Unusual Effects of Some Vegetable Oils on the Survival Time of Stroke-Prone Spontaneously Hypertensive Rats

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ABSTRACT: Preliminary experiments have shown that a diet containing 10% rapeseed oil (low-erucic acid) markedly shortens the survival time of stroke-prone spontaneously hypertensive (SHRSP) rats under 1% NaCl loading as compared with diets containing perilla oil or soybean oil. High-oleate safflower oil and high-oleate sunflower oil were found to have survival time-shortening activities comparable to that of rapeseed oil; olive oil had slightly less activity. A mixture was made of soybean oil, perilla oil, and triolein partially purified from high-oleate sunflower oil to adjust the fatty acid composition to that of rapeseed oil. The survival time of this triolein/mixed oil group was between those of the rapeseed oil and soybean oil groups. When 1% NaCl was replaced with tap water, the survival time was prolonged by ~80%. Under these conditions, the rapeseed oil and evening primrose oil shortened the survival time by ~40% as compared with n-3 fatty acid-rich perilla and fish oil; lard, soybean oil, and safflower oil with relatively high n-6/n-3 ratios shortened the survival time by roughly 10%. The observed unusual survival time-shortening activities of some vegetable oils (rapeseed, high-oleate safflower, high-oleate sunflower, olive, and evening primrose oil) may not be due to their unique fatty acid compositions, but these results suggest that these vegetable oils contain factor(s) which are detrimental to SHRSP rats.

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The stroke-prone spontaneously hypertensive (SHRSP) strain of rats develops the highest blood pressure among the commonly available rat strains and dies frequently of apoplexy particularly when NaCl is loaded. The dietary risk factors for this animal model are known to be low-protein and high-salt which are also suggested for human apoplexy. Although the genetic differences between the SHRSP strain and normotensive control strain (WKY) have not been fully defined, this animal model has been used widely for studies on essential hypertension, apoplexy, atherosclerosis, and age-related mental disorders.

Perilla seed oil from beefsteak plant *(Perilla frutescens)* has a fatty acid composition similar to that of linseed oil (~15% linoleic and ~60% α-linolenic acid) and is relatively common in east Asian countries. This oil has been shown to lengthen survival time of Donryu rats (a conventional strain) by ~10% (1) and those of SHRSP rats by ~15% (2) as compared with safflower oil (70% linoleic and <1% α-linolenic acid). We hypothesized that the observed beneficial effects of perilla oil on animal models of chronic diseases of the elderly (3) were due to its very low n-6/n-3 ratio. Rapeseed oil (~26% linoleic and ~8% α-linolenic acid) would be expected to have comparable beneficial effects because of its relatively low n-6/n-3 ratio and high oleate content. Low-erucic acid rapeseed oil, however, was found instead to shorten the survival time of SHRSP rats by >40% compared with soybean oil and perilla oil under 1% NaCl loading as drinking water (4).

Historically, a rapeseed strain containing both much smaller amounts of thyrotoxic sulfur compounds and erucic acid was selected, which was found to exhibit significantly less thyrotoxicity as expected. Later, however, the double-low rapeseed oil was reported to induce myocardial necrosis in a strain of the rat (5). After extensive studies, the incidence of myocardial necrosis was ascribed to the unique fatty acid composition of the rapeseed oil. A positive correlation with oleic and α-linolenic acid and a negative correlation with saturated fatty acids were proposed (6), and the necrosis was reproduced with a mixture consisting of soybean oil and triolein (7). Thus, the safety of double-low rapeseed oil for human use appears to have been established earlier as summarized by Dupont *et al.* (8).

However, the positive and negative correlations reported for myocardial necrosis (6) do not appear to apply to the observed shortening of survival time by these oils when fed to SHRSP rats (4), because perilla oil with similar proportions of saturated fatty acids and a greater proportion of α-linolenic acid than rapeseed oil extended the survival time of SHRSP rats significantly. The purpose of these studies was to examine if the survival time-shortening activity is due to the unique fatty acid composition of rapeseed oil, and if such an activity is seen in the absence of NaCl loading.

EXPERIMENTAL PROCEDURES

Diets and animals. A conventional basal diet containing 3.0% (w/w) oil (CE2; Clea Japan Co., Ltd., Tokyo, Japan) was made of fish meal, soybean meal, defatted powdered milk, wheat flour, corn, wheat bran, alfalfa meal, a vitamin mixture,

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Abbreviation: SHRSP, Stroke-prone spontaneously hypertensive rat strain.

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and a mineral mixture. This basal diet and test oil (fat) were mixed at a weight ratio of 9 to 1. The final oil content was 12.7 wt% (29 energy percentage). Fat and oils commercially available for human use were used, except for high-erucic rapeseed oil which was not for human use; rapeseed oil (low-erucic), soybean oil, safflower oil (high-linoleate), high-oleate safflower oil, high-oleate sunflower oil, perilla oil, evening primrose oil, lard, triolein (from high-oleate sunflower oil), fish oil, and their mixtures. The vitamin E content (total tocopherol) of the basal diet was 26 mg/90 g diet, and those of fats and oils (per 10 g) were 51 mg (fish oil), 18 mg (perilla oil), 6.4 mg (safflower oil), and 2.4 mg (olive oil).

Dietary pellets containing supplemented fats or oils were prepared by Clea Japan Co., Ltd., except for the fish oil diet which was prepared in our own laboratory and contained about 40% water as compared with the pelleted diets containing 8% water. Diets with peroxide values below 10 meq/kg were served. The food in the cage was replaced every day in the case of fish oil diet, and every two days in other diets, in order to keep the peroxide values of the ingested diets below 100 meq/kg. Rats were kept in an air-conditioned room, and the temperature was kept at 23±3°C.

Genetically SHRSP rats were kindly provided by Dr. Y. Suzuki (Professor, Kinki University School of Medicine, Osaka, Japan), and those with high systolic blood pressures were mated to maintain the strain. Blood pressure was measured using equipment from Ueda Manufacturing Co., Ltd., Tokyo, Japan (Model UR5000). Litters were distributed to different dietary groups as far as possible. In preliminary experiments, the number of rats in each group was ~6, and it was increased to >10/group for confirmatory experiments. Rats were weaned to the conventional diet (CE2) at 3 wk of age, and the test diets were given from 4 wk of age. Diet and tap water or 1% NaCl solution were given *ad libitum.* The fatty acid compositions of the diets, determined as methyl esters by gas–liquid chromatography, are shown in Table 1.

Fractionation of rapeseed oil components. In efforts to separate presumed survival time-shortening factor(s) in rapeseed oil, rapeseed oil (low-erucic) was mixed with 2 vol of acetone or ethanol, the mixture was cooled to −70°C, left overnight, and the precipitate was filtered off. After evaporation of the solvent, the residue (*ca.* 0.5% of rapeseed oil as acetone-soluble and 0.7% as ethanol-soluble) was dissolved in soybean oil at a 5-fold concentration (in 1:5 the amount of the treated rapeseed oil) (acetone- or ethanol-soluble in Table 2). A methanol-soluble fraction (0.6% of oil) was obtained by treating the oil at room temperature (methanol-soluble). Saponification of the oil was performed by a conventional method, and the unsaponifiable fraction (0.33% of oil) was dissolved in soybean oil at a 2.5-fold concentration (unsaponifiable fraction). The oil (5.8 kg) dissolved in 9.5 L of hexane and 0.5 L of diethyl ether was mixed with 1 kg of silicic acid (Merck, Darmstadt, Germany). The mixture was stirred overnight, and 5.22 kg of oil was recovered from the hexane/diethyl ether fraction (silicic acid-treated).

Statistical analysis. Statistical analyses of the survival times were performed by Log-Rank and Wilcoxon's test using a computer program (9), "Statistics Made Visual™" (SAS Institute Inc., Cary, NC).

RESULTS

Effect of oils on survival time of SHRSP rats under 1% NaCl loading. At first, we tried to answer the question whether the unique fatty acid composition of the rapeseed oil is responsible for the shortening of survival time of SHRSP rats. In Experiment 1 (Table 3), a mixed oil with a fatty acid composition similar to that of rapeseed oil was prepared by mixing

TABLE 1
Fatty Acid Composition of Diets[a]

Fatty acid	Rapeseed oil	Rapeseed oil (high-erucic)	Soybean oil	Mixed oil[b]	Triolein/soybean oil	Safflower oil (high-oleate)	Sunflower oil (high-oleate)	Olive oil	Safflower oil	Perilla oil	Fish oil/soybean oil	Lard	Evening primrose oil
14:0	0.1	—[c]	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.1	1.6	1.2	0.1
16:0	6.5	5.3	11.6	7.1	9.4	6.2	7.1	11.4	8.8	7.9	13.3	22.2	8.1
16:1	0.2	0.2	0.3	0.1	0.1	0.1	0.1	0.5	0.2	0.2	2.9	2.3	0.1
18:0	1.5	1.3	3.6	2.0	3.2	2.3	3.5	3.0	2.5	1.8	2.8	10.7	1.8
18:1	55.4	16.3	24.8	58.1	51.2	69.1	66.3	65.5	10.2	20.7	20.6	39.4	13.0
18:2n-6	25.6	18.3	51.0	25.1	31.7	20.2	20.0	16.1	68.8	27.1	28.8	20.7	67.7
18:3n-6	—	—	—	—	—	—	—	—	—	—	—	—	6.9
18:3n-3	8.2	6.7	6.3	6.6	3.1	1.1	1.5	1.3	1.6	51.0	3.1	1.3	1.0
18:4n-3	—	—	—	—	—	—	—	—	—	—	0.8	—	—
20:1	1.2	5.4	0.5	0.3	0.5	0.3	0.7	0.3	0.5	0.3	1.2	0.9	0.3
20:4n-6	0.1	—	0.1	—	—	—	—	0.2	0.1	0.1	1.3	0.2	0.4
20:5n-3	0.3	0.3	0.6	0.2	0.3	0.2	0.2	0.2	0.5	0.3	5.0	0.5	0.2
22:1	0.3	45.4	—	—	—	—	—	—	—	—	—	—	—
22:6n-3	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.5	0.6	0.5	18.6	0.6	0.4

[a]A conventional diet containing 3% oil (90 g) was mixed with 10 g of lard, oil or oil mixtures, and typical fatty acid composition of the diets is shown in weight percentage. Some variations in fatty acid compositions were noted among the lots of oils used. Fatty acid composition of triolein/soybean oil/perilla oil diet used in Experiment 5 was not presented but was very similar to that of rapeseed oil. Unsaturated fatty acids with 20- and 22-carbon chains in the diets supplemented with vegetable oils were originated mainly from fish meat in the basal diet and partly from unidentified components of the oils.

[b]Diet supplemented with a mixture of 75% high-oleate safflower oil, 12.5% safflower oil, and 12.5% perilla oil.

[c]Not detectable or trace amounts.

TABLE 2
Survival Times of SHRSP Rats Fed Different Fractions of Rapeseed Oil Under NaCl Loading

Dietary group Number of animals	Rapeseed oil $n = 12$	Soybean oil $n = 12$	Acetone-soluble[a] $n = 6$	Ethanol-soluble[a] $n = 6$	Methanol-soluble[a] $n = 6$	Unsaponifiable fraction[a] $n = 7$	Silicic acid-treated[a] $n = 9$
Survived day ± SD (survival rate)	104 ± 6	213 ± 98	>202 ± 17[b] (2:6)	>172 ± 28[b] (1:6)	>200 ± 34[b] (3:6)	>152 ± 10[b] (7:7)	113 ± 6

[a]See text for the preparation of these fractions from rapeseed oil.
[b]Experiments were stopped when the difference vs. rapeseed oil group became statistically significant, and the numbers of rats surviving/total rats when experiments were stopped are shown in parentheses. SHRSP: stroke-prone spontaneously hypertensive rat strain.

TABLE 3
Survival Times of SHRSP Rats Fed Different Oils Under 1% NaCl Loading[a]

	Dietary group and number of animals			
Experiment 1	Rapeseed oil (Rap), $n = 6$	Soybean oil (Soy), $n = 6$	Mixed oil (high-oleate safflower/safflower/perilla) $n = 6$	
Survived day (± SD)	146 ± 12	267 ± 5	144 ± 7	
P value by Log-rank		0.001 vs. Rap	0.900 vs. Rap	
Wilcoxon	0.001 vs. Rap	0.633 vs. Rap		
Log-rank		0.001 vs. Soy		
Wilcoxon			0.001 vs. Soy	
Experiment 2	Rapeseed oil (Rap), $n = 12$	Soybean oil (Soy), $n = 11$	High-oleate safflower oil, $n = 12$	
Survived day (± SD)	122 ± 5	235 ± 17	116 ± 5	
P value by Log-rank		0.000 vs. Rap	0.243 vs. Rap	
Wilcoxon		0.000 vs. Rap	0.153 vs. Rap	
Log-rank			0.000 vs. Soy	
Wilcoxon			0.000 vs. Soy	
Experiment 3	Rapeseed oil (Rap), $n = 6$	Soybean oil (Soy), $n = 6$	High-oleate sunflower oil (HOS), $n = 8$	High-erucic rapeseed oil, $n = 8$
Survived day (± SD)	136 ± 11	189 ± 14	135 ± 7	115 ± 4
P value by Log-rank		0.005 vs. Rap	0.940 vs. Rap	0.024 vs. Rap
Wilcoxon		0.006 vs. Rap	0.898 vs. Rap	0.045 vs. Rap
Log-rank			0.008 vs. Soy	0.000 vs. Soy
Wilcoxon			0.013 vs. Soy	0.001 vs. Soy
Log-rank				0.018 vs. HOS
Wilcoxon				0.035 vs. HOS
Experiment 4	Rapeseed oil (Rap), $n = 6$	Soybean oil (Soy), $n = 6$	Safflower oil (Saf), $n = 7$	Triolein/soybean oil (Tri), $n = 6$
Survived day (± SD)	132 ± 11	241 ± 33	204 ± 19	172 ± 19
P value by Log-rank		0.005 vs. Rap	0.004 vs. Rap	0.047 vs. Rap
Wilcoxon		0.006 vs. Rap	0.005 vs. Rap	0.071 vs. Rap
Log-rank			0.151 vs. Soy	0.046 vs. Soy
Wilcoxon			0.350 vs. Soy	0.102 vs. Soy
Log-rank				0.223 vs. Saf
Wilcoxon				0.248 vs. Saf
Experiment 5	Rapeseed oil (Rap), $n = 11$	Soybean oil (Soy), $n = 11$	Olive oil (Oli), $n = 12$	Triolein/soybean oil/perilla oil, $n = 12$
Survived day (± SD)	131 ± 7	212 ± 25	155 ± 19	172 ± 20
P value by Log-rank		0.004 vs. Rap	0.401 vs. Rap	0.096 vs. Rap
Wilcoxon		0.015 vs. Rap	0.854 vs. Rap	0.161 vs. Rap
Log-rank			0.015 vs. Soy	0.388 vs. Soy
Wilcoxon			0.035 vs. Soy	0.326 vs. Soy
Log-rank				0.434 vs. Oli
Wilcoxon				0.312 vs. Oli

[a]Litters of SHRSP rats (male) were distributed among different dietary groups and the test diets were fed from 4 wk of age. See Table 2 for other abbreviations.

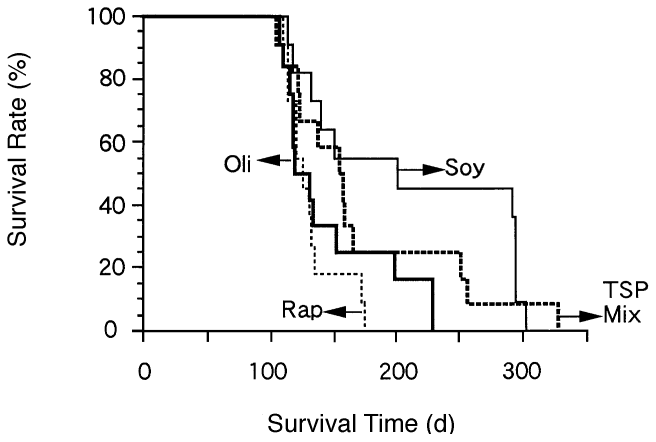

FIG. 1. Effect of vegetable oils on survival rate of stroke-prone spontaneously hypertensive (SHRSP) rats in the presence of 1% NaCl loading. A conventional basal diet containing 3% oil and test oil were mixed at a weight ratio of 9:1, and pelleted diet was fed to SHRSP rats from 4 wk of age. NaCl solution (1%) was given *ad libitum* as drinking water. Rapeseed oil (Rap, $n = 11$), olive oil (Oli, $n = 12$), soybean oil (Soy, $n = 11$), and triolein/soybean oil/perilla oil mixture (TSP Mix, $n = 12$) were examined. See Experiment 5 in Table 2 for detail of statistical analyses.

high-oleate safflower oil (75%), perilla oil (12.5%), and safflower oil (12.5%). The mixed oil was found to shorten the survival time by 45% compared to soybean oil similar to rapeseed oil. Earlier, we noted that perilla oil enhanced and safflower oil shortened the survival time of SHRSP rats by ~10% (1,2) compared with soybean oil. We suspected that the high-oleate safflower oil in the mixed oil was mainly responsible for the survival time-shortening activity of the mixed oil, since the high-oleate safflower oil was found to shorten the survival time similarly to the rapeseed oil (Experiment 2, Table 3). High-oleic sunflower oil was as effective as rapeseed oil, and high-erucic rapeseed oil reduced the survival time even more (Experiment 3). All the high-oleate vegetable oils examined (Experiments 1–3) were found to reduce the life expectancy of SHRSP rats.

When soybean oil and commercially available triolein, prepared from high-oleate sunflower oil, were mixed to adjust the oleate content to that of rapeseed oil, the linoleate content (32%) and α-linolenate content (3%) were slightly different from those of rapeseed oil (26% linoleate and 8% α-linolenate). The survival time of the triolein/soybean oil group was between those of the rapeseed oil and soybean oil groups (Experiment 4). The survival time of the safflower oil group was significantly less than that of the soybean oil group, as reported earlier (2). Because the number of animals was relatively small in Experiment 4, a similar experiment was performed using increased numbers of animals in Experiment 5 to test a triolein/soybean oil/perilla oil mixture. The survival times relative to that of soybean oil group (100) were 78% (triolein/soybean oil/perilla oil group), 72% (olive oil group), and 61% (rapeseed oil group) (Fig.1). The triolein/soybean oil/perilla oil mixture had almost the same fatty acid composition as that of rapeseed oil, but the survival time of this group tended to be longer than that of the rapeseed oil group, although the difference was not statistically significant.

In Experiment 5, the systolic blood pressure and pulse rate were measured at 4 and 8 wk of age and no significant differences among the dietary groups were detected (Table 4).

Effect of lard, vegetable oils, and a docosahexaenoic acid-rich fish oil on survival time of SHRSP rats in the absence of 1% NaCl loading. The survival times increased approximately by 80% when 1% NaCl solution was replaced with tap water. However, the survival time of the rapeseed oil group remained less, i.e., 61% of the soybean oil group (Experiment 6 in Table 5, Fig.2). Fatty acid composition of the evening primrose oil was much different from that of high-oleate vegetable oils (Table 1), but the survival time of this group was almost the same as that of the rapeseed oil group. The survival times of the perilla oil group and fish oil group were longer than that of the soybean oil group by 7 and 9%, respectively, but these differences were not statistically significant. In a similar experiment (Experiment 7), lard and safflower oil were found not to have such a marked survival time-shortening activity as observed with rapeseed oil, evening primrose oil, and other high-oleate vegetable oils (Table 3). Again, n-3 fatty acid-rich oils (fish oil and perilla oil) were beneficial for the survival of SHRSP rats as compared with lard and all other vegetable oils examined. The docosahexaenoate-rich fish oil, supplemented with soybean oil to meet the requirement for linoleate, gave the highest survival time.

Some attempts to separate the presumed survival time-shortening factor. Several fractions were prepared from rapeseed oil and tested to see if survival factor(s) could be removed

TABLE 4
Effect of Dietary Oils on Systolic Blood Pressure and Pulse Rate of SHRSP Rats under 1% NaCl Loading

	Dietary group[a] and number of animals			
	Rapeseed oil ($n = 11$)	Soybean oil ($n = 11$)	Olive oil ($n = 12$)	Triolein/soybean oil/perilla oil ($n = 12$)
At 4 wk of age				
Systolic blood pressure (mmHg)	157 ± 20	156 ± 23	154 ± 17	161 ± 21
Pulse rate (beat/min)	457 ± 75	431 ± 75	436 ± 70	416 ± 84
At 8 wk of age				
Systolic blood pressure (mmHg)	197 ± 16	198 ± 15	208 ± 15	192 ± 17
Pulse rate (beat/min)	382 ± 54	378 ± 36	366 ± 46	382 ± 45

[a]These dietary groups correspond to Experiment 5 in Table 2. See Table 2 for abbreviation.

TABLE 5
Survival Times of SHRSP Rats Fed Lard or Different oils Without NaCl Load

	Dietary group and number of animals				
Experiment 6	Rapeseed oil (Rap), $n = 12$	Evening primrose oil (Epo), $n = 12$	Soybean oil (Soy), $n = 11$	Perilla oil (Per), $n = 12$	Fish/soybean oil (Fis), $n = 12$
Survived day (± SD)	254 ± 12	261 ± 19	416 ± 16	445 ± 15	453 ± 16
P value by Log-rank		0.227 vs. Rap	0.000 vs. Rap	0.000 vs. Rap	0.000 vs. Rap
Wilcoxon		0.455 vs. Rap	0.000 vs. Rap	0.000 vs. Rap	0.000 vs. Rap
Log-rank			0.000 vs. Epo	0.000 vs. Epo	0.000 vs. Epo
Wilcoxon			0.000 vs. Epo	0.000 vs. Epo	0.000 vs. Epo
Log-rank				0.178 vs. Soy	0.078 vs. Soy
Wilcoxon				0.204 vs. Soy	0.106 vs. Soy
Log-rank					0.589 vs. Per
Wilcoxon					0.819 vs. Per
	Lard	Safflower oil	Perilla oil	Fish/soybean oil	
Experiment 7	$n = 11$	(Saf), $n = 11$	(Per), $n = 11$	(Fis), $n = 11$	
Survived day (± SD)	412 ± 10	404 ± 15	444 ± 13	488 ± 17	
P value by Log-rank		0.5854 vs. Lard	0.0219 vs. Lard	0.0006 vs. Lard	
Wilcoxon		0.8189 vs. Lard	0.0834 vs. Lard	0.0033 vs. Lard	
Log-rank			0.0217 vs. Saf	0.0010 vs. Saf	
Wilcoxon			0.0493 vs. Saf	0.0039 vs. Saf	
Log-rank				0.0153 vs. Per	
Wilcoxon				0.0545 vs. Per	

or isolated from the oil. Rapeseed oil was treated overnight at −70°C with acetone or ethanol, and the soluble fractions were mixed with soybean oil at a 5-fold concentration (in 1:5 the amount of the treated rapeseed oil). These fractions (acetone-soluble and ethanol-soluble in Table 2) did not shorten the survival time significantly. Similarly, methanol extracts of rapeseed oil (methanol-soluble), obtained at a room temperature, did not exhibit the survival time-shortening activity. Unsaponifiable materials from rapeseed oil were mixed with soybean oil at a 2.5-fold concentration (unsaponifiable fraction), and were found not to exhibit the activity.

Rapeseed oil (5.8 kg) in 9.5 L of hexane and 0.5 L of diethyl ether were mixed with 1 kg of silicic acid. The mixture was stirred overnight, and 5.22 kg of oil was recovered in the hexane/diethyl ether layer (silicic acid-treated). The survival time-shortening activity could not be removed. These experiments suggest that the survival time-shortening factor in rapeseed oil is relatively lipophilic, nonpolar in nature, and is closely associated with the triacylglycerols of the oil.

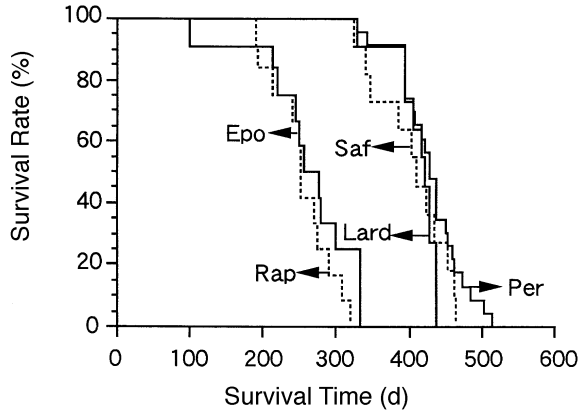

FIG. 2. Effect of vegetable oils on survival rate of stroke-prone spontaneously hypertensive (SHRSP) rats in the absence of 1% NaCl loading. A conventional basal diet containing 3% oil and test oil (lard) were mixed at a weight ratio of 9:1, and pelleted diet was fed to SHRSP rats from 4 wk of age, except for fish oil/soybean oil diet which was prepared in our laboratory (see text for details). Rapeseed oil (Rap, $n = 12$), evening primrose oil (Epo, $n = 12$), safflower oil (Saf, $n = 11$), perilla oil (Per, $n = 23$), or lard (Lard, $n = 11$) were examined. Tap water was given *ad libitum* as drinking water. See Table 4 for details of statistical analyses.

DISCUSSION

The survival time-shortening activity (4) was found not to be confined to rapeseed oil; high-oleate safflower oil, high-oleate sunflower oil, evening primrose oil, and olive oil exhibited comparable activities. Oleate esters have been implicated in certain disease conditions such as atherosclerotic plaques where cholesterol oleate accumulated as the major molecular species (10). However, in this case a high-oleate content itself does not appear to be crucial for this animal model because lard and a microbial oil containing 36–39% oleic acid were relatively safe, while evening primrose oil, containing only 13% oleic acid, was detrimental (Ref. 4 and Table 5). The survival time of the group fed a triolein/soybean oil mixture with an oleate content similar to that of rapeseed oil was between those fed soybean oil and rapeseed oil (Experiment 4 in Table 3). Similarly, the survival time of the group fed the triolein/soybean oil/perilla oil mixture, with a fatty acid composition very similar to that of rapeseed oil, was also found to be between those fed soybean oil and rapeseed oil, although the difference between the mixed oil group and rapeseed oil group was not statistically significant (Experiment 5 in

Table 3). Based on these results, it appears unlikely that the observed survival time-shortening activity is due to the unique fatty acid compositions of these oils. However, it should be noted that the triolein used to make up the two mixed oils was derived from high-oleate sunflower oil. If we presume that rapeseed oil and the other high-oleate vegetable oils contain detrimental factor(s) for SHRSP rats, then the factor(s) may have partially carried over from these oils through the purification steps.

Minor components other than triacylglycerol in high-oleic acid vegetable oils, such as olive oil and high-oleate safflower oil, have been implicated in a number of studies. A high-oleate safflower oil was shown to suppress mammary and colorectal carcinogenesis compared to high-linoleate safflower oil (11), whereas both olive oil and safflower oil were reported to stimulate colorectal carcinogenesis in contrast to perilla oil (12,13). Various preparations of olive oil are known to differ in their contents of minor components and the one used by Onogi *et al.* (12) might have contained a larger quantity of minor components which stimulated carcinogenesis, e.g., β-carotene which was recently shown in clinical trials to increase the incidence of certain types of cancers (14). In fact, olive oil had been shown earlier to suppress colon carcinogenesis (15).

If physiologically active minor components are present in some vegetable oils, they are lipophilic in nature and could affect the central nervous functions after being delivered to the brain through the blood brain barrier. In fact, we found unusual behavioral patterns in rapeseed oil-fed mice as compared with those fed several other kinds of vegetable oils. The phenomena could not be accounted for simply by the differences in the fatty acid compositions, or ratio of linoleate to α-linolenate (16). We have no evidence so far for the interpretation that the presumed survival time-shortening factor also affected the behavior of mice. However, some of the results of behavioral studies in rodents fed different vegetable oils are apparently inconsistent among different laboratories (17–19). The vegetable oils with significant survival-time shortening activities were fed to animals in some behavioral studies: rapeseed oil (18,20–22) and olive oil (17). Apparent discrepancies reported for the effects of vegetable oils on behavior of rodents may have originated, at least in part, from differences in minor components other than fatty acids in the oils.

Compared to Danish people, the incidence of thrombotic diseases in native Greenlanders (Inuit) was less than one-tenth, but that of apoplexy was slightly higher (23,24). Therefore, a long-term ingestion of large amounts of fish oil was suspected to be a risk factor for apoplexy. However, this has not been confirmed by epidemiologic studies (25); although n-3 fatty acid supplementation lengthens bleeding time, there is no evidence to suggest that it causes clinically significant bleeding episodes (25). Consistently, our present (Table 5) and previous studies (2,26) have shown that long-term feedings of fish oil and perilla oil at 10 w/w% of diet were beneficial for the suppression of the development of apoplexy in SHRSP rats.

Rapeseed oil (double-low) has been recently evaluated for possible nutritional benefits associated with its relatively low n-6/n-3 ratio, high-oleate content, and low level of saturated fatty acids. In fact, dietary rapeseed oil and olive oil resulted in decreased n-6 fatty acids with concomitant increases in oleic and n-3 fatty acids, which proved to be very effective for the secondary prevention of coronary heart disease (27). However, the observed beneficial effect of the combination of rapeseed oil and olive oil on coronary heart disease is interpreted to be due to their relatively low linoleic acid contents and n-6/n-3 ratios (28). We need to solve the problem of the survival-time shortening activity observed in SHRSP rats before advising people to consume a large amount of rapeseed oil and olive oil. At present, we need to evaluate carefully the potential merits and problems of high-oleate vegetable oils, including rapeseed oil, in human nutrition.

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