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Effects of long-term, light exercise under restricted feeding on age-related changes in physiological and metabolic variables in male Wistar rats

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Abstract

The effects of long-term, light exercise under restricted feeding on age-related changes in physiological and metabolic functions were examined in male Wistar rats. Adult (100 days old) rats were divided into sedentary (R10S) and exercise (R11E) groups, and given 10 and 11 g/day, respectively, of a 20% casein diet until they reached 900 days of age. Group R11E simultaneously underwent 3000 m/day of running exercise throughout the test period. As compared with the sedentary group, long-term, light exercise significantly increased body nitrogen retention and serum protein levels, decreased body fat and plasma insulin levels, prevented age-related decline in the basal metabolic rate, and reduced age-associated histopathological changes in the kidney and liver. Long-term, light exercise further enhanced the benefits of restricted feeding on age-related deterioration in physiological and metabolic variables and improved body composition, but did not prolong survival at 900 days of age.

Keywords: Dietary restriction; Long-term light exercise; Nitrogen balance; Basal metabolism; Aging rats

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1. Introduction

As compared with ad libitum feeding, in rats and mice, restricted feeding reduces the incidence of age-related diseases (Ross and Bras, 1965; Fujita et al., 1984; Lloyd, 1984) and reduces declines in physiological and metabolic functions (Masoro, 1984; Fujita and Ichikawa, 1986; Ichikawa and Fujita, 1987) with age, resulting in prolonged survival (Nolen, 1972; Ross, 1981). On the other hand, in ad libitum feeding rats, voluntary wheel running prolonged the survivals of rats (Goodrick, 1980). In addition, appropriate and regular exercise improved physiological and metabolic functions in animals (Brooks and White, 1978; Mazzeo and Horvath, 1986; Tobin and Beard, 1990) and humans (Poehlman et al., 1991; Hughes et al., 1993). However, most such investigations involved a special program consisting of moderate or heavy exercise with a higher consumption of oxygen over a shorter period of time. We know empirically that compared with sedentary daily life, normal physical activities in day-to-day life in humans must have some benefit in terms of maintaining and promoting health. Nevertheless, therein little experimental evidence of this, as a result of the difficulty of establishing an experimental model.

Laboratory rats are normally confined to cages that markedly restrict their physical activity, with resting energy expenditure accounting for 90% of the total daily energy expenditure, and daily physical activities accounting for the remaining 10% (Ichikawa and Fujita, 1987). This profile of energy expenditure in laboratory rats in cages is virtually identical to that of very sedentary elderly persons living in nursing homes (Fujita and Ohzeki, 1993) and hospitals. The purpose of this study is to prove experimentally whether in rats under restricted feeding, such long-term, light exercise equivalent to normal physical activity in day-to-day life in humans also produces benefits involving age-related changes in physiological and metabolic functions.

2. Materials and methods

2.1. Animals and diets

Male Wistar rats (Shizuoka Agricultural Cooperative for Laboratory Animals, Shizuoka, Japan) were fed a 20% casein diet (Table 1) ad libitum until maturity (100 days old), then divided into two groups. One group, group R10S (12 rats), received 10 g/day of the 20% casein diet, which is ~60% of the daily food consumption of rats fed ad libitum, and was kept under sedentary conditions in cages until 900 days of age. Group R11E (11 rats) were given 11 g/day of the same diet, with one gram of additional food given for increased energy expenditure by exercise, and also simultaneously underwent 3000 m/day of running-wheel exercise. Based on a preliminary study, the energy expenditures for this exercise were determined to be equivalent to ~1 g/day of the 20% casein diet used. The animals were kept in an air-conditioned room at 22 ± 2°C with a 12-h light period from

08:00 to 20:00 h. They were weighed once a week, and their food consumption was recorded daily.

2.2. Exercise

Group-R11E rats were housed individually throughout the test period in a structure especially designed for this kind of experiment. This structure consisted of a housing area of the same size as normal cages for the sedentary rats, a running wheel of 1 m/rotation, and an automatic feeding device electrically interlocked with the running wheel (Fig. 1). In this structure, group-R11E rats received daily a constant amount of food (11 g/day) and running exercise (3000 m/day) throughout the study.

2.3. Metabolic study and determination

The animals were kept for 3 consecutive days in individual metabolic cages, which were specially designed and were the same size as their normal cages, at 100-day intervals for the purpose of measuring nitrogen balance and basal metabolic rate (BMR), samples of urine and feces were collected. The nitrogen,

Table 1
Composition of 20% casein diet

Ingredients	Amount (%)
Casein[a]	20.0
L-Methionine[b]	0.3
Sucrose[a]	20.9
α-Cornstarch[a]	41.8
Vitamin mixture[c]	2.0
Mineral mixture[c]	
Macro-elements[d]	4.8
Micro-elements[d]	0.2
Cellulose powder[e]	5.0
Corn oil[a]	5.0

[a] From Oriental Yeast, Tokyo.

[b] From Wako Pure Chemical Industries, Osaka.

[c] From Oriental Yeast, Tokyo. Contents per 100 g of diet: retinyl acetate, 1000 IU; cholecalciferol, 200 IU; DL-α-tocopheryl acetate, 10.0 mg; menadione, 10.4 mg; thiamin · HCI, 2.4 mg; riboflavin, 8.0 mg; pyridoxine · HCI, 1.6 mg; cyano-cobalamine, 1.0 pg; D-biotin, 40 μg; folic acid, 0.4 mg; D-calcium pantothenate, 10.0 mg; nicotinic acid, 12 mg [these 11 ingredients are standard vitamins for rats and mice as reported by the American Institute of Nutrition (1977)]; ascorbic acid, 60 mg; *p*-amino benzoic acid, 10.0 mg; Inositol, 12.0 mg; and choline chloride, 400 mg.

[d] From Oriental Yeast. Contents per 100 g of diet: $CaHPO_4 \cdot H_2O$, 699 mg; KH_2PO_4, 1235 mg; NaH_2PO_4, 449 mg; NaCl, 224 mg; calcium lactate, 1684 mg; ferric citrate, 153 mg; $MgSO_4$, 344 mg; $ZnCO_3$, 5.28 μg; $MnSO_4 \cdot 6H_2O$, 5.76 μg; $CuSO_4 \cdot 5H_2O$, 1.44 pg; and KI, 0.48 μg.

[e] Composition as reported by Ebihara et al. (1979).

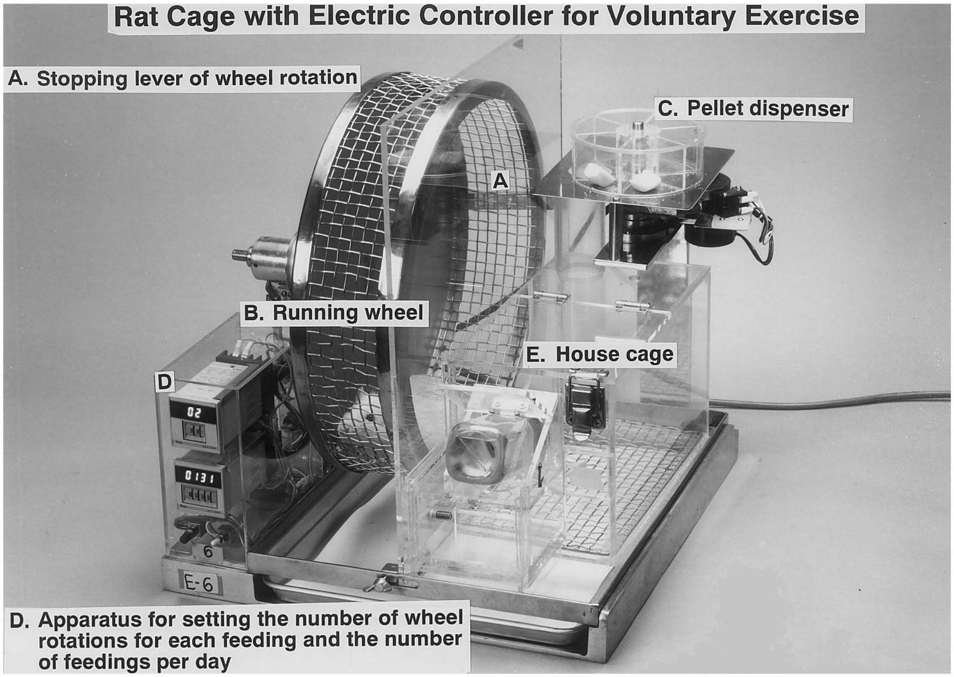

Fig. 1. The apparatus used for exercise loading in this study. Rats in group R11E were housed individually throughout the test period in the structure shown in this photograph, which consisted of a stopping lever of wheel rotation (A), a running wheel (B), an automatic feeding device interlocked with the running wheel (C), an electric controller (D), and a housing area (E). This structure functions as follows: whenever rats run a certain portion (e.g. one-fifth) of total daily scheduled exercise distance, the interlocking feeding device operates, and the same proportion (e.g. one-fifth) of the day's total food allotment is automatically supplied. When the animals have performed all of the day's scheduled exercise and received all of the day's food, the running wheel is electrically locked. Therefore, the rats in this study received a constant amount of food and running exercise throughout the test period.

energy, and fat contents of the urine, feces and food during the test period were determined by the Kjeldahl method, through the use of an automatic bomb calorimeter (Shimazu, Model CA-4, Japan), and by the hot ether extraction method (Laboman Geneco, Ex-Fat, USA), respectively. From the results, nitrogen balance and the apparent digestibility of food ingested were calculated. In the energy metabolic study, we continuously measured the oxygen and carbon dioxide levels in the expired air for 24 h using an automatic O_2–CO_2 analyzer (Nihon Denki SanEi, Model IH26, Japan), and calculated the BMR on the basis of the lowest value of oxygen consumption per hour. On day 900 of age, some biochemical constituents in the serum were measured using an automatic blood chemistry analyzer (Hitachi, Model 763, Japan). For some tissues, the histopathological changes were observed using a optical microscope at 900 days of age. The carcass was frozen, chopped, and dried at 100 ± 2°C and its water, fat, and protein contents were measured as described above. The dried carcass was also incinerated, and the weight was measured as crude ash.

2.4. Data analysis

In this study, groups R10S and R11E consisted of 12 and 11 rats, respectively, when the experiment began. However, five rats in each group died before reaching 900 days of age. Accordingly, all results other than survival and histopathological data are shown for the seven rats in group R10S and six in group R11E surviving at 900 days of age when assayed. The data was expressed as means ± S.D. and analyzed using the Student's *t*-test.

3. Results

3.1. Food consumption, body weight, and running performance

Results are shown in Fig. 2. All animals in both groups consumed more than 98% of the food given, and maintained a constant body weight throughout the test period. In terms of running performance, eight of the group-R11E rats attained 3000 m/day as scheduled within two months after the start of the experiment. However, since running distances for the remaining three rats were in the range between 2500 and 2700 m/day throughout the test period, the mean running distance for group-R11E rats ranged from 2800 to 3000 m/day. Results for survival are shown in Fig. 2. At 850 days of age, eight rats (66.6%) of the 12 in group R10S and nine rats (81.8%) of the 11 in group R11E survived. However, when assayed at 900 days of age, there were seven rats (58.3%) for group R10S, and six rats (54.5%) for group R11E.

3.2. Tissue weight

Table 2 shows the weights of various tissues at 900 days of age. Group R11E showed significantly increased mass for the gastrocnemius and lateral omohyoideus muscles, and tended to increase slightly the mass of the soleus and extensor digitorum longus muscles, as compared with those in group R10S. In contrast, the weight of visceral organs such as the liver, kidney, heart, small intestine, spleen, and adrenal gland showed no significant differences between the groups. Interestingly, the brain weight was significantly higher in the exercised rats than the sedentary rats.

3.3. Histopathological observation

The results are shown in Table 3. At 900 days of age, there were high incidences of fibrosis in the heart and leydig's cell tumor in the testis in both groups. However, incidences of hypercholangiole in the liver and glomerulopathy in the kidney were decreased by simultaneous exercise loading (45 and 73% for sedentary rats vs. 11 and 44% for exercised rats, respectively).

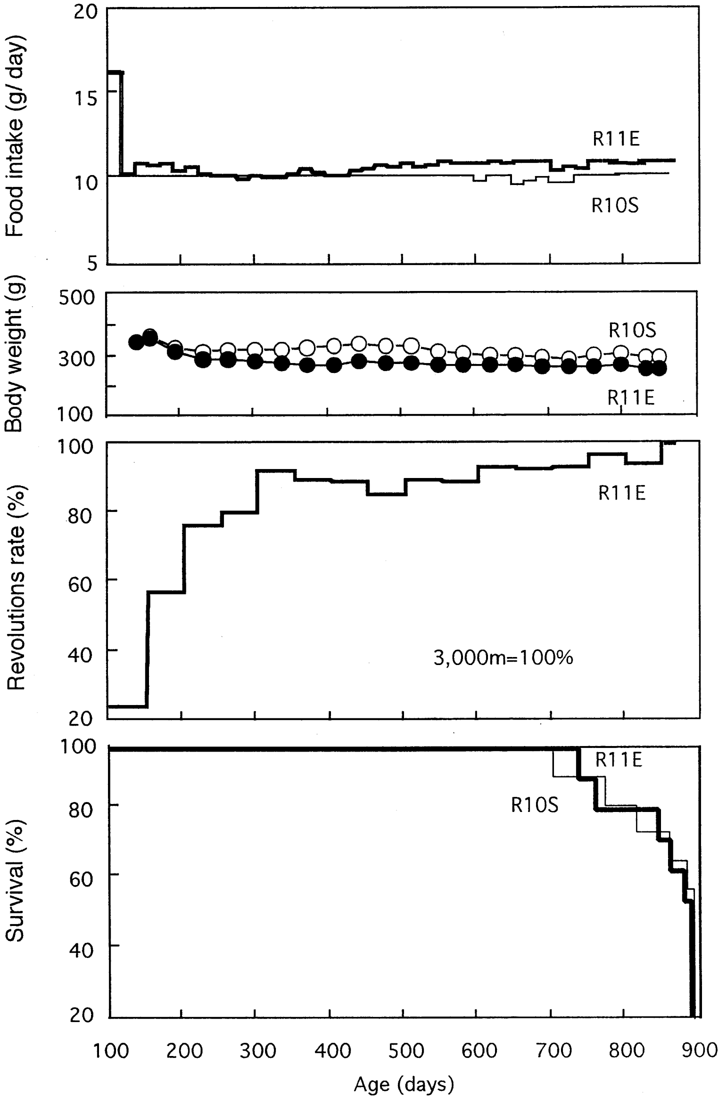

Fig. 2. Changes in food consumption, body weight, running performance, and survival. The R10S group was given 10 g/day of a 20% casein diet from 100 to 900 days of age under sedentary conditions. Group R11E received 11 g/day of the same diet, including 1 g/day of additional food for the increased energy expenditure for exercise, and simultaneously underwent 3000 m/day of wheel-running throughout the test period. Values are the mean of seven rats for group R10S (○) and 6 rats for group R11E (●) who were killed for measurement purposes at 900 days of age. Vertical bars indicate standard deviation. Figures in parentheses indicate the numbers of rats living at the time.

Table 2
Effects of long-term, light exercise under restricted feeding on some tissue weights at 900 days of age in male rats

Group[a]	Liver	Kidney	Heart	Lung
R10S	37.38 ± 12.39[b]	8.13 ± 2.14	3.29 ± 0.42	7.26 ± 2.18
R11E	34.37 ± 4.85	8.43 ± 0.55	3.65 ± 0.37	6.77 ± 2.23
	Small intestine	Spleen	Brain	Adrenal gland
R10S	11.98 ± 3.47	11.14 ± 14.05	6.90 ± 0.27	0.159 ± 0.021
R11E	12.79 ± 1.69	6.72 ± 5.29	7.84 ± 0.45***	0.171 ± 0.018
	GM[c]	LOM[d]	EDL[e]	SM[f]
R10S	3.62 ± 0.23	0.33 ± 0.04	0.41 ± 0.05	0.42 ± 0.04
R11E	4.58 ± 0.53**	0.44 ± 0.05*	0.45 ± 0.06	0 44 ± 0.08

[a] For details on groups, see the text.

[b] Mean ± S.D. of seven rats for group R10S and six rats for group R11E. Values expressed as g/kg BW.

[c] GI, gastrocnemius muscle.

[d] LOM, lateral omohyoideus muscle.

[e] EDL, extensor digitorum longus muscle.

[f] SM, soleus muscle.

* Significantly different from the value for group R10S at $P<0.05$.

** Significantly different from the value for group R10S at $P<0.01$.

*** Significantly different from the value for group R10S at $P<0.001$.

Table 3
Effects of long-term, light exercise under restricted feeding on histopathological changes in some visceral organs at 900 days of age in male rats

Organ	Histopathological findings	Group R10S[a], $n=11$	Group R11E[a], $n=9$
Lung	Pneumonia	0	0
Liver	Hypercholangiole	5 (45)[b]	1 (11)
	Hyperplastic nodule area	2 (18)	1 (11)
Kidney	Glomerulopathy	8 (73)	4 (44)
	Calcification	1 (9)	2 (22)
Stomach	Pyloric polyp	0	0
Pancreas	Islet cell tumor	0	0
Spleen	Lymphoma	1 (9)	0
Testis	Leydig cell tumor	7 (64)	8 (89)
Skin	Tumor	2 (18)	0

[a] For details on groups, see the text.

[b] Values are the numbers of rats, and the figures in parentheses are those values expressed as a percentage.

3.4. Body composition

The results of carcass analysis at 900 days of age are shown in Table 4. Dietary restriction prevented age-related changes in body composition between 100 and 900 days of age. On the other hand, long-term, light exercise in combination with restricted feeding significantly decreased the proportion of body fat and increased the proportion of body protein at 900 days of age, as compared with those for group R10S.

3.5. Nitrogen balance

Apparent digestibility remained almost constant from 92 to 94% for protein, 93–97% for lipids, and 91 to 93% for total energy in both groups throughout the test period, irrespective of age and exercise. The mean nitrogen balances for both groups were positive throughout the test period, and the values for group R11E were significantly higher than those in group R10S, irrespective of age, except at 500 days of age (Fig. 3). Long-term, light exercise enhanced further nitrogen retention in the body, as compared with the sedentary group.

3.6. Basal metabolic rate

Fig. 4 shows age-related changes in the BMR. In rats consuming a constant amount of food throughout their lives under restricted feeding, the mean BMR remained almost constant for group R10S and increased slightly for group R11E with advancing age. Consequently, the mean BMR at 900 days of age was significantly higher in group R11E than group R10S. When the BMR is expressed on the basis of urinary creatinine excretions, which roughly estimates skeletal muscle mass, values for both groups increased progressively with advancing age, and the values at 600 and 900 days of age for group R11E were significantly higher.

Table 4
Effects of long-term, light exercise under restricted feeding on body compositions at 900 days of age in male rats

Group[a]	Crude protein	Crude lipids	Water	Total ash
Initial[b]	21.78 ± 0.683	20.57 ± 2.71	54.23 ± 2.06	3.32 ± 0.49
Terminal[c]				
R10S	21.33 ± 1.63	18.92 ± 5.46	55.73 ± 4.56	4.02 ± 0.42
R11E	23.19 ± 1.11*	7.00 ± 5.43**	65.43 ± 4.03**	4.38 ± 0.53

[a] For details on groups, see the text.

[b] Initial and terminal indicate 100 and 900 days of age, respectively.

[c] Mean ± S.D. of seven rats for group R10S and six rats for group R11E. Values expressed as percentage (%).

* Significantly different from the value for group R10S at $P<0.05$.

** Significantly different from the value for group R10S at $P<0.01$.

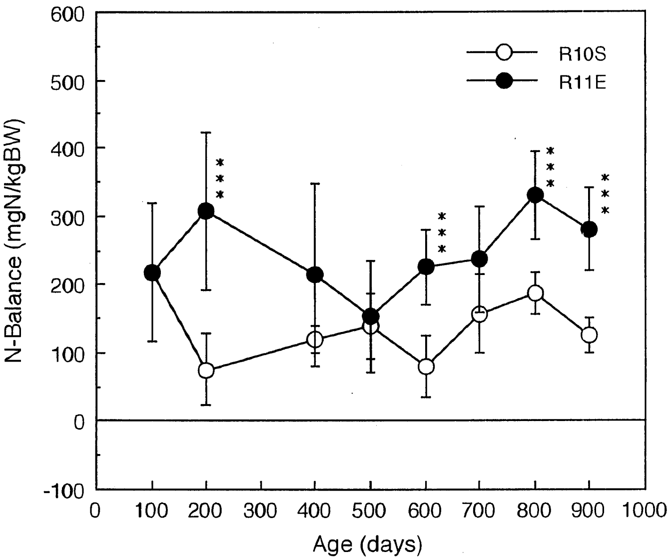

Fig. 3. Effects of long-term, light exercise under restricted feeding on age-related changes in nitrogen balances of male rats. Group R10S was given 10 g/day of a 20% casein diet from 100 to 900 days of age under sedentary conditions. Group R11E received 11 g/day of the same diet, including 1 g/day of additional food for the increased energy expenditure for exercise, and simultaneously underwent 3000 m/day of wheel-running throughout the test period. Nitrogen balance was examined for 3-day periods at intervals of 100 days from day 100. Values are the mean ± S.D. of seven rats for group R10S (○) and six rats for group R11E (●).

3.7. Blood chemistry

The results for some biochemical constituents in the serum at 900 days of age are shown in Table 5. In comparison with the sedentary rats, long-term, light exercise significantly increased the concentrations of total protein and significantly decreased plasma insulin levels. However, the exercise resulted in no significant changes in total cholesterol and triglyceride levels, because our rats of both groups were under restricted feeding conditions.

4. Discussion

In this study, as a model of light exercise virtually equivalent to normal physical activity in day-to-day life in humans, group-R11E rats were given 3000 m/day of running exercise with 11 g/day of a 20% casein diet, including 1 g/day of additional food to compensate for increased energy expenditures for exercise. Their body weight remained constant throughout the test period. In addition, the one gram of additional food was no more than 10% of the daily food intake of sedentary rats in cages, who also maintained their body weight throughout. Based on this, it may be safely said that 3000 m/day of running exercise in this study was certainly a light level.

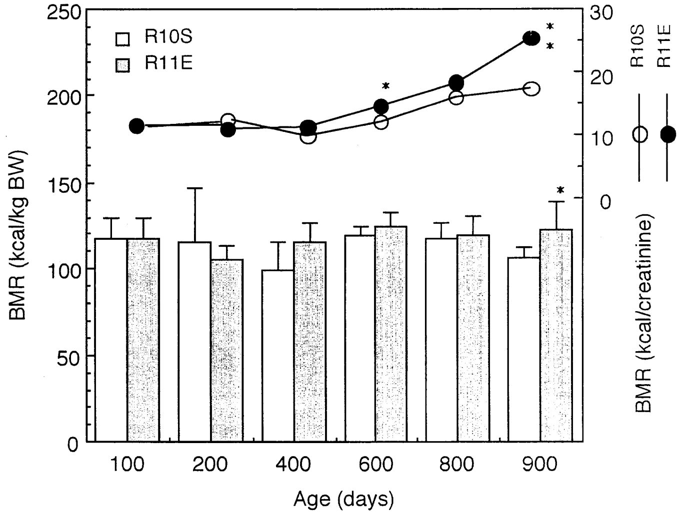

Fig. 4. Effects of long-term, light exercise under restricted feeding on age-related changes in the basal metabolic rate in male rats. For details on the groups, see the text. The oxygen and carbon dioxide levels in the expired air of rats in metabolic cages were continuously measured for 24 h using an automatic O_2–CO_2 analyzer at 200-day intervals, and the basal metabolic rate was calculated on the basis of the lowest value of oxygen consumption per hour. Values are the mean ± S.D. of seven rats for group R10S (○) and six rats for group R11E (●).

Table 5
Effects of long-term, light exercise under restricted feeding on some biochemical variables in the serum at 900 days of age in male rats

Group[a]	Total protein (g/100 ml)	Albumin (g/100 ml)	BUN[b] (g/100 ml)	Creatinine (g/100 ml)
R10S	5.44 ± 0.35[c]	2.31 ± 0.22	16.3 ± 2.80	0.39 ± 0.07
R11E	5.88 ± 0.10*	2.48 ± 0.10	19.7 ± 1.80*	0.43 ± 0.05
	Total cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)	Insulin[d] (pU/ml)	CPK[e] (pU/ml)
R10S	104.4 ± 31.9	82.4 ± 50.6	25.1 ± 4.5	575 ± 534
R11E	101.7 ± 15.6	61.2 ± 28.8	18.3 ± 4.8*	344 ± 436

[a] For details on groups see the text.

[b] BUN, blood urea nitrogen.

[c] Mean ± S.D. of seven rats for group R10S and six rats for group R11E.

[d] Immuno reactive insulin.

[e] CPK, creatine phosphokinase.

* Significantly different from the value for group R10S at $P < 0.05$.

Goodrick (1980) showed that in ad libitum feeding rats, voluntary wheel-running prolonged the mean and maximum survival times of rats. Edington et al. (1972) also found that light treadmill exercise (20 min/day at 10 m/min) from 120 days of age prolonged the mean survival time compared with that of sedentary rats. However, Holloszy et al. (1985) showed that exercise improved the mean survival time of rats, but did not extend the maximum life span. On the other hand, it has been well documented that compared with ad libitum feeding, restricted feeding improves age-related declines in immune functions. We (Utsuyama et al., 1996) observed previously that in rats under restricted feeding, long-term (100–700 days of age), moderate running exercise (5000 m/day) retarded the age-related decrement of immunological functions, as compared with sedentary rats in cages, while there was no difference in survival rate between sedentary and exercised rats at 700 days of age. In this study as well, the survival rate at 850 days of age was better in group R11E (82%) than in group R10S (67%), but there was no significant difference in survival rate between the two groups at 900 days of age: 58% for group R10S and 55% for group R11E. Long-term, light exercise did not prolong the survivals of rats before 900 days of age.

Compared with ad libitum feeding, in rats and mice, restricted feeding reduced the appearance and development of histopathological changes in tissues with advancing age (Yu et al., 1982; Iwasaki et al., 1988), resulting in a decrease in age-related diseases (Ross and Bras, 1965; Fujita et al., 1984; Lloyd, 1984). Appropriate physical activity and regular exercise also decrease the risk of degenerative diseases through increased energy expenditure, increased skeletal muscle mass, and/or improved physiological and metabolic functions. In this study, compared with the sedentary group, long-term, light exercise further reduced the incidence of histopathological changes in the liver and kidney. In addition, the exercise not only significantly increased nitrogen retention in the body and decreased body fat, resulting in improved body composition, but also resulted in increased serum protein and decreased plasma insulin levels. Long-term, light exercise in combination with restricted feeding produced multifarious benefits.

The idea that basal energy metabolism decreases with advancing age has been widely accepted. It has been shown that in ad libitum feeding rats, some chronic diseases such as tumor and renal disease appear and develop with advancing age; daily food consumption also starts to decrease from near 20 months of age without exceptions (Ichikawa and Fujita, 1987). At this time, the decline in the BMR may be one of the most reasonable metabolic adaptations to the decreased energy intake. Forsum et al. (1981) showed that energy restriction for one month in growing rats decreased significantly the BMR by 30%. On the other hand, age-related changes in body composition, particularly increased body fat and/or decreased body protein (Tauchi et al., 1971), are also related to decreased BMR in later life. In this study, animals in both groups consumed a constant amount of food throughout the test period, and group R10S showed no significant changes in body composition between 100 and 900 days of age. Mazzeo and Horvath (1986) showed that treadmill exercise for 3 months at an intensity of 75% of maximal oxygen capacity significantly decreased body fat levels regardless of age (6, 15 and

27 months of age). The exercised rats in the present study also showed significantly increased body protein and decreased body fat levels at 900 days, compared with group R10S. Consequently, the BMR remained almost constant between 100 and 900 days for group R10S and increased slightly with age for group R11E. However, when the BMR is expressed on the basis of urinary creatinine, which roughly estimates skeletal muscle mass, the values in both groups steadily increased with age, particularly in the R11E group. This suggested that the contribution of skeletal muscles to basal energy expenditures decreased with age, while that of tissues other than skeletal muscles relatively increased. In contrast to our results, Poehlman et al. (1992) showed that in older individuals, moderate exercise (300 kcal per session, three times/week) increased the resting metabolic rate by 9%, but light exercise (150 kcal per session three times/week) did not. They (Ballor and Poehlman, 1993) also showed that in adult rats, low-intensity (33% of the group's maximal running speed, 90 min/day, 5 days/week) and high-intensity (75%, 40 min/day, 5 days/week) exercise in combination with dietary restriction had no effect on resting metabolic rate. The difference between our and Poehlman's results may be due to differences in exercise duration: our rats underwent the exercise for such an extended period (800 days)-over half the normal life span of laboratory rats-but only 10 days (Poehlman et al., 1992) and 10 weeks (Ballor and Poehlman, 1993) in Poehlman's studies.

The present study showed that even with exercise as light as the equivalent of normal physical activity in day-to-day life in humans, if the exercise is continued steadily throughout the entire life span under adequate dietary conditions, exercise prevents age-related deteriorations in physiological and metabolic functions and body composition that may be caused by inactive daily living. From results, it was concluded that long-term, light running exercise does not lead to lifespan extension but which has potential to improve overall health.

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