Effects of dietary protein and fat on linoleic and α-linolenic acid metabolism and prostacyclin production in stroke-prone spontaneous hypertensive rats

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The effects of dietary proteins (casein or soybean protein) and fats (perilla oil high in α-linolenic acid or safflower oil high in linoleic acid) on the fatty acid composition of liver microsomes and the aortic prostacyclin production were studied in stroke-prone spontaneous hypertensive rats. The stimulating effects of casein compared to soybean protein on linoleic acid and α-linolenic acid metabolism and prostacyclin production were confirmed in this rat model. The ratio of (20:3n-6 + 20:4n-6)/18:2n-6, the linoleic acid desaturation index, of liver microsomes was reflected in that of aorta. In addition, there was a highly positive correlation between the linoleic acid desaturation index of phosphatidylcholine and the prostacyclin production of the aorta. Thus, the results of the present study indicated a significant role of dietary protein in the regulation of polyunsaturated fatty acids and hence, the eicosanoid production. The data showed a possible preferable effect of casein in relation to soybean protein. (J. Nutr. Biochem. 5:248–255, 1994.)

Keywords: casein; soybean protein; linoleic acid; α-linolenic acid; prostacyclin; SHR-SP

Introduction

Hypertension and thrombosis are well-known factors that lead to stroke. Diets high in protein, especially animal protein, and high in fat prevent the incidence of stroke.[1] The mechanism of preventing hypertension and thrombosis through interaction of prostaglandins (PGs) within the vascular tissues has been established. One of the PGs such as prostacyclin (PGI_2) causes vasodilation and prevents platelet aggregation, thus exerting a preventive effect on hypertension and thrombosis.[2] It has been reported that linoleic acid desaturation and PGI_2 synthesis decrease hypertension in humans and experimental animals, although the causal relationship between the two phenomena is not known.[3,4] The production of PGs depends not only on the supply of substrate fatty acids, polyunsaturated fatty acids (PUFAs), but also on the kinds of dietary PUFAs, either n-6 or n-3. The latter interferes with the production of the 2-series PGs.[5,6] Dietary protein, especially casein (CAS) and soybean protein (SOY), also influences desaturation of PUFAs in normal and diabetic rats.[7,8] However, no information is available regarding the effect of dietary protein on fatty acid desaturation and aortic PGI_2 production in stroke-prone spontaneous hypertensive rats (SHR-SP), a model suitable to examine hypertension and thrombosis. We describe here the interaction of dietary protein and fat on fatty acid desaturation of tissue phospholipids and aortic PGI_2 production in SHR-SP.

Methods and materials

Animals and diets

The diets were prepared according to the formula recommended by the American Institute of Nutrition[9] as follows (g/100 g diet): protein, 20; fat, 5; vitamin mixture, 1; mineral mixture, 3.5; choline bitartrate, 0.2; DL-methionine, 0.3; cellulose, 5; corn starch, 15; and sucrose to 100. Four experimental diets were prepared by combining two proteins with two fats. Either casein (CAS, Wako Pure Chemicals, Osaka, Japan) or soybean protein isolate (SOY, Fujipro R, Fuji Oil Co., Osaka, Japan) served as the protein source. Perilla oil (PER, edible grade; Ohta Oil Co., Okazaki, Japan) and safflower oil (SAF,

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Rinolu Yushi Co., Nagoya, Japan) were used as the fat source. Total PUFA contents of each of these fats were made comparable by mixing palm olein (Fuji Oil Co., Osaka, Japan) with safflower oil *(Table 1)*. Therefore, the composition of PUFAs was practically the sole variable. Vitamin and mineral mixtures (AIN-76) were purchased from Oriental Yeast Co., Tokyo, Japan.

SHR-SP were kindly provided by the late Prof. K. Okamoto, Kinki University School of Medicine (Osaka, Japan), and bred in our animal facility. The male rats, 4-weeks-old, were acclimatized for 1 week on a commercial non-purified diet (NMF, Oriental Yeast Co., Tokyo, Japan) in a room with controlled temperature (20 to 22° C) and a 12-hr light/dark cycle. The eight rats were each fed experimental diets ad libitum for 8 weeks.

The systolic blood pressure of prewarmed, conscious rats were measured weekly by the tail cuff method (Blood Pressure Monitor, MK-1000, Muromachi Kikai Co., Tokyo, Japan). On the last day of feeding, the rats were fasted for 5 hr (8:00 a.m. to 1:00 p.m.) and blood (9 mL) was collected under light diethylether anesthesia from the abdominal aorta in a syringe containing 1.0 mL of 3.8% trisodium-citrate. The blood plasma was used for lipid and amino acid analysis. The brain, liver, and thoracic aorta were excised immediately.

Lipid analyses

Lipids were extracted according to the method of Folch et al.[10] Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and cholesterol ester were separated by thin-layer chromatography.[11] The fatty acid compositions of liver microsomes, plasma, aorta and brain were analyzed by gas-liquid chromatography in a Silar 10 C column.[12] The ratio of the metabolites to parent molecules, (20:3n-6 + 20:4n-6)/18:2n-6 and 20:5n-3/18:3n-3 (eicosapentaenoic [EPA]/α-linolenic acid [ALA]), were used as a desaturation index for linoleic acid (LA) and ALA, respectively. Plasma and liver lipids were measured as described previously.[7,13]

Measurement of prostacyclin production

The thoracic aorta was excised, adhering blood cleaned off, and used for the measurement of PGI_2 production by a radioimmunoassay as 6-keto-$PGF_{1\alpha}$ (NEK-008, New England Nuclear, Boston, MA USA) under the condition of production being linear with respect to the tissue weight (25 mg) and incubation time (30 minutes) at 25° C.[8,14]

Measurement of plasma amino acid concentration

Plasma amino acids were measured by an automatic amino acid analyzer (JLC-300, Japan Electric Optical Laboratory, Tokyo, Japan) in samples deproteinized by sulfosalicylic acid.[15]

Statistics

An analysis of variance and the multiple comparison procedure of Tukey were adopted to test the statistical differences.[8,16] Differences with $P < 0.05$ were considered as statistically significant.

Table 1 Fatty acids composition of dietary fats

Dietary fat	Fatty acids (weight %)					
	14:0	16:0	18:0	18:1	18:2n-6	18:3n-3
Perilla oil	—	6.5	1.5	18.3	16.1	57.5
Safflower oil*	0.2	9.6	2.0	14.0	74.2	—

*Safflower oil : palm olein = 9 : 1, wt/wt.

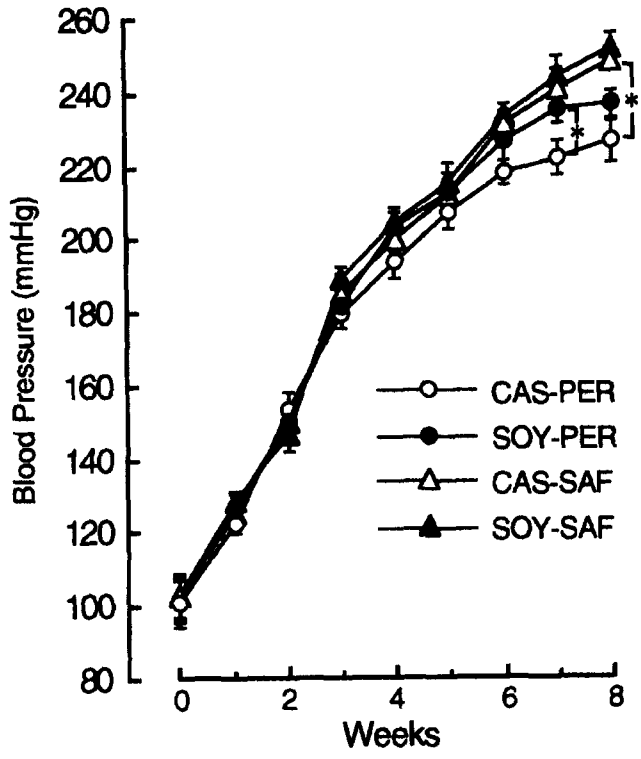

Figure 1 Effects of dietary proteins and fats on blood pressure in SHR-SP. Each point and vertical bar represent the mean ± SE for eight rats. CAS: casein, SOY: soybean protein, PER: perilla oil, SAF: safflower oil. *Significantly different at $P < 0.05$.

Results

Growth parameters

The food intake and body weight gain of the rats were similar among the groups (data not shown). The relative liver weight was significantly lower in the rats fed SOY diets than in those fed CAS diets, irrespective of the fat source (3.9 ± 0.1, 3.5 ± 0.1, 3.8 ± 0.1, and 3.5 ± 0.0 g/100 g body weight for CAS-PER, SOY-PER, CAS-SAF and SOY-SAF, respectively).

Blood pressure

The magnitude of the increase in systolic blood pressure during the 8 weeks feeding tended to be higher in the rats fed with SAF than in those fed with PER irrespective of the protein source. The difference between the CAS-PER group and the CAS-SAF group at 8 weeks, and between the CAS-PER group and the SOY-PER group at 7 weeks was significant *(Figure 1)*.

Concentrations of plasma and liver lipids

As shown in *Table 2*, the concentrations of plasma total and high density lipoprotein cholesterol were significantly lower in the SOY-PER group than in the CAS-PER group, while the protein-dependent difference was less marked when dietary fat was SAF. The concentration of plasma phospholipids was significantly lower in the rats fed PER than in those fed SAF. A similar trend was also observed in plasma triglyceride, but the difference was not statistically significant.

The concentrations of liver cholesterol and triglyceride were also significantly lower in the rats fed SOY except for cholesterol of the SAF groups *(Table 2)*. The concentration of liver phospholipid was similar among the groups.

Fatty acid compositions of liver microsomal phospholipids

The fatty acid compositions of liver microsomal PC, PE, and PI are shown in *Table 3*. In PC, the proportion of palmitic and stearic acids tended to be lower, while that of palmitoleic and oleic acids tended to be higher in the CAS than in the SOY groups when comparing the same fat groups. Consequently, the ratio of 16:1/16:0 and 18:1/18:0 in PC decreased significantly when the dietary protein was SOY, suggesting a decrease in Δ7 and Δ9 desaturation, respectively *(Figure 2)*. A similar, but a less marked trend, was observed with PE and PI. These results were similar to those observed with exogenous hypercholesterolemic (ExHC) rats.[17]

Dietary protein also affected the proportion of LA, while dietary fat affected that of arachidonic acid (AA) in PC. The proportion of LA was significantly lower in the CAS group than in the SOY group, while that of AA was not significantly influenced by dietary protein. Thus, the desaturation index for LA expressed as (20:3n-6 + 20:4n-6)/18:2n-6 tended to be higher in the CAS than in the SOY groups *(Figure 2)*. In the same protein groups, PER feeding, compared with SAF feeding, resulted in a lower percentage of AA, while the proportion of LA in PC remained unchanged *(Table 3)*. Thus, the LA desaturation index was significantly higher in the SAF than in the PER groups *(Figure 2)*.

The proportion of eicosapentaenoic (EPA), docosapentaenoic, and docosahexaenoic acids (DHA) in PC was influenced by the type of dietary fat. These ALA metabolites were significantly higher in the PER than in the SAF groups *(Table 3)*. Dietary protein also affected ALA metabolism, and the proportion of EPA tended to be higher in the CAS than in the SOY groups, while that of ALA was the same

Table 2 Effects of dietary proteins and fats on the concentrations of plasma and liver lipids in SHR-SP

Groups	Plasma (mmol/L)			
	Cholesterol	HDL-cholesterol	Phospholipid	Triglyceride
CAS-PER	1.81 ± 0.04	1.39 ± 0.03	1.45 ± 0.04	0.65 ± 0.07
SOY-PER	1.60 ± 0.04[a]	1.19 ± 0.05[a]	1.34 ± 0.03	0.54 ± 0.05
CAS-SAF	1.74 ± 0.03	1.52 ± 0.03	1.74 ± 0.04[a]	0.79 ± 0.11
SOY-SAF	1.64 ± 0.04	1.41 ± 0.04[b]	1.68 ± 0.03[b]	0.69 ± 0.07

Groups	Liver (μmol/g liver)		
	Cholesterol	Phospholipid	Triglyceride
CAS-PER	6.4 ± 0.3	44.1 ± 0.5	12.9 ± 0.8
SOY-PER	5.2 ± 0.1[a]	45.1 ± 0.8	8.9 ± 0.5[a]
CAS-SAF	5.8 ± 0.1	43.0 ± 0.8	10.7 ± 0.3[a]
SOY-SAF	5.2 ± 0.1	44.2 ± 1.3	7.3 ± 0.6[c]

Values represent means ± SE for eight rats. CAS: casein, SOY: soybean protein, PER: perilla oil, SAF: safflower oil.
[a,b,c]Significantly different from the CAS-PER, SOY-PER, and CAS-SAF group at $P < 0.05$, respectively.

Table 3 Effects of dietary proteins and fats on the fatty acid compositions of liver microsomal phospholipids in SHR-SP

Groups	Fatty acids										
	16:0	16:1	18:0	18:1	18:2 n-6	18:3 n-3	20:3 n-6	20:4 n-6	20:5 n-3	22:5 n-3	22:6 n-3
	weight %										
Phosphatidylcholine											
CAS-PER	24.4 ± 0.8	3.0 ± 0.1	20.4 ± 0.6	11.3 ± 0.6	11.3 ± 0.9	0.8 ± 0.0	1.1 ± 0.1	9.0 ± 0.5	11.7 ± 1.2	1.8 ± 0.1	3.9 ± 0.4
SOY-PER	25.1 ± 0.6	2.4 ± 0.2[a]	21.6 ± 0.5	9.3 ± 0.3[a]	14.9 ± 0.8[a]	0.8 ± 0.1	1.1 ± 0.0	9.3 ± 0.6	8.7 ± 0.7	2.1 ± 0.1	3.5 ± 0.3
CAS-SAF	21.7 ± 0.5[a]	2.2 ± 0.1[a]	20.8 ± 0.4	9.2 ± 0.3[a]	9.6 ± 0.7	—	0.6 ± 0.0[a]	30.6 ± 1.3[a]	—	0.2 ± 0.1[a]	1.1 ± 0.3[a]
SOY-SAF	23.6 ± 0.8	2.0 ± 0.2	23.0 ± 0.4[c]	8.2 ± 0.3	12.7 ± 0.8[c]	—	0.6 ± 0.1[b]	25.6 ± 2.0[b]	—	0.2 ± 0.0[b]	0.8 ± 0.1[b]
Phosphatidylethanolamine											
CAS-PER	18.5 ± 0.7	1.5 ± 0.2	26.5 ± 1.0	9.2 ± 0.7	5.0 ± 0.6	1.0 ± 0.1	0.4 ± 0.0	9.5 ± 0.6	12.9 ± 1.2	4.0 ± 0.3	9.5 ± 1.0
SOY-PER	21.5 ± 0.6[a]	1.2 ± 0.2	27.0 ± 0.7	7.9 ± 0.6	7.0 ± 1.2	1.1 ± 0.1	0.4 ± 0.0	10.0 ± 0.9	10.1 ± 0.9	4.7 ± 0.3	8.3 ± 0.8
CAS-SAF	17.6 ± 0.4	1.4 ± 0.1	25.1 ± 0.7	9.0 ± 0.5	7.5 ± 0.6	—	0.4 ± 0.0	28.3 ± 1.4[a]	0.2 ± 0.0[a]	0.7 ± 0.2[a]	3.0 ± 0.8[a]
SOY-SAF	20.4 ± 1.4	1.2 ± 0.2	27.4 ± 0.8	8.1 ± 0.5	8.8 ± 1.1	—	0.4 ± 0.0	25.0 ± 2.4[b]	—	0.7 ± 0.1[b]	2.2 ± 0.4[b]
Phosphatidylinositol											
CAS-PER	6.2 ± 0.6	0.5 ± 0.0	49.1 ± 1.6	3.6 ± 0.2	1.3 ± 0.1	0.3 ± 0.0	4.2 ± 0.2	20.9 ± 1.6	2.3 ± 0.2	6.0 ± 0.6	3.6 ± 0.5
SOY-PER	6.8 ± 0.7	0.5 ± 0.1	49.6 ± 1.3	2.7 ± 0.2[a]	1.5 ± 0.1	0.3 ± 0.0	3.8 ± 0.1[a]	22.9 ± 1.8	1.8 ± 0.1	5.4 ± 0.4	3.0 ± 0.4
CAS-SAF	5.6 ± 0.4	0.5 ± 0.0	47.7 ± 1.4	3.5 ± 0.3	1.9 ± 0.2	—	1.1 ± 0.1[a]	36.2 ± 2.0[a]	—	0.5 ± 0.1[a]	0.3 ± 0.2[a]
SOY-SAF	6.4 ± 0.6	0.5 ± 0.0	50.1 ± 1.6	2.8 ± 0.2	2.3 ± 0.3	—	1.1 ± 0.1[b]	33.8 ± 2.7[b]	—	0.5 ± 0.2[b]	0.2 ± 0.1[b]

Values represent means ± SE for eight rats. CAS: casein, SOY: soybean protein, PER: perilla oil, SAF: safflower oil.
[a,b,c]Significantly different from the CAS-PER, SOY-PER, and CAS-SAF group at $P < 0.05$, respectively.

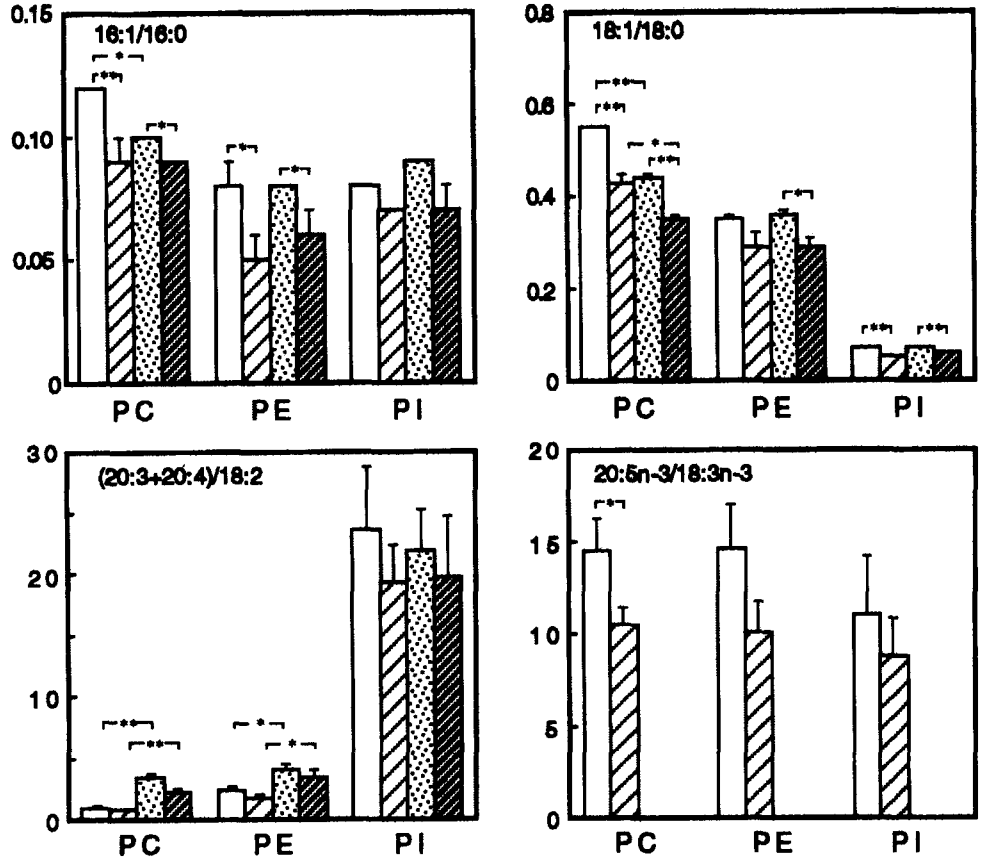

Figure 2 Effects of dietary proteins and fats on fatty acid ratios of liver microsomes in SHR-SP. PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol. □ : CAS-PER, ▨ : SOY-PER, ▩ : CAS-SAF, ■ : SOY-SAF. Each column and vertical bar represent the mean ± SE for eight rats. CAS: casein, SOY: soybean protein, PER: perilla oil, SAF: safflower oil, *,**Significantly different at $P < 0.05$ and $P < 0.01$, respectively.

(Table 3). Thus, the desaturation index for ALA expressed as EPA/ALA was significantly higher in the former than in the latter *(Figure 2).* In addition, the ratio of EPA/AA was significantly higher in the CAS group than in the SOY group (1.28 ± 0.08 and 0.95 ± 0.07 for CAS-PER and SOY-PER, $P < 0.01$, respectively). A similar dietary effect was observed in liver PE and PI. These results were similar to those observed with the normal rats.[8]

Fatty acid composition of plasma and aorta

The same trend observed in the liver microsomal PC was observed in the fatty acid composition of plasma PC *(Table 4).* In the cholesterol ester fraction, a relatively high proportion of AA and EPA was observed compared with the PC fraction *(Table 4).* The proportion of ALA in cholesterol ester in this rat model was lower when compared with that in ExHC rats[17] but similar to that in normal rats.

In aortic PC, the proportion of LA was not significantly different between the PER and SAF groups, while that of AA was significantly lower in the former than in the latter *(Table 4).* Therefore, the ratio of (20:3n-6 + 20:4n-6)/18:2n-6 was significantly higher in the SAF than in the PER groups (0.92 ± 0.04, 0.79 ± 0.07, 2.90 ± 0.34, and 2.07 ± 0.27 for CAS-PER, SOY-PER, CAS-SAF, and SOY-SAF, respectively). In addition, the ratio tended to be higher in the CAS group than in the SOY group. The proportion of EPA and DHA was higher in the rats fed PER than in those fed SAF, but the protein effect was not observed *(Table 4).*

Fatty acid composition of brain

The fatty acid compositions of brain PC and PI were comparable between the groups, except for the proportion of arachidonic acid, in contrast to other tissues *(Table 5).* In PE and PS, the proportions of AA, 22:4n-6, and 22:5n-6 were significantly high in the SAF group, while that of DHA was high in the PER group, reflecting the type of dietary fats. The protein-dependent difference in the fatty acid composition was not observed in these phospholipids.

Prostacyclin production by aorta

The results of the aortic production of PGI_2 are shown in *Figure 3.* The production of PGI_2, measured as 6-keto-$PGF_{1\alpha}$, was higher in the rats fed SAF than in those fed PER. There was also a tendency for greater production when CAS was the protein source than when SOY was the protein source, though not significant due to a relatively large deviation.

Table 4 Effects of dietary proteins and fats on the fatty acid compositions of plasma and aorta phosphatidylcholine and plasma cholesterol ester in SHR-SP

Groups	Fatty acids										
	16:0	16:1	18:0	18:1	18:2 n-6	18:3 n-3	20:3 n-6	20:4 n-6	20:5 n-3	22:5 n-3	22:6 n-3
	Weight %										
Plasma phosphatidylcholine											
CAS-PER	21.0 ± 0.2	1.8 ± 0.1	20.9 ± 0.3	10.5 ± 0.1	16.1 ± 0.4	1.0 ± 0.2	1.4 ± 0.0	9.6 ± 0.3	8.8 ± 0.2	2.7 ± 0.1	4.7 ± 0.1
SOY-PER	21.4 ± 0.3	1.4 ± 0.1[a]	22.2 ± 0.2[a]	8.3 ± 0.1[a]	18.4 ± 0.2[a]	0.6 ± 0.1	1.3 ± 0.0	10.2 ± 0.5	6.9 ± 0.3[a]	3.1 ± 0.1	4.7 ± 0.2
CAS-SAF	19.4 ± 0.4[a]	1.5 ± 0.0[a]	20.9 ± 0.2	7.7 ± 0.2[a]	14.0 ± 0.5[a]	0.2 ± 0.1	0.7 ± 0.0[a]	30.3 ± 0.7[a]	—	0.2 ± 0.1[a]	1.2 ± 0.3[a]
SOY-SAF	19.6 ± 0.3[b]	1.2 ± 0.0[c]	22.3 ± 0.2[c]	6.3 ± 0.1[b,c]	14.7 ± 0.5[b]	—	0.7 ± 0.1[b]	29.7 ± 0.5[b]	—	0.2 ± 0.0[b]	1.1 ± 0.1[b]
Cholesterol ester											
CAS-PER	5.4 ± 0.2	4.5 ± 0.2	0.4 ± 0.0	7.2 ± 0.3	16.3 ± 0.3	3.2 ± 0.1	0.4 ± 0.0	25.3 ± 0.6	33.5 ± 0.8	0.4 ± 0.1	1.4 ± 0.0
SOY-PER	5.6 ± 0.3	3.3 ± 0.2[a]	0.4 ± 0.0	5.9 ± 0.3	20.9 ± 0.6[a]	2.5 ± 0.1[a]	0.4 ± 0.0	28.0 ± 1.3	28.4 ± 0.6[a]	0.5 ± 0.1	1.5 ± 0.1
CAS-SAF	5.0 ± 0.3	2.9 ± 0.1[a]	0.3 ± 0.0[a]	4.1 ± 0.1[a]	14.3 ± 0.5[a]	—	0.2 ± 0.0[a]	69.6 ± 0.7[a]	—	0.4 ± 0.1	0.3 ± 0.1[a]
SOY-SAF	4.4 ± 0.2[b]	2.1 ± 0.2[c]	0.3 ± 0.0[c]	3.2 ± 0.1[b]	15.9 ± 0.5[c]	—	0.2 ± 0.0[b]	70.4 ± 0.9[b]	—	0.4 ± 0.1	0.4 ± 0.0[b]
Aorta phosphatidylcholine											
CAS-PER	32.0 ± 0.9	2.0 ± 0.1	13.2 ± 0.3	14.1 ± 0.3	11.3 ± 1.1	0.5 ± 0.1	1.4 ± 0.1	8.8 ± 0.6	2.7 ± 0.2	2.7 ± 0.6	9.1 ± 2.1
SOY-PER	30.5 ± 1.4	1.8 ± 0.1	13.5 ± 0.5	13.0 ± 0.2[a]	13.5 ± 1.4	0.8 ± 0.2	1.4 ± 0.1	8.6 ± 0.5	2.7 ± 0.3	2.6 ± 0.6	9.4 ± 2.2
CAS-SAF	27.0 ± 1.5	1.5 ± 0.2[a]	15.1 ± 0.4[a]	10.9 ± 0.3[a]	9.4 ± 1.8	—	0.7 ± 0.1[a]	22.9 ± 1.2[a]	—	1.6 ± 0.3	6.9 ± 2.1
SOY-SAF	26.9 ± 1.6	1.4 ± 0.1	15.1 ± 0.3[b]	10.4 ± 0.2[b]	12.2 ± 1.9	—	0.8 ± 0.1[b]	21.5 ± 1.6[b]	—	1.3 ± 0.3	6.0 ± 2.0

Values represent means ± SE for eight rats. CAS: casein, SOY: soybean protein, PER: perilla oil, SAF: safflower oil.
[a,b,c]Significantly different from the CAS-PER, SOY-PER, and CAS-SAF group at $P < 0.05$, respectively.

Table 5 Effects of dietary proteins and fats on the fatty acid compositions of brain phospholipids in SHR-SP

Groups	Fatty acids											
	16:0	16:1	18:0	18:1	18:2 n-6	18:3 n-6	20:3 n-6	20:4 n-6	22:4 n-6	22:5 n-6	22:5 n-3	22:6 n-3
	weight %											
Phosphatidylcholine												
CAS-PER	42.9 ± 0.6	1.0 ± 0.1	13.2 ± 0.1	33.4 ± 0.5	0.9 ± 0.1	1.6 ± 0.0	0.4 ± 0.0	3.3 ± 0.2	0.3 ± 0.0	—	0.1 ± 0.0	1.8 ± 0.2
SOY-PER	42.7 ± 0.4	1.0 ± 0.1	13.2 ± 0.1	32.9 ± 0.5	1.2 ± 0.2	1.6 ± 0.0	0.4 ± 0.0	3.4 ± 0.2	0.2 ± 0.1	—	0.2 ± 0.1	1.7 ± 0.2
CAS-SAF	42.2 ± 0.9	0.8 ± 0.1	13.1 ± 0.1	32.9 ± 0.4	1.1 ± 0.1	1.6 ± 0.0	0.3 ± 0.0	4.5 ± 0.3[a]	0.5 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	1.4 ± 0.1
SOY-SAF	42.5 ± 0.6	0.9 ± 0.1	13.1 ± 0.1	32.9 ± 0.5	1.0 ± 0.1	1.5 ± 0.0	0.3 ± 0.0[b]	4.3 ± 0.2[b]	0.5 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	1.4 ± 0.1
Phosphatidylethanolamine												
CAS-PER	4.5 ± 0.1	—	15.4 ± 0.2	23.1 ± 1.8	—	3.0 ± 0.1	0.6 ± 0.0	10.1 ± 0.2	4.2 ± 0.1	0.2 ± 0.0	1.1 ± 0.1	18.4 ± 0.6
SOY-PER	4.4 ± 0.1	—	15.3 ± 0.2	22.6 ± 1.6	—	3.0 ± 0.1	0.7 ± 0.0	10.2 ± 0.2	4.3 ± 0.1	0.2 ± 0.0	1.0 ± 0.1	18.8 ± 0.6
CAS-SAF	4.5 ± 0.2	—	15.6 ± 0.3	20.1 ± 1.6	—	2.8 ± 0.1	0.7 ± 0.0	12.7 ± 0.2[a]	6.2 ± 0.1[a]	2.1 ± 0.1[a]	0.2 ± 0.0	15.6 ± 0.5[a]
SOY-SAF	4.4 ± 0.1	—	15.7 ± 0.3	19.2 ± 1.8	—	2.8 ± 0.1	0.6 ± 0.0	12.6 ± 0.2[b]	6.1 ± 0.2[a]	2.1 ± 0.1[b]	0.2 ± 0.0	15.7 ± 0.6[b]
Phosphatidylinositol												
CAS-PER	4.8 ± 0.4	—	36.5 ± 1.0	4.9 ± 0.3	—	—	—	38.1 ± 1.5	1.1 ± 0.4	—	2.1 ± 0.9	7.1 ± 1.0
SOY-PER	5.6 ± 0.3	—	38.4 ± 0.7	5.1 ± 0.2	—	—	—	39.9 ± 1.0	0.4 ± 0.3	—	1.3 ± 0.6	6.7 ± 0.7
CAS-SAF	5.1 ± 0.2	—	37.7 ± 0.5	4.6 ± 0.1	—	0.5 ± 0.2	—	40.3 ± 0.5	1.2 ± 0.4	—	1.4 ± 0.5	5.6 ± 0.5
SOY-SAF	4.7 ± 0.3	—	38.2 ± 0.8	4.8 ± 0.2	—	0.4 ± 0.1	—	41.7 ± 0.8	1.3 ± 0.4	—	1.7 ± 0.4	5.6 ± 0.6
Phosphatidylserine												
CAS-PER	1.2 ± 0.1	—	41.7 ± 0.2	18.4 ± 0.6	—	1.3 ± 0.0	1.1 ± 0.0	3.9 ± 0.3	2.7 ± 0.1	0.4 ± 0.0	1.2 ± 0.1	24.9 ± 0.8
SOY-PER	1.2 ± 0.1	—	41.7 ± 0.3	18.2 ± 0.5	—	1.4 ± 0.1	1.2 ± 0.0	4.0 ± 0.1	2.6 ± 0.1	0.3 ± 0.0	1.2 ± 0.1	25.4 ± 0.8
CAS-SAF	1.2 ± 0.1	—	41.8 ± 0.3	16.5 ± 0.9	—	1.3 ± 0.1	1.0 ± 0.0	4.9 ± 0.2[a]	4.0 ± 0.1[a]	3.5 ± 0.2[a]	0.5 ± 0.1[a]	22.1 ± 0.7[a]
SOY-SAF	1.2 ± 0.1	—	42.3 ± 0.3	16.6 ± 0.4	—	1.3 ± 0.0	1.1 ± 0.0	4.8 ± 0.1[b]	4.0 ± 0.1[b]	3.3 ± 0.1[b]	0.5 ± 0.0[b]	21.8 ± 0.5[b]

Values represent means ± SE for 8 rats. Fatty acids less than 1% were omitted. CAS: casein, SOY: soybean protein, PER: perilla oil, SAF: safflower oil. [a,b]Significantly different from the CAS-PER and SOY-PER group at $P < 0.05$, respectively.

Relationship between desaturation index and prostacyclin production

A highly significant positive correlation was observed between LA desaturation index, the ratio of (20:3n-6 + 20:4n-6)/18:2n-6, in liver microsomal PC and in aortic PC, and between PGI_2 production by the aorta and the LA desaturation index in aortic PC *(Figure 4)*. As a result, there was also a statistically significant positive correlation between PGI_2 production by the aorta and the LA desaturation index in liver microsomal PC.

Plasma amino acid concentration

As shown in *Figure 5*, plasma concentrations of isoleucine, proline, serine, threonine, and valine were significantly higher in the rats fed CAS than in those fed SOY when dietary fat was PER, while the concentration of glycine was higher in the latter.

Discussion

The impaired desaturase-elongase system for PUFAs or the imbalance in the eicosanoid production may be related to the development of hypertension.[3,4,18] Thus, increased dietary intake of PUFAs, particularly n-3 fatty acids, is recommended for hypertensive animals more than normotensive animals.[19]

Dietary protein influences desaturation of PUFAs. CAS in relation to SOY promotes the desaturation of LA and ALA in normal rats.[7] The present study also confirmed the

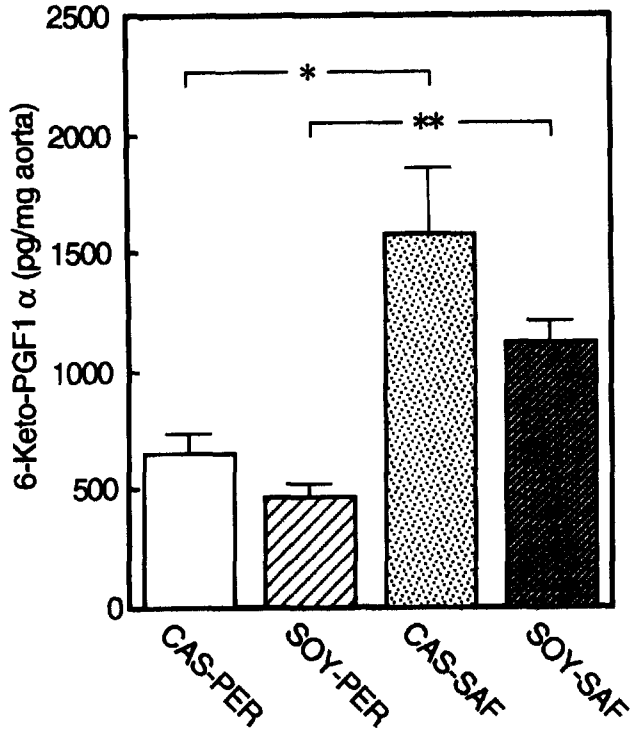

Figure 3 Effects of dietary proteins and fats on aortic PGI_2 production in SHR-SP. Each column and vertical bar represent the means ± SE for eight rats. PGI_2 was measured as 6-keto $PGF_{1\alpha}$. CAS: casein, SOY: soybean protein, PER: perilla oil, SAF: safflower oil. *,**Significantly different at $P < 0.05$ and $P < 0.01$, respectively.

regulatory action of dietary protein in the metabolism of the fatty acids, even in SHR-SP. From the results of the ratio of (20:3n-6 + 20:4n-6)/18:2n-6 and EPA/ALA of liver microsomal PC, CAS appeared to stimulate the desaturation of LA and ALA. Thus, CAS, compared with SOY, is thought to be useful for improving the impaired desaturase-elongase system of hypertensive animals.

PGI_2, which is produced from AA, causes vasodilation and inhibits thrombosis. The protein-dependent change in the fatty acid composition was also reproduced in the aorta, and the ratio of (20:3n-6 + 20:4n-6)/18:2n-6 of aortic PC was correlated significantly with the prostacyclin production, indicating a positive association between the LA desaturation and prostacyclin production. While the PGI_3 production from n-3 PUFAs was not determined, it is reasonable to consider that the same is true in that case, also.

Although we did not analyze the fatty acid composition of the platelets, the ratio of EPA/AA in liver microsomal PC was higher in the rats fed CAS than in those fed SOY. TXA_3 (thromboxane) from EPA does not exert an aggregatory effect in contrast to TXA_2 from AA. Thus, it is suggested that CAS may influence the balance of 3- and 2- series PGs in favor of preventing thrombosis, because the similar protein-effect was observed in various tissue lipids.

In brain PE, LA and EPA were very low, while DHA was markedly high as compared with that in liver, plasma, and aorta. However, the effect of dietary protein on the brain fatty acid composition was not observed. The fatty acid composition of brain phospholipids has in general been shown to be relatively resistant to this type of dietary fat compared with other tissues.[20]

Hypertension is one of the common aggravating factors for stroke and atherogenesis. While hypertension is a direct cause of arterionecro-thrombogenic stroke, it accelerates atherogenesis in cerebral arteries more than hyperlipidemia.[21] A significant inverse correlation has been noted between blood pressure and the ratio of low density lipoprotein to

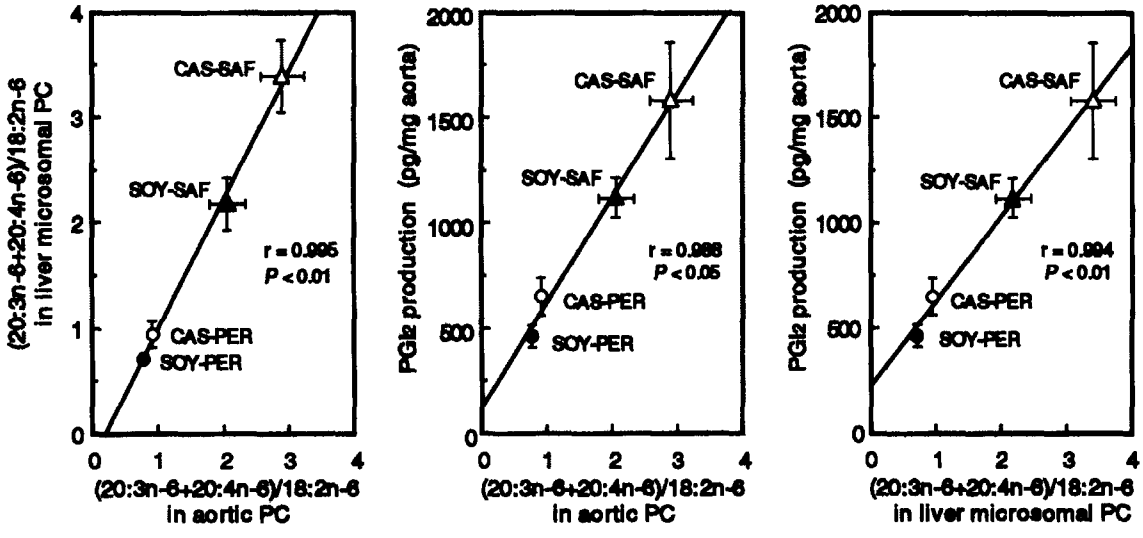

Figure 4 Relationship between aortic PGI_2 production and desaturation index in SHR-SP. Each point and vertical bar represent the mean ± SE for eight rats. CASE: casein, SOY: soybean protein, PER: perilla oil, SAF: safflower oil.

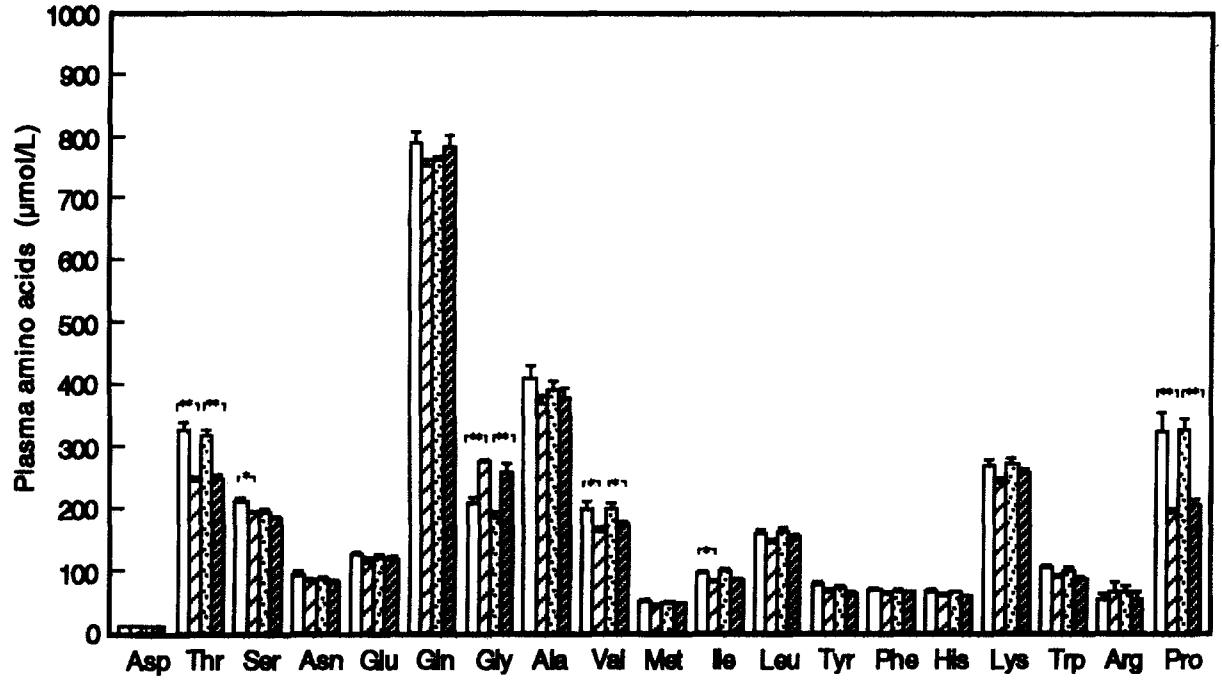

Figure 5 Effects of dietary proteins and fats on plasma amino acid concentrations in SHR-SP. □ : CAS-PER, ▨ : SOY-PER, ▩ : CAS-SAF, ▩ : SOY-SAF. Each column and vertical bar represent mean ± SE for eight rats. CAS: casein, SOY: soybean protein, PER: perilla oil, SAF: safflower oil. *,**Significantly different at $P < 0.05$ and $P < 0.01$, respectively.

high density lipoprotein (atherogenic index).[1] In this study, the concentration of plasma cholesterol was lower in the rats fed SOY than in those fed CAS. However, the concentration of plasma HDL cholesterol in the SOY group was also low, thus the atherogenic index was comparable between both protein groups. In addition, no correlation between the concentration of plasma cholesterol and that of amino acids was observed in contrast to the case of ExHC rats,[17] in which the concentrations of plasma arginine and glycine, one of the possible regulatory factors responsible for the plasma cholesterol level, were higher in the SOY-fed rats than in the CAS-fed rats, suggesting the effect of the amino acid composition of dietary proteins on plasma cholesterol.[22]

CAS or ALA attenuated the development of hypertension compared with SOY or LA, respectively. These results were consistent with previous reports.[1,23] The protein-dependent difference could be attributed in part to the difference in the amino acid composition. It has been reported that there was a significant negative correlation between dietary methionine and blood pressure or stroke incidence, and that tyrosine administration decreased blood pressure.[1] CAS contained these amino acids more than SOY (2.77 and 1.22 g/100 g, and 5.45 and 3.86 g/100 g for methionine and tyrosine, respectively). Thus, CAS may ameliorate hypertension in part by affecting the central blood pressure regulation through increasing the supply of these amino acids.[1]

In summary, the protein effect on the metabolism of n-6 and n-3 PUFAs was confirmed, even in SHR-SP. CAS promoted the desaturation of LA and ALA in liver microsomal and aortic phospholipids and the prostacyclin production by the thoracic aorta. There was a highly positive correlation between the prostacyclin production by the aorta and the LA desaturation index in liver microsomes and the aorta. These results indicate a significant role of dietary protein in the regulation of the fatty acid metabolism and prostacyclin production in SHR-SP.

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