

## Serum Lipid Concentrations and Mean Life Span Are Modulated by Dietary Polyunsaturated Fatty Acids in the Senescence-Accelerated Mouse<sup>1</sup>

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**ABSTRACT** The senescence-accelerated mouse (SAMP8) is an animal model used in studies of aging. This study was undertaken to investigate the effects of dietary PUFA on longevity (Experiment 1) and serum lipid concentrations (Experiment 2) in SAMP8 mice. Male mice were fed either an (n-3) PUFA-rich (9 g/100 g perilla oil) or an (n-6) PUFA-rich (9 g/100 g safflower oil) diet beginning at 6 wk of age. Experiment 1: The groups did not differ in body weight gain, but those fed perilla oil had significantly lower scores of senescence relative to those fed safflower oil ( $P < 0.05$ ). The mean life span of mice fed perilla oil was  $357 \pm 21$  d and of those fed safflower oil,  $426 \pm 24$  d ( $P < 0.05$ ). Pathological studies revealed that the incidence of tumors was significantly lower in the perilla oil group than in the safflower oil group ( $P < 0.05$ ). Approximately half the mice fed perilla oil had died after 10 mo, and the direct causes closely connected with death could not be specified. Experiment 2: The serum total cholesterol, HDL cholesterol, triglyceride and phospholipid concentrations were significantly lower in the perilla oil group than in the safflower oil group ( $P < 0.01$ ). A marked decrease of serum HDL cholesterol and apolipoprotein A-II (ApoA-II) concentrations in advanced age were observed in the mice fed perilla oil ( $P < 0.01$ ). Ten-month-old mice fed perilla oil had a significantly greater ratio of apolipoprotein A-I (ApoA-I) to ApoA-II than those fed safflower oil. Separation of HDL subfractions revealed that the smaller HDL species were much more abundant than the larger HDL species in both dietary oil groups. These findings suggest that dietary (n-3) and (n-6) PUFA differ in their effects on serum lipid metabolism which may modulate the mean life span of SAMP8 mice fed each dietary oil. J. Nutr. 130: 221-227, 2000.

**KEY WORDS:** • senescence-accelerated mouse (SAM) •  $\alpha$ -linoleic acid • linoleic acid • serum lipids • longevity

The available evidence indicates that higher cholesterol levels increase the risks of coronary heart disease and that cholesterol reduction results in corresponding decreases in risk. However, existing evidence does not strongly support the idea that cholesterol reduction increases overall nonvascular mortality or mortality from any specific cause (Gaziano et al. 1996). The association between low serum cholesterol levels and a definite benefit on total mortality is still unclear.

A murine model of accelerated senescence was developed by our group (Takeda et al. 1991): senescence-accelerated mouse (SAM) strains (SAMP1, P2, P3, P6, P7, P8, P9 and P10), and control SAMR mouse strains (SAMR1, R4 and R5) with normal aging characteristics. SAMP strains show earlier onset and irreversible advancement of senescence manifested

by clinical signs and gross lesions following a normal process of development, and each SAMP strain has a strain-specific age-associated disorder. These mice have been used as models to investigate the aging process, including senile amyloidosis (Higuchi et al. 1983), degenerative joint disease (Chen et al. 1989), senile osteoporosis (Matsushita et al. 1986), deficits in learning and memory (Yagi et al. 1988) and senile cataract (Hosokawa et al. 1988). Senile amyloidosis is one of the most characteristic age-associated disorders in the SAMP strains. The three molecular types (type A, B and C) of apolipoprotein A-II (apoA-II), with different amino acid substitutions at four positions, correlated with the susceptibility of each strain to senile amyloidosis (Higuchi et al. 1991). The strains (SAMP1, P2, etc.) with a high incidence and severe senile amyloidosis have type C apoA-II, whereas the strains with a low incidence and slight or mild amyloidosis have type A apoA-II (SAMP8 and P3) or type B apoA-II (SAMR1, R4 and P6). Mice are typical "HDL-animals" in that HDL is a major lipoprotein class and the serum cholesterol level is low (Chapman 1986). The total cholesterol levels in the type C or type A apoA-II strains, such as SAMP1 and SAMP8, are one-third

<sup>1</sup> Soybean protein isolate was a generous gift from the Fuji Oil Company, Osaka. This work was supported by a grant from the Ministry of Education, Science, Sports & Culture and the Ministry of Health and Welfare of Japan, and by a Special Research Grant from Koshien University.

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<sup>3</sup> Abbreviations used: Apo, apolipoprotein; CD, commercial diet; PL, phospholipid; SAM, senescence-accelerated mouse; TG, triglyceride.

**TABLE 1**  
Fatty acid composition of the oils<sup>1,2</sup>

Fatty acid	Safflower oil	Perilla oil	Commercial diet
<i>g/100 g total fatty acids</i>			
16:0	6.8	6.4	14.9
18:0	2.1	1.6	1.8
18:1(n-9)	13.9	17.8	21.9
18:2(n-6)	75.1	14.3	50.6
18:3(n-3)	0.3	58.5	3.9
(n-3)/(n-6)	0.00064	4.09	13.0

<sup>1</sup> All of the oils were stored at 4°C. Additional vitamin E (DL- $\alpha$ -tocopherol) was added to the oils as an antioxidant so that the final concentration in the mixed diets was 0.009%.

<sup>2</sup> Fatty acid analysis described previously (Umezawa et al. 1995).

Fatty acid composition of commercial diet is quoted from the collected works of Nihon CLEA (1997).

the levels in humans. We utilized the SAMP8 strain mice as the experimental animal in this study due to the accelerated senescence, with a low incidence of amyloidosis and an age-associated decrease in the serum cholesterol concentrations (Higuchi et al. 1991 and 1993). An age-associated or diet-induced further decrease in the serum levels of cholesterol in SAMP8 strains may affect senescence and life span. However, this is yet to be clarified.

Dietary triglycerides (TG) composed either of (n-6) PUFA or (n-3) PUFA differ in their effects on serum lipid levels. (n-6) PUFA lower cholesterol but not TG levels (Balasubramanian et al. 1985, Leaf and Weber 1988), while (n-3) PUFA lower serum cholesterol, especially VLDL cholesterol, and TG in rats (Ventura et al. 1989) and humans (Phillipson et al. 1985, Saynor et al. 1984). Additionally, dietary PUFA can affect disease pathogenesis and every cell of the immune system via regulation of the levels of eicosanoids (Hardardottir et al. 1992), cytokines (Meydani et al. 1991) and cell-activation enzymes (May et al. 1993).

This study was undertaken to investigate the effects of a high (n-3) PUFA and a high (n-6) PUFA diet on serum lipid levels and longevity using SAMP8 strain mice. The two different vegetable oils, perilla and safflower, used in this study have very different (n-3)/(n-6) ratios, but similar proportions of saturated and monoenoic fatty acids.

## MATERIALS AND METHODS

**Animals and diets.** SAMP8 mice were bred and raised in our laboratory under conventional conditions. Male mice at 6 wk of age were fed diets containing 9 g/100 g  $\alpha$ -linolenate-rich perillaseed oil [18:3(n-3)/18:2(n-6) = 4.09] or 9 g/100 g linoleate-rich safflower seed oil [18:3(n-3)/18:2(n-6) = < 0.01]. The validity of using these oils was assessed by examining the reproductive physiology and growth rates of offspring (Naito et al. 1990). The major fatty acids in the diets are shown in Table 1. The diets contained by weight: 25% soybean protein isolate, 0.3% DL-methionine, 32.7% corn starch, 25% sucrose, 2% cellulose powder, 5% mineral mixture (Umezawa et al. 1990), 1% vitamin mixture (Umezawa et al. 1990) and 9% oil. Diets with peroxide values below 30 meq/kg were routinely used. This study consisted of two experiments. The groups fed perilla oil included 21 (Experiment 1) and 12 (Experiment 2) mice, and the mice in the groups fed safflower oil included 20 (Experiment 1) and 14 (Experiment 2). In addition, 8 SAMP8 male mice at 10-mo-old fed commercial diet (CD) (CE-2; NIHON Clear, Tokyo, Japan) from 4 wk of age were used in Experiment 2. The CD contained 25.2% crude protein, 50.2% carbohydrate, 4.4% fat, 4.4% fiber and 7.0% ash

(collected works of Nihon CLEA, 1997). Mice were housed at about five per cage, allowed free access to diet and tap water and were maintained in a temperature-controlled room (24  $\pm$  2°C) with a 12-h light/dark cycle, throughout their lives in Experiment 1 and until they were 5-mo- or 10-mo-old in Experiment 2. All mice were maintained according to the policies and recommendations of The Kyoto University Animal Care and Use Committee.

### Experiment 1

Each mouse was weighed weekly beginning at wk 6 after birth. All mice were inspected at least twice daily. The mice that died spontaneously were removed from their cages and immediately necropsied.

**Grading score of senescence.** This system was designed to represent changes in the behavior and appearance of the mice that were considered to be associated with the aging process (Hosokawa et al. 1984). Briefly, the 11 categories measured included 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 to 3 mo, and the sum of the scores of the 11 categories was recorded.

**Histological examination.** The age at death of each mouse was recorded, and the abdominal skin, liver, kidneys, spleen, heart, lungs, stomach, thyroid, adrenals, gonads and aorta were fixed in 10% (v/v) neutral buffered formalin, embedded in paraffin, cut into 4- $\mu$ m sections and stained with hematoxylin and eosin.

### Experiment 2

Mice at 5- or 10-mo-old were food-deprived for about 15 h before collection of blood samples obtained by cardiac puncture following light anesthesia with ether. The serum was then stored at -45°C until analysis.

**Serum lipids and lipoprotein quantitation.** Serum total cholesterol levels were determined using an enzymatic procedure (Cholesterol C test; Wako Pure Chemical Industries, Osaka, Japan). HDL cholesterol was measured according to a modified heparin-manganese precipitation procedure (HDL cholesterol C test; Wako). TG levels were measured spectrophotometrically using acetylacetone (Triglyceride-test; Wako). Phospholipid (PL) levels were measured using a calorimetric kit (Phospholipid-test; Wako).

There are two major proteins, apolipoprotein A-I (ApoA-I) and ApoA-II in HDL. The levels of ApoA-I and ApoA-II in serum were determined using an immunoblotting method as described previously (Higuchi et al. 1995). Serum (50 nL) was applied to a 15 to 20% gradient sodium dodecyl sulfate-polyacrylamide mini gel, 84 mm wide  $\times$  90 mm high, and electrophoresis was carried out at 15 mA for 2.5 h. After electrophoresis, samples were electroblotted onto polyvinylidene difluoride membranes (Bio Rad Laboratories, Richmond, CA) using a semidry apparatus (Nihon Eido, Tokyo, Japan) at 150 mA for 2 h. ApoA-I and ApoA-II were detected after incubation of the membranes with monospecific rabbit anti-mouse ApoA-I and ApoA-II antiserum (diluted 1:4,000) by the avidin-biotinylated horseradish peroxidase complex method, using 3-3'-diaminobenzidine tetrahydrochloride as a substrate. The amounts of ApoA-I and ApoA-II were determined by comparing the intensity of bands with that of bands of the internal control, purified mouse ApoA-I and ApoA-II protein, using a Densitron (Jookoo, Tokyo, Japan).

**Nondenaturing gradient polyacrylamide gel electrophoresis.** To ascertain whether dietary oils affected the size distribution of HDL, nondenaturing polyacrylamide gradient gel electrophoresis was used (Higuchi et al. 1993). Gels containing a 2–15% linear polyacrylamide gradient were electrophoresed in 25 mmol/L of Tris and 192 mmol/L of glutamic acid. Prior to electrophoresis, serum samples (3  $\mu$ L) were stained for lipid by incubation at 4°C overnight with 2.5  $\mu$ L of freshly prepared Sudan Black B dye solution (5 parts 10 g/L of Sudan Black B in ethylene glycol-3 parts 400 g/L of sucrose). Electrophoresis was carried out at 25 mA for 2 h. The amounts of the HDL species were determined by comparing the intensity of bands with that of bands of the HDL<sub>3</sub> level in the safflower oil group, using a Densitron.

### Statistical analysis

Two-way ANOVA was used to compare results for the two types of dietary oils across the various ages of the mice. Comparisons between results for the two types of dietary oils in each age group were made using Tukey's test. Survival curves were estimated using the Kaplan-Meier test (Kaplan and Meier 1958), and a comparison of the curves was carried out by Log-Rank test analysis using the Statistical Analysis System (SAS Institute, Cary, NC). The significance of the differences with respect to the pathological findings due to age within each dietary oil group was analyzed using Fisher's Exact Test (Steel and Torrie 1980). Statistical significance was established when  $P < 0.05$ .

## RESULTS

### Experiment 1

**Growth and grading score of senescence.** Food consumption did not differ in the two vegetable oil-fed groups of SAMP8 (2–10-mo-old) mice ( $3.5 \pm 0.1$  g/d in mice fed safflower oil;  $3.4 \pm 0.2$  g/d in those fed perilla oil). Body weight gains from 6 wk to 15 mo did not differ (not shown).

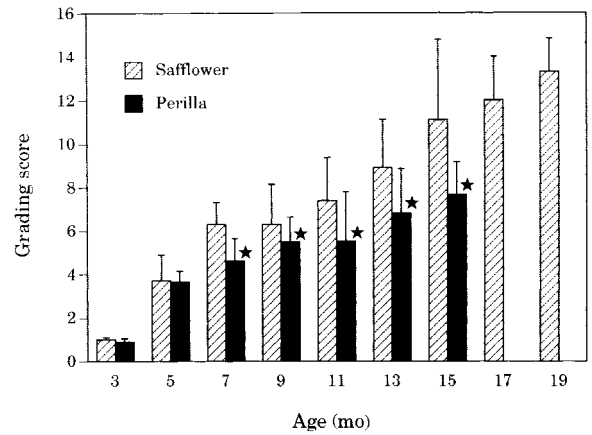
Grading scores that reflect senescence are shown in **Figure 1**. Increasing senescence scores with aging were found in both groups. The grading scores of mice fed perilla oil were significantly lower than those fed safflower oil after 7 mo of age ( $P < 0.05$ ).

**Survival curves.** The survival curve for the perilla oil group was shifted to the left (to a younger age) of the curve of the safflower oil group ( $P < 0.05$ ) (**Fig. 2**). The mean life span of the perilla oil group ( $357 \pm 21$  d) was significantly ( $P < 0.05$ , 17%) shorter than that of the safflower oil group ( $426 \pm 24$  d) ( $P < 0.05$ ).

**Pathological findings.** The primary pathological findings were inflammatory changes, such as abscess, pneumonia and ulcer, tumors (mostly malignant lymphomas), amyloidosis and contracted kidneys (**Table 2**). Abscess and overall inflammatory changes were significantly greater in 6–20-mo-old mice in the perilla oil group (abscess:7, pneumonia:5, ulcer:2) than in mice of the same age in the safflower oil group (abscess:2, ulcer:2, pneumonia:1) ( $P < 0.01$ ). However, in the perilla oil group, inflammatory changes were directly related to death for only 6 of 16 mice that exhibited them. Almost all the mice in the safflower oil group died as a result of tumors, amyloidosis and contracted kidneys, and the number of mice that had tumors was significantly higher than that in the perilla oil group ( $P < 0.05$ ). The mice in the safflower oil group exhibited thymic lymphoblastic lymphomas ( $n = 6$ ), nonthymic lymphoblastic lymphomas ( $n = 1$ ) and squamous cell carcinoma ( $n = 1$ ). Two of those in the perilla oil group had developed thymic lymphoblastic lymphomas. Severely contracted kidneys were observed in seven mice in the safflower oil group, and there were amyloid protein deposits in the kidneys. Others in the safflower oil group exhibited myocardial infarction ( $n = 1$ ), coagulative necrosis of the liver ( $n = 1$ ), congestion and edema of the lung ( $n = 1$ ) and volvulus ( $n = 2$ ). The direct causes of death of 6 of 15 perilla oil-fed mice that died after 10-mo-old could not be identified. Reasons for all deaths could be determined in the mice fed safflower oil.

### Experiment 2

**Serum lipids and cholesterol.** Serum concentrations of total cholesterol, HDL cholesterol, TG and phospholipids (PL) in mice fed two dietary oils at both 5- and 10-mo-old were significantly lower in the perilla oil group than in the safflower oil group ( $P < 0.01$ ) (**Table 3**). The concentrations

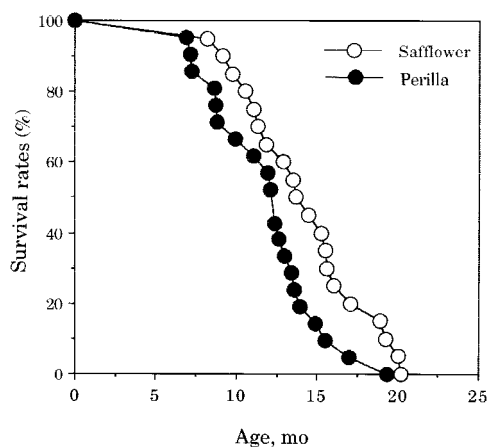


**FIGURE 1** Age-related changes of grading scores of senescence in senescence-accelerated mice (SAMP8) fed perilla oil and safflower oil diets. The values are means  $\pm$  SD.  $n = 21$  (P, perilla oil group) and 20 (S, safflower oil group) at 3, 4 and 5 mo, 20 (P and S) at 7 mo, 15(P) and 19(S) at 9 mo, 14(P) and 16(S) at 11 mo, 8(P) and 12(S) at 13 mo, 3(P) and 9(S) at 15 mo, 5(S) at 17 mo and 3(S) at 19 mo. ★Significantly different from the corresponding safflower oil group using Mann-Whitney U-test ( $P < 0.05$ ).

of total cholesterol and PL in both diet groups did not significantly differ between 5- and 10-mo-old mice. However, the serum HDL cholesterol concentrations were significantly lower in 10-mo-old mice than in 5-mo-old mice in both diet groups ( $P < 0.01$ ). The ratio of HDL cholesterol to total cholesterol in the perilla oil group was 55% lower at 10-mo-old compared with at 5 mo, while those in the safflower oil group was 33% lower at 10 mo than at 5 mo ( $P < 0.01$ ). The serum TG level in the safflower oil group only was significantly lower at 10-mo-old compared with 5 mo ( $P < 0.01$ ). Lipid concentrations at 10 mo in the CD group generally were between the safflower and perilla oil groups. Serum total protein (g/L) at 10 mo in the two oil groups did not differ ( $77.3 \pm 5.6$ , perilla oil group;  $76.8 \pm 7.5$ , safflower oil group).

**Serum Apo.** The serum levels of ApoA-I in both 5- and 10-mo-old mice were significantly lower in the perilla oil groups than in the safflower oil groups ( $P < 0.01$ ), and a difference with age in each dietary oil group was not observed (**Table 4**). The serum levels of ApoA-II were significantly lower in 10-mo-old mice than in 5-mo-old mice in both dietary oil groups ( $P < 0.05$ ): 50% lower in the perilla oil group and 29% lower in the safflower oil group. The ratio of ApoA-I to ApoA-II was significantly higher at 10 mo of age compared with 5 mo in both diet groups, and at 10 mo of age those in the perilla oil group were significantly higher than those in the safflower oil group ( $P < 0.05$ ). The serum levels of ApoA-I, ApoA-II and the ratio of ApoA-I to ApoA-II at 10 mo in the CD group were comparable to those of the perilla oil group.

**HDL size distribution.** Three distinct HDL size classes, referred to from the largest to the smallest as HDL<sub>1</sub>, HDL<sub>2</sub> and HDL<sub>3</sub>, were observed on gradient gels. Typical patterns in 10-mo-old mice are shown in **Figure 3A**. The predominant form observed in both dietary oil groups was HDL<sub>3</sub>, whereas HDL<sub>1</sub> was present at very low levels. Densitometric gel scanning of the peaks representing the three major species of HDL yielded the findings shown in **Figure 3B**. As observed with the serum levels of HDL cholesterol, the SAMP8 mice had a 55% lower HDL lipid stain intensity in response to the perilla oil diet compared with the intensity observed for the safflower oil diet group. However, there were clear differences between the



**FIGURE 2** Survival curves for senescence-accelerated mice (SAMP8) fed perilla or safflower oil. The numbers of mice in each group are the same as those in Figure 1. The mean life span of the perilla oil group ( $357 \pm 21$  d) was significantly shorter than that of the safflower oil group ( $426 \pm 24$  d) ( $P < 0.05$ ).

dietary oil groups on the different HDL species. The perilla oil group exhibited 58, 48 and 54% lower levels of HDL<sub>3</sub>, HDL<sub>2</sub> and HDL<sub>1</sub>, respectively, compared with the levels observed for the safflower oil group.

## DISCUSSION

In this study, the SAMP8 mice fed perilla oil did not have longer maximum life spans and had a shorter mean life span than those of mice fed safflower oil, despite the fact that the grading scores for senescence, represented by changes in behavior and appearance were significantly lower in the perilla oil group than in the safflower oil group. Therefore, clarifying the causes of death for the mice fed the two dietary oils would be interesting.

Routine postmortem examinations of the SAMP8 strain fed CD showed that amyloidosis was the most common autopsy

finding with an incidence of 39.1%, while tumors were second (32.6%), and the third was abscess (26.1%) (Takeda et al. 1997). All SAMP8 mice fed safflower oil developed tumors (42.1%), amyloidosis (31.5%) and/or contracted kidneys (36.8%), and these were directly related to death. However, it was found that replacing safflower oil with perilla oil in the diet suppressed the appearance of amyloidosis (18.8%) and tumors (12.5%) in SAMP8 mice; the inflammatory changes including abscess, pneumonia and ulcer were directly related to death in 37% of the mice fed perilla oil, although almost all mice fed perilla oil exhibited these findings (87.5%). The benefits of (n-3) PUFA in cancer (Dajani 1993) and kidney disorders (DeCaterina et al. 1993), as well as in cardiovascular disease (Schmidt and Dyerberg 1994), have been demonstrated. A diet supplemented with perilla oil suppressed tumor metastasis (Hori et al. 1987) and tumorigenesis (Okuyama et al. 1987), and reduced hypertension and apoplexy (Shimokawa et al. 1988), thrombosis (Watanabe et al. 1989) and allergic response (Hashimoto et al. 1988). Additionally, it was reported that rats fed perilla oil had extended mean survival times compared to that of those fed safflower oil (Yamamoto et al. 1991). The beneficial effects of perilla oil were evident in SAMP8 mice fed perilla oil, but it did not affect life span, and for 44% of the mice fed perilla oil, the direct cause of death could not be identified.

It has been reported that the type A ApoA-II gene is responsible for lower plasma cholesterol concentrations and smaller HDL size (Doolittle et al. 1990). In 10-mo-old male SAM mice fed CD, the SAMP8 mice with type A ApoA-II showed lower serum levels of total cholesterol (about two-thirds) and even lower levels of HDL cholesterol (about one-third) compared to those of SAMR1 mice with type B ApoA-II (Higuchi et al. 1995). In this study, 10-mo-old SAMP8 mice fed perilla oil had lower serum levels of total cholesterol (about half) and even HDL cholesterol (about one-quarter) than SAMR1 mice fed CD. This hypolipidemic effect of perilla oil may be due to inhibition of lipogenic enzymes in the liver (Iritani et al. 1980) and decreased formation of both chylomicrons in the intestine and VLDL in the liver (Nestel et al.

**TABLE 2**

*Pathological findings in SAMP8 mice fed diets containing perilla or safflower oil throughout their lives*

Dietary group	n <sup>1</sup>	Pathological findings											
		Inflammation <sup>2</sup>						Tumors	Amyloidosis <sup>4</sup>	Contracted kidneys <sup>5</sup>			
		Abscess		Pneumonia <sup>3</sup>		Ulcer							
<i>age, mo</i>													
		6-9	10-20	6-9	10-20	6-9	10-20	6-9	10-20	6-9	10-20	6-9	10-20
<i>mice, n</i>													
Perilla	16	0	7*	1	4	0	2	0	2*	0	3	0	3
Safflower	19	0	2	0	1	1	1	0	8	1	5	1	6

<sup>1</sup> Animals with advanced postmortem alterations were excluded.

<sup>2</sup> All inflammatory changes were significantly greater in 6-20-mo-old mice in the perilla oil group than in mice of the same age in the safflower oil group ( $P < 0.01$ ).

<sup>3</sup> Pneumonic changes were significantly greater in 6-20-mo-old mice in the perilla oil group than in mice of the same age in the safflower oil group ( $P < 0.05$ ).

<sup>4</sup> Carcasses with notable hepatic amyloidosis but not exhibiting inflammation or tumors were included.

<sup>5</sup> Carcasses with notable renal amyloidosis but not exhibiting inflammation or tumors were included. \*Significantly different from the corresponding safflower oil group. (Fisher's Exact Test,  $P < 0.05$ ). SAMP8, senescence-accelerated mouse.

TABLE 3

Serum lipid concentrations in SAMP8 mice fed diets containing perilla or safflower oil for 14 or 34 wk<sup>1</sup>

Diets	n	Total cholesterol	HDL cholesterol	HDL/Total cholesterol	Triglyceride	Phospholipid
		mmol/L		%		mmol/L
5 mo						
perilla oil	6	1.82 ± 0.20 <sup>b</sup>	1.32 ± 0.29 <sup>b</sup>	72.5 ± 6.80 <sup>a</sup>	0.49 ± 0.18 <sup>c</sup>	1.51 ± 0.11 <sup>b</sup>
safflower oil	8	2.68 ± 0.45 <sup>a</sup>	1.90 ± 0.26 <sup>a</sup>	70.8 ± 14.5 <sup>a</sup>	1.00 ± 0.42 <sup>a</sup>	2.53 ± 0.50 <sup>a</sup>
10 mo						
perilla oil	6	1.63 ± 0.37 <sup>b</sup>	0.53 ± 0.06 <sup>c</sup>	32.5 ± 4.17 <sup>c</sup>	0.36 ± 0.07 <sup>c</sup>	1.72 ± 0.27 <sup>b</sup>
safflower oil	6	3.01 ± 0.51 <sup>a</sup>	1.43 ± 0.06 <sup>b</sup>	47.5 ± 9.88 <sup>b</sup>	0.65 ± 0.26 <sup>b</sup>	2.77 ± 0.44 <sup>a</sup>
Two-way ANOVA						
Diet		<i>P</i> < 0.01	<i>P</i> < 0.01	<i>P</i> < 0.01	<i>P</i> < 0.05	<i>P</i> < 0.01
Age		NS	<i>P</i> < 0.01	<i>P</i> < 0.01	NS	NS
Interaction		<i>P</i> < 0.01	<i>P</i> < 0.01	NS	NS	NS
CD(10 mo) <sup>2</sup>	8	2.33 ± 0.29	0.81 ± 0.34	34.7 ± 5.1	0.77 ± 0.21	2.17 ± 0.22

<sup>1</sup> Values are means ± sd. Within a column, values with different superscript letters are significantly different (*P* < 0.05).

<sup>2</sup> CD, commercial diet (CE-2, NIHON CLEAR; Tokyo, Japan) were fed to senescence-accelerated mice (SAMP8) from 6 wk to 10 mo of age.

1984). Several studies revealed that reduction of HDL cholesterol levels accompanied by increased VLDL + LDL cholesterol levels in a diet result in susceptibility to diet-induced atherosclerosis. Though the findings were not published, 5-mo-old mice fed perilla oil had higher proportions of LDL cholesterol to total cholesterol and lower proportions of VLDL cholesterol to total cholesterol. Lower formation of VLDL-triacylglycerol (Harris et al. 1984) and VLDL ApoB (Nestel et al. 1984) by (n-3) PUFA was shown. However, the serum HDL cholesterol levels of SAMP8 mice fed safflower oil were twice those of SAMP8 mice fed CD, while their total cholesterol levels were nearly the same levels as those of SAMR1 mice fed CD, which were 1.4 times higher than those of SAMP8 mice fed CD.

HDL consists of two major proteins, ApoA-I and A-II. Interestingly, the levels of ApoA-I in mice fed perilla oil or safflower oil did not change between 5- and 10-mo-old, despite the HDL cholesterol levels being clearly lower at 10 mo than at 5 mo. However, both dietary oil groups at 10 mo had low levels of serum ApoA-II that were associated with HDL cholesterol levels. Transgenic mice with ApoA-II revealed that increased ApoA-II expression results in an elevated HDL

cholesterol concentration and a greater proportion of larger HDL particles (Hedrick et al. 1993). Lower HDL cholesterol concentrations in congenic mouse strains have been reported to be caused by lower ApoA-II concentrations (Wang et al. 1997). In SAM mice fed CD, the serum ApoA-II concentrations of SAMP8 mice with type A ApoA-II were about 0.30 g/L, those of SAMP1 mice with type C ApoA-II were about 0.20 g/L, and those of SAMR1 mice with type B ApoA-II were about 0.60 g/L (Higuchi et al. 1983, 1991). The serum ApoA-II concentrations in 10-mo-old SAMP8 mice fed the perilla oil or CD were comparable, while those in the safflower oil group were close to those observed in SAMR1 mice with normal aging characteristics. Generally, at higher ratio of ApoA-I/ApoA-II, HDL function more efficiently in reverse cholesterol transport (Mehrabian and Lusis 1992). In this study, perilla oil lowered HDL cholesterol levels but increased the ratio of ApoA-I/ApoA-II compared with the safflower oil.

An important factor influencing HDL size is the ratio of ApoA-I/ApoA-II in plasma, and the higher ApoA-II content of the larger HDL species which contain more lipids than smaller HDL (Doolittle et al. 1990). Dietary saturated fats resulted in an increase in HDL<sub>1</sub> in baboons and a decrease in

TABLE 4

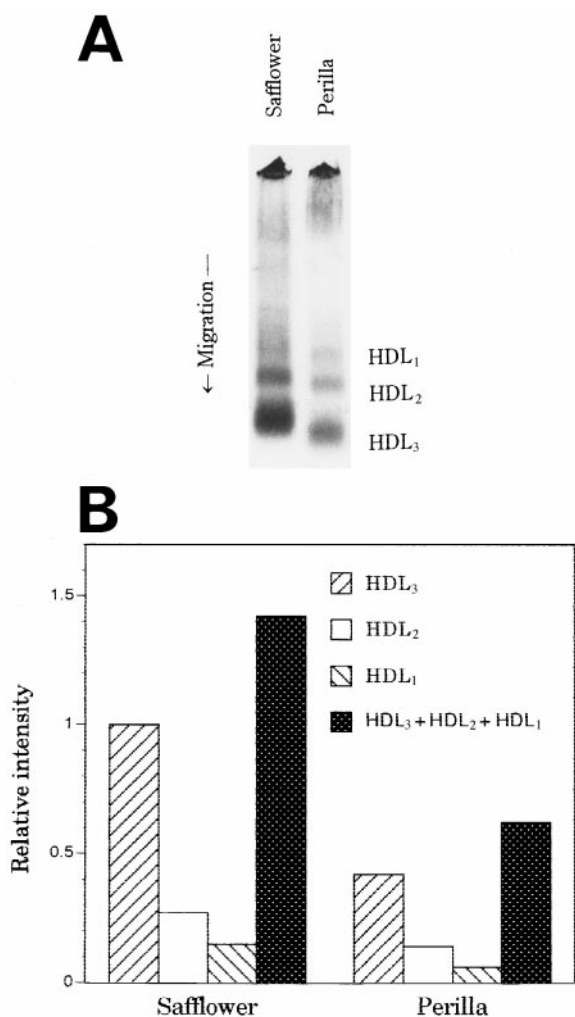
Serum levels of Apolipoprotein (Apo) A-I and Apolipoprotein A-II in SAMP8 mice fed diets containing perilla or safflower oil for 14 or 34 wk<sup>1</sup>

Diets	n	Apolipoprotein A-I	Apolipoprotein A-II	ApoA-I/ApoA-II
		g/L		
5 mo				
Perilla oil	6	1.22 ± 0.45 <sup>b</sup>	0.59 ± 0.05 <sup>a</sup>	2.04 ± 0.77 <sup>c</sup>
Safflower oil	8	1.73 ± 0.20 <sup>a</sup>	0.73 ± 0.16 <sup>a</sup>	2.43 ± 0.68 <sup>c</sup>
10 mo				
Perilla oil	6	1.33 ± 0.27 <sup>b</sup>	0.30 ± 0.05 <sup>c</sup>	4.36 ± 0.57 <sup>a</sup>
Safflower oil	6	1.80 ± 0.12 <sup>a</sup>	0.52 ± 0.05 <sup>b</sup>	3.53 ± 0.30 <sup>b</sup>
Two-way ANOVA <sup>3</sup>				
Diet		<i>P</i> < 0.01	<i>P</i> < 0.01	NS
Age		NS	<i>P</i> < 0.01	<i>P</i> < 0.01
CD (10 mo) <sup>2</sup>	8	1.39 ± 0.24	0.29 ± 0.02	4.77 ± 1.03

<sup>1</sup> Values are means ± sd.

<sup>2</sup> CD, commercial diet (CE-2, NIHON CLEAR; Tokyo, Japan) were fed to senescence-accelerated mice (SAMP8) from 6 wk to 10 mo of age.

<sup>3</sup> There were no significant interactions. Within a column, values with different superscript letters are significantly different (*P* < 0.05).



**FIGURE 3** The size distributions of serum HDL in 10-mo-old senescence-accelerated mice (SAMP8) fed perilla or safflower oil for 34 wk. Three  $\mu\text{L}$  of serum were prestained for neutral lipids using Sudan Black and electrophoresed to equilibrium on a 2–15% nondenaturing polyacrylamide gel. **A**. Representative migration patterns of HDL observed for SAMP8 mice fed perilla or safflower oil diets. **B**. Integration of the HDL<sub>1</sub>, HDL<sub>2</sub>, and HDL<sub>3</sub> peaks on densitometric scans of gradient gels, which provides an estimate of the neutral lipids comprising each HDL size class. These studies were performed in duplicate. One of three trials with consistent results is shown.

the amount of large HDL and an increase in small HDL in monkeys (Babiak et al. 1988). A soybean oil diet resulted in a larger proportion of small HDL in hamsters (Ahn et al. 1994). In human studies, heart disease is associated with low concentrations of HDL<sub>2</sub> and HDL<sub>3</sub> (Miller 1987). Consistent with these data, both HDL<sub>2</sub> and HDL<sub>3</sub> size classes were reduced by high fat diets in a mouse strain susceptible to diet-induced atherosclerosis (LeBoeuf et al. 1990). The consumption of perilla or safflower oil by SAMP8 mice resulted in a larger proportion of small HDL, and the proportions of smaller HDL subclasses did not differ significantly between the two dietary oil groups.

In humans, most guidelines recommend reducing plasma levels of cholesterol to decrease risks for coronary heart disease. On the other hand, in men receiving dietary and/or pharmacological treatments to lower cholesterol concentrations, mortality for coronary heart disease tended to be lower,

but total mortality was not affected by the treatments and deaths not related to illness, accident, suicide or violence, increased (Muldoon et al. 1990, LaRosa et al. 1990). SAMP8 mice have a genetic characteristic of low serum cholesterol levels, and the supplied dietary oils modified their serum lipid metabolism, causing their serum cholesterol concentrations to become still lower or higher. Those differences between the two diet groups may influence both the cause of death and mean life span. The relationship between serum lipids and longevity requires further study.

## ACKNOWLEDGMENTS

We thank members of the laboratory of Field of Regeneration Control, Institute for Frontier Medical Sciences, Kyoto University in conducting the study.

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