Dietary fat modulation of apoA-II metabolism and prevention of senile amyloidosis in the senescence-accelerated mouse

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Abstract Senescence-accelerated mouse-prone (SAMP1; SAMP1@Umz) is an animal model of senile amyloidosis with apolipoprotein A-II (apoA-II) amyloid fibril (AApoAII) deposits. This study was undertaken to investigate the effects of dietary fats on AApoAII deposits in SAMP1 mice when purified diets containing 4% fat as butter, safflower oil, or fish oil were fed to male mice for 26 weeks. The serum HDL cholesterol was significantly lower ($P < 0.01$) in mice on the diet containing fish oil (7.4 ± 3.0 mg/dl) than in mice on the butter diet (38.7 ± 12.5 mg/dl), which in turn had significantly lower ($P < 0.01$) HDL levels than mice on the safflower oil diet (51.9 ± 5.6 mg/dl). ApoA-II was also significantly lower ($P < 0.01$) in mice on the fish oil diet (7.6 ± 2.7 mg/dl) than on the butter (26.9 ± 7.3 mg/dl) or safflower oil (21.6 ± 3.7 mg/dl) diets. The mice fed fish oil had a significantly greater ratio ($P < 0.01$) of apoA-I to apoA-II, and a smaller HDL particle size than those fed butter and safflower oil. Severe AApoAII deposits in the spleen, heart, skin, liver, and stomach were shown in the fish oil group compared with those in the butter and safflower oil groups (fish oil > butter > safflower oil group, $P < 0.05$). These findings suggest that dietary fats differ in their effects on serum lipoprotein metabolism, and that dietary lipids may modulate amyloid deposition in SAMP1 mice.—Umezawa, M., K. Tatematsu, T. Korenaga, X. Fu, T. Matushita, H. Okuyama, M. Hosokawa, T. Takeda, and K. Higuchi. **Dietary fat modulation of apoA-II metabolism and prevention of senile amyloidosis in the senescence-accelerated mouse.** ***J. Lipid Res.*** **2003.** 44: **762–769.**

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Amyloidosis is the term given to a group of diseases characterized by extracellular deposits of amyloid substance in various tissues. Senile amyloidosis is the most characteristic age-related disorder in an inbred strain of mice, senescence-accelerated mouse-prone (SAMP1; SAMP1@Umz), established by Takeda et al. (1) as a model of accelerated senescence. A unique senile amyloid fibril (AApoAII) protein was isolated as extracellular deposits from the liver of SAMP1 mice (2). Amyloid deposits increased with advancing age in all tissues except bone and brain parenchyma (3, 4). Biochemical studies have shown that apolipoprotein A-II (apoA-II), a major apolipoprotein of plasma high density lipoprotein (HDL), is a serum precursor of murine senile amyloidosis (5, 6), and whole apoA-II is deposited as AApoAII without degradation (7, 8). AApoA-II is distinguishable from murine protein AA in secondary amyloidosis or mouse immunoglobulin components, and was found to be present universally in aged mice of various strains (9). The three known variants of apoA-II protein (Types A, B, and C) have different amino acid substitutions at four positions that are correlated with the susceptibility of inbred mice strains to senile amyloidosis (10). The SAMP1 strain, with a high incidence and severe senile amyloidosis, has Type C apoA-II (genotype *Apoa2*^{c}) with glutamine at position 5, whereas the senescence-accelerated mouse-resistant (SAMR1; SAMR1@Umz) strain, which has a very low incidence of amyloidosis, has Type B apoA-II (*Apoa2*^{b}) with proline at position 5 (11). The SAMP1 strain is characterized by low levels of serum apoA-II and HDL, small HDL particle size, age-associated decreases in serum levels of apoA-I and apoA-II, age-associated increases in the clearance rate of serum apoA-II

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Abbreviations: AApoAII, apolipoprotein A-II amyloid fibrils; SAM, senescence-accelerated mouse.

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and HDL, age-associated decreases in the hepatic levels of mRNA for the apoA-II gene, and decreased apoA-II protein synthesis compared with the SAMR1 strain (12).

Dietary triglycerides (TGs) composed either of saturated fatty acids (SFAs), n-6 PUFA, or n-3 PUFA differ in their effects on serum lipid levels. Saturated fat raises both cholesterol and TGs; n-6 PUFA lowers serum cholesterol, but not triglycerides; and n-3 PUFA lowers serum cholesterol, especially in VLDL and serum TG levels in rats (13–15) and humans (16). Clinical and pathologic studies in humans have shown that the incidence of amyloidosis is only 6% in patients with lepromatous leprosy in Mexico, in contrast to those in the United States who consume far more saturated fat in their diet, with a rate of 24% (17). The induction of AA-type amyloidosis in young CBA/J mice was enhanced when diets enriched with coconut oil (n-6 SFA) were substituted for diets containing n-3 or n-6 PUFA (18). SAMP1 mice failed to develop amyloidosis when fed a low-calorie diet (19). Although diet has not been extensively investigated as a risk factor for amyloidosis, we hypothesize that dietary factors modulate the rate at which β-pleated sheet fibrils accumulate in most forms of amyloidosis. Epidemiological and biochemical studies show a strong reduction in the incidence of Alzheimer's disease (AD) and dementia in patients treated with cholesterol-lowering drugs (20, 21) and reductions in the levels of AD β-amyloid peptides Aβ42 and Aβ40, both in vitro and in vivo (22). Recently, we found that diets enriched with n-3 PUFA markedly decreased the levels of serum cholesterol and apoA-II concentrations in SAMP strain mice with advancing age, compared with those with n-6 PUFA (23). This study was undertaken to examine the levels of AApoAII deposition in SAMP1 mice fed a diet in which the main source of fat was either *a*) butter, which is high in SFA; *b*) safflower oil, which is high in n-6 PUFA; or *c*) fish oil, which is high in n-3 PUFA.

We found that the dietary fats affected the course of the disease and also noted that the safflower oil diet in which the PUFA are n-6 fatty acids rather than n-3 fatty acids had a significantly beneficial effect on disease outcome, while the fish oil diet had the opposite effect, even though the fish oil diet also altered the levels of serum lipids and the apolipoprotein profile.

MATERIALS AND METHODS

Animals, diets, and sampling

SAMP1 and SAMR1, as controls with a very low incidence of senile amyloidosis, were obtained from our breeding colony under conventional conditions. The colony has been maintained by sister-brother breeding from SAMP1 and SAMR1 generously provided by Dr. Takeda of Kyoto University (1). Six-week-old male mice in each strain were fed diets containing butter, safflower oil, or fish oil. Safflower oil was added to the butter diet and fish oil diet to raise the level of essential fatty acids. Dietary fat in the butter group was a mixture of butter and safflower oil (3:1, by weight). In the safflower oil group, the sole source of dietary fat was safflower oil, and in the fish oil group, dietary fat was provided by a mixture of fish oil and safflower oil (3:1, by weight).

TABLE 1. Fatty acid composition of dietary fats

Fatty Acid	Butter Group	Safflower Oil Group	Fish Oil Group	CD
	g/100 g total fatty acids			
4:0	2.1			
6:0	1.5			
8:0	0.9			
10:0	2.3			
12:0	2.5			
14:0	8.0	0.5	3.7	0.4
14:1	0.7			
15:0	0.9			
16:0	22.7	8.6	7.8	15.4
16:1	1.3	0.1	6.5	1.3
17:0	0.5			
18:0	8.1	2.2	1.7	1.8
18:1(n-9)	20.0	10.4	9.9	22.7
18:2(n-6)	27.8	78.0	29.7	49.4
18:3(n-3)	0.5	0.1	0.3	3.4
20:4(n-6)	0.1	0.2	0.1	0.2
20:5(n-3)			19.0	1.8
22:6(n-3)			7.8	1.7

CD, commercial diet. Butter and safflower oil were stored at 4°C, and fish oil at −35°C. Additional vitamin E (DL-α-tocopherol) was added to the oils as an antioxidant so that the final concentration in the mixed diets was 0.018%.

The major fatty acids in these diets are shown in **Table 1**. Experimental diets contained, by weight: 25% casein, 38% corn starch, 25% sucrose, 2% cellulose powder, 5% mineral mixture, 1% vitamin mixture, and 4% fat (24). Diets were stored at −35°C to prevent fatty acid oxidation. Diets with peroxide values below 30 meq/kg were routinely used. Each group was fed a particular fat diet for 26 weeks. In addition, 8-month-old SAMP1 and SAMR1 male mice fed a commercial diet (CD) (CE-2; NIHON CLEAR, Tokyo, Japan) from 4 weeks of age were used. The CD contained 25.2% crude protein, 50.2% carbohydrate, 4.4% fat, 4.4% fiber, and 7.0% ash. Mice were housed 3–4 per cage, allowed free access to food and tap water, and were maintained in a temperature-controlled room (24 ± 2°C) with a 12 h light-dark cycle. All mice were maintained according to the policies and recommendations of the Koshien University Animal Care and Use Committee.

Mice at 32 weeks old were fasted for about 15 h before collection of blood samples obtained by cardiac puncture following light anesthesia with ether. The serum was then stored at −35°C until analysis.

Histological examination

The abdominal skin, liver, spleen, heart, and stomach of each mouse 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 (25). Green birefringence under a polarizing microscope was considered a positive indication of amyloid presence. The peroxidase-antiperoxidase method (26) using anti-AApoAII (4) was used for identification of different types of amyloid fibril proteins in the immunohistochemical study.

The intensity of the AApoAII amyloid deposition was determined semiquantitatively using the amyloid index (AI) as a parameter (27). The AI is the average of the degree of AApoAII deposition, graded 0 to 4 in the organs examined (liver, spleen, skin, heart, and stomach) in stained sections: 0, no AApoAII found; 1, minute amount of AApoAII deposits; 2, a small amount of AApoAII deposits only in the periportal areas of the liver, in the perifollicular regions of the spleen, in the interstitial tissues

covering less than 10% of the area of both ventricles, the atrioventricular septum, and both atria of the heart, only in the glandular portion and squamous-glandular junction of the stomach, and in less than 30% of the papillary layer of the dermis of the skin; 3, a moderate amount of AApoAII deposits in less than 30% of the area of the liver lobules, and less than 30% of the red pulp of the spleen, in the interstitial tissues covering 10–30% of the heart muscle area, in less than 50% of the lamina propria and submucosa of the squamous epithelia of the stomach, and in 30–80% of the area of the papillary layer of the dermis in the skin; 4, extensive AApoAII deposits in 30–80% of the area of the liver lobules, in 30–80% of the red pulp of the spleen, in the interstitial tissues covering more than 30% of the heart muscle area, in more than 50% of the lamina propria and submucosa of the squamous epithelia of the stomach, and in almost all parts of the papillary layer of the dermis and around the hair follicles and sebaceous glands of the skin.

HDL isolation, lipid extraction, and fatty acid analysis

HDL (1.063 g/ml < d < 1.219 g/ml) was prepared from the serum of SAMP1 mice fed the various dietary oils by preparative ultracentrifugation according to a procedure described previously (28). HDL was dialyzed against 0.15 M NaCl containing 0.05% EDTA adjusted to pH 8.0 at 4°C. Total lipids were extracted from the isolated HDL fraction with chloroform-methanol according to Blight and Dyer's method (29). Fatty acids were converted to methyl esters with 5% HCl-methanol, and quantified by capillary column gas-liquid chromatography (Shimadzu, Kyoto, Japan) using heptadecanoic acid as an internal standard, as described previously (30).

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 (HDL-C) was measured according to a modified heparin-manganese precipitation procedure (HDL-C C test, Wako). TG levels were measured spectrophotometrically using acetylacetone (Triglyceride test, Wako).

The serum levels of apoA-I and apoA-II were determined using an immunoblotting method as described previously (31). Fifty nanoliters of serum was applied to a 15% to 20% gradient SDS-polyacrylamide mini gel, 84 mm wide × 90 mm high, and electrophoresis was carried out at 15 mÅ 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 mÅ 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 PAGE

To ascertain whether dietary oils affected the size distribution of HDL, nondenaturing PAGE was used (32). Gels containing a 2–15% linear polyacrylamide gradient were electrophoresed in 25 mmol/l Tris and 192 mmol/l glutamic acid. Prior to electrophoresis, serum samples (3 μl) were stained for lipids by incubation at 4°C overnight with 2.5 μl of freshly prepared Sudan Black B dye solution (five parts 10 g/l Sudan Black B in ethylene glycol and three parts 400 g/l sucrose). Electrophoresis was carried out at 25 mÅ for 2 h. The HDL species were quantitated by comparing the intensity of bands with that of the HDL_3 bands in each dietary fat group using a Densitron.

Statistical analysis

ANOVA was used to compare results of the three diets. The AI of the various organs in the three groups were compared by the Mann-Whitney U test. Significance was established when $P < 0.05$.

RESULTS

Diet and weight gain

There were no significant differences in food intake among the various dietary groups in each strain. In SAMP1 mice, weight gains in the butter group were significantly greater than those in the safflower oil and fish oil groups ($P < 0.05$). Expressed as the mean percentage of initial weight ± SD at 20 weeks of age, the butter-fed mice gained 42.2 ± 14.9%, the safflower oil-fed mice gained 31.2 ± 9.8%, and the fish oil-fed mice gained 26.1 ± 4.6%. There was no significant difference in weight gain among dietary groups in SAMR1 mice.

Amyloid deposition

The severity of amyloid deposition (AI) in organs from SAMP1 mice at 32 weeks of age is shown in **Table 2**. In the fish oil group, most spleen, heart, skin, liver, and stomach specimens demonstrated amyloid deposits, and the AI in the fish oil group showed the highest levels among the three groups ($P < 0.01$). On the other hand, the grading score of amyloid deposition in the heart, liver, and stomach of the safflower oil group was significantly lower than in the butter and fish oil group ($P < 0.01$), and the AI of this group was the lowest level among the three groups. Furthermore, the butter group demonstrated more severe amyloid deposits than the safflower oil group ($P < 0.05$).

Lipid composition of the HDL

The fatty acid composition of the HDL extracted from the serum of mice fed either a butter, safflower oil, or fish oil diet is shown in **Table 3**. The differences in the ratios of oleate (18:1) and linoleate (18:2 n-6) among the diets are reflected in the differences in the fatty acid composition of the HDL lipids. However, in the fish oil group, there was a significant decrease in 20:4(n-6) and 22:4(n-6) (the fatty acids that can be produced from linoleate), despite the similar fatty acid composition of the butter and fish oil diets. The relative increase of eicosapentaenoate (EPA, 20:5n-3) and docosahexaenoate (DHA, 22:6n-3) in the fish oil diet led to a major increase of EPA and DHA in HDL lipids. However, the HDL lipids of mice fed the fish oil diet did not reflect the EPA-DHA ratio of the diet, and the HDL lipids were enriched in DHA. EPA and DHA were present in small amounts in the HDL of butter and safflower oil-fed animals even though these fatty acids were not in these synthetic diets. However, the CD fed prior to the start of the experiment did contain EPA and DHA.

TABLE 2. Influence of dietary fats on senile amyloid deposition in organs of SAMP1 mice

Diets	n	Grading Score of Amyloid Deposition in Organs					
		Spleen	Heart	Skin	Liver	Stomach	AI
Butter	8	0	1.20 ± 0.84	0.70 ± 0.97	0.60 ± 0.89	1.20 ± 1.09	0.74 ± 0.61
Safflower oil	12	0.08 ± 0.29[a]	0.46 ± 0.72[a]	0.91 ± 0.94	0.21 ± 0.39[a]	0.71 ± 0.75[a]	0.47 ± 0.43[a]
Fish oil	9	2.00 ± 1.12[a,b]	1.44 ± 0.73[a,b]	2.11 ± 0.33[a,b]	0.67 ± 1.32[b]	2.11 ± 1.05[a,b]	1.67 ± 0.63[a,b]
CD (29–36 weeks old)	11	0.72 ± 1.10	0.72 ± 0.78	0.45 ± 0.52	1.00 ± 0.89	1.54 ± 1.04	0.89 ± 0.61

AI, amyloid index; SAMP1, senescence-accelerated mouse-prone, SAMP1@Umz. Data are means ± SD. The AI is the average of the degree of deposition, graded 0 to 4 in the organs examined in stained sections. See text for a detailed account of AI. The AI of different groups of mice was compared using the nonparametric Mann-Whitney U test. CD (CE-2, NIHON CLEAR; Tokyo, Japan) was fed to SAMP1 mice throughout their life.

[a] $P < 0.05$ versus butter.

[b] $P < 0.05$ versus safflower oil.

Serum cholesterol and TG levels

Strain differences were evident between SAMR1 and SAMP1 mice in serum cholesterol and TG levels, which were both significantly higher in SAMR1 mice than in SAMP1 mice (**Table 4**). The concentrations of total and HDL-C in SAMR1 and those of total cholesterol in SAMP1 did not significantly differ between the butter and safflower oil groups. Administration of the fish oil diet resulted in significant decreases in total and HDL-C levels in SAMR1 strains ($P < 0.01$). In contrast to total cholesterol levels, which were higher in the butter group of SAMP1 mice than in the safflower oil group, the HDL-C levels were significantly lower in the butter group than in the safflower oil group ($P < 0.01$). Furthermore, the total and HDL-C levels in the fish oil group, especially the latter, showed marked decreases compared with those in the three groups ($P < 0.01$). The ratio of HDL-C to total cholesterol in the butter, safflower oil, and fish oil groups was 35%, 62%, and 17%, respectively. In SAMP1 mice, the TG levels of the fish oil group showed a significant decrease compared with the butter and safflower oil groups ($P < 0.01$). However, in SAMR1 mice, TG levels did not differ among the three groups.

TABLE 3. Fatty acid composition of HDL from the serum of SAMP1 mice fed butter, safflower oil, or fish oil

Fatty Acid	Butter Diet	Safflower Oil Diet	Fish Oil Diet
		% of total fatty acid	
16:0	14.9 ± 1.07	15.2 ± 0.60	14.5 ± 2.85
16:1	1.9 ± 0.20	1.3 ± 0.92	0.9 ± 0.40[a]
18:0	12.1 ± 1.41	11.1 ± 1.19	12.6 ± 1.77
18:1	13.6 ± 1.31	6.8 ± 0.79[a]	8.7 ± 1.03[a,b]
18:1n-7	2.5 ± 0.62	1.9 ± 0.33	0.7 ± 0.57[a,b]
18:2	15.3 ± 0.64	22.6 ± 1.13[a]	16.5 ± 3.12[b]
18:3n-6	0.9 ± 0.39	0.7 ± 0.25	0.9 ± 1.10
18:3n-3	0.5 ± 0.28	0.8 ± 0.32	1.7 ± 0.41[a,b]
20:0	0.3 ± 0.19	0.2 ± 0.09	ND[a,b]
20:1	0.5 ± 0.24	0.5 ± 0.29	ND[a,b]
20:4n-6	30.8 ± 2.10	33.1 ± 2.69	9.8 ± 2.08[a,b]
20:5n-3	0.7 ± 0.38	0.2 ± 0.21[a]	14.4 ± 4.35[a,b]
22:4n-6	2.5 ± 0.51	4.0 ± 0.71[a]	0.3 ± 0.50[a,b]
22:6n-3	3.6 ± 0.51	1.9 ± 0.22[a]	18.9 ± 4.10[a,b]

ND, not determined. Average ± SD for four mice in each dietary oil group is presented. Statistical significance of differences between the butter, safflower oil, and fish oil groups is shown.

[a] $P < 0.05$ versus butter.

[b] $P < 0.05$ versus safflower oil.

Serum levels of apolipoproteins

The fish oil group in SAMP1 mice showed a significant decrease in both serum apoA-I and apoA-II levels when compared with the butter and safflower oil groups ($P < 0.01$) (**Table 5**), especially apoA-II levels, which markedly decreased to one third of the levels in the other two groups. There was no dietary effect on apoA-I in SAMR1 mice, but in the butter group, apoA-II levels were significantly lower than those in the safflower and fish oil groups ($P < 0.05$). The ratio of apoA-I to apoA-II in SAMP1 mice tended to be higher than that in SAMR1 mice, and was markedly increased in the fish oil group compared with the butter and safflower oil groups ($P < 0.01$).

HDL size distribution

Three distinct HDL size classes, referred to from the largest to the smallest as HDL_1, HDL_2, and HDL_3, were seen on gradient gels. Typical patterns at 32 weeks old are shown in **Fig. 1A**. The predominant form observed in both mouse strains was HDL_3, whereas HDL_1 was present at very low levels. The migration of the HDL band was delayed in SAMR1 mice compared with that in SAMP1, and there was a tendency for the HDL band of mice fed the fish oil diet to show more rapid migration relative to that of mice fed the butter or safflower oil diet. Densitometric gel scanning of the peaks representing the three major species of HDL yielded the findings shown in Fig. 1B. There were clear differences in different HDL species among the dietary groups. The fish oil group exhibited decreases in the rates of HDL_2 and HDL_1 compared with the levels observed for the butter and safflower oil groups.

DISCUSSION

Our results revealed that dietary fats modulate amyloidogenesis in SAMP1 mice. A safflower oil diet ameliorated the severity of senile amyloidosis in SAMP1 mice, while the fish oil diet accelerated amyloidosis. Moreover, dietary fats significantly changed the serum lipid and lipoprotein levels. Therefore, clarifying serum lipid and lipoprotein metabolism in mice fed various dietary fats would be biomedically and therapeutically relevant in the study of amyloidosis.

TABLE 4. Serum lipid concentrations in SAMP1 and SAMR1 mice fed diets containing butter, safflower oil, or fish oil

Diets	n	SAMP1			SAMR1		
		Total Cholesterol	HDL Cholesterol	Triglycerides	Total Cholesterol	HDL Cholesterol	Triglycerides
		mg/dl					
Butter	8	109.1 ± 27.3	38.7 ± 12.5	147.0 ± 35.2	187.7 ± 53.8	87.1 ± 28.9	179.7 ± 43.8
Safflower oil	10	82.7 ± 23.2	51.9 ± 5.6[a]	116.7 ± 27.9	164.5 ± 22.3	73.7 ± 28.9	200.2 ± 55.1
Fish oil	8	39.2 ± 14.9[a,b]	7.4 ± 3.0[a,b]	90.0 ± 32.1[a,b]	105.1 ± 19.9[a,b]	43.8 ± 12.0[a,b]	207.1 ± 58.3
CD (28–32 weeks old)		93.0 ± 17.1	28.2 ± 3.5	252.1 ± 44.1	138.5 ± 22.9	82.5 ± 11.2	142.2 ± 47.8

SAMR1, senescence-accelerated mouse-resistant, SAMR1@Umz. Values are means ± SD. Five SAMR1 mice were used in each dietary group. CD (CE-2, NIHON CLEAR; Tokyo, Japan) was fed to SAMP1 and SAMR1 mice throughout their lives.

[a] $P < 0.05$ versus butter.

[b] $P < 0.05$ versus safflower oil.

The serum total cholesterol and TG levels in aged SAMP1 mice fed the fish oil diet were about half those observed in mice fed the safflower oil diet, the butter diet, and the chow diet. In particular, their serum HDL-C levels were 26% of those observed on chow diets. In mice fed the safflower oil and butter diets, HDL-C rose to about 1.8 and 1.3 times that the level of that in chow diet mice, respectively. In this study, fish oil decreased the concentrations of serum total and HDL-C both in the SAMP1 and SAMR1 strains; thus, functional differences in lipid metabolism between the safflower oil and fish oil diet were defined. Berr et al. reported that hepatic excretion of cholesterol is increased with safflower oil consumption (n-6 PUFA-rich), reduced with fish oil consumption (n-3 PUFA-rich), and intermediate with coconut oil consumption (SFA-rich) in rats (33). The hypocholesterolemic effect of dietary fish oil might be caused by docosahexaenoic acid on plasma cholesterol (34). The effects of fish oil are attributed to suppression of the rate-limiting enzyme of cholesterol synthesis and overall cholesterol synthesis in rats (14, 15, 35).

ApoA-II influences both the structural and functional properties of HDL. Purcell-Huynh et al. revealed a high correlation of the apoA-II gene with plasma concentrations of HDL-C (36). Overexpression of the apoA-II gene resulted in an elevated HDL-C concentration (37). Thus, the marked decrease in serum HDL-C and apoA-II in fish oil-fed mice may be related to the lower expression of apoA-II in the liver. Decreases in HDL-C concentrations and levels of hepatic mRNA for apoA-II in hamsters fed soybean oil in comparison with coconut oil support this hypothesis (38).

The mechanism by which apoA-II affects the HDL-C levels could involve, in part, its effect on HDL size. The SAMP1 mice fed the fish oil diet had higher levels of smaller sized HDL particles than those fed the safflower oil or butter diets. An important factor influencing HDL size is the ratio of apoA-I-apoA-II in plasma (39). Generally, HDLs rich in apoA-II function less efficiently in reverse cholesterol transport than do HDLs that are poor in apoA-II (40). The increased ratio of apoA-I-apoA-II in SAMP1 mice fed fish oil would facilitate more efficient transport of cholesterol than that in mice fed safflower oil or butter. Dietary polyunsaturated fats resulted in a decrease in large HDL particles and an increase in small HDL particles in monkeys (41), and a soybean oil diet induced a larger proportion of small HDL particles in hamsters (38). We found dramatic changes in the fatty acid composition in HDL particles in mice fed the fish oil diet compared with HDL in mice fed the safflower oil diet. ApoA-II protein may be less stable in smaller and n-3 PUFA-rich HDL particles, and more susceptible to fibril formation in SAMP1 mice fed the fish oil diet.

One possible explanation for the decreased serum apoA-II concentration with increasing age in SAMP1, as suggested by Naiki et al., is that serum ApoA-II clearance is accelerated with increasing age, and several organs trap more apoA-II in old SAMP1 mice than in young mice (42). Accordingly, changes in the metabolic environment, in addition to mutations in apoA-II, might affect senile amyloidogenesis. Acceleration of apoA-II metabolism may lead to more severe amyloid deposition in mice fed a diet enriched in fish oil.

Experimental and etiological studies indicate that di-

TABLE 5. Serum levels of apoA-I and apoA-II in SAMP1 and SAMR1 mice fed diets containing butter, safflower oil, or fish oil

Diets	n	SAMP1			SAMR1		
		ApoA-I	ApoA-II	A-I/A-II	ApoA-I	ApoA-II	A-I/A-II
		mg/dl					
Butter	8	69.4 ± 13.9	26.9 ± 7.3	2.84 ± 0.73	87.8 ± 7.5	47.2 ± 2.7	1.85 ± 0.27
Safflower oil	10	58.6 ± 11.3	21.6 ± 3.7	2.79 ± 0.42	95.0 ± 22.4	65.4 ± 11.6[a]	1.45 ± 0.19
Fish oil	8	30.7 ± 5.3[a,b]	7.6 ± 2.7[a,b]	4.64 ± 2.35[a,b]	90.2 ± 15.0	57.9 ± 7.5[a]	1.55 ± 0.19
CD (28–32 weeks old)		79.8 ± 9.7	27.8 ± 11.3	2.78 ± 1.3	93.8 ± 18.3	63.2 ± 8.3	1.49 ± 0.31

Values are means ± SD. Five SAMR1 mice were used in each group. CD (CE-2, NIHON CLEAR; Tokyo, Japan) was fed to SAMP1 and SAMR1 mice throughout their lives.

[a] $P < 0.05$ versus butter.

[b] $P < 0.05$ versus safflower oil.

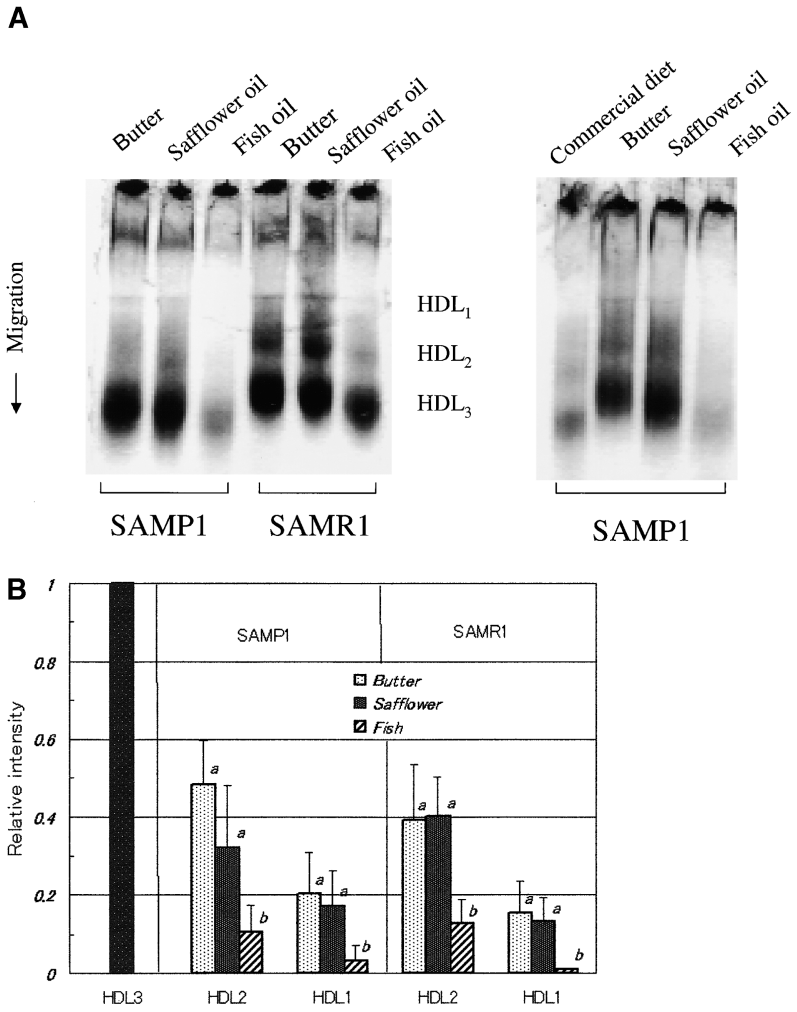

Fig. 1. The size distributions of serum HDL particles in senescence-accelerated mouse-prone (SAMP1; SAMP1@Umz) and senescence-accelerated mouse-resistant (SAMR1; SAMR1@Umz) mice fed butter, safflower oil, and fish oil diets. Three microliters of serum was prestained for neutral lipids using Sudan Black and electrophoresed to equilibrium in a 2–15% nondenaturing polyacrylamide gel. A: Representative migration patterns of HDL observed for SAMP1 and SAMR1 mice fed butter, safflower oil, and fish oil diets. The pattern for the chow diet is shown for SAMP1 mice only. B: Integration of the HDL_1, HDL_2, and HDL_3 peaks on densitometric scans of gradient gels provides an estimate of the neutral lipids comprising each HDL size class. These studies were performed in duplicate.

etary modification of lipid metabolism could change the susceptibility to amyloidosis. A fish oil diet may modify the prostaglandin profile of macrophages and may change cellular immune function and/or enhance the processing of serum amyloid A (18). A high-fat diet has been associated with the development of severe islet amyloidosis in mice (43). We evaluated whether AA amyloid protein could serve as a nidus for the deposition of AApoAII fibrils; however, we did not identify serum amyloid A protein in SAMP1 mice fed the three dietary fats in this study or detect AA amyloid deposition associated with AApoAII deposition in their organs.

Recent studies indicate that the prevalence of AD is reduced among people taking cholesterol-lowering medicines (20, 44). The higher serum cholesterol levels and the severity of amyloid deposition in aged butter-fed SAMP1 mice suggest a similar mechanism at work. Our finding that the lowering of serum cholesterol in mice fed the fish oil diet accelerated senile amyloid deposition was contrary to our expectations. Cholesterol depletion may increase membrane fluidity, impairing the internalization of amyloid precursor protein (APP) and increasing trafficking of APP through the nonamyloidogenic α-secretase pathway (45, 46). Although we do not have the evidence yet that apoA-II aggregates to form amyloid fibrils in the membrane, increasing amounts of highly unsaturated fatty acids derived from fish oil may change physical characteristics of the membranes, such as membrane fluidity and the distribution of lipid rafts, and may activate specific pathways involved in apoA-II fibrilogenesis (47, 48).

Our findings emphasize the importance of genetic-dietary interactions in the control of lipoprotein metabolism. It remains to be shown whether serum HDL or apoA-II clearance is accelerated by the fish oil diet or reduced HDL synthesis, and whether senile amyloid deposition in SAMP1 is due to characteristics of the fish oil diet generally, or more specifically involves n-3 polyunsaturated fatty acids. This information would be useful in determining the cause of amyloidogenesis.

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