Dietary Soybean Protein Compared with Casein Retards Senescence in the Senescence Accelerated Mouse[1,2]

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ABSTRACT The effects of replacing dietary casein with soybean protein on mean life span, mean life span of the last one-tenth of a group, grading scores of senescence and deposition of senile amyloid were investigated in senescence accelerated mice (SAM-P/1) compared with a control strain (SAM-R/1). SAM-R/1 mice fed the soybean protein-containing diet had mean life spans of 618 ± 42 d (males) and 578 ± 62 d (females), 58% (males) and 44% (females) longer than those of corresponding casein fed mice ($P < 0.01$). Similarly, in SAM-P/1 mean life-spans were 265 ± 16 d (males) and 307 ± 23 d (females) in the soybean diet group, 27% (males) and 30% (females) longer than in the casein diet groups ($P < 0.01$). The mean life span of the last one-tenth of each group fed soybean protein was significantly longer than the corresponding group fed casein. In SAM-R/1 mice, pathological studies revealed that severe secondary amyloid deposition (amyloid A protein) in the kidneys, spleen, stomach and liver was significantly suppressed, in males only, by replacing casein with soybean protein ($P < 0.01$). The occurrence of contracted kidneys caused by the infiltration of amyloid A protein was suppressed in SAM-R/1 mice fed the soybean protein-containing diet ($P < 0.05$). The deposition of senile amyloid in SAM-P/1 mice with aging was retarded by replacing casein with soybean protein ($P < 0.01$). These results indicate that dietary protein source is important in modulating the advance of senescence in SAM mice. J. Nutr. 123: 1905–1912, 1993.

INDEXING KEY WORDS:

- *senescence accelerated mouse* • *life span*
- *dietary protein* • *senile amyloid*
- *secondary amyloid*

A murine model of accelerated senescence, the senescence accelerated mouse (SAM)[4] strains (SAM-P/1, -P/2, -P/3, -P/6, -P/7, -P/8, -P/9 and -P/10), and control SAM-R mouse strains (SAM-R/1, -R/2 and -R/4) with normal aging characteristics, was developed in our laboratory (Chen et al. 1989, Matsushita et al. 1986, Shimada et al. 1992, Takeda et al. 1981, Takeshita et al. 1982, Yagi et al. 1988).

One of the most characteristic pathological findings in SAM is senile amyloidosis. Spontaneous age-associated, systemic amyloidosis occurs in some aged SAM-R mice, but it is very frequent and severe in SAM-P mice. In mice two types of amyloid proteins have been characterized: amyloid A protein, which occurs in experimentally-induced amyloidosis (Eriksen et al. 1976) and in the spontaneous amyloidosis observed in some strains of mice (Westermark et al. 1979); and spontaneous senile amyloid (AS_{SAM}) in SAM mice which we discovered and characterized. This amyloid protein is deposited extracellularly with advancing age in all tissues except the brain and bone marrow (Takeshita et al. 1982). A unique senile amyloid fibril protein, AS_{SAM}, has been isolated from SAM-P/1 mice (Matsumura et al. 1982). This protein differs from murine amyloid A protein, which is similar to the protein found in secondary amyloidosis in humans (Skinner et al. 1977).

In mice and hamsters amyloid A protein is deposited when the animals are fed a normal casein

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[4]Abbreviations used: AS_{SAM}, senile amyloid; SAM, senescence accelerated mouse; SPI, soy protein isolate.

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diet, but a low casein diet delays amyloid formation (Irwin et al. 1969). In our previous work, reducing the food intake of SAM-P/1 mice by 40% extended the mean and maximum survival times and reduced the grading score of senescence and the deposition of AS_{SAM} (Kohno et al. 1985, Umezawa et al. 1990). These findings suggested that the overall reduction of energy in the diet might increase longevity and reduce amyloid formation in SAM mice.

In this study we replaced dietary casein with soybean protein and examined its effects on longevity, grading score of senescence and deposition of amyloid in SAM mice.

MATERIALS AND METHODS

Mice and diets. Six-week-old SAM-R/1 and SAM-P/1 mice, born and reared in our laboratory under conventional conditions, (Takeda et al. 1981), were used. The basic composition of the diet was published previously (Umezawa et al. 1990). Briefly, the diets contained the following (g/100 g diet): nitrogen source, 25; DL-methionine, 0.3; mineral mixture (Clea Japan, Tokyo, Japan), 5; vitamin mixture (Clea Japan), 1; cellulose powder, 2; soybean oil, 9; sucrose, 25; and cornstarch, 32.7. The dietary nitrogen sources were casein (vitamin-free, ALACID™700-769, Nippon Proteins, Tokyo, Japan) and soybean protein isolate (SPI) (Fujipro R, Fuji Oil, Osaka, Japan). The amino acid composition of the test diets is shown in **Table 1**. The group fed casein as the protein source included 11 males and 7 females of the SAM-R/1 strain, and 12 males and 10 females of the SAM-P/1 strain. The mice in the group fed soybean protein included 13 males and 9 females of SAM-R/1, and 10 males and 8 females of SAM-P/1. All the mice were housed from four to six per cage, were given free access to diet and tap water and were maintained in a temperature-controlled room (24 ± 2°C) with a 12-h light:dark cycle, throughout their lives. The average amount of food ingested by each mouse was determined every other day until the animals reached 10 mo of age (for SAM-R/1), or 6 mo of age (for SAM-P/1). Each mouse was weighed monthly beginning in wk 6 postnatal. All mice were inspected at least twice daily (at the start and the end of the light phase of a light:dark cycle). All mice that died spontaneously were removed from their cages and necropsied immediately. Although autolysis occurred in some cases, it did not adversely affect the evaluation of the amyloid index. All animals were maintained according to the policies and recommendations of The Kyoto University Animal Care and Use Committee.

Grading score of senescence. The scoring system used to evaluate the degree of senescence has been described elsewhere (Hosokawa et al. 1984). This system was designed to represent changes in the behavior and appearance of the mouse considered to be associated with the aging process. The 11 categories in which the above changes are measured include reactivity, passivity, glossiness and coarseness of coat, hair loss, ulcers, periophthalmic lesions, cataracts, corneal ulcers, corneal opacity and lordokyphosis. Each category has five grades of intensity of characteristics or changes. Each mouse was examined by inspection and palpation every 2 mo, and the sum of the scores of the 11 categories was recorded.

TABLE 1

Amino acid composition of diets[1]

Amino acid	Diet group: Casein	Diet group: Soybean protein isolate (SPI)
	g/100 g diet	
Isoleucine	1.11	1.16
Leucine	2.01	1.82
Valine	1.32	1.16
Methionine*	0.60	0.32
Cystine*	0.09	0.30
Phenylalanine	1.11	1.21
Tyrosine*	1.18	0.96
Threonine	0.90	0.86
Tryptophan	0.28	0.30
Lysine*	1.69	1.47
Histidine	0.62	0.59
Arginine	0.85	1.84
Glycine*	0.45	1.00
Serine	1.24	1.07
Alanine*	0.66	1.00
Proline*	2.39	1.25
Aspartic acid*	1.56	2.68
Glutamic acid	4.92	5.00

[1]The amino acid content of the 25 g/100 g protein diets provides approximately 23% amino acids in the diet. *Difference in amino acid concentration between diets ≥10%.

Histological examination. The age at death of each mouse was recorded, and the abdominal skin, liver, kidneys, spleen, heart, lungs, stomach, thyroid, adrenals, gonads, sciatic nerve and aorta were fixed in 10% (v/v) neutral buffered formalin, embedded in paraffin, cut into 4-μm sections and stained with hematoxylin and eosin or with alkaline Congo red (Puchtler et al. 1962). A green birefringence under the polarizing microscope was considered to be a positive criterion for the presence of amyloid. The peroxidase-anti-peroxidase method of Sternberger (1979), as modified by Fujihara et al. (1980), was used for the identification of different types of amyloid fibril proteins in organs. Anti-AS_{SAM} and anti-murine protein amyloid A antisera were prepared as described previously (Higuchi et al. 1983). SAM-P/1 mice that died over 100 d of age and SAM-R/1 mice that died over 400 d of age were used in the immunohistochemical

study. The amounts of amyloid deposition in the liver, kidneys, spleen, heart, abdominal skin and stomach were graded in four levels from stained sections, and the scores were summed and divided by 6 to obtain the average intensity—"amyloid index" (Takeda et al. 1988)—of amyloid deposition in each animal.

Statistical analysis. A two-way ANOVA was used to compare results with the two types of protein source across the various ages of the mice. Comparisons between results with the two types of protein source at each age were made using Student's *t* test (Robert and James 1980). Survival curves were estimated by the Kaplan-Meier test (Kaplan and Meier 1958), and the curves were compared with the Wilcoxon test (Robert and James 1980). The significance of the differences with respect to the pathological findings within each dietary group was analyzed using Fisher's exact test (Steel and Torrie 1980). The grading scores of senescence in the dietary groups and the amyloid index of each organ in the two groups were compared with the use of Mann-Whitney's test (Robert and James 1980). Correlation between amyloid index and age was analyzed using Spearman's rank test (Robert and James 1980). The comparison of two regression lines relating amyloid index to age was determined by the analysis of covariance with the Statistical Analysis System (SAS Institute, Cary, NC). Statistical significance was established when $P < 0.05$. With respect to the body weights, survival curves, grading scores and amyloid indexes in each organ, the males and females were considered separately and also were compared to one another.

RESULTS

Growth curve and longevity. Food consumption (g/(mouse·d)) by the two dietary groups of SAM-R/1 (2–10 mo of age) and SAM-P/1 (2–6 mo of age) mice were 3.5 ± 0.1 (males, SAM-R/1), 3.4 ± 0.1 (females, SAM-R/1), 3.0 ± 0.1 (males, SAM-P/1) and 2.8 ± 0.2 (female, SAM-P/1). No significant differences were noted in food intake between the mice fed the casein and those fed the SPI diet. Both groups of SAM-R/1 male and female mice showed similar changes in body weight from 6 to 48 wk of age (**Fig. 1**). On the other hand, the SAM-P/1 male mice fed casein began to lose body weight after 20 wk of age and the corresponding females did so after 28 wk of age, but no weight loss occurred in the mice fed SPI. The survival curves for the SPI group in both strains differed significantly from those for the corresponding casein groups (**Fig. 2**, $P < 0.01$). There was no significant gender difference within each group. SAM-R/1 mice fed SPI had a mean life span of 618 ± 42 d (males) and 578 ± 62 d (females), while that of those fed the casein was 390 ± 53 d (males) and 401 ± 34 d (females). In SAM-P/1 the mean life spans were 265 ± 16 d (males) and 307 ± 23 d (females) in the SPI group and 208 ± 16 d (males) and 237 ± 26 d (females) in the casein group. The SPI group had a significantly longer mean life span than did the casein group in both strains ($P < 0.01$). There was no significant sex difference within

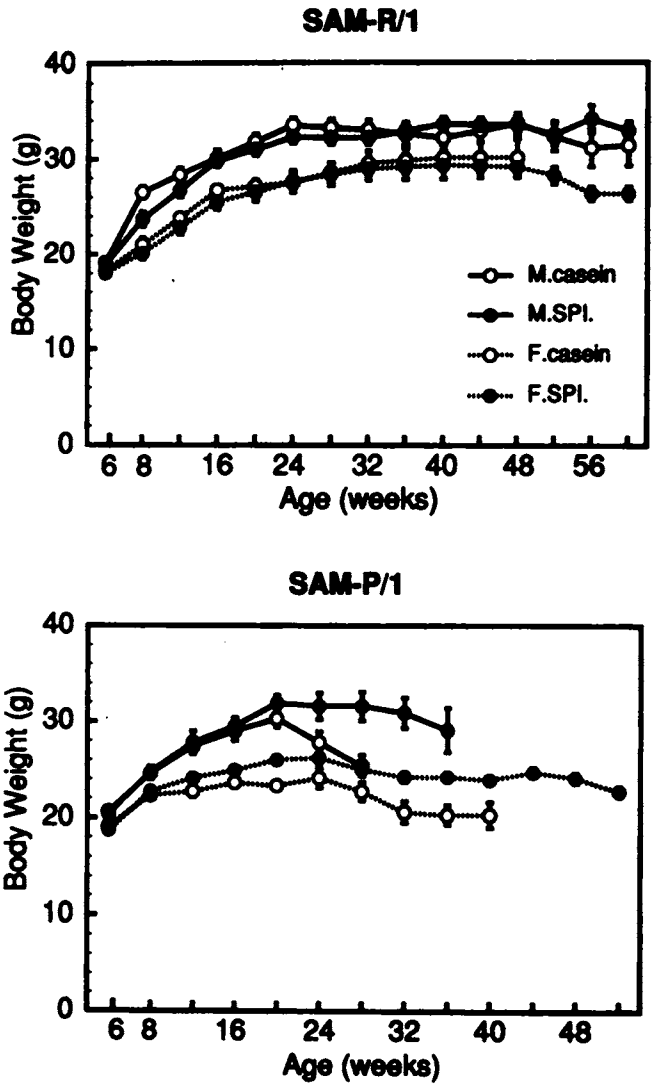

FIGURE 1 Body weights with advancing age in SAM-R/1 mice (*upper panel*) and SAM-P/1 mice (*lower panel*) fed casein or soybean protein isolate (SPI). Points are means ± SEM. The numbers of male mice fed casein were 11 in SAM-R/1 and 12 in SAM-P/1; those fed SPI were 13 in SAM-R/1 and 10 in SAM-P/1. For female mice fed casein, the numbers were 7 (SAM-R/1) and 10 (SAM-P/1) and for those fed SPI, 9 (SAM-R/1) and 8 (SAM-P/1). M = male, F = female. No statistically significant differences were noted in SAM-R/1 mice. In SAM-P/1 mice, two-way ANOVA of age and protein source showed significant (males: $P < 0.05$) age × protein source interaction, and the effects of protein source were significant (males and females: $P < 0.01$). The casein group values was significantly different (Student's *t* test) from the corresponding SPI group values (males: 24 and 28 wk of age, $P < 0.05$; females: 32, 36 and 40 wk of age, $P < 0.01$).

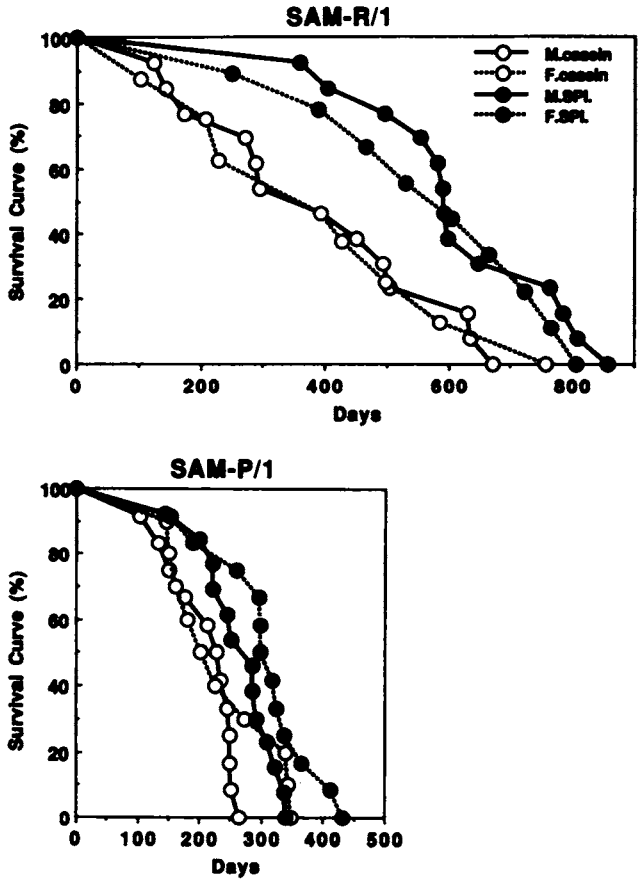

FIGURE 2 Survival curves for mice in the casein and SPI groups of SAM-R/1 (*upper panel*) and for those of SAM-P/1 (*lower panel*). The numbers of male mice fed casein were 11 in SAM-R/1 and 12 in SAM-P/1; those fed SPI were 13 in SAM-R/1 and 10 in SAM-P/1. For female mice fed casein, the numbers were 7 (SAM-R/1) and 10 (SAM-P/1) and for those fed SPI, 9 (SAM-R/1) and 8 (SAM-P/1). M = male, F = female. The survival curves for the SPI group in both strains differed significantly from those for the corresponding casein groups ($P < 0.01$). There was no significant differences between the sexes in the same diet group.

each group. The mean life span of the last one-tenth of each group to survive was 824 ± 16 d (SAM-R/1) and 402 ± 20 d (SAM-P/1) in the SPI groups and 688 ± 36 d (SAM-R/1) and 343 ± 2 d (SAM-P/1) in the casein groups. In both strains, the 10th percentile life span for the SPI group were significantly longer than for the casein group ($P < 0.05$). As regards the statistical analysis concerning the 10th percentile life span, the males and females were pooled, because there was no significant sex difference within a diet group.

Grading scores of senescence. Increasing senescence scores with aging in each experimental group are shown in **Figure 3**. There was no significant difference between the two dietary groups at each age in either strain. But male SAM-R/1 mice in the SPI group tended ($P < 0.10$) to have a lower increase in grading scores relative to those fed casein, and the

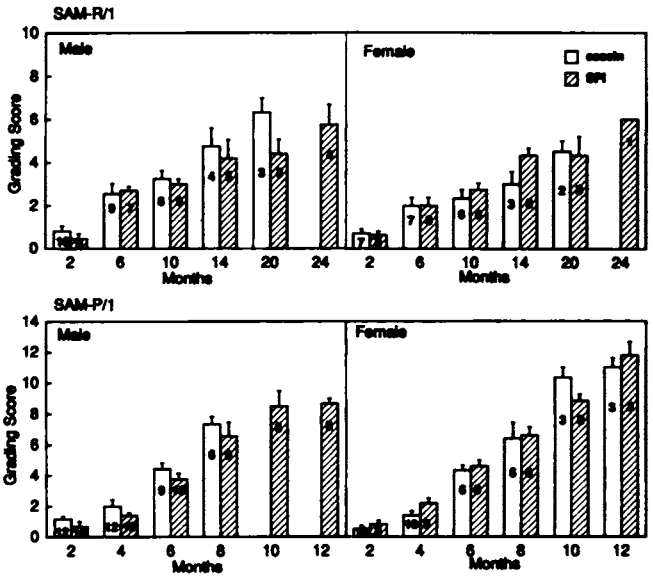

FIGURE 3 Changing of scores with age in casein and soybean protein isolate (SPI) fed groups of SAM-R/1 mice (*upper panel*) and SAM-P/1 mice (*lower panel*). The values are means ± SEM. The numbers in the columns are the numbers of mice. In both SAM-R/1 and SAM-P/1 mice, there were no significant differences in the scores at each month between the two diet groups or between the sexes.

grading scores of male SAM-R/1 mice fed casein tended ($P < 0.10$) to be higher than those of female SAM-R/1 mice fed casein.

Pathological findings. The primary pathological findings were inflammatory changes, tumors (mostly malignant lymphoma), amyloidosis and contracted kidneys (**Table 2**). Within the SAM-R/1 strain, more mice with amyloidosis in the casein group died after 16 mo of age than did those in the SPI group ($P < 0.05$). Amyloid A Protein deposition was more marked and extensive in the former than in the latter group. Severely contracted kidneys were observed in six of the eight casein fed mice that died after 16 mo of age ($P < 0.05$), and there were definite amyloid A protein deposits. More SAM-R/1 mice fed SPI than those fed casein had tumors, but the difference was not significant ($P < 0.10$). The SAM-R/1 mice fed SPI exhibited granulocytic neoplasms ($n = 3$), histiocytic neoplasms ($n = 2$) and nonthymic lymphomas ($n = 6$). Four of those fed casein appeared to have developed nonthymic lymphomas. There was no significant sex difference in the pathological findings of the two dietary groups of SAM-R/1 mice. In the SAM-P/1 strain, there were no significant differences in the pathologic findings between the two groups with the exception of inflammation. The 4–10 mo old SAM-R/1 and 4–6 mo old SAM-P/1 mice fed SPI had a significantly lower prevalence of inflammation than did those fed casein ($P < 0.05$). A number of aged mice fed SPI in both strains had inflammatory changes, but all of

TABLE 2

Pathological findings in SAM-R/1 and SAM-P/1 mice fed diets containing soybean protein or casein

Strains and dietary groups[1]	n[2]	Pathological findings								
		Inflammations			Tumors		Amyloidosis[3]		Contracted kidneys[4]	
		age, mo								
SAM-R/1		4–10	11–15	16–28	4–15	16–28	4–15	16–28	4–15	16–28
Casein	17	6	4	1	0	4	2	6	2	6
	(11, 6)	(4, 2)	(2, 1)	(1, 0)	(0, 0)	(1, 3)	(2, 0)	(5, 1)	(2, 0)	(5, 1)
SPI	22	1*	3	5	1	11	0	2*	1	2*
	(13, 9)	(0, 1)	(0, 3)	(4, 1)	(0, 1)	(8, 3)	(0, 0)	(2, 0)	(1, 0)	(1, 1)
SAM-P/1		4–6	7–10	11–14	4–6	7–14	4–6	7–14	4–6	7–14
Casein	22	8	5	1	0	0	0	9	0	2
	(12, 10)	(4, 4)	(5, 0†)	(0, 1)	(0, 0)	(0, 0)	(0, 0)	(4, 5)	(0, 0)	(1, 1)
SPI	18	1*	5	6*	0	0	0	12	0	5
	(10, 8)	(1, 0)	(3, 2)	(3, 3)	(0, 0)	(0, 0)	(0, 0)	(6, 6)	(0, 0)	(2, 3)

[1]Dietary groups: Casein or soybean protein isolate (SPI) 25 g/100 g diet were provided.
[2]Animals with advanced postmortem changes were excluded. The numbers in parentheses give the numbers of male mice (left) and female mice (right).
[3]Carcasses with notable hepatic amyloidosis, without any inflammatory or tumorous changes were included.
[4]Carcasses with notable renal amyloidosis, without any inflammatory or tumorous changes were included.
*Significant differences from the corresponding casein groups; †significant gender difference in the same dietary groups by Fisher's Exact Test ($P < 0.05$).

these mice also developed tumors, amyloidosis and/or contracted kidneys. Inflammatory changes were significantly greater in 7–10 mo old male SAM-P/1 mice fed casein than in females fed the same diet ($P < 0.05$).

Amyloid deposition. Amyloid A protein deposition was greater in the kidneys, spleen, stomach and liver of SAM-R/1 mice fed casein than in mice fed SPI (**Table 3**, $P < 0.01$). In the mice fed casein, there was a significant difference in the severity of amyloid A protein deposition between males and females; the females showed significantly lower amyloid A protein deposition than the males in the kidneys, spleen, stomach and liver. The average intensity of amyloid A protein deposition (amyloid index) in SAM-R/1 mice that died after 400 d of age is shown in **Figure 4**. Six mice that died before 400 d of age died younger than 300 d of age, and autopsy revealed that they died of inflammations. The casein fed group showed fairly high amyloid indexes irrespective of age. Within the SPI group, there was a slight increase in the amyloid A protein amyloid index in aged mice that died after 750 d of age, but these indexes were significantly lower than those of the casein fed group. Amyloid A protein deposition was not observed in the SAM-P/1

TABLE 3

Influence of dietary protein source on amyloid A (AA) protein deposition in organs of SAM-R/1 mice[1]

Group	n	Amyloid index in organs					
		Liver	Spleen	Heart	Skin	Kidney	Stomach
Male							
Casein	7	1.10 ± 0.09*†	2.71 ± 0.42*†	0.69 ± 0.21	0.13 ± 0.13	2.85 ± 0.26*†	2.21 ± 0.14*†
SPI	11	0	0.17 ± 0.11	0.23 ± 0.12	0	0	0.20 ± 0.13
Female							
Casein	5	0.60 ± 0.40	1.00 ± 0.45	0.50 ± 0.50	0.25 ± 0.25	1.00 ± 0.44	0.70 ± 0.43
SPI	8	0	0.14 ± 0.14	0.36 ± 0.24	0.14 ± 0.14	0.19 ± 0.19	0.13 ± 0.13

[1]Data are means ± SEM. The intensity in the deposition of amyloid A protein in organs for each animal were graded at four levels using immunohistochemically stained sections; 4, extremely heavy deposits; 3, heavy deposits; 2, moderate deposits; 1, light deposits; 0, no deposits. *Significant differences from the corresponding casein group by Mann-Whitney's test in mice that died over 400 d after birth ($P < 0.01$); †Significant differences from the corresponding female group by Mann Whitney's test ($P < 0.01$).

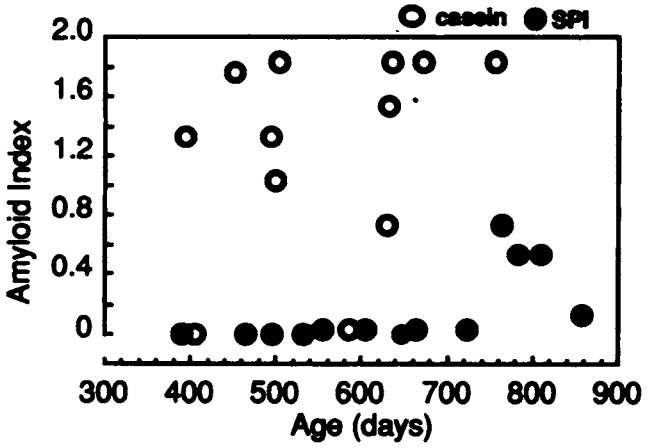

FIGURE 4 Influence of diet and age on amyloid A protein deposition in SAM-R/1 mice fed casein or soybean protein isolate (SPI). The amyloid index was calculated by adding the scores for each of the six organs (liver, kidney, spleen, heart, skin and stomach), and dividing the sum by 6 to obtain the average intensity of amyloid deposition for each mouse. The mice in the group fed casein included 7 males and 5 females, and those in the group fed SPI included 9 males and 4 females. The amyloid indexes of casein fed group were significantly lower in the females than in the males but those of SPI fed group showed no significant sex difference. There was no correlation between amyloid index and age in the casein group by Spearman's rank test (r = 0.49), but there was a significant correlation in the SPI group (r = 0.65, $P < 0.05$).

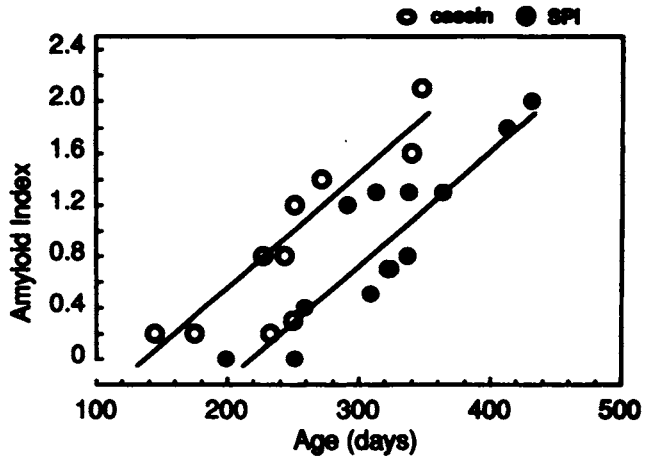

FIGURE 5 Influence of diet and age on senile amyloid (AS_{SAM}) deposition in SAM-P/1 mice fed casein or soybean protein isolate (SPI). The amyloid index was calculated by adding the scores for each of the six organs (liver, kidney, spleen, heart, skin and stomach), and dividing the sum by 6 to obtain the average intensity of amyloid deposition for each mouse. The mice in the group fed casein included 6 males and 4 females, and those in the group fed SPI included 7 males and 5 females. There was no significant sex difference for amyloid index within each group (Mann-Whitney's test). There were correlations between amyloid index and age in both groups by Spearman's rank test (casein: r = 0.87, $P < 0.01$; SPI: r = 0.88, $P < 0.01$), and the effect of the SPI diet on amyloid deposition (amyloid index) was significant by the covariate analysis ($P < 0.01$).

dietary groups, except in those mice that had inflammatory changes, pneumonia, or skin ulcers.

There were no significant differences in SAM-P/1 mice in the severity of AS_{SAM} deposition between the casein and the SPI fed groups, or between the males and females in each group (data not shown). As shown in **Figure 5**, the amyloid index (AS_{SAM}) increased with age in both groups of SAM-P/1 mice, but the onset of AS_{SAM} deposition was significantly later in the SPI group than in the casein group ($P < 0.01$). Depositions of AS_{SAM} in SAM-R/1 mice were not seen in either dietary group, except in 8 (6 males and 2 females) of the 14 aged mice that died after 600 d of age. Average amyloid indexes of AS_{SAM} in these aged mice were 0.42 ± 0.13 (3 male and 2 female mice fed casein) and 0.33 ± 0.09 (3 male mice fed SPI). It was observed that there was every possibility of AS_{SAM} and amyloid A protein depositions in the same organ, but there was no significant correlation between the severities of these two amyloid protein depositions (data not shown).

DISCUSSION

Previous work in our laboratory showed that 40% energy restriction in SAM-P/1 increased longevity (males: 47%, females: 40%). However, in SAM-R/1 mice with a normal aging process, a significant reduction in the grading score by energy restriction was not reflected by a prolongation of mean life span and the mean life span in last 10th of mice that survived (Kohno et al. 1985). In the present study, the soybean protein-containing diet significantly prolonged the mean life span (by 52%) and the life span of the 10th percentile survivors (by 20%) of SAM-R/1 mice in comparison with the casein diet. These findings indicate that replacing dietary casein with soybean protein had as much effect on longevity as did energy restriction.

A conspicuous pathologic feature of the SAM-R/1 mice fed casein was the marked extracellular protein amyloid A deposition in various organs, which is characteristic of secondary amyloidosis. Of the SAM-R/1 mice fed the casein-containing diet that died after 13 mo of age, 82% exhibited severely contracted kidneys, which were attributed to amyloid A protein deposition. We found that replacing casein with soybean protein in the diet suppressed the appearance of secondary amyloidosis in SAM-R/1 mice. The high incidence of nonthymic lymphomas and histiocytic neoplasms in aged SAM-R/1 mice is the primary characteristic of this strain (median survival time: 18.9 mo; Takeda et al. 1981 and 1991). The occurrence of those tumors in SAM-R/1 mice increased after 16 mo of age and did not show a significant

difference between the two sexes (unpublished data). More SAM-R/1 mice fed SPI than those fed casein had tumors. It seems likely that the reason for the higher incidence of tumors at the time of death in SAM-R/1 mice fed SPI is related to the fact that these mice live longer than do those fed casein. The soybean protein diet did not suppress or delay the occurrence of lymphomas or neoplasms. Our results indicate that a soybean protein diet may enable SAM-R/1 mice to age without either secondary amyloidosis or the side effects associated with food restriction, thereby facilitating full life span studies with this animal model.

In SAM-P/1 mice, the replacing of dietary casein with soybean protein resulted in a 30% increase in longevity, which is small compared with the 52% increase observed in SAM-R/1 mice. In SAM-P/1 mice fed either casein or soybean protein, the main pathological finding was senile amyloidosis, which is the primary characteristic of aged SAM-P/1 mice. Although the soybean protein diet did not decrease the severity of AS_{SAM} deposition relative to the casein diet, it did significantly delay the onset of AS_{SAM} deposition in organs. Moreover, in the SAM-P/1 mice fed casein, a number of young mice died of inflammations, unlike those fed soybean protein. The retardation of AS_{SAM} deposition and the inhibition of the occurrence of inflammations in the early days might be factors in the slight increase in longevity observed in the soybean diet-fed group of SAM-P/1 mice. On the other hand, the amyloid A protein deposition observed in the SAM-R/1 mice fed casein was rarely found in the SAM-P/1 mice fed the same diet. This difference between the two strains might be explained by strain differences in inducible amyloid deposition (Glenner et al. 1976), or by deaths of short-lived SAM-P/1 mice which preceded the occurrence of amyloidosis. The promoting effect of dietary casein on amyloidogenesis has been explained as solely an effect of protein intake (Glenner et al. 1976, Grayzel et al. 1934). The production of amyloid was delayed in hamsters by a low casein diet (Irwin et al. 1969) and was inhibited in mice by a protein-free diet (Kedar et al. 1973). It seems, therefore, that adequate protein intake is essential for the initiation of an amyloidogenic stimulus (Kedar et al. 1973). In the present study, despite ad libitum consumption of the diets, the soybean protein diet inhibited amyloid A protein amyloid synthesis in experimental animals. The mechanism of action by which soybean protein exerts its anti-amyloidogenic effect is not clear.

The question arises as to what occurs in the soybean protein-fed mice that contributes to reduced amyloidosis and increased longevity. There have been several reports on the effect of soybean protein diet in rats: hypocholesterolemic action (Tanaka et al. 1984), retardation of chronic nephropathy and increased longevity (Iwasaki et al. 1988, Masoro and Yu 1989) but few in mice. One possible reason for the difference between casein and soybean protein might be their relative amino acid compositions. Strain A mice fed a high cystine, low protein diet had secondary amyloidosis, but the incidence was lower in those fed a low cystine, low protein diet (Heston et al. 1945). The arginine to lysine ratio in soybean protein, which is twice that of casein, might be a determining factor in reducing cholesterol (Kritchevsky et al. 1982) and may have an effect in the present study.

The effect of soybean protein on longevity may be partly due to nonprotein materials present in this preparation. The roles of selenium in the antioxidant system in all tissues and in the resistance to infectious diseases have been noted (Machlin and Bendich 1987, Erskine et al. 1989). The selenium concentration of casein and SPI used in the present study was 0.22 and 0.32 μg/g protein, respectively. Although the selenium concentration of SPI is higher than in casein, it has been reported that the selenium of soybean proteins is largely in the form of selenomethionine bound to proteins. Therefore, the availability of selenium in SPI is slightly lower than that of casein (Yasumoto et al. 1983). Soybeans contain isoflavones that have estrogenic, fungitoxic, and antioxidant properties (Eldridge 1982) and saponins that have hypocholesterolemic activity (Oakenfull et al. 1979). Those two factors have been shown to play a role in the reduction of breast and colon cancers (Messina and Barnes 1991). Because soybean protein isolate contains much smaller amounts of isoflavones and saponins than does soybean (Eldridge 1982), we cannot state that the effects of SPI observed in our study are due to these components.

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