Table 5
Hepatic and serum sterol contents of SHRSP rats fed the test diets

	Can	Can/Alk	Soy	Soy/PS	Hyd.Soy	Olv	Epo
Serum (mg/ml serum)							
Cholesterol	0.59 ± 0.03^{a}	0.53 ± 0.02^{ab}	0.48 ± 0.02^{ab}	0.37 ± 0.04^{bc}	0.48 ± 0.02^{ab}	0.30 ± 0.06^{c}	0.29 ± 0.09^{c}
Campesterol	0.08 ± 0.00^{ab}	0.05 ± 0.00^{cd}	0.03 ± 0.00^{de}	0.09 ± 0.01^{a}	0.03 ± 0.01^{e}	0.01 ± 0.00^{e}	0.02 ± 0.01^{e}
β-Sitosterol	0.06 ± 0.00^{ab}	0.06 ± 0.01^{ab}	0.04 ± 0.00^{bc}	0.07 ± 0.01^{a}	0.04 ± 0.01^{bc}	0.02 ± 0.01^{c}	0.03 ± 0.01^{bc}
Total PS	0.14 ± 0.00^{a}	0.11 ± 0.01^{ab}	0.08 ± 0.00^{bc}	0.16 ± 0.02^{a}	0.07 ± 0.01^{c}	0.04 ± 0.01^{c}	0.05 ± 0.02^{c}
Total sterol	0.73 ± 0.03^{a}	0.64 ± 0.03^{a}	0.56 ± 0.02^{a}	0.53 ± 0.06^{abc}	0.56 ± 0.03^{b}	0.33 ± 0.06^{cd}	0.34 ± 0.11^{bcd}
PS/cholesterol	1.24 ± 0.01^{b}	0.21 ± 0.02^{bc}	0.16 ± 0.01^{cd}	0.44 ± 0.01^{a}	0.14 ± 0.02^{cd}	0.12 ± 0.01^{d}	0.16 ± 0.01^{cd}
Liver (mg/g liver)							
Cholesterol	1.44 ± 0.05^{ab}	1.69 ± 0.09^{a}	1.78 ± 0.01^{a}	1.22 ± 0.06^{b}	1.66 ± 0.09^{a}	1.64 ± 0.06^{a}	1.51 ± 0.09^{ab}
Campesterol	0.20 ± 0.01^{b}	0.17 ± 0.01^{bc}	0.14 ± 0.01^{cd}	0.30 ± 0.01^{a}	0.11 ± 0.01^{d}	0.09 ± 0.01^{d}	0.10 ± 0.01^{d}
β-Sitosterol	0.13 ± 0.01^{b}	0.11 ± 0.01^{b}	0.11 ± 0.01^{b}	0.20 ± 0.01^{a}	0.10 ± 0.01^{b}	0.10 ± 0.01^{b}	0.12 ± 0.01^{b}
Total PS	0.33 ± 0.02^{b}	0.28 ± 0.02^{bc}	0.25 ± 0.02^{bc}	0.50 ± 0.02^{a}	0.20 ± 0.03^{c}	0.20 ± 0.01^{c}	0.22 ± 0.02^{bc}
Total sterol	1.76 ± 0.07	1.97 ± 0.11	2.02 ± 0.11	1.72 ± 0.09	1.87 ± 0.07	1.83 ± 0.07	1.73 ± 0.12
PS/cholesterol	0.19 ± 0.00^{b}	0.14 ± 0.00^{cd}	0.12 ± 0.01^{cd}	0.29 ± 0.00^{a}	0.11 ± 0.01^{c}	0.11 ± 0.00^{d}	0.13 ± 0.00^{cd}

Values are means ± SEM ($n = 4$). Values in the same row not sharing a common superscript are significantly different ($p < 0.05$).

Table 6
Correlation coefficients (r) between the mean survival time and dietary or tissue sterol content in rats fed the test diets for 8 weeks

	Diet		Serum		Liver	
	r	p	r	p	r	p
Cholesterol	0.482	0.096	0.214	0.645	0.216	0.642
Brassicasterol	0.311	0.301	ND	ND	ND	ND
Campesterol	0.180	0.566	0.268	0.561	0.364	0.422
Stigmasterol	−0.183	0.550	ND	ND	ND	ND
β-Sitosterol	0.024	0.937	0.353	0.438	0.226	0.626
Total PS	0.054	0.861	0.302	0.511	0.325	0.478
Total sterol	0.081	0.792	0.267	0.563	0.643	0.119
PS/cholesterol	−0.016	0.960	0.281	0.541	0.220	0.635

Correlation coefficient was determined by linear regression analysis. ND, not detectable.

diet containing practically no significant amounts of endogenous cholesterol and phytosterols was used in other studies (Ratnayake et al., 2000a,b; Ogawa et al., 2003), while a conventional diet containing endogenous cholesterol and PS was used as a basal diet in our study. In our study, the effect of PS is likely to be masked to some extent because dietary cholesterol would competitively inhibit the absorption of PS in the intestine.

Among the vegetable oils with the survival-shortening activity in SHRSP rats (e.g. olive oil, corn oil, evening primrose oil, hydrogenated soybean oil, high-oleic safflower oil, high-oleic sunflower oil, and Can), Can has been studied most extensively. The unfavorable effects of Can, however, are not confined to SHRSP rats (Naito et al., 2000a,b,c,d, 2003; Du et al., 2001; Kameyama et al., 1996; Sauer et al., 1997; Innis and Dyer, 1999). In piglets fed a milk replacer containing Can, increased requirements for vitamin E were found after iron injection (Sauer et al., 1997). However, the hepatic vitamin E content was greater in the Can group than in the Soy group, indicating that tissue vitamin E level is not a critical factor for the survival-shortening activity in SHRSP rats. Although Can is a double-low type oil, it still contains reduced amounts of glucosinolates and their hydrolysis products such as isothiocyanates, oxazolidinethione, indole derivatives and other minor components (Bjeldanes et al., 1991). Therefore, effort to identify the presumed factors is warranted, even though the impact of the above observations on human nutrition is entirely unknown.

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Cholesterol Synthesis in Mice Is Suppressed but Lipofuscin Formation Is Not Affected by Long-Term Feeding of n-3 Fatty Acid-Enriched Oils Compared with Lard and n-6 Fatty Acid-Enriched Oils

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Hypocholesterolemic activity of dietary polyunsaturated fatty acids is observed after relatively short-term but not long-term feedings, and their long-term feedings are suspected to accelerate aging through tissue accumulation of lipid peroxides and age pigments (lipofuscin). To define the long-term effects of fats and oils in more detail, female mice were fed a conventional basal diet supplemented with lard (Lar), high-linoleic (n-6) safflower oil (Saf), rapeseed oil (Rap), high-α-linolenic (n-3) perilla oil (Per), or a mixture of ethyl docosahexaenoate and soybean oil (DHA/Soy) from 17 weeks to 71 weeks of age. The DHA/Soy and Per groups had decreased serum cholesterol levels compared with the Lar and Saf groups, but the difference between the Lar and Saf groups was not significant. The 3-hydroxy-3-methyglutary-CoA (HMG-CoA) reductase activity in the liver was also significantly lower in the Per and DHA/Soy groups. However, no significant difference in lipofuscin contents in the brain and liver was observed among the 5 dietary groups, despite significant differences in peroxidizability indices of the dietary and/or tissue lipids. These results indicate that n-3 fatty acid-rich oils are hypocholesterolemic by suppressing hepatic HMG-CoA reductase activity compared with animal fats and high-linoleic (n-6) oil, but tissue lipofuscin contents are not affected by a long-term feeding of fats and oils with different degree of unsaturation in mice.

Key words docosahexaenoic acid (DHA); perilla oil; lard; cholesterol; 3-hydroxy-3-methyglutary-CoA (HMG-CoA); age pigment

Hypercholesterolemia was assumed to be a major risk factor for atherosclerosis and related diseases, and raising the polyunsaturated to saturated (P/S) ratio of dietary fatty acids as well as reducing the intake of cholesterol had long been recommended for the prevention of atherosclerosis. However, we observed no significant decrease in serum cholesterol levels in mice after 17 weeks feeding of safflower oil as compared with lard.[1] Moreover, raising the P/S ratio of dietary fatty acids has been proven in long-term clinical studies (7—10 years) to be ineffective in lowering serum cholesterol and even to be risky for the prevention of coronary heart disease.[2—4] Instead, a nutritional intervention to decrease the intake of linoleic acid (n-6) and increase that of α-linolenic acid (n-3) and oleic acid was found to be highly effective for the secondary prevention of coronary heart disease[3] as reviewed elsewhere.[5,6] Many lines of evidence also support the proposal that n-3 fatty acids in fish oil, EPA and docosahexaenoic acid (DHA), are effective for the prevention of coronary heart disease as summarized by Lands.[7]

On the other hand, the uptake of oxidized low density lipoprotein (LDL) by macrophages is considered an early event in the progress of atherosclerosis,[8] and long-term feedings of n-3 fatty acids, particularly DHA with six double bonds, are suspected to enhance lipid peroxide accumulation in tissues and oxidized-LDL formation. This latter interpretation is apparently inconsistent with the observed beneficial effects of fish oils for the prevention of coronary heart disease. Although the regulatory mechanism of cholesterol synthesis has been revealed at the levels of gene expression, especially in the vitro system, the available data are not enough to predict tissue cholesterol levels under different dietary conditions. One purpose of the present experiments was to define the long-term feeding effects of different types of fats and oils on serum and hepatic cholesterol levels under the same dietary conditions.

Lipofuscin is an auto-fluorescent yellow pigment that accumulates within cytoplasmic granules of post-mitotic tissues during aging. The lipofuscin is, therefore, generally called an "age pigment".[9—11] It also accumulates in the central nervous system with pathological processes such as Alzheimer's disease.[12] The age pigment has been supposed to be cellular debris derived from lipid peroxides by free radical-induced oxidative stress, and thus regarded as one of the indices of lipid peroxidation in tissues.[13] However, the accumulation of age pigment in tissues of animals under different dietary fatty acids has rarely been determined. The second purpose of this study was to define the effect of long-term feeding of fats and oils on age pigment contents in mice.

MATERIALS AND METHODS

Animals and Diets Specific pathogen-free, female C57BL/6 mice at 5 weeks of age were purchased from SLC Japan, Inc., Tokyo. The mice were initially fed for up to 17 weeks of age with a conventional diet (CE2; Central Laboratory for Experimental Animals (Clea) Japan, Inc., Tokyo) containing 4.4% (w/w) lipids (lipids contained in the materials and supplemented soybean oil) and defined amounts of nutrients. Then, mice were divided randomly into five groups of 12 animals each. Average body weight of the 5 dietary groups was 24.6±0.2 g and the maximum deviation from the mean was 1.3%. They were housed in a room specified for

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special pathogen-free animals with a fixed room temperature (24±2 °C), humidity (55±5%) and lighting (from 06:00 to 18:00), and given free access to filtered water and an experimental diet. Lard (Lar), safflower oil (high-linoleic acid type, Saf), rapeseed oil (low-erucic Canola type, Rap), perilla oil (from seeds of beefsteak plant, Per) and a 1:9 mixture of DHA ethylester (kindly supplied by Harima Chemicals Inc., Tokyo and Shiseido Co., Tokyo) and soybean oil (DHA/Soy) were used. The amount of DHA in the DHA/Soy diet (2% energy) was set between the intakes of DHA and EPA by average Japanese (0.7% energy) and Greenland natives (5.1% energy).[14]

The basal diet (CE2) and experimental fat or oil was mixed at a weight ratio of 9 to 1. The final lipid content was calculated to be 14.0 wt% (31.4% energy), a level higher than that of the average Japanese (26% energy) but lower than the average American (*ca.* 37% energy). The fatty acid composition of the experimental diet is shown in Table 1. Peroxidizability index (PI)[15] was determined as follows; PI=(% monoenoate×0.025)+(% dienoate×1)+(trienoate×2)+(% tetraenoate×4)+(% pentaenoate×6)+(% hexaenoate×8). The experimental diets were purchased as pellets from Clea Japan Co., Ltd., and kept at 4 °C for less than 1 month, except for the DHA/Soy diet which was prepared in our laboratory and kept frozen at −20 °C for less than 1.5 weeks before serving. The diets were replaced every day in the case of the DHA/Soy diet, and every two days in other diets, conditions that were determined by preliminary experiments to keep the peroxide values of the served diets below 10 meq/kg.

Determination of Cholesterol Mice were sacrificed at 71 weeks of age after feeding of the test diets for 54 weeks, and tissue samples were stored at −80 °C until analysis. Total cholesterol was separated by silica gel thin-layer chromatography, the ester form was hydrolyzed with sodium methoxide, and free cholesterol plus an internal standard, β-sitosterol, were analyzed as dinitrobenzoyl derivatives by reversed-phase HPLC on a Wakosil ODS column as described by Kasama *et al.*[16] and Newkirk and Sheppard.[17]

Determination of Fatty Acid Composition in Dietary and Tissue Lipids Lipids were extracted from diets and tissue samples with chloroform/methanol according to Bligh and Dyer's method,[18] fatty acids were converted to methylesters, and analyzed by gas-liquid chromatography as described previously.[19]

Table 1. Fatty Acid Composition of Experimental Diet[a]

Fatty acid	Lar	Saf	Rap	Per	DHA/Soy
14:0	1.2	0.3	0.2	0.2	0.2
16:0	23.2	9.4	7.4	8.4	10.9
16:1	1.9	0.4	0.6	0.4	0.3
18:0	12.1	2.6	2.3	1.8	3.4
18:1n-9[b]	37.1	17.7	51.6	17.9	20.7
18:2n-6[b]	19.4	64.5	26.7	23.5	48.6
18:3n-3[b]	1.5	1.4	7.2	44.5	6.4
20:0	0.2	0.3	0.6	0.2	0.3
20:1	1.2	0.7	1.5	0.8	0.6
20:4n-6[b]	0.2	0.1	0.1	0.1	0.1
20:5n-3[b]	0.8	0.9	0.7	0.8	1.1
22:0	0.1	0.4	0.3	0.1	0.3
22:1	0.4	0.4	0.4	0.3	0.3
22:6n-3[b]	0.8	0.7	0.6	0.8	6.7
24:0	0.1	0.1	0.2	0.1	0.1
n-6/n-3 ratio	6.5	21.6	3.1	0.5	3.4
PI[c]	35	79	52	125	123

a) The fatty acid composition of the diet (% of total fatty acids) was analyzed by gas-liquid chromatography. b) The position of the double bond number numbered from the methyl terminus is designated as n-9, n-6 or n-3. c) Peroxidizability Index.

Determination of Hepatic HMG-CoA Reductase Activity A 10% (w/v) homogenate of mouse liver was prepared in 0.9% NaCl. The homogenates were centrifuged at 700×*g* for 5 min and then the supernatants were centrifuged at 12000×*g* for 30 min. Microsomal fractions were obtained by centrifugation of the supernatants at 105000×*g* for 60 min at 4 °C. These fractions were resuspended in 0.1 M sucrose/50 mM KCl/40 mM potassium-phosphate buffer (pH 7.4) containing 10 mM DTT, and the suspensions were used for assay of 3-hydroxy-3-methylglutary-CoA (HMG-CoA) reductase activity. HMG-CoA reductase activity was measured essentially as described by Kuroda and Endo.[20] Briefly, 20—100 μg of the microsomal protein was incubated with 2.55 mM DL-[3-^{14}C] HMG-CoA 144 MBq/mmol (Dupont, NEN) in 0.5 M potassium-phosphate buffer containing 10 mM NADPH, 100 mM DTT and 100 mM EDTA for 15 min at 37 °C. Hydrochloric acid (2 N) was added to stop the reaction, and samples were further incubated for 15 min at 37 °C. The incubated mixture was applied to silica gel 6G TLC plates (Merck). Plates were developed in acetone–benzene (1:1), and the area corresponding to mevalonate (*Rf* 0.2—0.6) was scraped off and mixed with 20 ml of Clearsol *II* (Nacalai Tesque). Radioactivity was measured using a scintillation counter LSC-5100 (Aloka, Tokyo). HMG-CoA reductase activity was expressed as nanomol of [^{14}C]-mevalonate produced per min per mg of microsomal protein.

Determination of Yellow Fluorescent Lipofuscin in Tissues Yellow fluorescent lipofuscin in tissues was determined as described elsewhere.[21,22] Briefly, 50 mg of the lyophilized tissues was homogenized in 9.0 ml of PBS containing 0.1% SDS. The homogenate was centrifuged at 105000×*g* for 60 min at 25 °C. The supernatant was condensed to 1/10 volume by ultrafiltration through a Diaflo R ultrafiltration membrane (PM-10 Amicon Corporation, Ireland) in order to remove low molecular weight materials (below 10 kDa). The condensed solution was made up to 9.0 ml with the same bufferized solution. Fluorescence spectra and intensities of the solution were recorded with a Hitachi 650-60 fluorescence spectrophotometer (Hitachi Co., Ltd. Tokyo) equipped with a xenon-lamp. The instrument was standardized with a solution of 0.1 μM quinine sulfate in 0.1 N sulfuric acid to give a fluorescence intensity of 1.00 at 450 nm when excited at 350 nm. The relative fluorescence intensity (Rfi) of the extract against that of quinine sulfate was obtained, and an Rfi unit was calculated by multiplying Rfi by volume (ml) of the solution.

Statistical Analysis Data were represented as means± S.D. Statistical analysis of data was performed using Bonferroni's multiple comparison (Stat View J-4.11; Abacus Concepts, Inc., Berkeley, CA, U.S.A.).

RESULTS

Serum and Hepatic Cholesterol Total serum cholesterol in the DHA/Soy, Per, Rap, Saf and Lar groups were 84.6, 85.3, 97.2, 110.8 and 114.7 mg/dl, respectively (Fig. 1). The levels of DHA/Soy and Per groups were significantly

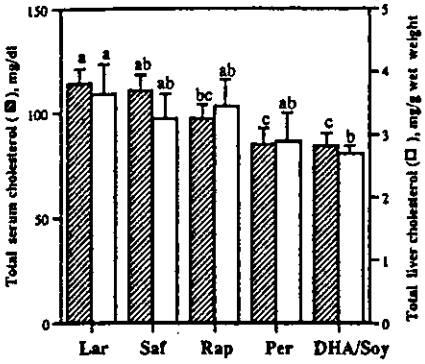

Fig. 1. Effect of Dietary Fat and Oil on the Total Serum and Liver Cholesterol

Mice were sacrificed at 71 weeks of age. A conventional diet was fed for 17 weeks prior to experimental diets. Values are means±S.D. of 6 mice. The bars with different superscripts are significantly different from each other by ANOVA at $p<0.05$. Oblique and open columns represent the total serum cholesterol and total liver cholesterol, respectively.

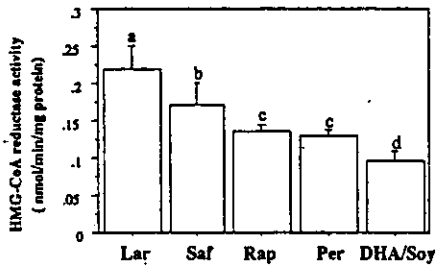

Fig. 2. Effect of Dietary Fat and Oil on HMG-CoA Reductase Activity

Mice were sacrificed at 71 weeks of age after feeding test diets for 51 weeks. Values are means±S.D. of 6 mice. The columns with different superscripts are significantly different from each other by ANOVA at $p<0.05$.

lower than those of the Saf and Lar groups, but there were no significant differences among the Per, DHA/Soy and Rap groups. It is emphasized that a long-term feeding of animal fat (Lar) and high-linoleic acid vegetable oil (Saf) brings about no significant difference in serum cholesterol levels in mice or in rats. Hepatic cholesterol levels roughly paralleled the serum cholesterol levels.

Hepatic HMG-CoA Reductase Activity The hepatic HMG-CoA reductase activities in the Lar, Saf, Rap and Per groups were higher by 144.0%, 87.9%, 47.3% and 41.8%, respectively, compared with that of the DHA/Soy group (Fig. 2). Although the serum and hepatic cholesterol levels were not significantly different between the Lar and Saf groups, the difference in the HMG-CoA reductase activities of the two groups was significant. The hepatic HMG-CoA reductase activities, however, roughly paralleled the serum and hepatic cholesterol levels (in the serum, $r=0.77$, $p<0.0001$; in the liver, $r=0.56$, $p=0.0014$).

Hepatic and Brain Lipofuscin Contents The lipofuscin contents in the brain were not affected by the diets (Fig. 3). In the liver, lipofuscin was not detectable in any of the dietary groups. In the brain, the lipofuscin contents were not correlated with PI values of the dietary lipids ($r=0.49$, $p=0.18$) nor with those of tissue lipids ($r=0.69$, $p=0.20$).

Fatty Acid Composition of Brain and Liver Fatty acid composition of brain phospholipid is kept relatively constant under the dietary conditions. The arachidonate (20:4n-6) levels in the Per and DHA/Soy group were slightly but statistically significantly lower than in the other dietary groups. No significant difference was observed in the DHA contents or PI values of the 5 dietary groups, despite a great difference in these parameters in the diets (Table 2).

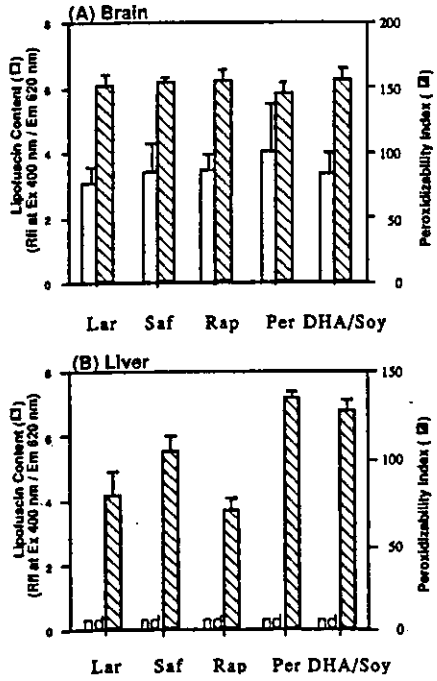

Fig. 3. Lipofuscin Content and Peroxidizability Index

Brain (A) and liver (B) from mice fed a test diet for 51 weeks were analyzed ($n=6$). Statistical analyses were performed using one-way AVONA. Relative fluorescein intensity (Rfi) was taken as lipofuscin content, and is expressed by open columns. Peroxidizability index of tissue fatty acids is expressed by oblique columns.

Table 2. Fatty Acid Composition of Brain Phospholipid

Fatty acid	Dietary group				
	Lar	Saf	Rap	Per	DHA/Soy
	% (wt/wt) of total fatty acids				
16:0 DMA	2.1±0.1	2.0±0.2	2.0±0.2	2.1±0.2	2.0±0.3
16:0	18.3±0.7	17.5±0.5	18.0±1.2	18.2±1.4	18.4±1.3
16:1	0.8±0.1	0.5±0.2	0.5±0.1	0.7±0.2	0.6±0.2
18:0DMA	3.6±0.2	3.7±0.1	3.6±0.2	3.6±0.1	3.5±0.1
18:1DMA	2.1±0.5^{b}	1.7±0.2^{ab}	1.7±0.1^{ab}	1.6±0.0^{a}	1.7±0.2^{ab}
18:0	20.2±0.5	20.7±1.0	21.0±0.9	21.4±1.2	21.1±1.0
18:1	21.3±0.6^{ab}	20.2±0.6^{a}	21.4±0.6^{b}	21.6±0.7^{b}	20.8±0.5^{ab}
18:2n-6	0.6±0.0^{a}	1.2±0.2^{b}	0.7±0.1^{a}	0.8±0.1^{a}	1.4±0.4^{b}
20:0	0.3±0.2	0.4±0.0	0.2±0.2	0.3±0.2	0.4±0.1
20:1	2.1±0.3	2.1±0.2	2.2±0.3	2.2±0.4	1.9±0.3
20:3n-6	0.5±0.1	0.4±0.1	0.5±0.4	0.5±0.1	0.8±0.2
20:4n-6	8.5±0.5^{b}	8.9±0.3^{b}	8.7±0.5^{b}	7.3±0.3^{a}	7.8±0.3^{a}
22:0	0.3±0.3	0.3±0.3	0.4±0.1	0.3±0.3	0.2±0.3
22:4n-6	2.6±0.2^{ab}	3.0±0.1^{b}	2.6±0.2^{ab}	2.4±0.8^{ab}	2.2±0.3^{a}
22:5n-6	0.1±0.2	N.D.	0.1±0.3	N.D.	N.D.
22:5n-3	0.1±0.1^{a}	0.1±0.1^{a}	0.1±0.1^{a}	0.5±0.1^{b}	0.2±0.2^{a}
22:6n-3	13.0±0.7	12.7±0.5	13.1±0.9	12.5±1.0	14.0±1.1
24:0	0.8±0.2	0.9±0.3	0.5±0.4	0.8±0.1	0.6±0.4
24:1	2.9±1.3	3.5±1.3	2.5±0.2	3.2±0.9	2.5±0.8
n-6/n-3 Ratio	0.9±0.0^{b}	1.1±0.0^{c}	1.0±0.1^{b}	0.9±0.0^{a}	0.9±0.1^{a}
PI	151±8	153±5	154±10	145±11	156±10
Total fatty acids (mg/g tissue)	35.4±4.0	35.6±2.1	37.1±1.1	34.8±3.1	35.3±4.1

Values (%) for the fatty acid composition of total brain phospholipids are means±S.D. (n-6). Values with different superscripts are significantly different from each other at $p<0.05$. N.D., not detectable.

Table 3. Fatty Acid Composition of Hepatic Total Lipids

Fatty acid	Dietary group				
	Lar	Saf	Rap	Per	DAH/Soy
	% (wt/wt) of total fatty acids				
14:0	0.4 ± 0.1^{b}	0.3 ± 0.1^{a}	0.4 ± 0.1^{b}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}
16:0DMA	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
16:0	26.2 ± 2.4^{c}	22.2 ± 3.0^{b}	20.4 ± 1.1^{ab}	17.8 ± 1.0^{a}	23.2 ± 1.6^{bc}
16:1	2.8 ± 0.5^{c}	0.9 ± 0.4^{a}	1.8 ± 0.6^{b}	1.2 ± 0.4^{ab}	1.3 ± 0.4^{ab}
18:0	5.1 ± 0.8^{ab}	6.2 ± 1.0^{bc}	4.2 ± 0.5^{a}	6.8 ± 1.2^{c}	5.4 ± 0.4^{abc}
18:1	37.4 ± 6.6^{b}	14.5 ± 1.6^{a}	41.6 ± 4.2^{b}	18.9 ± 1.6^{a}	18.5 ± 2.6^{a}
18:2n-6	16.7 ± 2.9^{a}	43.3 ± 1.9^{d}	20.9 ± 3.4^{ab}	24.9 ± 1.3^{b}	34.9 ± 2.5^{c}
18:3n-6	0.3 ± 0.3^{a}	1.0 ± 0.3^{b}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}
18:3n-3	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	2.4 ± 0.6^{ab}	17.5 ± 5.1^{c}	2.2 ± 0.4^{ab}
20:1	0.3 ± 0.1^{bc}	0.1 ± 0.1^{a}	0.4 ± 0.1^{c}	0.2 ± 0.1^{ab}	0.2 ± 0.1^{ab}
20:3n-6	0.5 ± 0.0^{a}	0.9 ± 0.2^{b}	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	0.7 ± 0.1^{ab}
20:4n-6	3.8 ± 0.5^{a}	5.3 ± 0.7^{b}	3.0 ± 0.2^{a}	3.0 ± 0.9^{a}	2.9 ± 0.6^{a}
20:5n-3	0.8 ± 0.2^{a}	0.4 ± 0.2^{a}	0.8 ± 0.2^{a}	3.1 ± 0.5^{c}	1.5 ± 0.2^{b}
22:0	0.1 ± 0.0	tr.	0.1 ± 0.1	tr.	0.1 ± 0.1
22:1	N.D.	N.D.	N.D.	tr.	tr.
22:4n-6	0.1 ± 0.0^{a}	0.2 ± 0.1^{a}	tr.	N.D.^{a}	0.1 ± 0.0^{bc}
22:5n-3	0.5 ± 0.2^{a}	0.3 ± 0.1^{a}	0.4 ± 0.0^{a}	1.1 ± 0.1^{c}	0.7 ± 0.1^{b}
22:6n-3	4.1 ± 1.0^{b}	3.6 ± 0.8^{ab}	2.5 ± 0.2^{a}	4.2 ± 0.9^{b}	7.3 ± 0.8^{c}
24:0	N.D.	tr.	N.D.	N.D.	tr.
24:1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.1
n-6/n-3 Ratio	3.8 ± 0.5^{b}	11.0 ± 1.9^{c}	4.1 ± 0.4^{b}	1.2 ± 0.3^{a}	3.3 ± 0.4^{b}
PI	77 ± 16^{a}	103 ± 10^{b}	68 ± 8^{a}	133 ± 4^{c}	126 ± 7^{c}
Total fatty acids (mg/g tissue)	106 ± 15^{ab}	95 ± 19^{a}	133 ± 15^{b}	88 ± 18^{a}	98 ± 13^{a}

. Values (%) are means±S.D. (n-6). Values with different superscripts are significantly different from each other in the line at $p<0.05$. N.D., not detected; tr., trace.

Fatty acid composition of hepatic lipids roughly reflected that of the experimental diets when total n-6 and n-3 fatty acids were summed up (Table 3). The proportions of total saturated fatty acids in the Lar group, total monounsaturated acids in both the Rap and Lar groups, total n-6 polyunsaturated acids in the Saf group and total n-3 polyunsaturated acids in the Per group were significantly higher than those of other groups. The proportion of DHA in the DHA/Soy group was greater than in the other dietary groups, and the n-6/n-3 ratio of the Saf group was much higher while that of the Per group was lower than in the other groups. The PI values in the Per and the DHA/Soy groups were greater than in the other dietary groups, and total fatty acids in the Rap group were significantly higher than in the other groups except for the Lar group.

DISCUSSION

The regulatory mechanisms of cholesterol biosynthesis have been revealed at the levels of gene expression.[23—25] In cultured cells, transcription factors (sterol responsive element binding proteins, SREBP 2 and SREBP 1c) are up-regulated by saturated fatty acid (S) and monounsaturated fatty acid (M) but are down-regulated by polyunsaturated fatty acids.[26,27] Highly unsaturated fatty acids such as EPA (20:5n-3), DHA (22:6n-3) and arachidonic acid (20:4n-6) are more effective than linoleic acid (18:2n-6) in regulating the gene expression. Thus, the cholesterol synthesis appears to be regulated by the degree of unsaturation and chain length *in vitro*. On the other hand, cholesterol is known to suppress cholesterol synthesis partly by suppressing the maturation of SREBP.[28] These mechanisms are expected to work *in vivo* to maintain tissue cholesterol levels. However, tissue cholesterol is known to vary depending on such factors as the amounts and the type of dietary fatty acids as well as the period of dietary manipulation.[29—31] So far, available data have not been sufficient to describe long-term effects of dietary fats and oils with a different degree of unsaturation and different n-6/n-3 balance even in animal studies.

Hypocholesterolemic activity of dietary linoleic acid compared with animal fats, which has been observed after relatively short feeding periods, was not observed after long-term feedings, *e.g.*, >1/10 the life span. The DHA/Soy diet as well as the Per diet was more hypocholesterolemic after long-term feedings than the Lar and Saf diets in a mouse strain (Fig. 1). These results are consistent with others, which compared combinations of these fats and oils under the same conditions.[29—31] Thus, the earlier recommendations to increase the intake of high-linoleic vegetable oils and decrease that of animal fats for the prevention of chronic diseases such as thrombotic diseases[32] needs to be re-evaluated, particularly because longer-term dietary intervention brought about no significant beneficial effects on plasma cholesterol nor on atherosclerotic diseases.[1,4] The observed effects of dietary fats and oils on tissue cholesterol levels were roughly accounted for by the activities of HMG-CoA reductase, a rate-limiting enzyme of cholesterol synthesis as reported by other groups in rats.[23,33] The serum and hepatic cholesterol levels in different dietary groups are consistent with those predicted from *in vitro* studies in cultured cells.[34,35]

Beside genes related to cholesterol synthesis, dietary fatty acids are known to affect the expression of genes for other related proteins such as LDL receptor,[36] LCAT[37] and PPAR-α that can regulate bile acid synthesis and β-oxidation.[29,38,39] For example, α-linolenic acid is a preferred substrate for the mitochondrial β-oxidation system in comparison to linoleic, saturated and monounsaturated acids in the rat.[40] Although DHA is a relatively poor substrate for the mitochondrial β-oxidation system, it can quickly undergo β-oxidation in rat or mouse peroxisomes after proliferation.[41—44]

Hypercholesterolemia itself has long been considered the major risk factor for elderly-onset diseases but this has now been questioned[5]; plasma cholesterol level was reported to be negatively correlated with all causes of mortality and cancer death after follow up for 10 years.[45,46] The levels of prenyl intermediates rather than plasma cholesterol appear to be important factors for carcinogenesis and atherogenesis.[5,47]

The free radical theory of aging and thrombotic diseases was based mainly on a comparison of dietary fats and oils with different degrees of unsaturation. The degree of unsaturation was positively correlated with autoxidizability in the air atmosphere and presumed markers for these diseases, *e.g.*, oxidized LDL and lipofuscin. n-3 Fatty acids such as ALA and DHA have an additional double bond compared with n-6 fatty acids with the same carbon number, and long-term feedings of n-3 fatty acids are suspected to increase lipid peroxides and their secondary products, *e.g.*, lipofuscin. We employed the SDS extraction method[22] for determination of yellow fluorescent lipofuscin. Tissue accumulation of lipofuscin is likely to reflect the differential rates of its turnover; the rate of degradation may be relatively faster and/or antioxidative capacity to suppress its formation may be greater in the liver. Faster turnover rates of hepatic cells compared with

brain neurons may also affect tissue lipofuscin contents. Alternatively, the fluorescent materials in these tissues except for the brain may be different from the so-called yellow fluorescent lipofuscin,[9-11] a presumed index of aging and lipid peroxidation. In this method, we have observed that lipofuscin was well detected in the brain, but not in the liver of mice. Despite a great difference in the peroxidizability of dietary fats and oils, no significant difference was observed in the brain lipofuscin contents of the 5 dietary groups. We have shown that rats fed DHA-rich oil and perilla oil exhibit superior learning ability in brightness-discrimination learning tests compared with a high-linoleic safflower oil group.[48] These observations are inconsistent with the lipid peroxide theory of aging and atherogenesis.

Finally, we would like to comment that the percent of fat energy of the diet (31.4%) was set between those of average Japanese (*ca.* 26%) and average Americans (*ca.* 37%), and the amount of DHA in the DHA/Soy diet (2%) was far below the dietary levels in Greenland natives (5.1%).[14] Although excessive intake of n-3 fatty acids is suspected to increase apoplexy, the higher incidence of apoplexy observed in Greenland natives compared with Danes[14] is rather ascribed to their lower intake of Vitamin C required for the synthesis of collagen and elastin to strengthen blood vessels. No appreciable symptom of apoplexy was observed in the present experiments.

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菜種油摂取による脳卒中易発症高血圧自然発症ラットの短命化の要因に関する基礎的研究

内藤由紀子，大原直樹

Phytosterols play a role in canola oil-induced shortening of lifespan in stroke prone spontaneously hypertensive rats

Yukiko NAITO, Naoki OHARA

High blood level of cholesterol is one of the risk factors for cardiovascular diseases, and foods of which some ingredients are capable of decreasing the level of cholesterol have been chosen and recommended for daily meal. Plant sterols (phytosterols) in vegetable oils, fruits and vegetables possess such an effect, and phytosterol-enriched margarine and dietary oils have become commonly consumed. However, it has been reported in stroke prone spontaneously hypertensive rats (SHRSP) that the intake of a diet containing canola oil shortened lifespan, though the mechanism underlying such an unfavorable effect is unknown. We have studied about the several physiological changes in SHRSP fed a diet containing 10% canola oil as the only dietary fat, and attempted to find causal substances. Soybean oil has been used as control, because the oil is contained in the normal diets for rats. The diet containing 10% canola oil shortened lifespan of SHRSP. The diet also increased blood pressure, accelerated blood coagulation and increased blood lipids. Similar increases in blood pressure and blood lipid level were also found in spontaneously hypertensive rats and normotensive Wistar Kyoto rats. In the SHRSP, erythrocytes became fragile with activated Na^+, K^+-ATPase, and phytosterols in those membranes were found increased compared with those of the animals fed a diet containing soybean oil. Then, we examined the fragility of erythrocyte obtained from SHRSP fed a diet containing 10% canola oil with 0.06% cholesterol, which is one of the essential membrane components and is liable to be replaced with phytosterols. The erythrocyte obtained were less fragile than that in SHRSP fed a diet containing 10% canola oil.

Additionally, a diet containing phytosterol-enriched canola oil showed a further shortening of lifespan as compared with the diet containing 10% canola oil. Since canola oil is originally high in phytosterol content compared with soybean oil, this finding suggests an essential role of phytosterols in the life-shortening observed.

In this paper, pathophysiological changes accompanying the shortening of life that might be implicated in the mechanisms for an accelerated onset of stroke 'by canola oil or phytosterol-rich oils' are also discussed.

1. はじめに

1) 心臓血管系疾患とコレステロール

近年，食生活の変化や環境ストレスなどに起因する心臓血管系疾患患者の増加が注目されている．特に，血中コレステロール量の増大は，動脈硬化症，狭心症，高血圧症，脳卒中のような心臓血管系疾患発症のリスク指標として重要であることが広く知られている．そのため，生活習慣病の予防を目的とした，血中コレステロールレベルの上昇を防ぐ食物および食品を取り入れた食生活に

Safety Testing Laboratory

対する社会的関心が高まり，それが推奨されている．

その中で，植物油中の植物ステロール（phytosterol）が，食物中コレステロールの消化管吸収を抑え，血中コレステロールレベルを低下させることに注目が集まっている[1]．植物ステロールは，菜種油，大豆油等の植物油中に多く含まれ，植物油以外では，果物や野菜（いちじく，落花生，ゴマなど），豆腐やマーガリンなどの加工食品にも含まれている．さらに植物ステロールを積極的に添加したマーガリンや食用油等の食品の有効性に関する研究事例も多数報告されるようになり[2-4]，植物ステロールを含む食品が一般的に広く使用されるようになってきた．

植物ステロールは体内で合成されることはなく，また，通常それ自体は消化管からの吸収率が低く，有害作用もほとんどないとされている[5]．植物ステロールと構造が類似しているコレステロールは，摂取量の40％以上が小腸から吸収されるのに対し，植物ステロールは5％以下である．一方，前述のように，植物ステロールは，小腸からのコレステロール吸収を阻害することによって，血中コレステロールレベルを低下させる作用を有し[6]，この作用が生活習慣病の予防効果の一因であるとみられる．

2）植物油の特性

わが国では，全食用植物油のうち，菜種油の需要が最も多く，全消費量の約40％（約89万トン）を占めている（1999年，農林水産省食品油脂調べ）．また菜種油は日常消費される多様な製品（マーガリン，マヨネーズ，ドレッシング，フライドポテト，唐揚げ，揚げあられ，パン・ケーキ類，ドーナツおよびインスタントラーメン等）中に含まれている．

菜種油の脂肪酸組成を，広く用いられているもう一種の植物油である大豆油と比較した（表1，社団法人日本植物油協会）．菜種油中に最も多く含まれる脂肪酸はオレイン酸で，約60％を占める．次いでリノール酸（約20％），リノレン酸（約10％）の順に多い．現在摂取されている菜種油は，心臓毒性作用を有するエルシン酸含有率を，品種改良によって2％未満にした油（キャノーラ油）であり，本実験でもこの油を用いた．一方，大豆油にはリノール酸が最も多く含まれ（約50％），次にオレイン酸（約20％），パルミチン酸，リノレン酸（それぞれ約10％）の順である．また，飽和脂肪酸は，菜種油（約6％）よりも大豆油（約15％）に多く含まれている．飽和脂肪酸については，血中コレステロールおよび中性脂肪量の増加作用を有することが，また，不飽和脂肪酸については，n-9系一価不飽和脂肪酸（オレイン酸）が血中コレステロール量低下作用および心臓病発症低下作用を有すること，n-6系多価脂肪酸（リノール酸）を過剰摂取すると脂質代謝の

表1　大豆油および菜種油に含まれる脂肪酸組成（％）

	Fatty acid	Soybean oil	Canola oil
14:0	Myristic acid	0.1	0
16:0	Palmitic acid	11.0	4.0
16:1	Palmitooleic acid	0	0.2
18:0	Stearic acid	3.6	1.7
18:1	Oleic acid	23.4	58.8
18:2	Linoleic acid	54.0	21.4
18:3	Linolenic acid	7.0	11.3
20:0	Arachidic acid	0.3	0.5
20:1	Eicosenoic acid	0.2	1.4
22:0	Behenic acid	0.4	0.3
22:1	Erucic acid	0	0.4

バランスが崩れることが知られている．さらに，$n-6$系不飽和脂肪酸/$n-3$系不飽和脂肪酸比が高くなると，血栓が形成されやすくなることが報告されている[7]．

菜種油および大豆油に含まれるステロール（コレステロールおよび植物ステロール）含量を比較した（表2）[8]．菜種油中の総植物ステロール量（705 mg/100 g）は，大豆油（286 mg/100 g）の約2.5倍である．また，菜種油中のβ-シトステロール（380 mg/100 g）およびカンペステロール（250 mg/100 g）は，どちらも大豆油中の含量（それぞれ168 mg/100 gおよび61 mg/100 g）よりも多く，一方，スティグマステロールは菜種油にはほとんど含まれないが（2 mg/100 g），大豆油には比較的多く含まれている（53 mg/100 g）．摂取された植物ステロールは，コレステロールよりも優先して胆汁酸ミセルに取り込まれるため，ミセル中のコレステロールが減少し，小腸で吸収されるコレステロールが少なくなる．ミセル中の植物ステロールはほとんど吸収されず，また，ミセルに取り込まれなかったコレステロールは便として排泄される．

脂肪酸組成やステロール含量に注目した場合，菜種油は健康に良い油であり，植物ステロールが多く含まれているため，生活習慣病の予防に有効であると考えられてきた．

3）植物油摂取におけるSHRSPおよびSHRの特徴

前項で示した菜種油の特徴にも関わらず，脳卒中易発症高血圧自然発症ラット（SHRSP）にある種の植物油のみを唯一の脂肪源として摂取させると，生存日数が短縮するという報告[9-12]があり，その中で，菜種油は生存日数を短縮する油の一つとされている[10-12]．

SHRSPの生存日数の短縮に関与すると考えられる物質はまだ特定されていないが，植物油中の脂肪酸の特異的組成[9]，未知物質[10-12]および植物ステロール[13]が疑われている．脂肪酸組成については，α-リノレン酸／リノール酸比が高い場合にSHRSPの生存日数が延長するという報告があるが，菜種油とは脂肪酸組成が異なるオリーブ油およびコーン油も，SHRSPの生存日数を短縮させる作用を持つこと[14]や，リパーゼ処理した菜種油では生存日数短縮作用が消失するものの，脂肪酸組成は菜種油とほとんど差がないこと[12]から，脂肪酸組成が生存期間短縮の要因である可能性は低いと考えられる．

菜種油摂取による生存日数短縮が報告されたSHRSPは，Wistar系ラットからの選択交配によって分離された，遺伝的に高血圧を発症する高血圧自然発症ラット（SHR）の亜系である．加齢に伴う血圧の上昇がSHRより急速で，収縮期圧の最大値はSHRに比べ，一般に40～50 mmHg高く，25週齢以降には脳血管障害（脳出血および脳梗塞）を併発して死亡する[15]．この脳血管障害の発症率は，SHRSPの方がSHRよりも有意に高い．SHRSPは，約20週齢で血圧が約250 mmHgとなり，心肥大，臓器（脳，心臓，腎臓，腸間膜など）の血管の炎症を伴うこと，SHRよりも脳血管にアテロームが発生しやすいことが特徴である．また，これらの動物では肝臓でのコレステロール生合成能が低下しており，血中コレステロール量が少ないことが知られている．

表2　大豆油および菜種油に含まれるステロール類（mg/100g oil）

	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β-Sitosterol	Total phytosterol
Soybean oil	1	4	61	53	168	286
Canola oil	2	73	250	2	380	705

4）研究の概要

汎用されている菜種油が，実験動物のみならずヒトに対しても有害作用を示すならば，それは重大な社会問題である．菜種油摂取によって生じる実験動物の生理機能等の変化およびSHRSPでみられた生存日数短縮の原因と機序を調べることは，私たちの食生活と密接に関わっている植物油の安全性について理解を深めるために，また，食品の安全性を保証する上で重要な研究課題であると考え，検討を続けている．そこで，これまで得られた結果をここでまとめることにした．

菜種油摂取による生存日数の短縮が報告されたSHRSPと，SHRおよびこれらの選抜親系統である正常血圧Wistar Kyoto（WKY）ラットの3系統のラットを用い，10％菜種油摂取によるSHRSPの生存日数短縮の背景にある生理学的ならびに血液学的機能変化を調べ，原因物質を探索した．また，一般症状観察，体重，摂餌量，摂水量および血圧測定，腎機能検査，血液学，血液生化学および病理学検査，血管反応性および細胞膜脆弱性検査，膜結合酵素活性の測定を行った．特にSHRSPにみられるコレステロール合成能低下は，先に述べた植物ステロールによるコレステロール吸収の阻害と相俟って，SHRSPの血中コレステロールレベルを下げ，生理機能に影響を及ぼす可能性が大きい．本研究は，菜種油中の植物ステロールがSHRSPの生存日数短縮に関与する可能性に注目し，それを証明しようとした．

ラット用の通常飼料には，脂肪分として主に大豆油が含まれることから，大豆油を摂取させた動物を対照動物とした．

2．実験結果

1）血圧に対する影響

菜種油を異なる系統のラットに，異なる条件[SHRSP（7週間摂取，食塩負荷あり），WKYラット（26週間摂取，食塩負荷あり）[16]，SHR（26週間摂取，食塩負荷あり）[16]，WKYラット（13週間摂取，食塩負荷なし）[17]，SHRSP（4週間摂取，食塩負荷なし）[18]]で摂取させた場合，いずれの系統あるいは条件においても，昇圧が促進されることが明らかとなった（図1および表3）．SHRSPでは，昇圧亢進が脳卒中発症に大きく影響すると考えられる．

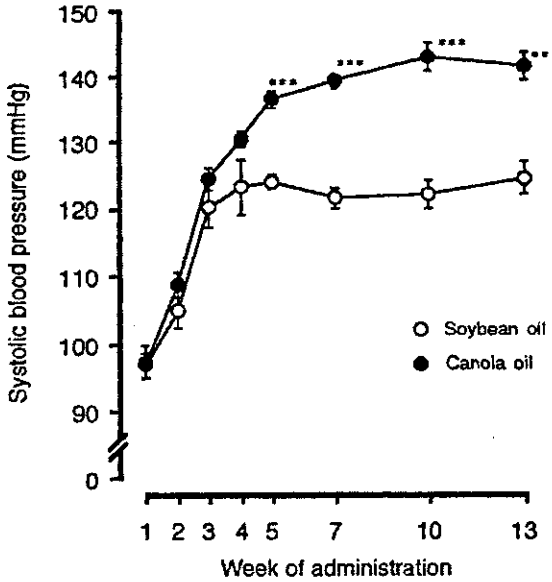

図1　13週間10％大豆油または菜種油含有飼料を摂取したWistar Kyoto（WKY）ラットの収縮期血圧の変化

血圧は，毎週1回Tail-cuff法で測定した．
シンボル（○，大豆油群；●，菜種油群）は，4～5例の平均および標準誤差を表す．
** $p<0.01$および*** $p<0.001$，大豆油群と比較して有意差あり（unpaired t-test）．

2）血液に対する影響

菜種油摂取動物で血小板数の減少（26週間摂取SHRおよびWKYラット，13週間摂取WKYラット）および好中球数の増加（26週間摂取SHRおよび13週間摂取WKYラット）が認められた[16,17]（表3）ことから，菜種油摂取により血管傷害が誘発され，損傷した部位への粘着による血小板数減少，炎症性反応に伴う好中球の動員が推測された．また，13週間摂取WKYラットのPTおよびAPTTには影響が認められなかったが[17]，7週間および4週間投与SHRSPのPTおよびAPTTには，それぞれ短縮傾向あるいは短縮が認められ[19]（表3），血栓形成の促進が示唆された．

3）血液生化学に対する影響

菜種油摂取動物の血中総コレステロール，遊離コレステロールおよびトリグリセリド濃度の増大

表3　菜種油を摂取したラットでみられる変化

Animal strain	SHRSP	SHR	WKY	WKY
Feeding period (week)	7	26	26	13
NaCl-loading (with/without)	with	with	with	without
Body weight	↓	→	→	→
Food intake	→	→	→	→
Water intake	→	→	→	→
Blood pressure	↑	↑	↑	↑
Urinalysis				
Urinary volume	→	(↑)	(↑)	→
Na^+	→	(↑)	(↑)	→
K^+	→	(↑)	(↑)	→
Cl^-	→	(↑)	(↑)	→
Hematology				
RBC	→	→	→	↑
Hematocrit	→	→	→	↑
WBC	→	→	→	↑
Neutrophil	→	↑	→	↑
Lymphocyte	→	→	→	→
Platelet	→	↓	↓	↓
PT	↓			→
APTT	→			→
Blood chemistry				
BUN	→	→	→	→
Glucose	→	↓	↓	↓
Total cholesterol	↑	↑	↑	↑
Free cholesterol		↑	↑	↑
Triglyceride	↑	↑	↑	↑
HDL		↑	↑	→
Phospholipids	↑	↑	↑	↑
NEFA	→	→	→	↓
Na^+	→	→	→	→
K^+	→	↓	↓	↓
Cl^-	→	→	→	→
Ca^{++}	→			→
Hepatic enzyme				
G6PDH				↑
Catalase				↓
SOD				↓

↑, increase compared with soybean oil group

↓, decrease compared with soybean oil group

→, no differences between canola oil group and soybean oil group

( ), tendency

not measured

が，7週間摂取SHRSP．26週間摂取SHR，および26および13週間摂取WKYラットで認められたことから，菜種油摂取は血漿中脂質濃度を増加させることが明らかとなった[16,17]（表3）．これは，動脈硬化症を起こしやすい系統であるSHRやSHRSPにおいては，高血圧関連症状発症の早期化につながる．

4）腎機能に対する影響

菜種油を26週間摂取したSHRおよびWKYラットで，尿量および電解質の増加傾向が認められたが有意な変化ではなく[16]，13週間摂取WKYラットでは変化が認められなかった[17]（表3）ことから，菜種油は腎機能に対して著しい影響を及ぼさないと考えられた．

5）血管反応に対する影響

26週間菜種油を摂取したSHRおよびWKYラットの摘出灌流腸間膜血管床標本および胸部大動脈リング標本を用いて血管反応を検討した実験では，血管内皮由来血管弛緩因子（一酸化窒素）の産生あるいは遊離機能，血管平滑筋のノルエピネフリン誘発収縮およびアセチルコリン誘発弛緩反応および交感神経終末のノルエピネフリン貯蔵量に変化は認められなかった[16]．また，4週間菜種油を投与したSHRSPの摘出血管では，種々のオータコイド（アンジオテンシンII，アラキドン酸，ATP．エンドセリン-1，ノルエピネフリンおよびセロトニン）に対する血管収縮反応およびプロスタノイド（プロスタサイクリンおよびトロンボキサンA_2）の産生は影響を受けなかった[18]．したがって，内因性の血管作動物質に対する血管反応の変化は昇圧の要因でないと考えられた．しかし，カリウムフリー栄養液中での血管標本収縮が菜種油投与動物で亢進したこと，および胸部大動脈のNa^+, K^+-ATPase活性が上昇したことから，イオン透過性の変化に基づく平滑筋の興奮性の亢進など，間接的に血圧上昇に結びつく生理機能の変化が示唆された．

6）Na^+，K^+-ATPase活性および赤血球膜浸透圧抵抗性に対する影響

4週間の菜種油投与によりSHRSPの脳，心臓，腎臓および胸部大動脈におけるNa^+, K^+-ATPase活性は上昇（表4）し[18]．赤血球膜の浸透圧抵抗性は低下した（図2）[19]．また，菜種油を摂取したSHRSPでは，細胞膜機能の維持に関わる膜中コレステロール量の総ステロール量に対する割合が低下した（表5）[8]．これらのことから，菜種油を摂取したSHRSPでは，細胞膜の脆弱化が全身で起こる可能性があること，また，これには菜種油摂取により吸収され，細胞膜中に蓄積した植物ステロール量の増加が関与することが示唆された．

7）生存日数に対する影響

SHRSPの生存日数は，菜種油摂取により短縮した（図3）[8]．また，大豆油特有の植物ステロール（スティグマステロール）を大豆油中含有量に相当する量だけ添加した菜種油を摂取した動物では，さらに生存日数が短縮した（図4）．菜種油

表4　10％大豆油または菜種油を4週間強制経口投与した脳卒中易発症高血圧自然発症ラット（SHRSP）のNa^+, K^+-ATPase活性

	Soybean oil	Canola oil
Brain	63.9± 6.9	92.4± 7.7*
Heart	39.4± 8.4	80.1± 17.5*
Kidney	19.1± 3.0	34.0± 6.5*
Aorta	72.7	95.2

Values are means ± S. E. (nmol/mg protein/min) for 10 samples, except aorta. Values of aorta represent the activities obtained from pooling samples.

*$p<0.05$, significantly different from the values of soybean oil group (unpaired t-test)

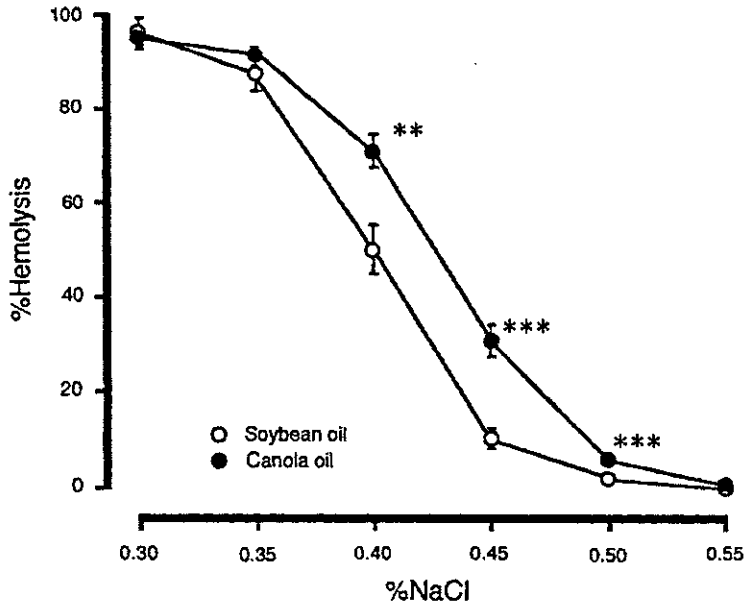

図2　4週間大豆油または菜種油を摂取した脳卒中易発症高血圧自然発症ラット（SHRSP）の溶血性

大豆油または菜種油は，摂餌量の10％に相当する量を強制経口投与した．
リン酸緩衝液の希釈系列（0.1～0.85％塩化ナトリウム相当）を調製した．動物から得た血液を各濃度の溶液に加えて混和し，30分間静置した．遠心分離後上清を得，540 nmにおける吸光度を測定し，溶血率を算出した（0.1％溶液を100％溶血とした）．
シンボル（○，大豆油群：●，菜種油群）は，10例の平均および標準誤差を表す．
** $p<0.01$ および *** $p<0.001$，大豆油群と比較して有意差あり（unpaired t-test）．

表5　10％大豆油または菜種油を4週間強制経口投与した脳卒中易発症高血圧自然発症ラット（SHRSP）の赤血球膜中ステロール量

Group		Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β-Sitosterol	Total phytosterol
Soybean oil	Mean	414	n. d.	2.79	0.255	2.19	5.23
	±S.E.	38.7		0.184	0.0242	0.163	0.354
Canola oil	Mean	415	0.135	6.49***	n. d.	3.09***	9.72***
	±S.E.	9.77	0.0376	0.173		0.0899	0.264

Values are means ± S. E. (nmol/mg erythrocyte membranes) for 10 animals.

n. d., < 0.1 nmol/mg erythrocyte membranes

***$p<0.001$, significantly different from the values of the soybean oil group (unpaired t-test)

にスティグマステロールを添加した場合でも，摂取した動物の生存日数が菜種油を摂取した動物と違いがなければ，菜種油特有の植物ステロールが生存日数短縮に関与すると推察されるが，短縮が認められた．したがって，生存日数短縮には，菜種油に特有な植物ステロールが関与するのではなく，植物ステロールの総量が関与すると考えられた．

3. 考察とまとめ

SHRSPに菜種油を唯一の脂肪源として摂取させるとみられる生存日数の短縮には，菜種油中から体内に取り込まれる植物ステロールの量が関与することが明らかになった．Na^+, K^+-ATPase活性の変化がみられたことから，細胞膜中に植物ステロールが蓄積することによって細胞膜中の機能タンパク質であるナトリウムポンプが影響を受けたと推測された．植物ステロールは，吸収後細胞膜中のコレステロールと置換することによって膜結合酵素活性に影響を与えることや[20]，膜コレステロールが減少すると，本来の膜結合酵素の機能不全が誘発されること[21-24]が報告されている．このとき膜は脆弱化し，脳卒中につながる血管傷害とも関連する可能性が考えられた．SHRSPでは植物ステロールの吸収が亢進し，排泄能が低下しているため，植物ステロールが体内に蓄積されやすいことが報告されている[25]．したがって，この系統の動物に多量の植物ステロールを含有する菜種油を摂取させると，体内の植物ステロール量がさらに増加すると考えられる．また，菜種油摂取によってみられた血漿中脂質レベルの上昇が，血管傷害を促進することも疑われた．図5に示したように，Na^+, K^+-ATPase活性の上昇，赤血球膜脆弱化，血液凝固時間の短縮および血中脂質の増加は，アテローム形成を含む血管傷害，出血および血栓形成に関与する変化と考えられる．主な要因は，過剰の植物ステロールの蓄積と考えられ，上記の変化を通じて生存日数の短縮をもたらすことが明らかになった．

本研究の結果から，植物ステロールは菜種油摂取によるSHRSPの短命化に関わる原因物質の一つであることが示唆された．近年，高コレステロール血症を防ぐ目的で，植物油の摂取が推奨されてはいるが，血中植物ステロールレベルの上昇による細胞膜の脆弱化等の影響がヒトでも認められないとは限らない．欧米では，アテローム性動脈硬化症の発症を予防する目的で，飽和脂肪酸やコレステロールの摂取量を削減する食事療法が注目

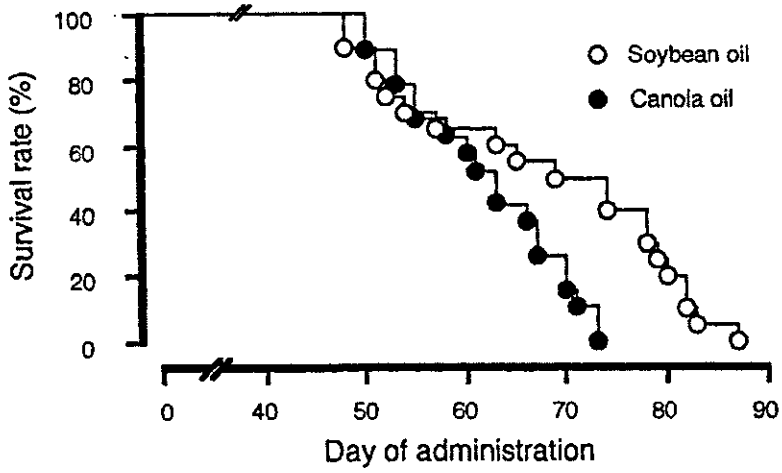

図3　10％大豆油または菜種油含有飼料を摂取した脳卒中易発症高血圧自然発症ラット（SHRSP）の生存曲線

動物には，1%食塩水を飲水として自由摂取させた．
○および●は，それぞれ大豆油群および菜種油群を表す．両群間に有意差あり（$p < 0.05$, Log-rank test, Tarone-Ware test）．

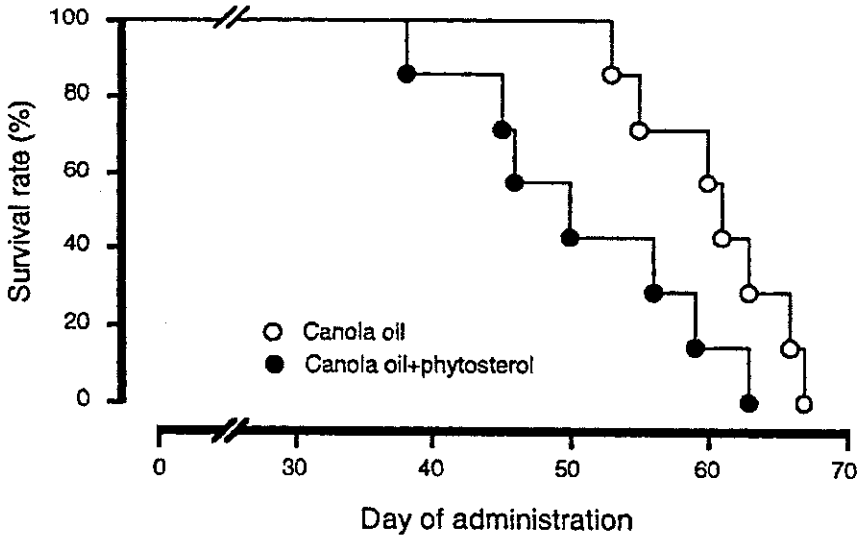

図4　10％菜種油または植物ステロール添加10％菜種油含有飼料を摂取した脳卒中易発症高血圧自然発症ラット（SHRSP）の生存曲線

動物には，1％食塩水を飲水として自由摂取させた．

○および●は，それぞれ10％菜種油群およびスティグマステロール添加10％菜種油群を表す．両群間に有意差あり（$p < 0.05$，Log-rank test. Tarone-Ware test）．

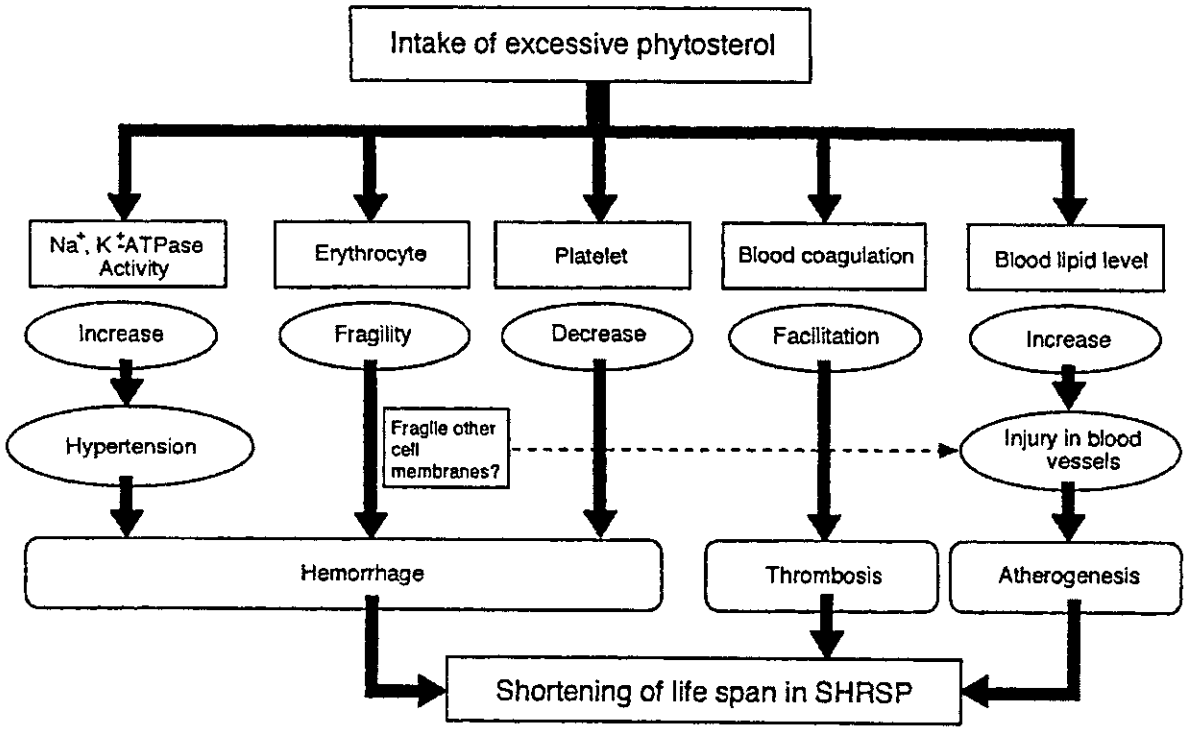

図5　まとめ

され，この療法を取り入れた際の乳幼児における血中コレステロール，コレステロール前駆物質および植物ステロール量の変化に関する研究結果が報告されている[26]．母乳や調合乳に代えて，脱脂乳と菜種油等の植物油を与えられた乳幼児では，血中コレステロールレベルやコレステロール生合成に変化はみられないが，血中植物ステロールレベルが上昇する．Mellies らも，同様の結果を報告している[27]．また，魚，卵および牛乳アレルギーの子供では，食事制限によって動物性脂肪の摂取量が減り，代わりに植物性脂肪の摂取量の増加およびコレステロールの摂取量の減少がみられると同時に，血中の植物ステロールレベルが上昇していることが報告されている[28]．この研究では，動物性脂肪の代替として用いられる菜種油中に植物ステロールが多いことが，コレステロール吸収の抑制および血中コレステロールレベルの低下と平行してみられる血中植物ステロールの上昇にかかわると結論づけており，このような状態ではコレステロールの生合成が代償性に促進されるが，それでもなおコレステロールレベルは低い．血中植物ステロール量が増加した状態で，SHRSP を対象にした本研究でみられたことと同様の細胞膜の脆弱化がヒトでも起こるか否か明らかではない．しかし，植物ステロールの長期大量摂取が発達期にある子供にどのような影響を及ぼすかについてはほとんど情報がないため，今後詳細に検討する必要があると考えられる．また，フィトステロール血症患者においては，植物ステロールの消化管吸収亢進および胆汁や糞便への排泄能低下がみられるため，菜種油のような植物ステロールを多く含む食用油を摂取すると，より多くの植物ステロールが蓄積され，フィトステロール血症でみられる症状の一つである結節性黄色腫の悪化を促進する可能性がある．したがって，フィトステロール血症患者などステロールの吸収排泄に異常のある病態[29]では，植物油の過剰摂取に注意が必要と考えられる．一方，われわれの日常の食生活では，摂取する脂肪源が常に同一になることはほとんど考えられないため，菜種油からの植物ステロール摂取に過敏となる必要はないと考えられるが，食用油を含め，多様な食品から知らず知らずのうちに摂取している植物ステロールが，われわれの生理機能に，あるいは健康に影響を及ぼすことは理解しておくことが肝要である．

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