Changes in Platelet Aggregation and Lipid Metabolism in Rats Given Dietary Lipids Containing Different n-3 Polyunsaturated Fatty Acids

Norihiro YAMADA,*,*** Jun SHIMIZU, Masahiro WADA, Toshichika TAKITA and Satoshi INNAMI

Department of Nutrition, Faculty of Agriculture, Tokyo University of Agriculture, Tokyo 156-8502, Japan
(Received October 17, 1997)

Summary We compared the effects of different n-3 polyunsaturated fatty acids (PUFA) on platelet aggregation and lipid metabolism in rats. α-Linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were used as n-3 PUFA sources. The rats were fed diets containing 10% lipids (polyunsaturated/saturated fatty acid (P/S) ratio = 1.0; n-3/n-6 = 0.02 for the control group, 0.2 for the test groups) for two weeks. The platelet counts, platelet aggregation, and production of thromboxane A₂ (TXA₂), plasma total cholesterol (TC) and triacylglycerols (TG) were not different between the ALA group and the control group, but showed a decreasing tendency for the EPA group and significant decreases for the DHA group. The production of prostacyclin in the aorta was significantly decreased in all of the n-3 PUFA groups when compared with that in the control group. Liver TC and TG concentrations were significantly decreased in the DHA group when compared with those in the control group. Based on the above, it is assumed that the physiological action exerted by n-3 PUFA differs by type and that DHA is a more effective n-3 PUFA, both for suppressing platelet aggregation and for modulating lipid metabolism in the plasma and liver of rats.

Key Words α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, platelet aggregation

Dyerberg and Bang (1) conducted an epidemiological study on the Inuits and reported that fish oil exhibited a platelet aggregation-suppressing effect. It has since been confirmed by animal experiments (2, 3) and clinical studies (4–6) that eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are contained

* To whom correspondence should be addressed.
** Present address: Department of Food Nutrition, Nagoya Bunri Junior College, Nagoya 451-0077, Japan.
abundantly in fish oil, as well as their precursor compound, \( \omega \)-linolenic acid (ALA), are effective for suppressing platelet aggregation. It has also been found that some physiological functions including lipid metabolism modulation are affected to different degrees depending on the type of \( n \)-3 PUFA \((7, 8) \). In fact, DHA has been reported to be most effective for suppressing platelet aggregation in in vitro experiments among \( n \)-3 PUFA \((9, 10) \). There are a limited number of studies that have attempted to quantitatively or relatively evaluate the in vivo relation between different \( n \)-3 PUFA types and the platelet function. Morisaki et al \((2) \) found that EPA and DHA were equally effective for suppressing platelet aggregation when compared with the control, while Ikeda et al \((11) \) reported that there was no difference among ALA (control), EPA and DHA in their action for suppressing platelet aggregation. Thus, the findings obtained by in vitro and in vivo studies on the relation between different \( n \)-3 types and suppression of platelet aggregation do not concur; moreover, the results obtained in vivo have not always been in agreement.

We \((12) \) previously carried out an experiment using fish oil as an \( n \)-3 source and safflower oil as an \( n \)-6 source and found that platelet aggregation was significantly suppressed even at the \( n \)-3/\( n \)-6 ratio of 0.2. The \( n \)-3/\( n \)-6 ratio of 0.2 may possibly be the minimum level in rats. We therefore carried out an experiment with the P/S ratio of dietary lipids set at 1 and the \( n \)-3/\( n \)-6 ratio at the minimum level of 0.2 in order to make a quantitative and/or relative evaluation of the platelet aggregation suppressing activity of different \( n \)-3 PUFA types and to obtain data on lipid metabolism for reference.

**MATERIALS AND METHODS**

**Experimental animals, diet and rearing method.** Following a week on a basal diet, five-week-old male Sprague-Dawley strain rats (Clea Japan, Inc., Tokyo, Japan) were individually housed in stainless steel apartment cages in a room \((23 \pm 1^\circ C\) temperature, \(50 \pm 5\%\) humidity) kept on a 12h light/dark cycle (lighting: 08:00–20:00), and given the test diet for two weeks. The feed was supplied at 17:00 and removed at 09:00 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-76\textsuperscript{TM}), 1.0\% vitamin mixture (AIN-76\textsuperscript{TM}), 0.2\% choline bitartrate and 5\% cellulose powder. The test lipids used in the experiments were prepared by mixing lard (Hayashi Chemicals Co., Ltd., Tokyo, Japan), Extra linoleic-90, Extra \( \omega \)-linolenic-90 (Nippon Oil & Fat Co., Ltd., Tokyo, Japan) and EPA-95E and DHA-95E (Harima Chemicals Inc., Tokyo, Japan), and added to the experimental diets to a level of 10\%.

Table 1 shows the representative fatty acid compositions, P/S ratio and \( n \)-3/\( n \)-6 ratios of the test lipids. The control group was allotted the \( n \)-3/\( n \)-6 ratio of 0.02.

**Separation of platelets and extirpation of aorta.** At the completion of the rearing period, the rats were laparotomized under anesthesia with pentobarbital sodium (Pitman-Moore, Inc., Mundelen, USA), and blood was collected from the
Table 1. Fatty acid compositions of experimental lipids.

| Fatty acid | Control | ALA | EPA | DHA |
|------------|---------|-----|-----|-----|
| C16:0      | 18.6    | 19.0| 18.9| 18.7|
| C18:0      | 10.2    | 10.0| 10.3| 10.4|
| C18:1      | 34.7    | 33.8| 34.3| 34.5|
| C18:2 n-6  | 30.3    | 25.8| 25.5| 25.5|
| C18:3 n-3  | 0.5     | 5.0 | 0.5 | 0.5 |
| C20:5 n-3  | —       | —   | 4.6 | 0.2 |
| C22:6 n-3  | —       | —   | —   | 4.8 |

P/S ratio  1.0  1.0  1.0  1.0
n-3/n-6 ratio 0.02 0.2 0.2 0.2

abdominal aorta with a silicon tube attached to a needle; 7.4 mL of the blood was placed in a polyethylene tube containing 0.6 mL of 77 mM EDTA·2K (pH 7.4) to measure platelet aggregation, and serum was obtained from the remaining sample to measure thromboxane B₂ (TXB₂), a stable metabolite of TXA₂.

Following the counting of platelets with a particle counter (PC-8; Elma Co., Ltd., Tokyo, Japan), the blood was centrifuged at 160 × g at room temperature for 10 min to obtain a platelet-rich plasma (PRP). PRP was then centrifuged at room temperature at 600 × g for 10 min to separate the platelets. The separated platelets were suspended in calcium-free serum (containing 1.2 mg of EDTA·2K per 1 mL) from rats fed the basal diet to the point where the platelet count in the serum was equal to that in the blood. The residue of PRP after separation was centrifuged at room temperature at 2,000 × g for 10 min to obtain platelet-poor plasma (PPP). The aorta extending from immediately behind the heart to the dichotomous region was extirpated and the fat was removed.

Analytical procedure. Plasma total cholesterol (TC), phospholipids (PL) and HDL-cholesterol (HDL-chol) were determined with a Test Wako kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and plasma triacylglycerols (TG) with a Clintec TG-S kit (Iatron Laboratories, Inc., Tokyo, Japan). Lipids in plasma, liver, platelets and aorta were extracted according to the method of Folch et al (13). Liver TC and PL were determined using the Test Wako kit (Wako Pure Chemical) and liver TG using the Clintec TG-S kit (Iatron). Fatty acid compositions of the plasma lipids and platelet and aorta PL were measured according to the methods described in a previous report (12). Platelet aggregation was measured by nephelometry with an aggregometer (SA-8; Mebanix Co., Ltd., Tokyo, Japan). The platelet suspension was stimulated with collagen that had been dissolved in physiological saline containing 43 mM CaCl₂ to give a final concentration of

Vol 44, No 2, 1998
3 µg/mL. The amounts of platelet TXA₂ and aorta prostacyclin (PGI₂) were determined by slightly modified methods of Lee et al. (14) and Mann et al. (15), 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 TXB₂ and 6-keto-prostaglandin F₁₂ (6-keto-PGF₁₂), a stable metabolite of PGI₂, were determined using an enzyme immunoassay (EIA) kit (Amersham International plc Co., Ltd., Buckinghamshire, England).

Statistical analyses. Significant intergroup differences in the means were studied by Duncan’s multiple range test (16) undertaken with an SAS computer package. The significance level was 5%.

RESULTS

Platelet counts and platelet aggregation

No significant intergroup differences were apparent in body weight gain, food intake or food efficiency (data not shown). Figure 1 shows the platelet counts and platelet aggregation. While the platelet counts in the ALA group were hardly different from those in the control group, they were significantly decreased in the EPA and DHA groups. In comparison with the control group, platelet aggregation was not significantly suppressed in the ALA and EPA groups but was significantly suppressed in the DHA group. Among the n-3 PUFA groups, however, no significant difference was apparent.
Table 2. Concentrations of plasma and liver lipids in rats fed different n-3 fatty acids for 2 weeks.1

| Items                   | Control      | ALA          | EPA          | DHA          |
|-------------------------|--------------|--------------|--------------|--------------|
| Plasma lipids (mmol/L)  |              |              |              |              |
| Total-cholesterol       | 2.07 ± 0.15a | 2.01 ± 0.07ab| 1.73 ± 0.10bc| 1.61 ± 0.08c |
| HDL-cholesterol         | 0.48 ± 0.06  | 0.54 ± 0.03  | 0.58 ± 0.06  | 0.51 ± 0.03  |
| Triacylglycerols        | 3.21 ± 0.57a | 2.77 ± 0.39ab| 1.73 ± 0.19b | 1.66 ± 0.15b |
| Phospholipids           | 2.46 ± 0.18a | 2.36 ± 0.16ab| 1.99 ± 0.12bc| 1.90 ± 0.11c |
| Liver lipids (μmol/g wet wt) |          |              |              |              |
| Total-cholesterol       | 6.94 ± 0.33a | 6.83 ± 0.35a | 7.30 ± 0.37a | 5.68 ± 0.32b |
| Triacylglycerols        | 30.0 ± 2.8a  | 26.7 ± 2.7a  | 25.9 ± 3.2a  | 11.7 ± 1.8b  |
| Phospholipids           | 24.2 ± 0.68  | 25.4 ± 0.86  | 25.0 ± 0.79  | 26.0 ± 0.31  |

1 Means ± SE of 8 rats. Means in the same line not sharing a common superscript letter are significantly different (p < 0.05).

**Plasma and liver lipids**

The results are shown in Table 2. The plasma TC and PL concentrations were significantly lower in the EPA and DHA groups than in the control group, while hardly any difference was noted in the ALA group. The concentrations in the DHA group were significantly lower than the values in the ALA group. There was no significant intergroup difference in HDL-chol. The plasma TG concentration was not substantially lower in the ALA group but was significantly lower in the EPA and DHA groups as compared to that in the control group. Liver TC and TG in the DHA group were significantly lower than the values in the control, ALA and EPA groups.

**Fatty acid compositions of plasma lipids and platelet and aortic PL fractions**

The results are shown in Table 3. Although the proportion of C18:2 (n-6) in the plasma was not affected by the difference in n-3 source, the corresponding proportion in the platelet PL fraction was significantly increased in the DHA group and that in the aortic PL fraction significantly increased in the EPA group when compared with those in the control group. The proportion of C18:3 (n-3) was significantly greater in the plasma and aortic PL fraction of the ALA group than in that of the control group. The proportion of C20:4 (n-6) was significantly decreased in the platelet and aortic PL fractions in all the n-3 PUFA groups in comparison with that of the control group. The proportion of C20:4 (n-6) in the aortic PL fraction in the DHA group was significantly lower than even that of the ALA group. The proportion of C20:5 (n-3) was significantly increased in the plasma and in both the platelet and aortic PL fractions in the EPA group when compared with levels in the control, ALA and DHA groups, while that of C22:6 (n-3) was significantly greater in the plasma as well as in both the platelet and
Table 3. Fatty acid composition of plasma lipids and phospholipid fractions in platelet and aorta of rats fed different n-3 fatty acids for 2 weeks.1

| Tissue | Fatty acid and ratio | Control | ALA   | EPA   | DHA   |
|--------|----------------------|---------|-------|-------|-------|
|        | (weight %)           |         |       |       |       |
| Plasma | 16:0                 | 25.0 ± 0.65 | 24.1 ± 0.45 | 24.7 ± 0.58 | 24.0 ± 0.38 |
|        | 18:0                 | 8.55 ± 0.45 | 9.02 ± 0.20 | 9.31 ± 0.40 | 9.65 ± 0.65 |
|        | 18:1                 | 29.0 ± 1.15 | 26.5 ± 0.46 | 24.2 ± 1.25 | 24.5 ± 1.05 |
|        | 18:2 n-6             | 15.6 ± 1.25 | 15.9 ± 0.95 | 15.0 ± 0.85 | 16.7 ± 0.65 |
|        | 18:3 n-3             | 0.17 ± 0.01 | 1.28 ± 0.16 | 0.20 ± 0.04 | 0.22 ± 0.02 |
|        | 20:4 n-6             | 9.89 ± 1.25 | 9.44 ± 1.05 | 7.80 ± 0.95 | 7.54 ± 0.65 |
|        | 20:5 n-3             | 0.11 ± 0.04 | 0.65 ± 0.09 | 3.27 ± 0.17 | 1.44 ± 0.32 |
|        | 22:6 n-3             | 0.91 ± 0.08 | 1.77 ± 0.12 | 2.75 ± 0.32 | 6.29 ± 0.75 |
|        | P/S                  | 0.80 ± 0.05 | 0.84 ± 0.06 | 0.90 ± 0.04 | 0.96 ± 0.03 |
|        | n-3/n-6              | 0.05 ± 0.00 | 0.16 ± 0.01 | 0.34 ± 0.03 | 0.34 ± 0.02 |
| Platelet| 16:0                 | 30.5 ± 1.45 | 29.1 ± 0.65 | 29.5 ± 0.95 | 30.8 ± 1.45 |
|        | 18:0                 | 18.6 ± 0.48 | 15.4 ± 0.95 | 17.4 ± 1.15 | 15.8 ± 0.25 |
|        | 18:1                 | 11.2 ± 0.35 | 13.3 ± 1.25 | 11.8 ± 0.75 | 11.8 ± 0.38 |
|        | 18:2 n-6             | 5.65 ± 0.25 | 6.45 ± 0.75 | 6.55 ± 0.34 | 7.80 ± 0.45 |
|        | 18:3 n-3             | 1.57 ± 0.47 | 2.41 ± 0.45 | 2.46 ± 0.35 | 2.40 ± 0.57 |
|        | 20:4 n-6             | 12.8 ± 0.95 | 9.47 ± 1.15 | 9.25 ± 0.56 | 8.98 ± 1.15 |
|        | 20:5 n-3             | 0.11 ± 0.05 | 1.64 ± 0.50 | 3.15 ± 0.38 | 1.27 ± 0.13 |
|        | 22:6 n-3             | 0.15 ± 0.10 | 0.06 ± 0.06 | 0.05 ± 0.05 | 0.45 ± 0.16 |
|        | P/S                  | 0.39 ± 0.02 | 0.39 ± 0.03 | 0.39 ± 0.02 | 0.39 ± 0.03 |
|        | n-3/n-6              | 0.12 ± 0.03 | 0.25 ± 0.04 | 0.38 ± 0.04 | 0.26 ± 0.05 |
| Aorta  | 16:0                 | 27.9 ± 0.95 | 29.7 ± 0.95 | 26.1 ± 1.75 | 30.4 ± 0.63 |
|        | 18:0                 | 16.1 ± 0.55 | 17.5 ± 0.65 | 18.1 ± 0.65 | 16.9 ± 0.44 |
|        | 18:1                 | 14.7 ± 0.46 | 14.5 ± 0.65 | 14.5 ± 0.65 | 15.8 ± 0.44 |
|        | 18:2 n-6             | 5.14 ± 0.46 | 6.10 ± 0.35 | 7.69 ± 1.25 | 6.75 ± 0.48 |
|        | 18:3 n-3             | 0.78 ± 0.13 | 2.01 ± 0.85 | 0.44 ± 0.07 | 0.74 ± 0.13 |
|        | 20:4 n-6             | 13.2 ± 0.56 | 11.3 ± 0.66 | 10.9 ± 0.66 | 9.15 ± 0.50 |
|        | 20:5 n-3             | 0.66 ± 0.11 | 0.98 ± 0.13 | 2.11 ± 0.16 | 1.19 ± 0.14 |
|        | 22:6 n-3             | 1.29 ± 0.27 | 1.46 ± 0.12 | 2.26 ± 0.25 | 3.05 ± 0.20 |
|        | P/S                  | 0.46 ± 0.05 | 0.47 ± 0.06 | 0.55 ± 0.08 | 0.44 ± 0.03 |
|        | n-3/n-6              | 0.14 ± 0.02 | 0.25 ± 0.04 | 0.30 ± 0.01 | 0.30 ± 0.01 |

1 Means ± SE of 8 rats. Means in the same line not sharing a common superscript letter are significantly different (p < 0.05).
Different n-3 PUFA and Platelet Aggregation and Lipid Metabolism

Fig. 2. Production of platelet TXA₂ and aortic PGI₂ in rats fed different n-3 fatty acids for 2 weeks. TXA₂ was measured as TXB₂ and PGI₂ as 6-keto-PGF₁α. Means±SE of 8 rats. Means in the same bar not sharing a common superscript letter are significantly different (p<0.05).

Concentrations of platelet TXA₂ and aortic PGI₂

The results are shown in Fig. 2. The concentration of TXA₂ in the platelets hardly decreased in the ALA group but decreased significantly in the EPA and DHA groups when compared with that in the control group. The PGI₂ concentration in the aorta decreased significantly in all of the n-3 PUFA groups when compared with that in the control group. The concentrations in the EPA and DHA groups were even significantly lower than that in the ALA group.

DISCUSSION

The decrease of platelet counts by the intake of n-3 PUFA has been confirmed by an epidemiological study conducted among the Inuits (1) and also by an experimental study of rats fed diets containing EPA (17). We have already reported that the platelet count decreases with an increasing n-3/n-6 ratio (12). Since the platelet count was significantly less than that in the control group only in the EPA and DHA groups in the present experiments, the action of fish oils to decrease platelet count was considered attributable to the additional effect of EPA and DHA.

No statistically significant difference in platelet aggregation was apparent.
among the three n-3 PUFA groups. In this point, our results seem to substantiate
the reports by Ikeda et al (11). However, while platelet aggregation in the ALA
group was not different from that in the control group, a suppressive tendency and
a significant suppression were observed respectively in the EPA and DHA groups
when compared with the control group. This result was substantially similar to that
of Morisaki et al (2) whose experiment took no account of the P/S ratio or the
n-3/n-6 ratio (ALA was not used). It was not made clear why the aggregation
suppression pattern of Ikeda et al (11) and that of the authors’ did not concur,
although the P/S ratio and the n-3/n-6 ratio were set at definite levels, but the
differences in the basal oil used and the n-3/n-6 ratio were considered relevant. At
any rate, it seems that ALA is somewhat inferior to EPA or DHA in respect to
platelet aggregation suppressing activity. Thus, the relation ALA < EPA ≤ DHA
holds in terms of the degree of platelet aggregation suppressing activity. A number
of reports have pointed out that DHA exhibits a stronger activity than EPA in
suppressing platelet reaction in in vitro experiments using human platelets (9, 10).
From these results, it is assumed that platelet aggregation suppressing activity is
strongest in DHA, followed by EPA and then ALA. This may be explained by
the following activities of DHA that has been incorporated into the platelet phos-
pholipids: (a) changing the property of the platelet membrane (18), (b) inhibiting
phospholipase A2 (PLA2) activity by means of DHA-phosphatidylethanolamine
(DHA-PE) (19), (c) inhibiting cyclooxygenase activity (20, 21), (d) acting anta-
gonistically on TXA2 receptor (9) and (e) suppressing production of the platelet-
activating factor (PAF) (22). It is therefore assumed that the decrease in platelet
counts, decreased production of TXA2 due to diminished PLA2 and cyclooxygenase
activities, change in its receptor activity and reduced production of PAF are related,
to a great extent, to the platelet aggregation suppressing activity of DHA. Although
the results of our in vivo experiment did not exactly concur with those of the in
vitro experiments in respect to the intensity of platelet aggregation suppressing
activity, they were not greatly apart. Further investigation on the experimental
conditions is warranted. The concentration of TXA2 in serum was significantly
decreased only in the EPA and DHA groups, and this may account for the sup-
pressed platelet aggregation that was apparent only in the EPA and DHA groups
when compared with the control group. While there was no significant difference
among the n-3 PUFA groups in the proportion of arachidonic acid (AA) in the
platelet PL fraction, the concentration of TXA2 decreased only in the above two
groups. This suggests that the decreased production of TXA2 in these groups was
largely due to inhibited conversion from AA to TXA2 rather than to the inhibited
uptake of AA into PL. It is known that cyclooxygenase activity is inhibited by EPA
(23) and DHA (20, 21) and PLA2 activity by DHA-PE (19). Marangoni et al (24)
have reported that DHA is retained longer than EPA in blood components. DHA
is therefore assumed to exert its physiological action longer than EPA. In fact, the
proportion of DHA in serum, platelets and aortic PL in the DHA group was
significantly larger than the corresponding contents of ALA and EPA in the

J Nutr Sci Vitaminol
respective groups.

The production of PGI2 in the aorta was less in all of the n-3 PUFA groups than in the control group, and was significantly less in the EPA and DHA groups than in the ALA group. Ikeda et al (11) reported that the production of PGI2 in the aorta was less in DHA-fed animals than in ALA-fed animals. When the proportion of AA in the aortic PL fraction is taken into account, the decrease in the production of PGI2, similar to the case of TXA2, was considered largely attributable to the inhibited production of PGI2 from AA rather than to the inhibited uptake of AA into PL.

Past reports have assigned a strong plasma TG lowering action to EPA and a strong plasma TC lowering action to DHA (11, 25), but EPA and DHA were equally effective for lowering plasma TC and TG in the present experiments. This discrepancy may be due to the differences in the duration of feeding of the test diets and the dietary P/S and n-3/n-6 ratios.

Ikeda et al (11) have reported that the liver TC concentration was lower in DHA-fed animals than in those given ALA or EPA, and that the fecal neutral and acidic sterol excretions were not affected by difference in the source of n-3 PUFA. Since our finding on liver TC coincided with that of Ikeda et al (11), we assumed that DHA might help decrease cholesterol synthesis in the liver.

Similarly, the liver TG concentration was lower only in the DHA group. Ikeda et al (11) have also reported that DHA was more effective for lowering liver TG than either ALA or EPA. It is therefore assumed that DHA also decreases TG synthesis in the liver.

The results of the present experiments have shown that EPA and DHA were more effective than ALA for suppressing platelet aggregation. EPA and DHA were also more effective than ALA for improving plasma lipids, DHA being the more potent PUFA of the two. The liver TC and TG concentrations were decreased only in the DHA group. Based on the above, it is believed that the physiological action differs according to the type of n-3 PUFA, and that DHA is a more effective n-3 PUFA both for suppressing platelet aggregation and modulating lipid metabolism in the plasma and liver of rats.

REFERENCES

1) Dyerberg J, Bang OH. 1979. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet 1: 433-435.
2) Morisaki N, Shinomiya M, Matsuoka M, Saito Y, Kumagai A. 1983. In vivo effects of cis-5,8,11,14,17-20:5(n-3) and cis-4,7,10,13,16,19-22:6(n-3) on serum lipoproteins, platelet aggregation, and lipid metabolism in the aorta of rats. Tohoku J Exp Med 141: 397-405.
3) Watanabe S, Suzuki E, Kojima N, Kojima R, Suzuki Y, Okuyama H. 1989. Effect of dietary α-linolenate/linoleate balance on collagen-induced platelet aggregation and serotonin release in rats. Chem Pharm Bull 37: 1572–1575.
4) Terano T, Hirai A, Hamazaki T, Kobayashi S, Fujita T, Tamura Y, Kumagai A. 1983.
Effect of oral administration of highly purified eicosapentaenoic acid on platelet function, blood viscosity and red cell deformability in healthy human subjects. *Atherosclerosis* 46: 321–331.

5) Nagakawa Y, Orimo H, Harasawa M, Morita I, Yashiro K, Murota S. 1983. Effect of eicosapentaenoic acid on the platelet aggregation and composition of fatty acid in man. *Atherosclerosis* 47: 71–75.

6) Carol MW, Melvin JS, Jean W. 1991. Eicosapentaenoic acid ethyl ester as an antithrombotic agent: comparison to an extract of fish oil. *Biochem Biophys Acta* 1081: 33–38.

7) Drevon CA, Baksaaas I, Kyokan HE, eds. 1993. Omega-3 Fatty Acids: Metabolism and Biological Effects. Birkhausey Verlag, Berlin.

8) Yasugi T, Nakamura H, Soma M, eds. 1993. Advances in Polyunsaturated Fatty Acid Research. Excerpta Medica, Tokyo.

9) Swann PG, Venton LV, Breton GCL. 1989. Eicosapentaenoic acid and docosahexaenoic acid are antagonists at the thromboxane A₂/prostaglandin H₂ receptor in human platelets. *FEBS Lett* 243: 244–246.

10) Gaudette DC, Holub BJ. 1990. Albumin-bound docosahexaenoic acid and collagen-induced human platelet reactivity. *Lipids* 25: 166–169.

11) Ikeda I, Wakamatsu K, Inayoshi A, Imaiizumi K, Sugano M, Yazawa K. 1994. α-Linolenic, eicosapentaenoic and docosahexaenoic acids affect lipid metabolism differently in rats. *J Nutr* 124: 1898–1906.

12) Yamada N, Takita T, Wada M, Kanke Y, Innami S. 1996. Effects of dietary n-3/n-6 and polyunsaturated fatty acid/saturated fatty acid ratios on platelet aggregation and lipid metabolism in rats. *J Nutr Sci Vitaminol* 42: 423–434.

13) Folch J, Lees M, Sloane-Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497–509.

14) Lee JH, Fukumoto M, Nishida H, Ikeda I, Sugano M. 1989. The interrelated effects of n-6/n-3 and polyunsaturated/saturated fatty acid ratios on regulation of lipid metabolism in rats. *J Nutr* 119: 1893–1899.

15) Mann NJ, Warric GE, O'Dea K, Knapp HR, Sinclair AJ. 1994. The effect of linoleic, arachidonic and eicosapentaenoic acid supplementation on prostacyclin production in rats. *Lipids* 29: 157–162.

16) Duncan DB. 1975. T-Tests and intervals for comparison suggested by the data. *Biometrics* 31: 339–359.

17) Takeuchi H, Nagaosa T, Muramatsu K. 1990. Effects of a dietary supplementation of n-3 eicosapentaenoic acid on the platelets and spleen in rats. *Agric Biol Chem* 54: 2437–2440.

18) Brown ER, Subbaiah PV. 1994. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on human skin fibroblasts. *Lipids* 29: 825–829.

19) Shikano M, Masuzawa Y, Yazawa K, Takayama K. 1994. Complete discrimination of docosahexaenoate from arachidonate by 85 kDa cytosolic phospholipase A₂ during the hydrolysis of diacyl- and alkenylacylglycerophosphoethanolamine. *Biochem Biophys Acta* 1212: 211–216.

20) Lands WEM, Le Tellier PR, Rome LH, Vanderhoek JY. 1973. Inhibition of prostaglandin biosynthesis. *In: Advances in the Biosciences* (Bergstrom S, Bernhard S, eds), Vol 9, p 15–28. Pergamon Press, New York.

21) Needleman P, Minkes M, Raz A. 1976. Thromboxanes: selective biosynthesis and distinct biological properties. *Science* 193: 163–165.

22) Shikano M, Masuzawa Y, Yazawa K. 1993. Effect of docosahexaenoic acid on the J Nutr Sci Vitaminol
generation of platelet-activating factor by eosinophilic leukemia cells, Eol-1. *J Immunol* **150**: 3525–3533.

23) Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H. 1979. Triene prostaglandins: Prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc Natl Acad Sci USA* **76**: 944–948.

24) Marangoni F, Angeli MT, Colli S, Eligini S, Tremoli E, Sirtori CR, Galli C. 1993. Changes of n-3 and n-6 fatty acids in plasma and circulating cells of normal subjects, after prolonged administration of 20:5 (EPA) and 22:6 (DHA) ethyl esters and prolonged washout. *Biochem Biophys Acta* **1210**: 55–62.

25) Kobatake Y, Kuroda K, Jinnouchi H, Nishide E, Innami S. 1984. Differential effects of dietary eicosapentaenoic and docosahexaenoic fatty acids on lowering of triglyceride and cholesterol levels in the serum of rats on hypercholesterolemic diet. *J Nutr Sci Vitaminol* **30**: 357–372.