

## Dietary Canola and Soybean Oil Fed to SHRSP Rat Dams Differently Affect the Growth and Survival of Their Male Pups<sup>1</sup>

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**ABSTRACT** Canola oil (Can), as well as some other oils, shortens the survival of SHRSP rats compared with soybean oil (Soy). Although detrimental factors other than phytosterols have not been identified, they are likely to be hydrophobic and transmittable to pups. To test this possibility, female SHRSP rats (F0) were fed a diet supplemented with Can or Soy and mated at 11 wk of age. The growth of suckling pups (F1) from the Can-fed dams was significantly retarded compared with that of pups from the Soy-fed dams. Half of the male pups (F1) were weaned to the same diet as their dams (Can→Can and Soy→Soy groups) and the rest were weaned to the other diet (Can→Soy and Soy→Can groups). The survival rate of the male pups (F1) was significantly lower in the Can→Can group than in the Soy→Can group, and in the Can→Soy group than in the Soy→Soy group, indicating that the oils fed to dams differently affected the growth and survival of pups. There were fewer pups per dam in the Can-fed dams (F0) than in the Soy-fed dams, and in the dams (F1) of the Can→Can and Soy→Can groups than in those of the Can→Soy and Soy→Soy groups. Although Can is nutritionally detrimental to SHRSP rats compared with Soy, no direct evidence has been obtained thus far relating these observations to human nutrition. *J. Nutr.* 134: 1347–1352, 2004.

**KEY WORDS:** • SHRSP rat • canola oil • soybean oil • survival • reproduction

Vegetable oils contain various minor components such as fat-soluble vitamins, phytosterols, isoflavonoids, tocopherols, and environmental chemicals (1). Fat-soluble substances are generally secreted into breast milk and are likely to affect the pups' physiology (2). The spontaneously hypertensive rat, stroke prone (SHRSP)<sup>3</sup> strain, derived from the SHR and Wistar-Kyoto (WKY) strains, develops hypertension and dies of stroke frequently, particularly when salt is added to their drinking water. SHRSP rats exhibit various other anomalies such as renal injury (3), peroxidative injury (4), developmental disorders (5), and reproductive physiologic disorders (6,7). Using this strain, we showed that dietary perilla seed oil, flaxseed oil, and fish oil with very low (n-6)/(n-3) ratios prolong survival by ~10% compared with safflower and soybean oils with high (n-6)/(n-3) ratios (8,9); however, canola oil (Can), with a relatively low (n-6)/(n-3) ratio (~2.5), markedly shortens survival (~40% in the absence of NaCl in the drinking water) compared with soybean oil (Soy). In addition, several other vegetable oils (e.g., olive oil, corn oil, high-oleic safflower oil, high-oleic sunflower oil, evening primrose oil, hydrogenated Soy, and hydrogenated Can) were

shown in our laboratory and by others to shorten survival similarly to Can (10–12). Decreased platelet number (13), increased red cell fragility (14), severe renal injury involving lesions in blood vessels (15), and elevated blood pressure (16) are associated with dietary Can. Antinutritional activities of Can were observed in other strains of rats (16) and mice (17), and in other species. For example, platelet number was decreased and mortality was increased in iron-injected piglets fed a milk replacer diet that contained Can (18,19).

Extensive effort has been made to identify the antinutritional factor associated with some vegetable oils. Ratnayake et al. (20,21) found that the phytosterol content of oils is involved in shortening survival because Can and corn oil, with higher phytosterol contents than Soy, exhibit such activities. Olive oil is an exception, however, in that it has the lowest phytosterol content but shortens survival the most among the oils examined. A purified phytosterol fraction from Can shortened survival when it was added to a Soy diet at 2 times the concentration of the Can diet, suggesting that the phytosterol content is a factor contributing to the shortening of survival (20). However, olive oil was not a single exception for the proposed association between survival and phytosterol content. Indeed, we found other exceptions (10,12), and Ogawa et al. (22) found that an amount of phytosterol comparable to that of Can is not sufficient to reproduce the activity of Can, indicating that factors other than the major phytosterols are involved in shortening survival.

We postulated that the factors that shorten survival are

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<sup>3</sup> Abbreviations used: Can, canola oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; SHRSP, spontaneously hypertensive rat, stroke prone; Soy, soybean oil; WKY, Wistar-Kyoto.

TABLE 1

Effect of dietary oils fed to dams on reproductive variables in rat dams (F0) fed Can or Soy diet and pups (F1) weaned to the same diet (Can→Can and Soy→Soy) or to the other diet (Can→Soy and Soy→Can), and on the survival of the 4 dietary groups of male pups (F1)

Generation	F0		F1			
	Can	Soy	Can→Can	Soy→Can	Can→Soy	Soy→Soy
Dietary group	Can	Soy	Can	Soy	Can	Soy
Diet for F0	Can	Soy	Can	Soy	Can	Soy
Diet for F1			Can		Soy	
Delivered dams/mated female, <i>n/n</i>	30/41	30/39	17/27	22/25	22/27	23/25
Male pups, <i>n</i>	106	140	57	85	107	100
Female pups, <i>n</i>	130	126	84	94	106	113
Total pups, <i>n</i>	236	266	141	179	213	213
Pups/dam, <sup>1</sup> <i>n</i>	7.9	8.9*	8.2	8.1	9.7	9.3

<sup>1</sup> 2-way ANOVA: the effect of the F1 diet was significant ( $P = 0.016$ ), whereas those of the F0 diet ( $P = 0.409$ ) and their interaction ( $P = 0.801$ ) were not. \* Different from Can,  $P = 0.037$  (Student's *t* test).

likely to be hydrophobic and transmittable to the next generation. Here, we examined this possibility by feeding Can and Soy to SHRSP rats through 2 generations.

## MATERIALS AND METHODS

**Animal and diets.** The conventional basal diet (CE-2, standardized for the proliferation and maintenance of rats and mice) was composed of soybean meal, fishmeal, skimmed milk, corn, wheat, wheat bran, alfalfa meal, a vitamin mixture, and a mineral mixture (The Central Laboratory for Experimental Animals Japan, Clea Japan). The basal diet consisted of 24.8% protein, 4.4% fat, 3.5% dietary fiber, 7% ash, and minerals and vitamins.<sup>4</sup> The basal diet (CE-2) and Can or Soy were mixed at a 9:1 ratio and the mixture was pelleted (Clea Japan). These test diets were stored at  $-20^{\circ}\text{C}$  for <3 mo. Vegetable oils available for human consumption were purchased from local markets. The test diets supplied were replaced every 2 d to keep the peroxide values of the served food below 100 mEq/kg. The pelleted conventional diet was more stable to peroxidation than the pelleted semipurified diet (data not shown).

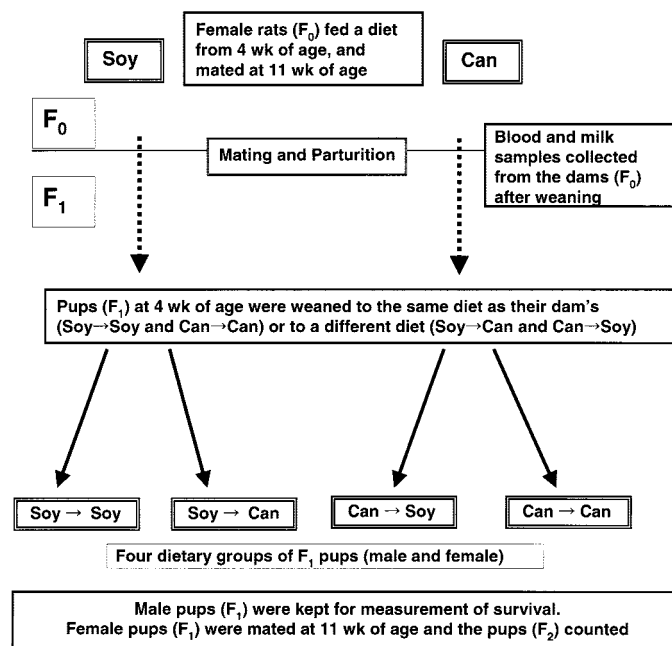
SHRSP rats were obtained from Seack Yoshitomi and maintained in our laboratory. Rats from the same litter were randomly divided into different dietary groups and kept under specific pathogen-free conditions. The temperature and humidity in the room were maintained at  $23 \pm 3^{\circ}\text{C}$  and  $50 \pm 3\%$ , respectively, with a 12-h day:night light cycle. For survival tests, the number of rats was 10–12 in each dietary group, and a 5 g/L NaCl solution was given as drinking water (NaCl-loaded). This study was approved by Ethical Committee of the Graduate School of Pharmaceutical Sciences, Nagoya City University.

**Reproductive physiology.** Female rats at 4 wk of age were randomly divided into the Can and Soy groups (F0,  $n = 9-12$ ). At 11 wk of age, they were mated with male SHRSP rats fed the basal diet for 1 wk. The rats were kept for 4 d at a male:female ratio of 1:2 and then the male/female combinations were changed for another 4 d (Fig. 1). Parturition and lactation were observed every 12 h. Dams and pups were fed the same diet for 3 wk after parturition and the weight gain of the pups was estimated. At 4 wk of age, the pups (F1) in each dietary group were randomly divided into 2 groups; half were weaned to the same diet as their dams (Can→Can and Soy→Soy groups) and the other half were weaned to the other diet (Can→Soy and

Soy→Can groups), and the survival of the male pups (F1) of the 4 dietary groups was monitored. The female SHRSP rats (F1) of the corresponding 4 dietary groups were mated at 11 wk of age. These experiments were repeated 2 or 4 times to obtain sufficient data for statistical analysis.

**Collection of breast milk.** Eight hours after separation from their pups, the dams at 3 wk of lactation were pretreated i.p. with 5 U of oxytocin (Wako Chemicals) and anesthetized with pentobarbiturate; their milk was collected with a Pasteur pipette and then frozen at  $-70^{\circ}\text{C}$  until analysis.

**Lipid analysis.** The fatty acid compositions of the test diets, serum lipids, and milk lipids were analyzed after extraction of total lipids according to the method of Bligh and Dyer (23). Fatty acids were converted to their methyl esters by treatment with 1.37 mol/L HCl in methanol and were quantified by GC using a capillary column (DB-225, J&W Scientific) (12). Heptadecanoic acid was used as an



**FIGURE 1** Protocol for the assessment of the reproductive physiology of rat dams (F0) fed Can or Soy diet and pups (F1) weaned to the same diet (Can→Can and Soy→Soy) or to the other diet (Can→Soy and Soy→Can), and of the survival of the 4 dietary groups of male pups (F1).

<sup>4</sup> The basal diet contained the following minerals and vitamins (/kg): calcium (12.5 g), phosphorus (10.6 g), magnesium (3.4 g), potassium (11.1 g), manganese (113 mg), iron (316 mg), copper (8.6 mg), zinc (52 mg), sodium (3.8 g), retinol (4.3 mg),  $\alpha$ -tocopherol (110 mg), thiamine (15.8 mg), riboflavin (15 mg), pyridoxine (13 mg), vitamin B-12 (0.035 mg), ascorbic acid (280 mg), pantothenic acid (30.5 mg), niacin (17.3 mg), folic acid (1.6 mg), choline (2.65 g), biotin (0.391 mg) and inositol (2.08 g).

internal standard. Sterols were determined as trimethylsilyl ether derivatives by GC as described by Ratnayake et al. (20).

**Statistical analyses.** Data are presented as means  $\pm$  SEM. Statistical analyses of the survival rates were performed by Log-rank test, which is interpreted to reflect relatively more of the difference in the late phase than of the early phase of the survival curves, and by Wilcoxon signed rank tests (nonparametric) reflecting more of the difference in the early phase. Student's *t* test was used for the comparison of 2 groups, i.e., the difference in the numbers of pups/dam between the Can and Soy groups (F0), and the difference in the fatty acid and sterol compositions of diets and tissue lipids between the 2 dietary groups. The difference in the number of pups/dam of the 4 dietary groups (F1) was analyzed by 2-way ANOVA with F0 diet and F1 diet as factors. Difference in the weights of male and female pups (F1) was analyzed by two-way ANOVA with sex and diet as factors. The computer program KyPlot ver. 2.0 (Kyence) was used. Probability values  $< 0.05$  were considered to indicate significant difference.

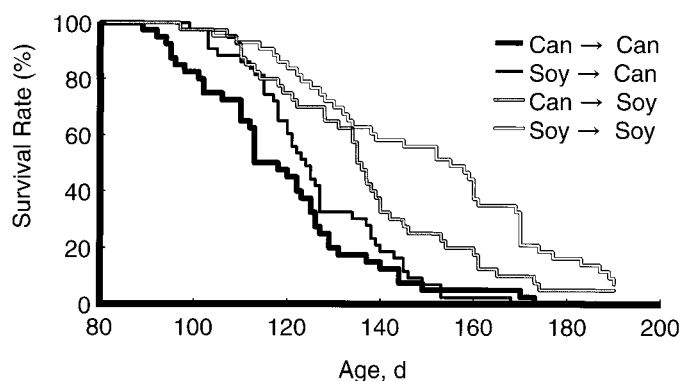
## RESULTS

**Reproductive physiology.** Rats in the 2 dietary groups became pregnant at similar rates (Table 1). The number of pups (F1) per dam was lower in the Can group than in the Soy group ( $P < 0.05$ ).

The pups grew normally. However, the weight gains of the male and female pups (F1) before weaning at 4 wk of age were lower in the Can group than in the Soy group ( $P < 0.01$ , Table 2), even though the mean number of pups per dam was lower in the Can group (Table 1). There were fewer pups (F2) per dam (F1) in the Can $\rightarrow$ Can and Soy $\rightarrow$ Can groups than in the Can $\rightarrow$ Soy and Soy $\rightarrow$ Soy groups ( $P < 0.05$ , Table 1).

**Survival of male pups (F1).** The mean survival time of the male pups (F1) increased in the order of Can $\rightarrow$ Can, Soy $\rightarrow$ Can, Can $\rightarrow$ Soy and Soy $\rightarrow$ Soy groups (Fig. 2, Table 3). The effect of dietary oil fed to dams (Can or Soy) on the survival of the pups (F1) was analyzed for the following 2 combinations. The difference in the survival rates of the Can $\rightarrow$ Can and Soy $\rightarrow$ Can groups was significant in the Wilcoxon test but not in the Log-rank test (Table 3). The difference in the survival rates of the Can $\rightarrow$ Soy and Soy $\rightarrow$ Soy groups was significant in both tests.

**Fatty acid and sterol compositions of dams' serum and milk lipids.** The serum of the Can and Soy groups contained similar concentrations of fatty acids (Table 4). The serum lipids of the Can group contained significantly more octadecenoic and less linoleic acid than those of the Soy group, reflecting the dietary fatty acid compositions. Interestingly, the proportion of arachidonic acid and the arachidonate/linoleate ratio were significantly higher in the Can group than in the Soy group, even though the Can diet contained less linoleic acid than the Soy diet (Table 4). Similarly, the proportions of



**FIGURE 2** Effect of feeding Can or Soy diets to rat dams (F0) on the survival of their male pups (F1) weaned to the same diet as their dams (Can $\rightarrow$ Can and Soy $\rightarrow$ Soy) or to the other diet (Can $\rightarrow$ Soy and Soy $\rightarrow$ Can) and given a 5 g/L NaCl solution as drinking water. The results of statistical analyses of the survival rates are presented in Table 3.

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as the (EPA plus DHA)/ $\alpha$ -linolenate ratio were greater in the Can group than in the Soy group.

The dams' milk from the 2 dietary groups (F0) contained similar concentrations of fatty acids. The fatty acid composition of the milk was very similar to that of each diet, except that the medium-chain fatty acids (10:0, 12:0, and 14:0), which were likely synthesized in the dams, were relatively higher. Because the proportions of 20- and 22-carbon highly unsaturated fatty acids were relatively small in the milk, the dietary effects on the elongation and desaturation reactions were not apparent in milk lipids.

The basal diet itself contained cholesterol and phytosterols; hence the difference in sterol concentrations between the 2 diets was smaller than that between the 2 oils (Table 5). Stigmasterol and brassicasterol were present in the diets but were not detectable in the serum and milk. The proportion of phytosterols in the total sterol fraction and the phytosterol/cholesterol ratio were greater in the Can diet than in the Soy diet, but in the milk lipids, the differences in these variables between the 2 dietary groups were less pronounced than in the diets. The phytosterol/cholesterol ratio decreased in the order of diets  $>$  serum lipids  $>$  milk lipids. The phytosterols were not concentrated into milk lipids compared with diet because the phytosterol/cholesterol ratio of the milk from the Can group (0.16) was even smaller than that of the milk of dams fed the control Soy diet (1.5). Similarly, the phytosterol/energy ratio of the milk from the Can group ( $\sim 25 \mu\text{g}/\text{kJ}$ ), calculated based on the energy contents of cows' and human

**TABLE 2**

Body weights of male and female F1 pups fed Can or Soy diets from weaning until 4 wk of age<sup>1</sup>

Age, d	Male		Female		2-way ANOVA, <i>P</i> -value		
	Can ( <i>n</i> = 53)	Soy ( <i>n</i> = 67)	Can ( <i>n</i> = 65)	Soy ( <i>n</i> = 59)	Sex	Diet	Interaction
7	12.1 $\pm$ 0.2	12.4 $\pm$ 0.2	11.6 $\pm$ 0.2	12.2 $\pm$ 0.2	0.032	0.001	0.487
14	22.6 $\pm$ 0.2	23.7 $\pm$ 0.2	21.6 $\pm$ 0.2	23.1 $\pm$ 0.2	0.001	$< 0.001$	0.437
21	33.8 $\pm$ 0.4	36.6 $\pm$ 0.4	32.5 $\pm$ 0.3	35.3 $\pm$ 0.4	$< 0.001$	$< 0.001$	0.992
28	49.7 $\pm$ 0.7	55.0 $\pm$ 0.6	46.6 $\pm$ 0.5	51.2 $\pm$ 0.7	$< 0.001$	$< 0.001$	0.546

<sup>1</sup> Values are means  $\pm$  SEM.

TABLE 3

Survival time of 4 dietary groups of male F1 rats fed Can or Soy diets from weaning until 4 wk of age<sup>1,2</sup>

Dietary group		Can→Can	Soy→Can	Can→Soy	Soy→Soy
Mean survival time, <i>d</i>		119 ± 3	126 ± 2	134 ± 4	150 ± 4
vs. Can-Can	Log-rank <sup>2</sup>		0.251	0.002	<0.001
	Wilcoxon <sup>3</sup>		0.033	0.001	<0.001
vs. Soy-Can	Log-rank			0.023	<0.001
	Wilcoxon			0.067	<0.001
vs. Can-Soy	Log-rank				0.003
	Wilcoxon				0.012

<sup>1</sup> Values are means ± SEM, *n* = 36–38/group.

<sup>2</sup> The Log-rank test reflects relatively more of the late phase than of the early phase of survival curves.

<sup>3</sup> The Wilcoxon test reflects more of the early phase.

milk) was even less than that of the milk of dams fed the control Soy diet (100 µg/kJ).

### DISCUSSION

Phytosterols shorten the survival of SHRSP rats (20,21), but a 5-fold greater amount of phytosterol is required to produce an effect similar to the survival-shortening effect of

Can (23). The SHRSP rat strain, as well as the parent WKY strain, develops phytosterolemia more easily than some other rat strains (24,25), and the subcutaneous administration of phytosterols at ~0.5 mg/kg daily for up to 48 d affects the fertility of male rats (26). However, the administration of phytosterol esters for 2 generations did not affect the reproduction of male and female rats (Wistar) at 8% of diet, a

TABLE 4

Fatty acid compositions of diets and dam's milk collected at 3 wk of lactation and of dam's serum at 6 wk of age in rats fed the Can or Soy diet<sup>1–3</sup>

	Diet		Serum		Milk	
	Can	Soy	Can	Soy	Can	Soy
<i>g/100 g total fatty acids</i>						
Saturated fatty acids						
10:0	—	—	—	—	6.3 ± 1.0	6.2 ± 1.3
12:0	—	—	—	—	4.2 ± 0.6	3.7 ± 0.6
14:0	0.3	0.3	—	—	2.7 ± 0.4	2.2 ± 0.3
16:0	9.3	13.2	14.5 ± 0.4	16.4 ± 0.3**	11.5 ± 1.6	13.8 ± 2.3
18:0 (dimethylacetal)	—	—	0.3 ± 0.1	0.3 ± 0.1	1.8 ± 0.4	3.4 ± 0.7*
18:0	2.1	4.1	12.7 ± 0.3	12.0 ± 0.6	0.2 ± 0.0	0.1 ± 0.0*
20:0	0.5	0.3	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:0	0.2	0.3	—	—	—	—
24:0	—	—	0.3 ± 0.1	0.0 ± 0.0*	—	—
Monounsaturated fatty acids						
14:1	0.0	0.1	—	—	—	—
16:1	0.5	0.4	0.0 ± 0.0	0.3 ± 0.1**	0.6 ± 0.1	0.6 ± 0.1
18:1(n-9)	49.3	24.1	22.4 ± 0.6	12.8 ± 0.9**	48.5 ± 6.9	24.4 ± 4.6**
18:1(n-7)	—	—	1.1 ± 0.3	0.5 ± 0.1*	—	—
20:1	1.3	0.5	—	—	1.0 ± 0.1	0.4 ± 0.1**
22:1	—	—	—	—	0.1 ± 0.0	0.1 ± 0.0
24:1	—	—	0.8 ± 0.1	0.7 ± 0.2	—	—
(n-6) PUFA						
18:2(n-6)	27.8	49.4	20.5 ± 0.4	33.7 ± 0.8**	24.1 ± 3.3	45.1 ± 7.9*
18:3(n-6)	0.6	0.0	0.4 ± 0.1	0.6 ± 0.0*	0.3 ± 0.1	0.5 ± 0.1
20:3(n-6)	—	—	—	—	0.2 ± 0.0	0.4 ± 0.1
20:4(n-6)	—	—	20.9 ± 0.5	17.4 ± 1.1*	0.7 ± 0.1	1.0 ± 0.2
22:4(n-6)	—	—	—	—	0.1 ± 0.0	0.1 ± 0.0
(n-3) PUFA						
18:3(n-3)	6.6	5.7	1.4 ± 0.1	2.0 ± 0.2**	5.5 ± 0.7	4.8 ± 0.8
20:5(n-3)	—	—	1.5 ± 0.1	1.0 ± 0.0**	0.4 ± 0.0	0.4 ± 0.1
22:5(n-3)	0.9	0.8	—	—	0.2 ± 0.0	0.2 ± 0.1
22:6(n-3)	0.8	0.7	2.8 ± 0.1	2.3 ± 0.1**	0.4 ± 0.1	0.5 ± 0.1
Total FA, mg/mL			5.33 ± 0.35	5.99 ± 0.32	108.9 ± 14.5	108.1 ± 18.8

<sup>1</sup> Values are means ± SEM, *n* = 18 (serum) or 10 (milk); —, not detectable.

<sup>2</sup> Asterisks indicate different from Can: \* *P* < 0.05; \*\* *P* < 0.01 (Student's *t* test).

<sup>3</sup> The total fatty acid concentration in the diets was 12.4 g/100 g (*n* = 2).

TABLE 5

*Sterol concentrations of diets and dams' milk collected after 3 wk of lactation and dams' serum taken at 6 wk of age in rats (F0) fed the Can or Soy diet<sup>1,2</sup>*

Diet group	Diet		Serum		Milk	
	Can	Soy	Can	Soy	Can	Soy
	$\mu\text{mol/g diet}$		$\text{mmol/L}$			
Cholesterol	1.77	1.69	1.70 $\pm$ 0.21	1.52 $\pm$ 0.22	1.25 $\pm$ 0.14	1.26 $\pm$ 0.19
Brassicasterol	0.18	—	—	—	—	—
Campesterol	1.28	0.42	0.22 $\pm$ 0.03	0.13 $\pm$ 0.03*	0.11 $\pm$ 0.02	0.07 $\pm$ 0.01
Stigmasterol	0.12	0.17	—	—	—	—
$\beta$ -Sitosterol	2.40	1.72	0.21 $\pm$ 0.03	0.16 $\pm$ 0.03	0.08 $\pm$ 0.01	0.07 $\pm$ 0.01
Total phytosterol	1.63	0.95	0.47 $\pm$ 0.03	0.33 $\pm$ 0.04*	0.20 $\pm$ 0.02	0.15 $\pm$ 0.02
Total sterol	2.31	1.61	2.36 $\pm$ 0.11	2.04 $\pm$ 0.16	1.57 $\pm$ 0.10	1.54 $\pm$ 0.17
Phytosterol/Cholesterol	2.38	1.46	0.25 $\pm$ 0.01	0.19 $\pm$ 0.01**	0.15 $\pm$ 0.01	0.11 $\pm$ 0.01

<sup>1</sup> Values are means  $\pm$  SEM,  $n = 18$  (serum) or 10 (milk); —, not detectable.

<sup>2</sup> Asterisks indicate different from Can: \*  $P < 0.05$ ; \*\*  $P < 0.01$  (Student's  $t$  test).

concentration that is much higher than that in the Can diet (0.16%) (27,28). The data presented in Table 5 support the interpretation that factors other than the major phytosterols are involved in the retarded growth and shortened survival of pups from dams fed the Can diet.

Eicosanoids derived from arachidonic acid and linoleic acid play pivotal roles in growth and reproductive physiology (29). Although the linoleic acid/ $\alpha$ -linolenic acid ratio of the Can diet was smaller than that of the Soy diet, the proportion of arachidonic acid in serum lipids was greater in the Can group than in the Soy group (Table 4), indicating that the retardation of growth of the F1 pups from the Can-fed dams was not due to a lack of eicosanoid precursors.

In piglets fed a milk replacer containing Can, increased requirements for vitamin E after iron injection were revealed (19). However, the hepatic vitamin E content was greater in the Can group than in the Soy group (unpublished observations), indicating that the tissue vitamin E level is not a critical factor for the shortening of survival in SHRSP rats. Although the Can used was a double-low variety of rapeseed oil with reduced contents of erucic acid and glucosinolates, it still contains hydrolysis products of glucosinolates such as isothiocyanates, oxazolidinethione, indole derivatives, and other minor components (30).

After death, cerebral bleeding was observed in most of male SHRSP rats from both dietary groups (10). The color of the lung was darker in the Can-fed rats, particularly in those with shorter survival times, compared with that of the Soy-fed rats, which was indicative of hemorrhage and/or hemostasis. In kidney, impaired blood vessels and glomerular structures were observed microscopically and the severity of nephropathy symptoms was greater in the Can group than in the Soy group (15 and unpublished observations). All of these observations could be related to survival, and the causes of death appeared to be complex.

A beneficial effect of Can was shown in the Lyon Diet Heart Study (31); Can and olive oil were effective in the secondary prevention of coronary heart diseases, possibly due to the reduced intake of linoleic acid and increased intakes of oleic acid and  $\alpha$ -linolenic acid. Although the use of relatively crude, high-erucic rapeseed oil has been associated epidemiologically with increased pulmonary adenocarcinoma in China (32), no other lines of evidence have been presented to date to suggest detrimental effects of Can on human health. However,

the unusual effects of Can in rodents (10,13,17,20) and piglets (18,19), as well as those observed in the present experiments, warrant further studies to identify the detrimental factors other than phytosterols and/or to produce Can with reduced shortening of survival in SHRSP rats, because its fatty acid composition seems to be beneficial in human nutrition.

#### LITERATURE CITED

1. Sauerwald, T. U., Demmelmair, H., Fidler, N. & Koletzko, B. (2000) Polyunsaturated fatty acid supply with human milk. Physiological aspects and in vivo studies of metabolism. *Adv. Exp. Med. Biol.* 478: 261–270.
2. Kreckhoffs, D.A.J.M., Brous, F., Hornstra, G. & Mensink, R. P. (2002) Effects on the human serum lipoprotein profile of  $\beta$ -glucan, soy protein and isoflavones, plant sterol and stanols, garlic and tocotrienols. *J. Nutr.* 132: 2494–2505.
3. Matsunaga, M., Komuro, T., Yamamoto, J., Hara, A., Morimoto, K. & Yamori, Y. (1980) Renal function, plasma rennin, and spontaneous diuresis in an advanced stage of hypertension in rats. *Jpn. Heart J.* 21: 737–751.
4. Tomita, I., Sano, M., Serizawa, S., Ohta, K. & Katou, M. (1979) Fluctuation of lipid peroxides and related enzyme activities at time of stroke in stroke-prone spontaneously hypertensive rats. *Stroke* 10: 323–326.
5. Ueno, K. I., Togashi, H., Mori, K., Matsumoto, M., Ohashi, S., Hoshino, A., Fujita, T., Saito, H., Minami, M. & Yoshioka, M. (2002) Behavioural and pharmacological relevance of stroke-prone spontaneously hypertensive rats as an animal model of a developmental disorder. *Behav. Pharmacol.* 13: 1–13.
6. Yamada, N., Kido, K., Tamai, T., Mukai, M. & Hayashi, S. (1981) Hypertensive effects on pregnancy in spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP). *Int. J. Biol. Res. Pregnancy* 2: 80–84.
7. Shibukawa, T., Horie, R., Kitao, M. & Yamori, Y. (1990) Stroke-prone spontaneously hypertensive rats as a model for toxemia of pregnancy and aggravating and preventive effects of maternal modifications during pregnancy on offspring's growth. *Jpn. Circ. J.* 54: 644–652.
8. Okuyama, H., Kobayashi, T. & Watanabe, S. (1996) Dietary fatty acids—the n-6/n-3 balance and chronic elderly disease. Excess linoleic acid and relative n-3 deficiency syndrome seen in Japan. *Prog. Lipid Res.* 35: 409–457.
9. Shimokawa, T., Moriuchi, A., Hori, T., Saito, M., Naito, Y., Kabasawa, H., Nagae, Y., Matsubara, M. & Okuyama, H. (1988) Effect of dietary alpha-linolenate/linoleate balance on mean survival time, incidence of stroke and blood pressure of spontaneously hypertensive rats. *Life Sci.* 43: 2067–2075.
10. Huang, M.-Z., Naito, Y., Watanabe, S., Kobayashi, T., Kanai, H., Nagai, H. & Okuyama, H. (1996) Effect of rapeseed and dietary oils on the mean survival time of stroke-prone spontaneously hypertensive rats. *Biol. Pharm. Bull.* 19: 554–557.
11. Huang, M.-Z., Watanabe, S., Kobayashi, T., Nagatsu, A., Sakakibara, J. & Okuyama, H. (1997) Unusual effects of some vegetable oils on the survival time of stroke-prone spontaneously hypertensive rats. *Lipids* 32: 745–751.
12. Miyazaki, M., Huang, M.-Z., Takemura, N., Watanabe, S. & Okuyama, H. (1998) Early mortality effect of partially hydrogenated vegetable oils in stroke-prone spontaneously hypertensive (SHRSP) rats. *Nutr. Res.* 18: 1049–1056.
13. Naito, Y., Yoshida, H., Nagata, T., Tanaka, A., Ono, H. & Ohara, N. (2000) Dietary intake of rapeseed oil or soybean oil as the only fat nutrient in spontaneously hypertensive rats and Wistar Kyoto rats—blood pressure and pathophysiology. *Toxicology* 146: 197–208.
14. Naito, Y., Konishi, C. & Ohara, N. (2000) Blood coagulation and

osmolar tolerance of erythrocytes in stroke-prone spontaneously hypertensive rats given rapeseed oil or soybean oil as the only dietary fat. *Toxicol. Lett.* 117: 209–215.

15. Miyazaki, M., Takemura, N., Watanabe, S., Hata, N., Misawa, Y. & Okuyama, H. (2000) Dietary docosahexaenoic acid ameliorates, but rapeseed oil and safflower oil accelerate renal injury in stroke-prone spontaneously hypertensive rats as compared with soybean oil, which is associated with expression for renal transforming growth factor-beta, fibronectin and renin. *Biochim. Biophys. Acta* 1483: 101–110.

16. Naito, Y., Kasama, K., Yoshida, H. & Ohara, N. (2000) Thirteen-week dietary intake of rapeseed oil or soybean oil as the only dietary fat in Wistar Kyoto rats—change in blood pressure. *Food Chem. Toxicol.* 38: 811–816.

17. Kameyama, T., Ohhara, T., Nakashima, Y., Naito, Y., Huang, M-Z., Watanabe, S., Kobayashi, T., Okuyama, H., Yamada, K. & Nabeshima, T. (1996) Effects of dietary vegetable oils on behavior and drug responses in mice. *Biol. Pharm. Bull.* 19: 400–404.

18. Innis, S. M. & Dyer, R. A. (1999) Dietary canola oil alters hematological indices and blood lipids in neonatal piglets fed formula. *J. Nutr.* 129: 1261–1268.

19. Sauer, F. D., Farnworth, E. R., Belanger, J. R., Kramer, J. G., Miller, R. B. & Yamashiro, S. (1997) Additional vitamin E required in milk replacer diets that contain canola oil. *Nutr. Res.* 17: 259–269.

20. Ratnayake, W.M.N., L'Abbé, M. R., Mueller, R., Hayward, S., Plouffe, L., Hollywood, R. & Trick, K. (2000) Vegetable oils in phytosterols make erythrocytes less deformable and shorten the life span of stroke-prone spontaneously hypertensive rats. *J. Nutr.* 130: 1166–1178.

21. Ratnayake, W.M.N., Plouffe, L., Hollywood, R., L'Abbé, M. R., Hidirglou, N., Sarwar, G. & Mueller, R. (2000) Influence of sources of dietary oils on life span of stroke-prone spontaneously hypertensive rats. *Lipid* 35: 409–420.

22. Ogawa, H., Yamamoto, K., Kamisako, T. & Meguro, T. (2003) Phytosterol additives increase blood pressure and promote stroke onset in salt-loaded stroke-prone spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* 30: 919–924.

23. Bligh, E. G. & Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37: 911–917.

24. Ikeda, I., Nakagiri, H., Sugano, M., Ohara, S., Hamada, T., Nonaka, M. & Imaizumi, K. (2001) Mechanisms of phytosterolemia in stroke-prone spontaneously hypertensive and WKY rats. *Metabolism* 50: 1361–1368.

25. Scoggan, K. A., Gruber, H. & Larivière, K. A. (2003) Missense mutation in the *Abcg5* gene causes phytosterolemia in SHR, stroke-prone SHR, and WKY rats. *J. Lipid. Res.* 44: 911–916.

26. Malini, T. & Vanithakumari, G. (1991) Antifertility effects of beta-sitosterol in male albino rats. *J. Ethnopharmacol.* 35: 149–153.

27. Whittaker, M. H., Frankos, V. H., Wolterbeek, A. P. & Waalkens-Berendsen, D. H. (1999) Two-generation reproductive toxicity study of plant stanol esters in rats. *Regul. Toxicol. Pharmacol.* 29: 196–204.

28. Waalkens-Berendsen, D. H., Wolterbeek, A. P., Wijnands, M. V., Richold, M. & Hepburn, P. A. (1999) Safety evaluation of phytosterol esters. Part 3. Two-generation reproduction study in rats with phytosterol esters—a novel functional food. *Food. Chem. Toxicol.* 37: 683–696.

29. Innis, S. M. (1986) Essential fatty acids in growth and development. *Prog. Lipid. Res.* 30: 39–103.

30. Bjeldanes, L. F., Kim, J. Y., Grose, K. R., Bartholomew, J. C., & Bradfield, C. A. (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Proc. Natl. Acad. Sci. U.S.A.* 88: 9543–9547.

31. de Lorgeril, M., Renaud, S., Mamelle, N., Salen, P., Martin, J. L., Monjaud, I., Guidollet, J., Toubout, P. & Delaye, J., (1994) Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 343: 1454–1459.

32. Shields, P. G., Xu, G. X., Blot, W. J., Fraumeni, J. F., Jr., Trivers, G. E., Pellizzari, E. D., Qu, Y. H., Gao, Y. T. & Harris, C. C. (1995) Mutagens from heated Chinese and U.S. cooking oils. *J. Natl. Cancer Inst.* 87: 836–841.