A.S., Tsvetkova, M.A., Uzunova, P.I., Glbova, T.D., Peneva, V.I., Sokolova, T., Ribarov, S.R., Nikolov, N.A., 1992. Lipid peroxidation in emotional stress in rats: correlation with parameters of open field behavior. Bull. Exp. Biol. Med. 113, 19–21.

Stevenson, J.A.F., Box, B.M., Feleki, V., Beaton, J.R., 1966. Bouts of exercise and food intake in the rat. J. Appl. Physiol. 21, 118–122.

Weindruch, R., Walford, R.L., 1988. The Retardation of Aging and Disease by Dietary Restriction. Thomas, Springfield, IL.

Wurdeman, R., Berdanier, C.D., Tobin, R.B., 1978. Enzyme overshoot in starved-refed rats: role of the adrenal glucocorticoid. J. Nutr. 108, 1457–1461.

Yamaguchi, K., Matsuoka, A., 1981. Effects of a high fat diet and electric stress on insulin response to hydrocortisone and catecholamines in isolated pancreatic islets. Horm. Metab. Res. 13, 9–13.

Yamaguchi, K., Matsuoka, A., 1982. Effects of a high fat diet and electric stress on adenylate cyclase activity and insulin release in isolated islets of Langerhans. Horm. Metab. Res. 14, 117–121.

Yu, B.P., Masoro, E.J., McMahan, C.A., 1985. Nutritional influences on aging of Fischer 344 rats. 1. Physical, metabolic and longevity characteristics. J. Gerontol. 40, 657–670.