Effects of Dietary *n*-3/*n*-6 and Polyunsaturated Fatty Acid/Saturated Fatty Acid Ratios on Platelet Aggregation and Lipid Metabolism in Rats

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Summary We studied the effects of dietary lipids on platelet aggregation and lipid metabolism in rats by varying the *n*-3/*n*-6 ration while maintaining the polyunsaturated fatty acid/saturated fatty acid (P/S) ratio fixed, and vice versa. After two weeks, the platelet counts decreased as the dietary *n*-3/*n*-6 ratio rose, and platelet aggregation was sufficiently suppressed at the ratio of 0.2. Differences in the dietary P/S ratio, however, did not affect either the platelet counts nor platelet aggregation. As the dietary *n*-3/*n*-6 ratio rose, the proportion of arachidonic acid (AA) in the plasma and the phospholipids (PL) of the platelets and aorta decreased gradually, whereas the proportion of eicosapentaenoic acid (EPA) in each tissue increased gradually. The proportion of EPA was higher in the platelets than in the aorta, while that of docosahexaenoic acid (DHA) was higher in the latter. The production of platelet thromboxane A₂ (TXA₂) and aortic prostacyclin (PGI₂) showed sharp declines, from the values for the *n*-3/*n*-6 ratio of 0.02 (control) to those for 0.5. These results suggest that the *n*-3/*n*-6 ratio of dietary fats necessary to ensure the suppression of platelet aggregation in normal rats would be at least 0.2 and no more than 0.5.

Key Words *n*-3/*n*-6 ratio, platelet aggregation, arachidonic acid, eicosanoids, eicosapentaenoic acid, docosahexaenoic acid

It is well known that fish oils rich in *n*-3 polyunsaturated fatty acids (PUFA) exhibit an antithrombotic action through the suppression of platelet aggregation in humans (1,2). Animal experiments have shown that fish oils are more effective in improving serum lipids than vegetable oils rich in *n*-6 PUFA (3-5). It has also been confirmed that the administration of perilla seed oil containing α -linolenic acid (C18:3 *n*-3, α -LnA) in abundance, refined eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) suppresses platelet aggregation in rats (6,7). There are also

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reports (8, 9) that the oral administration of refined EPA ethylester causes the ratio of EPA to arachidonic acid (AA) in platelet PL to increase and platelet aggregation to decrease in human. Sinclair (2), however, pointed out that the excessive intake of fish oils might induce various physiological abnormalities in the body, and claimed the importance of a well-balanced intake of *n*-6 and *n*-3 PUFA. Although many studies have since been conducted regarding the relation between physiological function or metabolic function and the *n*-3/*n*-6 ratio (10–15), no substantial studies have focused on the relation of the dietary *n*-3/*n*-6 ratio with platelet aggregation except for those by Takahashi et al. (16) and Surette et al. (17). The former group did not observe any correlation between them. The latter group reported that platelet aggregation in hamsters was slightly suppressed when the *n*-3/*n*-6 ratio was 0.56 ($p < 0.08$). In this study, we examined the effect of dietary fats on platelet aggregation in rats by varying the *n*-3/*n*-6 ratio or the P/S ratio. In the first and second experiments, the *n*-3/*n*-6 ratio was varied while the P/S ratio was kept at 1.0. In the third experiment, the *n*-3/*n*-6 ratio was kept at 0.2 or 0.5 and the P/S ratio was changed to 0.5, 1.0 and 1.5. Further, in connection with the above, the effects of different *n*-3/*n*-6 ratios on fatty acid composition, the eicosanoid formation in platelets and the aorta, and plasma lipid concentrations were also investigated.

MATERIALS AND METHODS

Experimental animals, diet and rearing method. Following one week of preliminary rearing on a basal diet, five-week-old male Sprague-Dawley strain rats (Tokyo Experimental Animals Co., Ltd., Tokyo) were housed in individual stainless steel apartment cages. The animal room was maintained at $23 \pm 1^\circ\text{C}$, $50 \pm 5\%$ humidity and a light/dark cycle of 12 h (lighting: 0800–2000). The animals were fed experimental diets for two weeks. The feed was supplied at 1700 and removed at 0900 the following morning. The animals were allowed water *ad libitum*. The basal diet was composed of 20% casein, 0.3% DL-methionine, 60% sucrose, 10% lard, 3.5% mineral mixture (AIN-76TM), 1.0% vitamin mixture (AIN-76TM), 0.2% choline bitartrate and 5% cellulose. The test fats used in the experiments were prepared by mixing lard, safflower oil, coconut oil (Hayashi Chemicals Co., Ltd., Tokyo) and fish oil (Nippon Oil & Fat Co., Ltd., Tokyo), and were added to the experimental diets at 10% level. Table 1 shows the fatty acid compositions, P/S ratios and *n*-3/*n*-6 ratios in each experiment. In experiments 1 and 2, the *n*-3/*n*-6 levels of 0.03 and 0.02 were designated as controls, respectively.

Separation of platelets and extirpation of aorta. On completion of the rearing period, rats were laparotomized under anesthesia with pentobarbital sodium (Pitman-Moore, Inc., Mundelen, USA), and blood was collected from the abdominal aorta using a polyethylene tube containing 77 mm of 7.5% EDTA·2K (pH 7.4) and a glass test tube. Following the counting of platelets using a particle counter (PC-8; Elma Co., Ltd., Tokyo), the blood was centrifuged at $160 \times g$ at room

Table 1. Fatty acid compositions of experimental fats.

Groups		Fatty acids (%)								Test oils ¹ (%)				
<i>n</i> -3/ <i>n</i> -6 ratio	P/S ratio	12:0	14:0	16:0	16:1	18:0	18:1	18:2 (<i>n</i> -6)	20:5 (<i>n</i> -3)	22:6 (<i>n</i> -3)	LD	SO	CO	FO
Exp. 1														
0.03 (Control)	1.0	0.1	1.1	19.3	2.3	9.3	35.6	28.9	—	—	7.1	2.9	—	—
0.5	1.0	2.0	3.4	18.0	4.0	7.7	28.7	20.0	4.5	2.6	4.7	1.9	0.5	3.0
1.0	1.0	3.2	4.1	16.8	5.7	5.6	23.7	15.1	8.1	4.3	3.3	1.4	0.7	4.6
2.1	1.0	4.0	6.1	15.9	6.1	4.6	19.4	9.3	10.8	6.2	1.9	0.8	1.0	6.3
4.8	1.0	6.3	8.0	14.8	7.2	3.1	14.6	3.8	13.4	7.7	0.5	0.2	1.3	8.1
Exp. 2														
0.02 (Control)	1.0	0.1	1.7	19.6	2.2	9.7	35.0	29.5	0.1	—	7.1	2.9	—	—
0.2	1.0	0.9	2.1	18.6	3.0	8.7	32.0	25.7	2.0	1.9	6.0	2.5	0.2	1.2
0.5	1.0	2.0	3.4	17.8	3.9	7.5	28.4	20.5	4.5	3.0	4.7	2.0	0.5	2.7
1.0	1.0	3.3	4.9	16.7	4.7	6.0	24.0	14.8	7.4	3.9	3.5	1.5	0.8	4.3
Exp. 3														
0.2	0.5	25.9	11.7	10.5	1.3	2.7	11.7	24.0	2.4	1.2	8.8	1.2	—	—
0.2	1.0	16.9	8.1	9.8	1.7	2.5	12.5	34.3	3.4	1.0	7.0	3.0	—	—
0.2	1.5	11.7	6.3	9.4	2.1	2.4	13.1	40.8	4.0	2.2	5.6	4.4	—	—
0.5	0.5	24.3	11.6	10.9	2.3	2.6	11.4	18.5	4.5	2.5	—	—	3.4	6.6
0.5	1.0	14.6	8.3	10.7	3.2	2.5	12.5	26.6	6.4	3.8	—	—	1.2	8.8
0.5	1.5	9.2	6.7	10.7	4.3	2.7	13.0	20.8	7.5	4.3	—	—	—	10.0

¹ LD: lard, SO: safflower oil, CO: coconut oil, FO: fish oil.

temperature for 10 min to obtain platelet-rich plasma (PRP). PRP was then centrifuged at $600 \times g$ at room temperature for 10 min to separate the platelets. The separated platelets were suspended in calcium-free serum from rats fed the basal diet (containing 1.2 mg of EDTA $\cdot$ 2K per 1 ml of serum) so as to make the platelet counts in the suspension equal to the counts in the blood. The residue of PRP after separation was centrifuged at $2,000 \times g$ at room temperature for 10 min to obtain platelet-poor plasma (PPP). The abdominal aorta, extending from the topmost region to the dichotomous region, was extirpated and removed of fat.

Analytical methods. Platelet aggregation was measured by nephelometry using an aggregometer (SA-8; Mebanix Co., Ltd., Tokyo). The platelet suspension was stimulated with collagen (final concentration $3 \mu\text{g/ml}$, dissolved in physiological saline containing 43 mM CaCl_2). Plasma total-cholesterol (TC), phospholipids (PL) and HDL-cholesterol (HDL-Chol) concentrations were measured by Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and plasma triacylglycerols (TG) by Clintec TG-S (Iatron Laboratories, Inc., Tokyo). Lipids were extracted from the plasma, platelets and aorta according to the method of Folch et al. (18). To platelets and the aorta specimen, 25-fold (w/w) of CM mixture was respectively added. The former mixture was subjected to ultrasonic treatment, and the latter was homogenized and allowed to stand overnight to extract lipids. PL fractions were separated, and the fatty acid compositions were analyzed according to the method of Taniguchi et al. (19). Gas chromatography was conducted with a GC-12A (Shimadzu Corporation, Kyoto, Japan) using $0.25 \text{ mm} \times 40 \text{ m}$ Silar-5CP stainless steel capillary columns (Chromatotec Co., Ltd., Tokyo), at a column temperature of 200°C , inlet temperature of 250°C and using nitrogen gas (2.2 ml/min) as the carrier. Fatty acids were identified based on the relation between the homologous carbon number and the retention time or comparison with the retention time of the authentic samples. The production of thromboxane A_2 (TXA_2) in platelets and prostacyclin (PGI_2) in the aorta were determined by slightly modified methods of Lee et al. (10) and Mann et al. (20), respectively. The aorta, approximately 2 cm long, was incubated for 15 min in 4 ml of Krebs Ringer-bicarbonate buffer (KRB, pH 7.4) supplemented with 5.5 mM glucose, and further incubated for 3 h in 4 ml of this mixture. The resultant product was diluted and used as a sample. The concentrations of thromboxane B_2 (TXB_2), a stable metabolite of TXA_2 , and 6-keto-prostaglandin $\text{F}_{1\alpha}$ (6-keto- $\text{PGF}_{1\alpha}$), a stable metabolite of PGI_2 , were determined using an enzyme immunoassay (EIA) kit (Amersham International plc Co., Ltd., Buckinghamshire, England).

Statistical analysis. Significant intergroup differences were studied by Duncan's multiple range test (21). The effects of the level of P/S ratio and n -3/ n -6 ratio and the interaction were evaluated by two-way ANOVA using a SAS computer package. Differences were considered significant at $p < 0.05$.

RESULTS

Exp. 1

No significant intergroup differences were observed in body weight gain or food intake. Table 2 shows the platelet counts and platelet aggregation. The platelet counts in the groups allotted an *n*-3/*n*-6 ratio of 0.5 or higher were significantly decreased as compared to those of the control group, but no difference was observed among the 0.5 group and the groups allotted ratios higher than 0.5. Platelet aggregation was significantly suppressed in groups with ratios of 0.5 and higher as compared to the control group.

Exp. 2

No significant intergroup differences were observed in body weight gain or food intake. Table 3 shows the plasma lipid concentrations. TG and PL concentrations decreased significantly at the *n*-3/*n*-6 ratio of 0.2, and the TC concentration showed a significant decrease at 0.5 as compared to concentrations in the control group.

HDL-Chol concentrations in the groups allotted *n*-3/*n*-6 ratios of 0.2 or higher were significantly higher than those in the control group, but the values in the groups with higher *n*-3/*n*-6 levels remained substantially constant. Table 2 shows the platelet counts and platelet aggregation. Platelet counts were smaller in the groups allotted higher *n*-3/*n*-6 levels. Platelet aggregation in the 0.2 and higher ratio groups was significantly suppressed and kept the same level as compared to the control group. Table 4 shows the fatty acid compositions of the total plasma lipids

Table 2. Effects of dietary *n*-3/*n*-6 ratios on platelet counts and platelet aggregation.¹

Items		<i>n</i> -3/ <i>n</i> -6 ratios				
		0.03	0.5	1.0	2.1	4.8
Exp. 1	Platelet counts ($\times 10^4/\mu\text{l}$)	70.6 $\pm$ 1.0 ^a	59.2 $\pm$ 2.2 ^b	61.2 $\pm$ 2.3 ^b	58.4 $\pm$ 1.3 ^b	57.4 $\pm$ 1.4 ^b
	Platelet aggregation (%)	68.8 $\pm$ 1.0 ^a	61.4 $\pm$ 1.1 ^{bc}	58.5 $\pm$ 2.7 ^b	64.1 $\pm$ 1.6 ^c	63.9 $\pm$ 0.9 ^c
Items		<i>n</i> -3/ <i>n</i> -6 ratios				
		0.02	0.2	0.5	1.0	
Exp. 2	Platelet counts ($\times 10^4/\mu\text{l}$)	79.6 $\pm$ 1.8 ^a	74.9 $\pm$ 1.3 ^{ab}	74.4 $\pm$ 2.4 ^{ab}	71.8 $\pm$ 1.3 ^b	
	Platelet aggregation (%)	71.8 $\pm$ 2.1 ^a	58.9 $\pm$ 4.9 ^b	57.4 $\pm$ 5.0 ^b	56.8 $\pm$ 3.9 ^b	

¹ M $\pm$ SE of eight rats. Means in the same line not sharing a common superscript letter are significantly different ($p < 0.05$).

Table 3. Effects of dietary *n*-3/*n*-6 ratios on concentrations of plasma lipids.¹

Plasma lipids (mg/dl)	<i>n</i> -3/ <i>n</i> -6 ratios			
	0.02	0.2	0.5	1.0
Total-cholesterol	76.5±4.5 ^a	70.2±2.2 ^a	61.1±3.2 ^b	58.8±2.5 ^b
HDL-cholesterol	27.7±2.8 ^a	41.9±2.5 ^b	40.9±2.8 ^b	42.2±2.1 ^b
Triacylglycerols	179±20.7 ^a	109±9.4 ^b	79±8.8 ^{bc}	58±3.1 ^c
Phospholipids	290±9.5 ^a	180±9.4 ^b	154±4.0 ^c	140±4.4 ^c

¹M±SE of eight rats. Means in the same line not sharing a common superscript letter are significantly different ($p < 0.05$).

and the PL fractions of the platelets and aorta. The proportion of C18:2 (*n*-6) in the PL fractions of the platelets and aorta in the 0.2 group significantly increased as compared to that in the control group. The proportion of C20:4 (*n*-6) in the plasma and PL fraction of the platelets showed a gradual decrease as the ratio was elevated. The proportion of C20:4 (*n*-6) in the PL fraction in the aorta showed a decreasing tendency in the group allotted the *n*-3/*n*-6 ratio of 0.5, and was significantly decreased in the 1.0 ratio group as compared to that in the 0.02 or 0.2 groups. As the *n*-3/*n*-6 ratio of dietary lipids was elevated, the incorporation of *n*-3 PUFA into the platelets and aorta increased. However, the incorporation patterns of individual *n*-3 PUFA were different among tissues. A comparison between the platelets and aorta revealed that the proportion of C20:5 (*n*-3) was larger than that of C22:6 (*n*-3) in the platelets and vice versa in the aorta, irrespective of the *n*-3/*n*-6 ratio. The P/S ratio in plasma was around 1.0 in all the groups, which was the same as that for the dietary lipids; whereas the ratios in the PL fractions of the platelets and aorta were around 0.3, and the ratio in the platelets was significantly reduced when the *n*-3/*n*-6 ratio was 1.0. The *n*-3/*n*-6 ratio in all the tissues increased gradually as the dietary *n*-3/*n*-6 ratio was elevated. The ratio of [20:3 (*n*-6) + 20:4 (*n*-6)]/[18:2 (*n*-6)], regarded as the desaturation index, for the linoleic acid metabolism decreased in the plasma as the *n*-3/*n*-6 ratio increased. The value of platelet PL significantly decreased in the *n*-3/*n*-6 0.2 group as compared to that in the control group, but no changes were observed at higher *n*-3/*n*-6 levels. No significance was observed in the index of the aorta PL. Figure 1 shows the changes in TXA₂ and PGI₂ production in the platelets and aorta, respectively. The decrease in production of both eicosanoids was sharp for the groups with ratios from 0.02 to 0.5, but was slight with no significance from 0.5 to 1.0.

Exp. 3

There were no significant intergroup differences in body weight gain or food intake. Table 5 shows the platelet counts and platelet aggregation, indicating that the P/S ratio had no effect on them.

Table 4. Effects of dietary *n*-3/*n*-6 ratios on fatty acid composition of plasma lipids, and phospholipid fractions in platelets and aorta (%).¹

Tissues	Fatty acids and ratios	<i>n</i> -3/ <i>n</i> -6 ratios			
		0.02	0.2	0.5	1.0
Plasma	16:0	22.0±0.6	21.5±1.0	21.9±0.8	22.7±0.7
	18:0	11.2±0.7 ^a	10.6±0.7 ^a	8.8±0.3 ^b	8.2±0.4 ^b
	18:1	22.7±2.4 ^a	16.9±1.2 ^b	18.3±1.3 ^{ab}	17.6±1.2 ^b
	18:2 <i>n</i> -6	12.9±1.3	13.6±1.3	11.8±0.9	10.5±0.2
	20:3 <i>n</i> -6	0.3±0.0 ^a	0.9±0.2 ^b	0.7±0.3 ^{ab}	0.1±0.0 ^a
	20:4 <i>n</i> -6	18.1±2.1 ^a	16.1±1.5 ^a	10.0±1.3 ^b	8.3±0.5 ^c
	20:5 <i>n</i> -3	ND ²	2.3±0.2 ^a	6.4±0.5 ^b	8.4±0.4 ^c
	22:5 <i>n</i> -3	ND	0.6±0.2 ^a	1.6±0.3 ^b	2.1±0.1 ^b
	22:6 <i>n</i> -3	1.7±0.2 ^a	5.0±0.3 ^b	6.8±0.4 ^c	7.8±0.3 ^d
Platelets	P/S	0.89±0.02	1.00±0.02	0.98±0.06	0.91±0.01
	<i>n</i> -3/ <i>n</i> -6	0.05±0.01 ^a	0.25±0.01 ^b	0.66±0.05 ^c	0.97±0.03 ^d
	Desaturation index ³	1.56±0.30 ^a	1.38±0.26 ^{ab}	0.94±0.14 ^{ab}	0.81±0.06 ^b
	16:0	33.4±0.7 ^a	32.7±1.3 ^a	34.9±0.8 ^a	37.6±0.2 ^b
	18:0	19.3±0.4 ^a	18.6±0.7 ^{ab}	17.2±0.6 ^b	19.6±0.3 ^a
	18:1	11.0±1.1 ^a	14.4±1.2 ^b	11.9±0.9 ^{ab}	9.7±0.2 ^a
	18:2 <i>n</i> -6	6.3±0.1 ^{ab}	9.3±0.5 ^c	6.7±0.2 ^a	5.7±0.2 ^b
	20:3 <i>n</i> -6	0.4±0.0	0.5±0.1	0.4±0.0	0.6±0.0
	20:4 <i>n</i> -6	12.4±1.2 ^a	6.9±0.9 ^b	5.8±0.5 ^{bc}	4.1±0.1 ^c
Aorta	20:5 <i>n</i> -3	ND	1.4±0.2 ^a	3.3±0.2 ^b	3.8±0.2 ^c
	22:5 <i>n</i> -3	ND	0.4±0.2 ^a	1.0±0.2 ^b	1.2±0.2 ^b
	22:6 <i>n</i> -3	ND	0.5±0.3 ^a	1.6±0.1 ^b	1.9±0.3 ^b
	P/S	0.35±0.03 ^a	0.33±0.04 ^a	0.30±0.01 ^{ab}	0.25±0.01 ^b
	<i>n</i> -3/ <i>n</i> -6	0.04±0.00 ^a	0.18±0.03 ^b	0.48±0.02 ^c	0.67±0.04 ^d
	Desaturation index	2.05±0.21 ^a	0.78±0.09 ^b	0.92±0.09 ^b	0.81±0.01 ^b
	16:0	33.7±1.1	31.1±0.7	32.3±1.4	31.8±0.7
	18:0	22.4±0.6 ^a	20.3±0.7 ^b	23.0±0.6 ^a	22.6±0.4 ^a
	18:1	13.8±0.5 ^a	12.7±0.4 ^{ab}	10.4±1.9 ^b	11.8±0.5 ^{ab}
	18:2 <i>n</i> -6	5.0±0.2 ^a	6.9±1.1 ^b	5.4±0.4 ^{ab}	4.2±0.2 ^a
	20:3 <i>n</i> -6	0.6±0.2	0.7±0.1	0.7±0.1	0.7±0.0
	20:4 <i>n</i> -6	7.3±0.9 ^a	7.2±0.4 ^a	5.9±0.7 ^{ab}	4.4±0.4 ^b
	20:5 <i>n</i> -3	ND	0.8±0.1 ^a	2.0±0.1 ^b	2.6±0.1 ^c
	22:5 <i>n</i> -3	0.2±0.1 ^a	1.2±0.1 ^b	1.6±0.2 ^b	1.3±0.0 ^b
	22:6 <i>n</i> -3	0.9±0.4 ^a	3.2±0.3 ^b	3.6±0.4 ^b	3.2±0.1 ^b
	P/S	0.24±0.03 ^a	0.33±0.02 ^b	0.30±0.02 ^{ab}	0.25±0.01 ^a
	<i>n</i> -3/ <i>n</i> -6	0.07±0.03 ^a	0.36±0.04 ^b	0.57±0.05 ^c	0.78±0.03 ^d
	Desaturation index	1.62±0.20	1.26±0.16	1.27±0.19	1.27±0.16

¹ M±SE of eight rats. Means in the same line not sharing a common superscript letter are significantly different ($p < 0.05$).

² ND=not detected.

³ Desaturation index = [20:3 *n*-6 + 20:4 *n*-6]/18:2 *n*-6.

Fig. 1. Effects of dietary n -3/ n -6 ratios on production of platelet TXA₂ and aortic PGI₂. TXA₂ was measured as TXB₂. PGI₂ was measured as 6-keto-PGF_{1α}. Black line: TXB₂, dotted line: 6-keto-PGF_{1α}. $M \pm SE$ of eight rats. Means in the same curve not sharing a common superscript letter are significantly different ($p < 0.05$).

Table 5. Effects of dietary P/S and n -3/ n -6 ratios on platelet counts and platelet aggregation.¹

P/S	Platelet counts ($\times 10^4/\mu l$)	Platelet aggregation (%)
	n -3/ n -6=0.2	
0.5	55.5 $\pm$ 1.9	62.4 $\pm$ 2.0
1.0	53.6 $\pm$ 2.1	59.9 $\pm$ 3.8
1.5	54.1 $\pm$ 1.2	61.5 $\pm$ 3.0
	n -3/ n -6=0.5	
0.5	51.0 $\pm$ 1.4	61.5 $\pm$ 2.8
1.0	52.0 $\pm$ 1.3	59.8 $\pm$ 3.9
1.5	50.3 $\pm$ 1.2	63.0 $\pm$ 2.6
ANOVA		
P/S	NS ²	NS
n -3/ n -6	0.01	NS
P/S $\times$ n -3/ n -6	NS	NS

¹ $M \pm SE$ of eight rats.

²NS=not significant.

DISCUSSION

The decrease in platelet counts induced by *n*-3 PUFA intake has already been established by the epidemiological studies of Eskimos (1,2) and by the experimental study on refined EPA administration to rats (22).

In this study, we also observed a decrease in platelet counts attributable to the inclusion of *n*-3 PUFA in diets. The data showed that *n*-3 PUFA is a more important factor than the P/S ratio in affecting platelet counts.

Platelet aggregation was also unaffected by an increase in the P/S ratio, and was found to be sufficiently suppressed when the *n*-3/*n*-6 ratio was 0.2. This is assumedly due to the fact that TXA₂ production in the platelets was significantly decreased when the *n*-3/*n*-6 ratio was 0.2. This assumption is supported by the finding that the proportion of AA in platelet PL was also significantly decreased at an *n*-3/*n*-6 ratio of 0.2. Surette et al. (17) reported that, in hamsters, the proportion of AA decreased ($p < 0.05$), but the suppression of platelet aggregation was weak ($p < 0.08$) at the *n*-3/*n*-6 ratio 0.56 although the P/S ratio was not identical. In the groups with higher *n*-3/*n*-6 levels, however, despite decreases in platelet counts and TXA₂ production, there were no noticeable differences in platelet aggregation. The reason for this remains unclear at present, but some defense mechanism may have worked to prevent the suppression of platelet aggregation. Although the P/S ratio in plasma lipids was around 1.0, the values in the PL fractions of the platelets and aorta were nearly as low as 0.3, indicating that the incorporation of PUFA into the PL of both tissues is comparatively low. However, this low level of incorporation is considered to be in no way definite, and to vary according to the duration of feeding. The proportion of AA in the PL fraction of the aorta tended to decrease at the *n*-3/*n*-6 ratio of 0.5 and significantly decreased at the ratio of 1.0 as compared to that at the ratios of 0.02 or 0.2. This result suggests that the variation pattern of PGI₂ production in the aorta is not necessarily similar to that of the proportion of AA. Lee et al. (10) found that, when rats were fed a diet containing cholesterol and perilla oil as the *n*-3 source under the condition of a P/S ratio of about 1.2, the proportion of AA in the phosphatidylcholine (PC) fraction in the aorta decreased at a *n*-6/*n*-3 ratio of 4 (*n*-3/*n*-6 ratio of 0.25) or lower, and that a similar behavior was observed in the production of PGI₂. There are some discrepancies between the results of Lee et al. and those reported here, but they are considered attributable to differences in some of the test conditions. Takahashi et al. (16) also reported that these formations were reduced accompanying increases in the *n*-3/*n*-6 ratio, although the P/S ratio was not fixed. EPA was more abundant than DHA in the PL fraction of platelets, nearly three times as much when the *n*-3/*n*-6 ratio was 0.2. Both EPA and DHA suppress platelet aggregation (7), but they behave differently in platelets; the former inhibiting the incorporation of AA into PL but not the latter (23). In contrast to the PL fraction of platelets, the proportion of DHA was larger than that of EPA in the PL fraction of the aorta. This finding agrees with the

results obtained by Takahashi et al. (16). DHA in the PL fraction of the aorta increased drastically at the n -3/ n -6 ratio of 0.2, and higher n -3/ n -6 ratios failed to produce further remarkable elevation of DHA. These results suggest that EPA and DHA are playing important roles in the platelets and aorta, respectively. Unlike the behavior in platelets, DHA is reported to inhibit the incorporation of AA into PL in endothelial cells (24) and is converted to hydroxy-docosahexaenoic acid by lipoxygenase (25,26). Hadjiagapiou and Spector (24) observed that 15% of DHA was retroconverted to EPA and docosapentaenoic acid (22:5 n -3, DPA) *in vitro* in cultured bovine endothelial cells. It is still unclear which of the above DHA functions acts potently in the aorta. Since the value of the desaturation index for the linoleic acid metabolism in the platelet PL fraction decreased significantly at the n -3/ n -6 ratio of 0.2 and no further changes were observed when the ratio was increased, Δ 6 desaturase activity in the platelets is assumed to be sufficiently suppressed at the ratio of 0.2, which agrees with the results for platelet aggregation.

The concentrations of plasma TC and TG decreased as the n -3/ n -6 ratio increased. It has already been confirmed by Schrijver et al. (15) that these concentrations decrease as the n -6/ n -3 ratio of dietary lipids is lowered (i.e. n -3/ n -6 ratio is raised) in rats free of cholesterol loading.

Based on these results, it is suggested that platelet aggregation is not affected by differences in the P/S ratio of dietary lipids and is suppressed by increasing the dietary n -3/ n -6 ratio, with 0.2 and no more than 0.5 being sufficient in normal rats.

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