Free Fatty Acid Fractions from Some Vegetable Oils Exhibit Reduced Survival Time-Shortening Activity in Stroke-Prone Spontaneously Hypertensive Rats

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ABSTRACT: Previously, we demonstrated that several vegetable oils that included low-erucic rapeseed oil markedly shortened the survival time (by ~40%) of stroke-prone spontaneously hypertensive (SHRSP) rats as compared with perilla oil, soybean oil, and fish oil. We considered that a factor other than fatty acids is toxic to SHRSP rats, because the survival time-shortening activity could not be accounted for by the fatty acid compositions of these oils. In fact, a free fatty acid (FFA) fraction derived from lipase-treated rapeseed oil was found to be essentially devoid of such activity. A high-oleate safflower oil/safflower oil/perilla oil mixture exhibited a survival time-shortening activity comparable to that of rapeseed oil, but the activity of this mixed oil was also reduced by lipase treatment. A partially hydrogenated soybean oil shortened the survival time by ~40%, but a FFA fraction derived from lipase-treated partially hydrogenated soybean oil shortened it by 13% compared with soybean oil. Fatty acid compositions of the rapeseed oil and a FFA fraction derived from lipase-treated rapeseed oil were similar, but those of hepatic phospholipids of rats fed the oil and FFA were slightly but significantly different. These results support the interpretation that the survival time-shortening activity exhibited by some vegetable oils is due to minor components other than fatty acids, and that an active component(s) were produced in or contaminated soybean oil during the partial hydrogenation processes.

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Perilla seed oil and fish oil rich in n-3 fatty acids have beneficial effects on animals used as models of chronic diseases of the elderly (1). Perilla oil prolonged the survival time of Donryu rats (a conventional strain) and stroke-prone spontaneously hypertensive (SHRSP) rats (a model of human stroke) by ~10 and ~15%, respectively, compared with safflower oil (2,3). The observed beneficial effects of these oils were attributed to their low n-6/n-3 ratios. Rapeseed oil (low-erucic) had been assumed to be beneficial for chronic diseases because of its relatively high oleate and α-linolenate, and low linoleate contents. Dietary rapeseed (low erucic) and olive oils were very effective for the secondary prevention of coronary heart disease (4). Unexpectedly, however, rapeseed oil shortened the survival time of SHRSP rats (by 40%) compared with soybean, safflower, perilla and fish oils (5–7).

Although an old-type rapeseed contained very high amounts of erucic acid and thyrotoxic sulfur compounds, oil made from a newly developed strain of rapeseed (double-low) contains much lower amounts of these compounds. However, Vles *et al*. (8) reported that even the double-low rapeseed oil induced myocardial necrosis in Sprague-Dawley rats. Subsequently, the incidence of myocardial necrosis was attributed to the unique fatty acid composition of double-low rapeseed oil because the incidence was positively correlated with oleic and α-linolenic acids and negatively correlated with saturated fatty acids (9). In regard to survival time-shortening activity, the positive and negative correlations observed for myocardial necrosis were not applicable because perilla oil, which prolonged the survival time of SHRSP rats, contained a similar proportion of saturated fatty acids and a higher proportion of α-linolenic acid than rapeseed oil (6).

The survival time-shortening activity was not restricted to rapeseed oil; other high-oleate vegetable oils such as high-oleate safflower, high-oleate sunflower, and olive oils were also active in this respect. A high-oleate content by itself was not responsible for the survival-time shortening activity because lard and a microbial oil containing 36-39% oleic acid were relatively safe in this animal model, and evening primrose oil containing as little as 15% oleic acid exhibited activity similar to that of the oils which reduced longevity. Therefore, the observed survival time-shortening activities of these vegetable oils could not be accounted for by their unique fatty acid compositions. We postulated the presence in these oils of a factor(s) that are toxic to SHRSP rats (6). Here, we report that FFA fractions derived from some of these oils had a lower survival time-shortening activity, which may help in identifying and developing the means to eliminate the active component(s).

MATERIALS AND METHODS

Diets and animals. A conventional basal diet containing 2.7% (w/w) oil (CE-2; Clea Japan Co., Ltd., Tokyo, Japan) was made of fish meal, soybean meal, defatted powdered milk,

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Abbreviations: FFA, free fatty acids; SHRSP, a strain of stroke-prone spontaneously hypertensive rats; SPF, specific pathogen-free; TLC, thin-layer chromatography.

wheat flour, corn, wheat bran, alfalfa meal, a vitamin mixture, and a mineral mixture. This basal diet was mixed with a vegetable oil or a derived free fatty acid (FFA) fraction in a 9:1 weight ratio. The final oil content was 12.7 wt% (29 energy %). The following vegetable oils, commercially available for human use, were used; rapeseed (low-erucic), soybean, safflower (high-linoleate), high-oleate safflower, perilla, sesame, and partially hydrogenated soybean (melting point: 30°C) oils. The source for the preparation of oleic acid ethyl ester (Wako Pure Chemical Industry, Osaka, Japan) is unclear. The fatty acid composition of the diets is shown in Table 1. Diets supplemented with these oils were kept at 4°C for less than 1 mon. Diets were replaced every 2 d to keep the peroxide values of the fed diets below 100 meq/kg.

A conventional strain of SHRSP rats, which were kindly provided by Dr. T. Suzuki (Kinki University School of Medicine, Osaka, Japan), was used in the experiment shown in Figure 1 and a specific pathogen-free (SPF) strain of SHRSP rats, which were purchased from Seack Yoshitomi Co. (Fukuoka, Japan), was used in other experiments. Blood pressure was measured every 2 wk using a photoelectric tail-cuff procedure after prewarming the rats at 35°C for 5 min in a thermostatic cage (Model UR5000; Ueda Manufacturing Co. Ltd., Tokyo, Japan). Male littermates were, as far as possible, equally distributed in different dietary groups. Rats were weaned to the conventional diet (CE-2) at 3 wk of age, and the test diets were given from 4 wk of age. The diet and 1% NaCl solution were given *ad libitum*. In the experiment shown in Figure 1, rats were kept in an air-conditioned room at 23 ± 3°C. In other experiments, rats were kept in a room specified for SPF animals; temperature and humidity were 23 ± 2°C and at 50 ± 2%, respectively.

Lipase and detergent treatment of vegetable oils. Vegetable oils and a partially hydrogenated soybean oil were dissolved at 4°C in polyvinyl alcohol solution (4.5 L) containing 1.8% polyvinyl alcohol 117 and 0.2% polyvinyl alcohol 205 (kindly provided by Kuraray Co., Ltd., Osaka, Japan). Sucrose fatty acid ester (2%) was used only for the preparation of detergent-treated hydrogenated soybean oil without added lipase (Experiment 3, Table 3). Then 0.1 M phosphate buffer, pH 8.0 (3 L), with or without 0.1% (wt/vol) Lipase AY 30 (90,000 I.U./3 g/3 L; Amano, Nagoya, Japan), was added and the mixture was stirred at room temperature or 35°C for 1 wk. Triacylglycerols in rapeseed oil, partially hydrogenated soybean oil, and a mixture of high-oleate safflower oil/safflower oil/perilla oil were hydrolyzed to FFA in 90.6, 86.3, and 91.7% of the total ester bonds, respectively. After the reaction, hexane (7.5 L) and NaCl (3 kg) were added, the hexane layer was separated, and the solvent was evaporated under reduced pressure to obtain FFA fractions or detergent-treated oils.

Lipid analysis. SHRSP rats ($n = 6$) in the rapeseed oil, soybean oil or partially hydrogenated soybean oil group were used for lipid analysis after 60 d of feeding. The SHRSP rats were anesthetized with pentobarbital (Nembutal, 50 mg/kg, i.p.; Dainabot, Osaka, Japan) after fasting for 18 h. Blood samples were collected from an abdominal vein using a syringe containing 3.8% (wt/vol) sodium citrate, and plasma was prepared by low-speed centrifugation. The thoracic aorta was excised, and the attached fat tissues were removed with the aid of a microscope. Samples were kept frozen at −80°C until lipid analysis. The total lipids were extracted from the samples according to the method of Bligh and Dyer (10), and triacylglycerols and phospholipids were separated by silica

TABLE 1
Fatty Acid Composition of the Experimental Diets[a] (% of total fatty acids)

Fatty acids[b]	Soybean oil	Rapeseed oil	Ethyl oleate/ soybean oil perilla oil[c]	Hydrogenated soybean oil (HSO)	FFA from lipase-treated rapeseed oil[d]	FFA from lipase-treated mixed oil[d,e]	FFA from lipase-treated HSO[d]	Sesame oil
14:0	0.3	0.3	0.8	0.9	0.6	0.3	0.4	0.3
16:0	12.2	7.8	9.6	13.1	10.4	9.2	11.1	10.6
16:1	0.3	0.4	0.5	1.0	0.4	0.4	0.5	0.5
18:0	3.5	2.0	2.1	5.3	2.3	2.0	6.0	4.2
*c*18:1	22.5	49.8	50.3	31.8	50.3	48.0	36.4	35.3
*t*18:1	n.d.[f]	n.d.	n.d.	14.1	n.d.	n.d.	15.6	n.d.
*tt*18:2	n.d.	n.d.	n.d.	0.9	n.d.	n.d.	1.8	n.d.
18:2n-6	51.6	27.9	26.7	27.3	27.0	27.3	22.2	44.6
18:3n-3	6.6	7.7	6.8	1.5	3.5	9.2	2.7	1.2
20:0	0.4	0.5	0.4	0.3	0.8	0.3	0.4	0.4
20:1	0.6	1.5	0.5	0.8	0.9	1.3	0.7	0.7
20:5n-3	0.6	0.6	0.7	1.3	1.1	0.6	0.6	0.6
22:0	0.4	0.3	0.4	0.6	1.3	0.3	0.5	0.3
22:1	0.2	0.3	0.4	0.4	0.4	0.3	0.2	0.4
22:6n-3	0.8	0.8	0.8	0.7	1.0	0.8	0.9	0.9

[a]Fatty acids are designated by the number of carbons; the number of double bonds, and the position of the first double bond numbered from the methyl terminus is indicated as n-3 or n-6, and *cis* or *trans* isomers are designated as *c* or *t*.
[b]Ninety grams of conventional diet that contained 2.7% oil was mixed with 10 g of oil or of free fatty acids (FFA) fraction from lipase-treated oils.
[c]Oleic acid ethyl ester/soybean oil/.perilla oil mixture was prepared by mixing oleic acid ethyl ester (57.2%), soybean oil (27.8%), and perilla oil (14.8%).
[d]See Materials and Methods section for the preparation of FFA fractions from lipase-treated oils.
[e]This mixed oil consisted of high-oleate safflower (75%), safflower (12.5%), and perilla oils (12.5%).
[f]n.d., not detected.

gel thin-layer chromatography (TLC). Petroleum ether/diethyl ether/acetic acid (80:30:1, by vol) was used as the developing solvent. Spots were located under a ultraviolet light after plates were sprayed with 0.005% (wt/vol) primuline solution (Nacalai Tesque Co., Kyoto, Japan) and the lipids were extracted from the adsorbent using Bligh and Dyer mixture. Fatty acids were analyzed as methyl esters by gas–liquid chromatography on a capillary column coated with DB-225 (0.2 mm, 30 m length; J&W Scientific, Folsom, CA), using heptadecanoic acid as an internal standard. Column temperature was programmed from 165 to 205°C at a rate of 3°C/min, then to 210°C at a rate of 1°C/min, and then to 222°C at a rate of 0.5°C/min. Injector and detector temperatures were both 250°C. The *trans*- and *cis*-18:1 contents were determined using $AgNO_3$–TLC in conjunction with gas–liquid chromatography. The $AgNO_3$-TLC analyses were performed on precoated silica gel TLC plates that had been immersed in a 10% (wt/vol) solution of $AgNO_3$ in acetonitrile, dried horizontally, and activated at 110°C for 1 h. The plates were developed with 0.75% ethanol in chloroform. The lipid bands were sprayed with primuline solution and then identified under an ultraviolet UV light. The separated fractions were scraped off the plate, and the lipids were extracted with chloroform/hexane (1:1, vol/vol) and analyzed by gas–liquid chromatography (7,11).

Statistical analysis. Data are presented as means ± SD. Statistical analysis of the survival time data was performed by log-rank and Wilcoxon signed rank method (a nonparametric method) using a computer program JMP 3.0, Statistic Made Visual (SAS Institute, Cray, NC). Statistical analysis of other data was performed by analysis of variance using Bonferroni's multiple comparison or repeated measures analysis of variance (Stat View J-4.11; Abacus Concepts Inc., Berkeley, CA).

RESULTS

Effect of oils and derived FFA fractions on survival time of SHRSP rats. Rats in all groups grew normally, and there were no differences in average body weights (data not shown) and systolic blood pressures (Fig. 1) among the rats in the rapeseed oil, soybean oil, and partially hydrogenated soybean oil groups and rats fed the FFA derived from lipase-treated rapeseed oil up to 13 wk of age. Rapeseed oil shortened the survival time of SHRSP rats by 35% compared to soybean oil (Fig. 2). In contrast, the FFA fraction derived from lipase-treated rapeseed oil did not exhibit such a survival time-shortening activity, although fatty acid compositions were not altered significantly by the lipase-treatment (Table 1). Partially hydrogenated soybean oil was as detrimental as rapeseed oil (Experiment 1, Table 2, and Fig. 2). When a triolein/soybean oil/perilla oil mixture with the same fatty acid composition as that of rapeseed oil was fed to SHRSP rats, the survival time of this mixed oil group was between those of the rapeseed oil and soybean oil groups (6). Similarly, the survival time of the oleic acid ethyl ester/soybean oil/perilla oil mixture group was between the rapeseed oil and soybean oil groups (Experiment 1, Table 1).

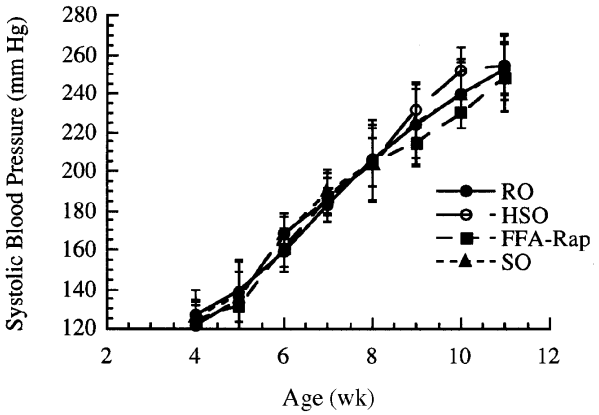

FIG. 1. Systolic blood pressure of stroke-prone spontaneously hypertensive (SHRSP) specific pathogen-free (SPF) rats under 1% NaCl loading. A diet containing 10% (w/w) rapeseed oil (RO), hydrogenated soybean oil (HSO), soybean oil (SO), or a FFA fraction derived from lipase-treated rapeseed oil (FFA-Rap) was fed to SHRSP rats from 4 wk of age. The differences among the dietary groups were not significant in the repeated measures ANOVA.

To minimize the standard deviation among experiments, we changed in subsequent experiments from using a conventional strain of SHRSP rats kept in a temperature-controlled room to SPF SHRSP rats kept in temperature, humidity, and light-controlled conditions. The difference in the mean survival times between the rapeseed oil and soybean oil groups was smaller in SPF SHRSP (Experiment 2, Table 2) than in the conventional SHRSP rats (Experiments 1, Table 2); the former had a higher blood pressure (~40 mm Hg) and appeared to be more sensitive to dietary conditions (e.g., 1%

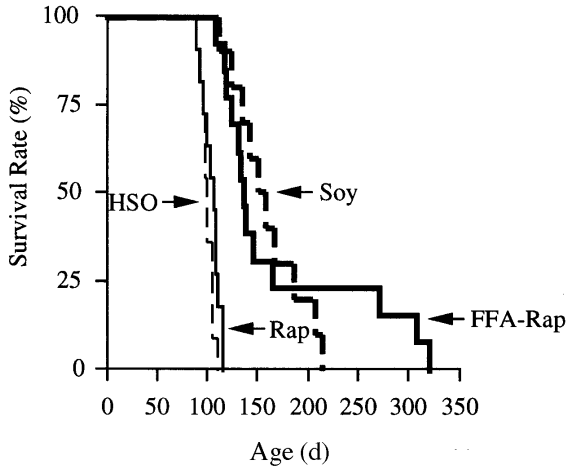

FIG. 2. Survival time-shortening activities of some vegetable oils and a free fatty acid (FFA) derived from lipase-treated rapeseed oil. Diets supplemented with different oils were fed to SHRSP rats (conventional) from 4 wk of age. NaCl solution (1%) was given *ad libitum* as drinking water. Partially hydrogenated soybean oil (HSO, $n = 11$), rapeseed oil (Rap, $n = 11$), soybean oil (Soy, $n = 10$), and a FFA derived from lipase-treated rapeseed oil (FFA-Rap, $n = 11$) were examined. Results of statistical analysis were presented in Experiment 1, Table 2. For other abbreviations see Figure 2.

TABLE 2
Survival Times of Conventional or SPF SHRSP Rats Fed Different Oils Under 1% NaCl Loading[a]

	Dietary group and number of animals				
Experiment 1 (SHRSP, conventional)	Rapeseed oil (Rap) (n = 11)	Soybean oil (Soy) (n = 10)	Hydrogenated soybean oil (HSO) (n = 11)	FFA from lipase-treated rapeseed oil (FFA-Rap) (n = 11)	Oleic acid ethyl ester/ soybean oil/perilla oil (n = 11)
Survived Day (±SD)	104 ± 3	160 ± 11	100 ± 2	145 ± 13	118 ± 6
P value by log-rank		0.0000 vs. Rap	0.1285 vs. Rap	0.0000 vs. Rap	0.0485 vs. Rap
Wilcoxon		0.0000 vs. Rap	0.2348 vs. Rap	0.0000 vs. Rap	0.0905 vs. Rap
Log-rank			0.0000 vs. Soy	0.0000 vs. Soy	0.0028 vs. Soy
Wilcoxon			0.0000 vs. Soy	0.0000 vs. Soy	0.0018 vs. Soy
Log-rank				0.0000 vs. HSO	0.0027 vs. HSO
Wilcoxon				0.0000 vs. HSO	0.0032 vs. HSO
Log-rank					0.0957 vs. FFA-Rap
Wilcoxon					0.0239 vs. FFA-Rap
Experiment 2 (SHRSP, SPF)	Rapeseed oil (Rap) (n = 11)	Soybean oil (Soy) (n = 11)	Sesame oil (Ses) (n = 11)	FFA from lipase-treated hydrogenated soybean oil (FFA-HSO) (n = 11)	FFA from lipase-treated mixed oil (n = 11)
Survived Day (±SD)	106 ± 3	130 ± 11	129 ± 8	124 ± 2	143 ± 3
P value by log-rank		0.0433 vs. Rap	0.0005 vs. Rap	0.0000 vs. Rap	0.0013 vs. Rap
Wilcoxon		0.0335 vs. Rap	0.0002 vs. Rap	0.0000 vs. Rap	0.0002 vs. Rap
Log-rank			0.6535 vs. Soy	0.7857 vs. Soy	0.7839 vs. Soy
Wilcoxon			0.1069 vs. Soy	0.0707 vs. Soy	0.0606 vs. Soy
Log-rank				0.9260 vs. Ses	0.0404 vs. Ses
Wilcoxon				0.4678 vs. Ses	0.0012 vs. Ses
Log-rank					0.0000 vs. FFA-HSO
Wilcoxon					0.0001 vs. FFA-HSO
Experiment 3 (SHRSP, SPF)	Rapeseed oil (Rap) (n = 9)	Soybean oil (Soy) (n = 9)	Detergent-treated rapeseed oil (Det-Rap) (n = 10)	Detergent-treated hydrogenated soybean oil (n = 9)	
Survived Day (±SD)	105 ± 3	121 ± 8	102 ± 2	98 ± 2	
P value by log-rank		0.0278 vs. Rap	0.0105 vs. Rap	0.0234 vs. Rap	
Wilcoxon		0.0466 vs. Rap	0.0329 vs. Rap	0.0225 vs. Rap	
Log-rank			0.0057 vs. Soy	0.0003 vs. Soy	
Wilcoxon			0.0165 vs. Soy	0.0020 vs. Soy	
Log-rank				0.2621 vs. Det-Rap	
Wilcoxon				0.5667 vs. Det-Rap	

[a]SPF, specific pathogen-free; SHRSP, stroke-prone spontaneously hypertensive rats.

NaCl loading). Despite the differences in substrains of SHRSP and in animal care conditions, a statistically significant difference was observed in the mean survival times between the soybean oil and rapeseed oil groups. Previously, we noted that a mixed oil (75% high-oleate safflower oil, 12.5% perilla oil, and 12.5% safflower oil) with a fatty acid composition similar to that of rapeseed oil shortened the survival time by 45% compared to soybean oil (6). A FFA fraction obtained by lipase treatment of the same mixture was essentially devoid of the survival time-shortening activity (Experiment 2, Table 2). The survival times of sesame oil and soybean oil groups were similar but tended to be shorter than that of rats fed the FFA fraction derived from a lipase-treated mixed oil (Experiment 2, Table 2, and Fig. 3). Previously, we noted that both hydrogenated soybean and rapeseed oils had survival time-shortening activities comparable to that of rapeseed oil (7). The FFA fraction derived from lipase-treated hydrogenated soybean oil prolonged the survival time compared with rapeseed oil. However, the survival time of rats fed the FFA fraction derived from lipase-treated hydrogenated soybean oil was significantly shorter than that of rats fed the FFA fraction derived from lipase-treated mixed oil (Experiment 2, Table 2).

The lipase treatment used for the preparation of FFA fractions involves both lipase and detergent action. The subsequent experiments (Experiment 3, Table 2) examined whether detergent treatment without added lipase removes the presumed survival time-shortening factor. Rapeseed and partially hydrogenated soybean oils were treated with detergents in the absence of lipase. The detergent treatment alone did not reduce the survival time-shortening activity of the oils (Experiment 3, Table 2).

Effect of oils and derived FFA fractions on tissue lipid composition. Lipid contents in plasma, aorta, liver, and heart were determined for the rats fed the rapeseed oil, FFA derived from lipase-treated rapeseed oil, partially hydrogenated soybean oil, or soybean oil. No significant difference was observed in the contents of plasma cholesterol, plasma triacylglycerols, plasma phospholipids, aortic cholesterol, aortic

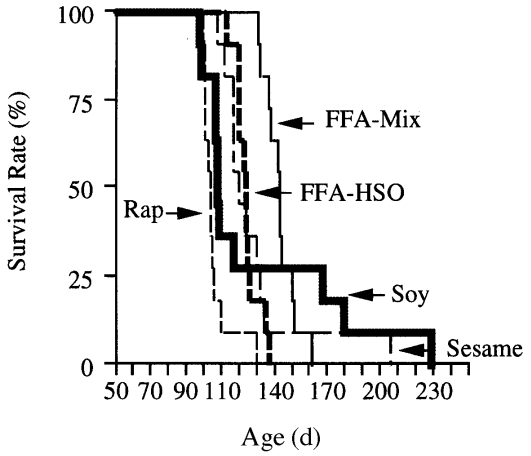

FIG. 3. Survival time-shortening activities of some vegetable oils and FFA fractions derived from lipase-treated oils. Rapeseed oil (Rap, n = 11), soybean oil (Soy, n = 11), sesame oil (Sesame, n = 11), a FFA derived from lipase-treated partially hydrogenated soybean oil (FFA-HSO, n = 11), and a FFA derived from lipase-treated mixed oil (FFA-Mix, n-11) were fed to SHRSP rats (SPF). Results of statistical analysis were presented in Experiment 2, Table 2. For other abbreviations see Figure 1.

phospholipids, liver phospholipids, liver neutral lipids, heart phospholipids, and heart neutral lipids among the four dietary groups (data not shown). The fatty acid compositions of tissue phospholipids are shown in Tables 3 and 4. *Trans* fatty acids were found in significant amounts only in the hydrogenated soybean oil group, and the compositions of other fatty acids in tissue phospholipids were affected relatively little by these diets. Interestingly, in plasma phospholipids and hepatic phospholipids, the proportion of arachidonic acid was significantly higher in the group fed the FFA derived from lipase-treated rapeseed oil than in the group fed the rapeseed oil (Tables 3 and 4). In contrast, no significant differences in fatty acid compositions among the four dietary groups were observed in the aortic phospholipids and triacylglycerols of all tissues examined (data not shown).

DISCUSSION

No survival time-shortening activity was observed in the FFA fractions from lipase-treated rapeseed oil (Fig. 1 and Experiment 1, Table 2) and mixed oil having a fatty acid composition similar to that of rapeseed oil (Experiment 2, Table 2). The chemical forms of the oils were different, e.g., triacylglycerol vs. free fatty acid, but fatty acid compositions were similar. Triacylglycerols are absorbed as 2-monoacylglycerols and FFA after pancreatic lipase hydrolysis, and the absorption rate of FFA is known to be higher than that of triacylglycerols (12). In the present experiments, no statistical differences were observed in tissue lipid contents and growth rates between the groups fed oils and those fed the FFA fractions derived from the lipase-treated oils. Our interpretation is that the presumed toxic factor(s) in these oils were inactivated by the lipase or they were hydrolyzed to a more hydrophilic compound(s) that remained in the detergent layer, or both. Lipase action was probably involved to obtain FFA fractions essentially devoid of survival time-shortening activity, because the activity was not diminished by treating oils with detergent-containing buffer without lipase.

The presumed toxic factor(s) in rapeseed oil could not be

TABLE 3
Fatty Acid Composition of Plama Phospholipids in SHRSP Rats (% of total fatty acids)

	Dietary group and number of animals			
Fatty acids	Rapeseed oil (n = 6)	FFA from lipase-treated rapeseed oil (n = 6)	Hydrogenated soybean oil (n = 6)	Soybean oil
14:0	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
16:0	20.9 ± 0.9	21.3 ± 0.8	20.0 ± 1.7	21.1 ± 1.3
16:1	1.1 ± 0.5	1.5 ± 0.5	1.3 ± 0.5	1.3 ± 0.4
18:0	29.3 ± 2.7[a]	28.2 ± 0.7[b]	24.4 ± 1.3[b]	29.8 ± 1.5[a]
*c*18:1	10.5 ± 1.5[a]	8.6 ± 0.5[a]	8.6 ± 2.6[b]	6.7 ± 1.4[b]
t18:1	n.d.[a]	trace	6.1 ± 0.6[a]	n.d.
18:2n-6	10.9 ± 1.4[a,b]	9.7 ± 0.5[b]	12.4 ± 1.4[a]	12.7 ± 1.6[a,b]
18:3n-6	0.2 ± 0.2	0.4 ± 0.4	0.2 ± 0.5	0.3 ± 0.2
18:3n-3	0.4 ± 0.2	0.5 ± 0.2	0.6 ± 0.4	1.2 ± 0.9
20:0	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2
20:3n-6	0.7 ± 0.4[b]	0.6 ± 0.1[b]	1.5 ± 0.8[a]	1.0 ± 0.5[a,b]
20:4n-6	14.9 ± 1.4[a,b]	18.4 ± 1.4[a]	13.1 ± 1.8[b]	15.3 ± 1.2[b]
22:0	0.9 ± 0.5	0.7 ± 0.1	0.8 ± 0.2	0.7 ± 0.1
22:4n-6	0.4 ± 0.3	0.5 ± 0.2	0.9 ± 0.1	0.7 ± 0.3
22:5n-3	0.4 ± 0.5	0.3 ± 0.3	1.0 ± 0.3	0.9 ± 0.3
22:6n-3	3.7 ± 0.9	4.1 ± 0.5	5.0 ± 1.4	3.9 ± 0.5
24:0	1.9 ± 0.4	1.7 ± 0.2	1.9 ± 0.5	1.8 ± 0.2
24:1	3.1 ± 0.8[a]	2.9 ± 0.4[a]	1.9 ± 0.5[b]	1.6 ± 0.4[b]
PL level (mg/dL)	68.6 ± 9.0	70.3 ± 4.1	68.6 ± 9.5	71.6 ± 6.9

[a]n.d., not detected; PL, phospholipids; for other abbreviations see Tables 1 and 2. Values with different roman superscripts are significantly different from each other at $P < 0.05$.

TABLE 4
Fatty Acid Composition of Hepatic Phospholipids in SHRSP Rats[a] (% of total fatty acids)

Fatty acids	Dietary group and number of animals			
	Rapeseed oil (n = 6)	FFA from lipase-treated rapeseed oil (n = 6)	Hydrogenated soybean oil (n = 6)	Soybean oil (n = 6)
14:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:0	16.1 ± 1.2	14.9 ± 1.0	16.5 ± 0.8	16.45 ± 0.5
16:1	0.2 ± 0.1[a]	0.2 ± 0.1[a]	0.3 ± 0.0[a]	0.1 ± 0.1[b]
18:0DMA[b]	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.1	0.2 ± 0.0
18:1DMA	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
18:0	27.8 ± 1.8[a]	26.6 ± 1.1[a]	23.4 ± 0.6[b]	28.9 ± 1.5[a]
*c*18:1	9.1 ± 0.7[a]	7.9 ± 0.8[a]	8.3 ± 0.9[a]	4.7 ± 1.0[b]
*t*18:1	n.d.	n.d.	4.0 ± 0.9[a]	n.d.
18:2n-6	11.8 ± 0.4[b]	11.4 ± 0.8[b]	13.8 ± 1.2[a]	14.5 ± 1.5[a]
18:3n-6	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
18:3n-3	0.4 ± 0.1[a]	0.3 ± 0.0[a,b]	0.2 ± 0.0[c]	0.3 ± 0.0[b]
20:0	0.3 ± 0.1[a]	0.2 ± 0.0[a]	0.1 ± 0.0[b]	0.1 ± 0.0[b]
20:1	0.3 ± 0.0[a]	0.2 ± 0.1[a,b]	n.d.	0.1 ± 0.0[b,c]
20:3n-6	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	0.2 ± 0.3
20:4n-6	21.8 ± 2.8[b]	27.4 ± 0.8[a]	20.3 ± 1.3[b]	23.1 ± 1.2[b]
20:5n-3	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
22:0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
22:4n-6	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
22:5n-6	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.2 ± 0.1
22:5n-3	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.2
22:6n-3	8.7 ± 0.5[a,b]	10.1 ± 0.8[a]	9.9 ± 1.5[a,b]	8.4 ± 0.5[b]
24:0	0.6 ± 0.1[b,c]	0.5 ± 0.0[c]	0.7 ± 0.0[a,b]	0.8 ± 0.1[a]
24:1	0.5 ± 0.1[a]	0.5 ± 0.1[a]	0.3 ± 0.0[b]	0.2 ± 0.1[b]
PL level (mg/g liver)	19.2 ± 0.7	21.9 ± 1.4	18.7 ± 2.2	20.4 ± 4.2

[a]Values with different roman superscripts are significantly different from each other at $P < 0.05$.

[b]DMA, dimethylacetal. For other abbreviations see Tables 1–3.

eliminated or inactivated by partial hydrogenation (7). Unexpectedly, partially hydrogenated soybean oil exhibited a survival time-shortening activity similar to rapeseed oil, suggesting that a toxic factor(s) are generated in or contaminate partially hydrogenated soybean oil during the process of production (Fig. 1 and Ref. 7). The survival time of rats fed the FFA fraction derived from lipase-treated partially hydrogenated soybean oil was significantly shorter than that of rats fed the FFA fraction derived from a mixture of high-oleate safflower oil/safflower oil/perilla oil (Table 2), suggesting that *trans* fatty acids may be partially responsible for the survival time-shortening activity. The lipase treatment was also effective for the preparation of FFA fraction from hydrogenated oils (Table 2).

Several other studies have shown or suggested the existence of minor components other than triacylglycerol in rapeseed oil. Among sulfur compounds, isothiocyanates and oxazolidinethione do not appear to be responsible for the survival time-shortening activity, because butyl isothiocyanate (8 mg/kg of soybean oil), phenethyl isothiocyanate (1 mg/kg), and allyl isothiocyanate (1 mg/kg) did not shorten the survival time at concentrations comparable to those in rapeseed oil (5). Rapeseed oil has a significantly higher phytosterol content than soybean oil (13). However, unsaponifiable compounds from rapeseed oil did not exhibit survival time-shortening activity and no significant amounts of phytosterols were found in tissue lipids (data not shown). We suggest that the toxic factor(s), if any, are different from those described above and are sensitive to lipase action.

Mean survival time of experimental animals has not been a common endpoint for the determination of the safety or nutritional adequacy of fats and oils. By using this measure, we raised a question on the safety of some of the common oils and oil products in human nutrition, although the applicability of the results to human nutrition is entirely unknown. Besides shortened survival time, we observed accelerated renal injury in SHRSP rats fed rapeseed oil and partially hydrogenated oil (Miyazaki, M., Watanabe, S., Oikawa, T., Morazumi, K., Fuzinami, T., and Okuyama, H., unpublished data). Other groups have also raised questions on the safety of some common vegetable oils using different endpoints; for example, increased mortality after injection of iron into piglets fed a rapeseed oil-supplemented, tocopherol-restricted diet (14), and reduced platelet counts in piglets fed rapeseed oil- or high-oleate vegetable oil-supplemented diets (15,16). The problem of myocardial necrosis reported in rapeseed oil-fed Sprague-Dawley rats remains unresolved (8,9). The lipase-treatment and successful preparation of FFA fraction essentially devoid of survival time-shortening activity of the original oils described in this paper may help in solving these unresolved problems raised by other laboratories.

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