Chronic Food Restriction Modulates the Advance of Senescence in the Senescence Accelerated Mouse (SAM)[1]

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ABSTRACT The effects of chronic food restriction on grading scores of senescence, deposition of senile amyloid (AS_{SAM}), mean life span and 10th decile were investigated by using animal models for accelerated senescence (SAM-P/1) and for normal aging (SAM-R/1). The experimental groups consisted of control (ad libitum fed), 80% (fed 80% of control intake), and 60% (fed 60% of control intake) groups. The grading score of SAM-P/1 mice was significantly improved in the 60% group, but not in the 80% group, compared to the control group. The grading score of SAM-R/1 mice, however, was significantly less than that in the control group in both the 60 and 80% groups. In SAM-P/1 mice liver, skin and testis, the severity of senile amyloid deposition was significantly less with 40% food restriction (60% group) than in the control group. A restriction of 20% (80% group) had no influence on amyloid deposition. A definite tendency to prolong mean life span (24.3%) and 10th decile (65.9%, mean life span of the last 10th of survivors of a group) was observed in the 60% group of SAM-P/1 mice, but the changes were not statistically significant. In the 80% group of SAM-P/1 mice and also in either restriction group of SAM-R/1 mice, however, such a tendency was not evident. These results indicate that 40% food restriction modulates the advance of senescence in these mice. J. Nutr. *115*: 1259–1266, 1985.

INDEXING KEY WORDS Senescence Accelerated Mouse • senile amyloid • grading score • food restriction

Several pairs of AKR strain mice were donated by the Jackson Laboratory (Bar Harbor, ME) to our laboratory in 1968. We continued the sister-brother mating of these mice and became aware of the presence of certain litters in which most of the mice showed a moderate to severe loss of activity, alopecia and lack of glossiness, skin coarseness, periophthalmic lesions, increased lordokyphosis of the spine and shortened life span despite the relatively low incidence of thymic lymphoma. Among them we selected and maintained four substrains with severe exhaustion as "senescence prone" (P-series) and three substrains with a normal aging process as "senescence resistant" (R-series). The former four series are -P/1, -P/2, -P/3 and -P/4. The latter three series

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are -R/1, -R/2 and -R/3. Judging from findings in the survivors and for the Gompertzian function together with the growth pattern in body weight, the aging pattern in this model seems to be due to an accelerated senescence rather than a premature aging or senescence. Thus, this P-series was named "Senescence Accelerated Mouse" (SAM) (1). The substrains of P and R series have been designated SAM-P/1, -P/2, -P/3 and -P/4, and SAM-R/1, -R/2 and -R/3, respectively (2). Among pathological findings, spontaneous age-associated amyloidosis is one of the most characteristic findings in these mice (3). A unique amyloid fibril protein, AS_{SAM}, has been isolated from the liver of the SAM-P/1 mouse (4).

The present studies were undertaken to observe the effect of total food intake on the changes in grading score with advancing age, evaluated by the "Grading Score System" (2), severity of senile amyloid (AS_{SAM}) deposition and aging dynamics such as mean life span and 10th decile (the mean life span of the last 10th of survivors of a group) (5), by using the animal model of accelerated senescence, SAM-P/1, and control SAM-R/1 with normal aging.

METHODS

Animals. Four-week-old SAM-P/1 and SAM-R/1 mice reared in our laboratory under conventional conditions at 24 ± 2°C with artificial lighting in which 24-h cycle consisted of 14 light hours (0800–2200 h) and 10 dark hours (2200–0800 h) were separated into three groups: a control group, a group fed 80% of control intake and a group fed 60% of control intake. Mice were housed five per cage; there were four cages per group. To prepare a regimen close to that maintained by humans, each mouse in the control group was allowed ad libitum access to food from 0900 to 1100 h and from 1800 to 2000 h. The average food consumption per animal was determined twice a day. Food intake from the morning meal was not significantly different from that from the evening meal. Groups fed 80 and 60% were provided twice a day with the amount of diet representing approximately 80 or 60% of the food intake of the control group for the preceding meal. To allow ready access to food by each mouse and to minimize individual differences in food intake, the amount of food was divided roughly into five equal parts when given to the mice in the restricted groups. During a 10-d preliminary period, the control group consumed the same amount of food as was consumed by mice given access to food at all times (data not shown). All food given to the restricted groups was consumed within 2 h. Based on body weight gain during the preliminary test, a few mice considered to be dominant animals were excluded from the experimental group. Therefore, after the preliminary period, 3–5 mice were housed in one cage, because the dominant animals were not replaced by other mice. Composition of the control diet is shown in table 1 (6).

The control group included 10 males and 10 females of strain SAM-R/1 and 10 males and 10 females of SAM-P/1. The mice in the group fed 80% included 10 males and 10 females of SAM-R/1 and 9 males and 10 females of SAM-P/1, and those in the group fed 60% included 9 males and 9 females of SAM-R/1 and 8 males and 7 females of SAM-P/1. Each mouse was observed at least twice a day for signs of any gross abnormality. Cannibalism was rare and did not influence the results of the study. From these data, mean life span and 10th decile (5) were calculated. Each mouse was weighed once a week.

Evaluation of senescence. Evaluation of senescence was performed every 2 mo during the experiment, according to the "Grading Score System" (1, 2). This system was designed to represent changes in behavior and appearance. There are eleven categories that include reactivity, passivity, glossiness, coarseness, hair loss, ulcer, periophthalmic lesions, cataract, corneal ulcer, corneal opacity and lordokyphosis. In general, each category has five grades corresponding to the intensity of the characteristic or changes. For example, grade 0 represents no particular changes and grade 4 represents the most severe changes. Each grade in each category is clearly defined and the details have been reported elsewhere (1, 2).

Systematic and extensive studies with the grading score system have shown that if the

TABLE 1

Composition of control diet

Component	Amount
	%
Casein	25.0
DL-Methionine	0.3
Cornstarch	34.1
Sucrose	23.4
Cellulose powder	2.0
Soybean oil	9.0
Salt mix[1]	5.0
Vitamin mix[2]	1.0
Choline chloride	0.2
Soybean oil plus vitamins[3]	0.1

[1]Salt mixture provided (in milligrams per 100 g diet): NaCl, 540.440; $K_3C_6H_5O_7 \cdot H_2O$, 1184.444; K_2HPO_4, 386.667; $CaHPO_4 \cdot 2H_2O$, 1777.778; $CaCO_3$, 817.778; $MgCO_3$, 204.444; $FeC_6H_5O_7 \cdot 3H_2O$, 80.000; $CuSO_4 \cdot 5H_2O$, 0.889; $MnSO_4$, 6.222; $K_2Al_2(SO_4)_4 \cdot 24H_2O$, 0.444; KI, 0.222; $CoCl_2 \cdot 6H_2O$, 0.444; $ZnCO_3$, 0.222; NaF, 0.004 (6). [2]Panvitan Powder, Takeda Chemical Industries, Ltd., Osaka, Japan. Vitamin mixture contained the following in milligrams per 100 g diet: thiamin · HNO_3, 1.0; riboflavin, 1.5; pyridoxine · HCl, 1.0; nicotinic acid, 10.0; calcium pantothenate, 5.0; folic acid, 0.5; cyanocobalamin, 0.001; ascorbic acid, 37.5; *dl*-α-tocopherol, 1.0; and in IU per 100 g diet: retinyl palmitate, 2500; ergocalciferol, 200. [3]Vitamin A 2500 IU/100 g of diet as synthetic ester and vitamin E 1.0 IU/100 g of diet as α-tocopheryl acetate were added to soybean oil.

validity of the system is based on irreversibility and universality of the changes in each category with advancing age, most categories are valid for evaluation of the degree of senescence. This grading score system is a unique, useful and convenient method for evaluation of the degree of senescence in mice (2).

Histological examination. Age at death of each mouse was recorded, and the liver, spleen, testis and skin were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 4-μm sections and stained with hematoxylin and eosin or alkaline Congo red (7). Green birefringence under the polarizing microscope was considered to be a positive criterion for the presence of amyloid. Deposition of AS_{SAM} in these organs was observed by using the PAP (peroxidase-antiperoxidase) methods of Sternberger (8), as modified by Fujihara et al. (9). Anti-AS_{SAM} antiserum was prepared as described previously (10). Mice that died at 8–12 mo after birth were used for the immunohistochemical study. Ten randomly chosen areas of each tissue section were photographed, and the positively stained areas were estimated histometrically, by using an image analyzer (Model MOP-AM03; Kontron, Munich, West Germany). The degree of deposition was expressed as the percentage of the total area of the tissue examined in which there was deposition.

Statistical analysis. Analysis of variance (one-way classification), followed by Duncan's new multiple-range test (11), was employed to compare the means of grading score, mean life span and 10th decile in animals of each experimental group. Statistical significance of incidence of amyloidosis in each experimental group was analyzed by Fisher's Exact Test (12).

RESULTS

Body weight. The mean body weight for each group is plotted in figure 1. The mean body weight of the group fed 60% of control intake was lower than that of the controls and group fed 80%. The difference between the group fed 60% and the controls or group fed 80% was larger in males than in females, of both SAM-R/1 and SAM-P/1 strain. The difference in body weight became evident after 5 wk of feeding. At which time the mean body weight of the group fed 60% of control intake was not over 20 g.

Grading score. Increases in grading score with advancing age in each experimental group are shown in figure 2. In both SAM-P/1 and SAM-R/1 mice food restriction, especially the 40% restriction (group fed 60%), suppressed the increase in grading score with advancing age. At 6 and 8 mo, grading scores for the groups fed 60% of control intake were similar for the two strains. To compare groups within strains, the grading score of SAM-R/1 mice was significantly less in both the groups fed 60 and 80% of controls than that in the control group, after 8 mo of consuming the diets. The grading score of SAM-P/1 mice was significantly improved (lowered) in the group fed 60%, but not in the group fed 80%,

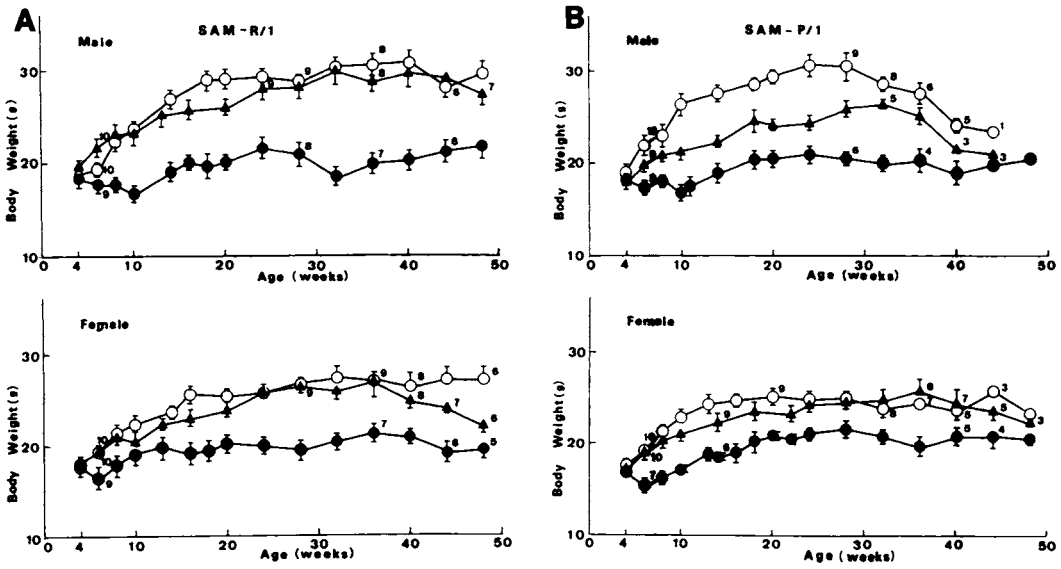

Fig. 1 Changes in body weight with advancing age in 1A SAM-R/1 (senescence-resistant) mice (top, male; bottom, female) and 1B SAM-P/1 (senescence-prone) mice (top, male; bottom, female). Each point is the mean ± SEM. ○—○ Control group, ▲—▲ group fed 80% of control intake, ●—● group fed 60% of control intake. The numeral listed on the right side of the body weight value shows the number of animals examined. Data obtained when the mice were 4 wk of age represent the mean weights of 10 mice in each group.

compared to the control group; that is, the grading score in the group fed 60% was significantly less than that in the control group after 6, 8 and 10 mo of consuming the diets.

Gross appearance. The SAM-R/1 mice in the groups fed 60 and 80% looked younger than did the controls after 8 mo of consuming the diets. In SAM-P/1 mice, the 60% group looked young compared to the control of 80% groups; glossiness of the hair was well preserved, and loss and coarseness of the hair were not evident as they were in the case of SAM-R/1 mice after 6 mo of consuming the diets.

Pathological findings. As shown in table 2, the main pathological findings that were probably closely related to death were inflammatory changes, tumors (mostly malignant lymphoma) and amyloidosis. In the SAM-P/1 strain, the number of mice with amyloidosis in the group fed 60% was significantly less than in the control group ($P < 0.05$), and there was a tendency toward a greater number of deaths due to inflammatory changes.

Amyloid deposition. As shown in table 3, severity of senile amyloid deposition in liver, skin and testis of SAM-P/1 mice was significantly decreased in the group fed 60%, compared to that in the control group of SAM-P/1 mice. In the spleen of the group fed 60%, the mean value (0.30%) tended to be less than that of the control group (9.41%), but it was without statistical significance. In the group fed 80%, the severity of amyloid deposition in the liver and testis tended to be lower than that in the control group, albeit not statistically significantly. In the case of the spleen and skin, however, there was no decrease in the severity of senile amyloid deposition by 20% food restriction. Only in the case of the skin was there a statistically significant difference in severity of amyloid deposition between the groups fed 80 and 60%; the group fed 60% showed the lesser degree of amyloid deposition.

In no experimental group was there AS_{SAM} deposition in liver, skin, spleen and testis of SAM-R/1 mice that died at 8–12 mo after birth.

Mean life span and 10th decile. As shown in table 4, the mean life span of the 60% group of SAM-P/1 mice, 413.8 d, was 24%

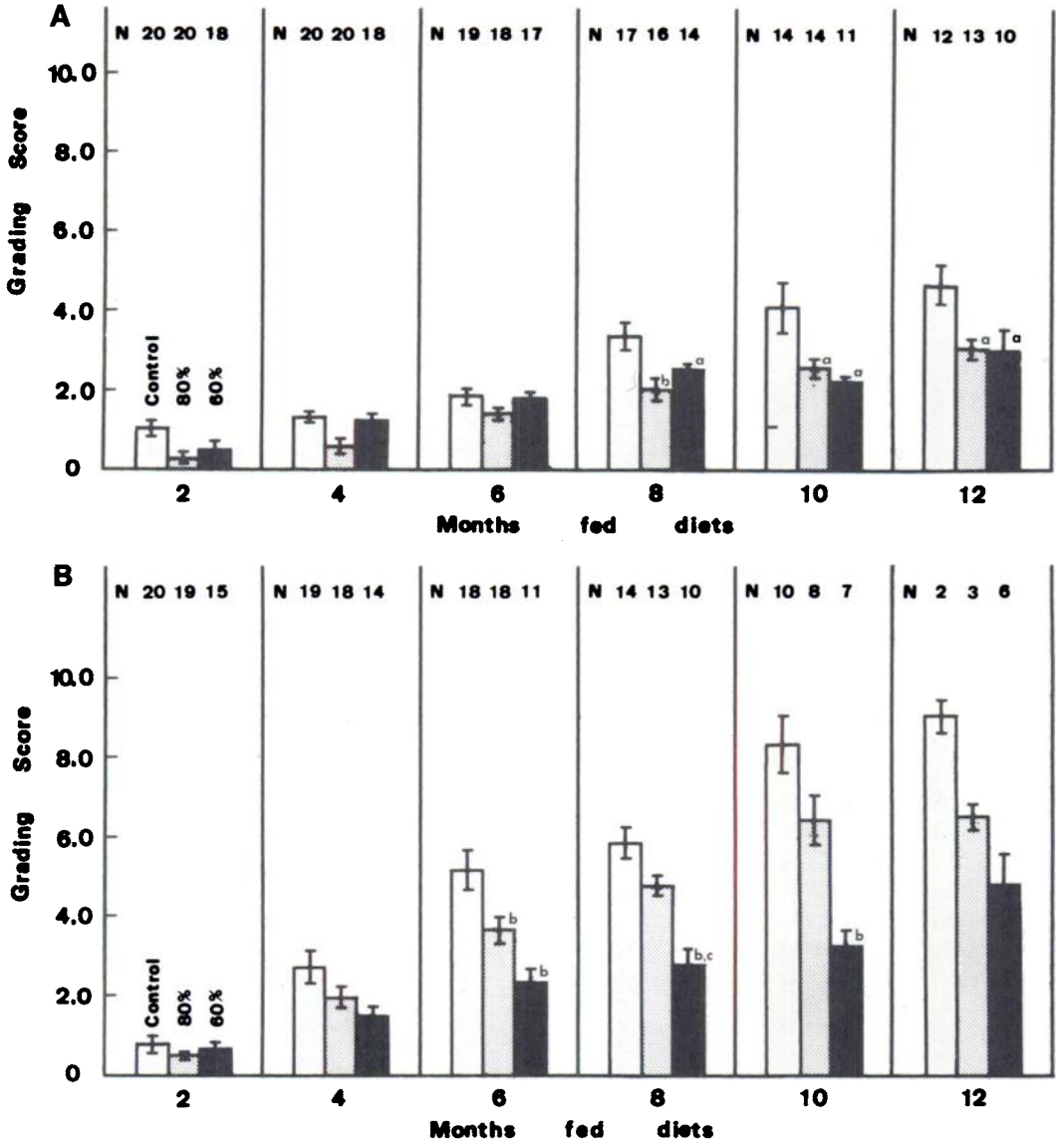

Fig. 2 Changes in grading score with advancing age in male and female mice. 2*A* SAM-R/1 (senescence-resistant) mice. 2*B* SAM-P/1 (senescence-prone) mice. Significant differences from corresponding control groups ($^{a}P < 0.05$, $^{b}P < 0.01$), and between groups fed 80 and 60% of control intake ($^{c}P < 0.05$). Vertical line represents standard error for each mean value. N: Number of animals examined each month.

longer than that of the control group. The 10th decile of the 60% group in SAM-P/1 mice was 737.5 d or 65.9% higher than that of the control group. The 10th decile in the 80% group was 29% higher than that of the control group. Although a definite tendency toward a greater mean life span and 10th decile was noted in the 60% group, compared to the control group, the values were not significantly different. In SAM-R/1 mice, however, food restriction had no significant effect on mean life span or 10th decile. When calculating these parameters, the mice that died within 6 mo after birth

TABLE 2

Pathological findings related to death in SAM-R/1 (senescence-resistant) and SAM-P/1 (senescence-prone) mice

Strain and group[1]	n[2]	Findings related to death			
		Inflammation	Tumor[3]	Amyloidosis[4]	Unknown
SAM-R/1					
Control	16	9	2	1	4
80%	15	8	3	1	3
60%	13	9	1	0	3
SAM-P/1					
Control	19	6	0	11	2
80%	16	9	1	4	2
60%	11	7	1	2*	1

[1]Groups: controls were fed ad libitum, the 80% group was meal fed 80% of the control intake and the 60% group was meal fed 60% of the control intake. [2]Animals with advanced postmortem changes were excluded. [3]Except for one mouse in the control group in SAM-R/1 (pulmonary carcinoma) and one mouse in the group fed 80% in SAM-R/1 (breast carcinoma), malignant lymphoma was observed. [4]Carcasses with notable renal and hepatic amyloidosis, without any inflammatory or tumorous changes were included. *Significant differences from the corresponding control groups by Fisher's Exact Test (12) ($P < 0.05$).

(one SAM-P/1 mouse in the control group, one SAM-R/1 mouse and one SAM-P/1 mouse in the group fed 80% and one SAM-R/1 mouse and two SAM-P/1 mice in the group fed 60%) were excluded from the count because causes of death in these mice were not related to the aging process.

DISCUSSION

Rats (13–20), mice (21–23) and hamsters (22) given restricted diets have shown extended mean and maximum survival times. Furthermore, decreased incidence or delayed onset of several diseases of old age have been reported in food-restricted animals (23, 24).

In the present work, a new inbred strain of accelerated senescence mice, termed SAM-P/1 and developed in our laboratory, was used to observe the effects of food restriction on advancement of senescence, senile amyloid deposition and mean life span and 10th decile. SAM-R/1 mice with normal aging were used as the control.

We housed three to five mice in one cage and, as described in the methods section, eliminated dominant animals. Standard errors of the body weight at all ages (fig. 1) attest to this. Standard errors of body weight in the food-restricted groups were nearly equal to those of SAM-P/1 and SAM-R/1 mice fed ad libitum and to those reported previously (1).

A "Grading Score System," which has an objective validity for evaluation of the degree of senescence (2), was adopted to observe the effects of food restriction on advancement of senescence. A 40% restricted diet was needed to suppress the advancement of senescence in SAM-P/1; a 20% restriction was without effect. In the SAM-R/1, a 20% restriction was as effective as the 40% restriction.

Amyloid deposition in skin, liver and testis was significantly less when a 40% food restriction was enforced than when the mice were fed ad libitum. In the spleen, marked, but not statistically significant, reduction in amyloid deposition was also evident in the 40% restricted group. Among the organs checked, liver and testis showed tendencies, (not statistically significant), for less senile amyloid deposition, when a 20%

TABLE 3

Effect of food restriction on senile amyloid deposition in SAM-P/1 mice[1,2]

Organ	Group	n	Area of deposition
			%
Liver	Control	7	1.51 ± 0.43
	80%	4	0.36 ± 0.14
	60%	6	0.16 ± 0.10*
Skin	Control	5	2.51 ± 0.66
	80%	5	2.35 ± 0.30
	60%	5	0.31 ± 0.30*†
Spleen	Control	5	9.41 ± 5.66
	80%	5	8.74 ± 5.95
	60%	5	0.30 ± 0.19
Testis	Control	4	5.48 ± 2.45
	80%	4	0.35 ± 0.24
	60%	4	0.25 ± 0.17*

[1]Data are means ± SEM for the number of mice under n. [2]Significant differences *from the corresponding control groups ($P < 0.05$), and †between groups fed 80% and groups fed 60% ($P < 0.05$) in mice that died from 8 mo to 12 mo of age.

TABLE 4

Mean life span and 10th decile[1]

Strain and group	*n*	Life span	10th decile[2]
		d	
SAM-R/1			
Control	20	444.4 ± 32.5	668.0 ± 24.1
80%	18	473.9 ± 32.7	645.7 ± 9.8
60%	17	413.9 ± 36.5	659.0 ± 12.6
SAM-P/1			
Control	19	332.8 ± 16.8	444.5 ± 36.5
80%	19	342.8 ± 24.5	572.0 ± 109.0
60%	13	413.8 ± 50.9	737.5 ± 17.5

[1]Data are means ± SEM for the number of mice under *n*. [2]Tenth decile is the mean life span of the last 10th of survivors of a group.

food restriction was enforced than when the mice were fed ad libitum. These results indicate that responsiveness of amyloid deposition to food restriction differs with the organ.

Aging dynamics in each experimental group were analyzed by using two parameters; mean life span and 10th decile, factors considered to be pertinent expressions of senescence (5). The mean life span and 10th decile in the group fed 60% of SAM-P/1 mice showed a marked prolongation: 24.3 and 65.9% of the corresponding control group, respectively; however, these changes were not statistically significant. These results coincided well with the reduction in severity of senile amyloid deposition and grading score in this experimental group (fig. 2*B*). In SAM-R/1 mice having a normal aging process, however, a significant reduction in the grading score observed in the groups fed 80 and 60% compared to the control group was not reflected in a prolongation of mean life span and 10th decile. This result observed in SAM-R/1 mice is not in agreement with data from other strains of rodents (13–23).

Food restriction was effective in controlling the aging process of a unique murine model of accelerated senescence, the SAM-P/1 mouse. The mechanisms involved in inhibition of the aging process, by means of dietary restrictions, are now under investigation in our laboratory.

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