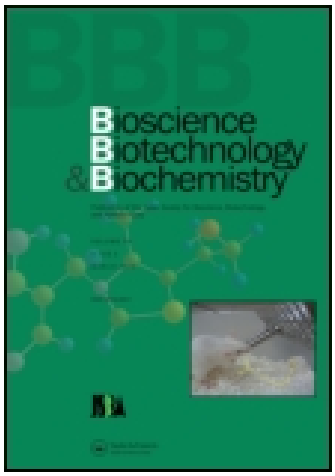


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# Lymphatic Absorption of Seal and Fish Oils and Their Effect on Lipid Metabolism and Eicosanoid Production in Rats

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# Lymphatic Absorption of Seal and Fish Oils and Their Effect on Lipid Metabolism and Eicosanoid Production in Rats

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**Eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) were distributed mainly in the sn-1 and 3 positions of seal oil triglyceride and in the sn-2 position of fish oil triglyceride. In Expt. 1, the structural distribution of EPA and DHA in lymph triglyceride of rats given seal or fish oils was similar to the distribution in the administered oils. In Expt. 2, seal oil-rich or fish oil-rich fats having constant polyunsaturated/monounsaturated/saturated fatty acids and n-6/n-3 polyunsaturated fatty acids ratios were fed to rats for 3 weeks. Seal oil more effectively reduced plasma and liver triglyceride than fish oil. Ratio of the productions of aortic prostacyclin and platelet thromboxane $A_2$ stimulated by thrombin was significantly higher in rats fed seal oil than in those fed fish oil. The results suggested that the different intramolecular distribution of EPA and DHA in dietary fat affected lipid metabolism differently in rats.**

**Key words:** fish oil; lipid metabolism; prostacyclin; seal oil; thromboxane



Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids contained in marine oils are known to have several important physiological and pharmacological functions.[1-5] Most studies of EPA and DHA have been done with fish oils in which these n-3 polyunsaturated fatty acids (PUFAs) are mainly at the second position (sn-2) in the triglyceride. Epidemiological studies in Greenland Inuit who live on mammals such as seals found a low rate of coronary heart diseases.[6] EPA and DHA in seal triglyceride are known to be mainly at the 1 (sn-1) and 3 (sn-3) positions. There are several studies showing that differences in intramolecular distribution of fatty acids in triglyceride may influence physiological functions of the fatty acids.[7-10] However, these studies were mainly done using vegetable and animal oils. Although Christensen *et al.* recently reported that metabolism of chylomicrons originated from dietary seal oil was different from that from fish oil,[11] little information is available on the effects of the intramolecular structure of triglycerides rich in EPA and DHA on lipid metabolism.

It is reported that EPA and DHA at the 1 and 3 positions of triglyceride are resistant to hydrolysis by pancreatic lipase[12] and have a lower absorption rate.[13] However, we observed that EPA and DHA given as trieicosapentaenoyl glycerol and tridocosahexaenoyl glycerol did not show lower absorbability, although the hydrolysis of these triglycerides by pancreatic lipase was slow.[14] Christensen *et al.* observed slower recovery in mesenteric lymph of EPA and DHA given as seal oil compared to fish oil.[15] Therefore, the influence of the location of EPA and DHA in triglyceride on their lymphatic absorption is still obscure. Although it is known that fatty acid at the 2 position of dietary triglyceride is well conserved during absorption processes,[16] there is little data on the positional distribution of EPA and DHA in lymph triglyceride after oral administration of seal or fish oils.

In this study, lymphatic transport and physiological effects of dietary seal and fish oils on lipid metabolism were examined in rats.

## Materials and Methods

*Materials.* Seal oil was from harp seals. Fish oil was a mixture of orbital fat of tuna and sardine oils. These oils were purified to edible oil grade. The peroxide values of seal and fish oils were 8.6 and 6.9, respectively. The fatty acid compositions of fish and seal oils and intramolecular distribution of the fatty acids are shown in Table I. Palm oil was provided by Fuji Oil, Osaka. High oleic safflower oil and safflower oil were provided by Rinoru Oil Mills, Tokyo. Fatty acid-free bovine serum albumin fraction V was obtained from Miles Inc., Kankakee IL, U.S.A. Sodium taurocholate (purity >98%) was from Nacalai Tesque, Kyoto.

*Experiment 1.* Male Sprague-Dawley rats, weighing about 308 g (Seiwa Experimental Animals, Fukuoka), were fed on a non-purified diet (Type NMF, Oriental Yeast Co., Tokyo) and given drinking water *ad libitum* for 5 days. Under nembutal anesthesia, a cannula was inserted into the left thoracic channel of each rat for collecting lymph and also a catheter was put into the stomach.[14,17] After the surgery, rats were placed in restraining cages in a warm recovery room. They were given a normal osmotic solution containing 139 mM glucose and 85 mM NaCl as drinking water, and it was infused at a rate of 3 ml/h *via* the gastric tube until the end of this experiment. Next morning, after collection of 2 h lymph for a blank, 3 ml of emulsified seal or fish oil was administered to the rats *via* the gastric tube. The emulsions contained 200 mg seal or fish oil, 50 mg fatty acid-free bovine serum albumin, and 200 mg sodium taurocholate were prepared by sonication. After the administration of the emulsion, lymph was collected in a tube containing EDTA at 3 h intervals unitl 9 h at a single collection from 9 to 24 h.

*Experiment 2.* Male Sprague-Dawley rats, 4 weeks old weighing about 129 g (Seiwa Experimental Animals, Fukuoka), were divided into 3 groups of six animals. The rats were kept in an air-conditioned from (21–24°C, light on 08:00–20:00). The experimental diets were prepared according to the recommendation of the American Institute of Nutrition,[18] and

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*Abbreviations*: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid; VLDL, very low density lipoprotein; TLC, thin-layer chromatography; GLC, gas-liquid chromatography.

**Table I.** Fatty Acid Composition of Fish and Seal Oils

| Fatty acids | Fish oil | | | Seal oil | | |
|---|---|---|---|---|---|---|
| | Total | sn-2 | sn-1,3 | Total | sn-2 | sn-1,3 |
| | mol% | | | | | |
| 14:0 | 4.9 | 3.2 | 4.2 | 4.0 | 6.1 | 1.9 |
| 14:1 | 0.5 | 0.0 | 0.4 | 3.3 | 5.1 | 1.2 |
| 16:0 | 10.4 | 8.4 | 11.3 | 1.8 | 3.5 | 1.5 |
| 16:1 | 11.3 | 8.5 | 12.4 | 18.4 | 35.2 | 11.1 |
| 16:2 | 2.3 | 3.3 | 2.9 | 1.6 | 3.1 | 1.2 |
| 16:3 | 1.5 | 1.4 | 2.0 | 1.0 | 1.3 | 1.0 |
| 16:4 | 2.1 | 2.2 | 1.9 | 1.8 | 1.9 | 1.8 |
| 18:0 | 1.9 | 0.9 | 2.6 | 0.1 | 0.2 | 0.2 |
| 18:1(n-9) | 12.8 | 8.5 | 16.3 | 13.7 | 20.8 | 11.9 |
| 18:1(n-7) | 3.8 | 2.2 | 5.1 | 2.2 | 2.4 | 2.2 |
| 18:2(n-6) | 1.5 | 1.3 | 1.7 | 2.5 | 6.4 | 1.0 |
| 18:3(n-3) | 1.1 | 1.0 | 1.3 | 1.6 | 3.7 | 0.9 |
| 18:4(n-3) | 4.4 | 4.7 | 4.3 | 6.2 | 4.2 | 7.7 |
| 20:1 | 2.4 | 2.2 | 3.3 | 3.0 | 0.8 | 4.0 |
| 20:4(n-6) | 1.5 | 1.8 | 1.3 | 0.5 | 0.1 | 0.6 |
| 20:4(n-3) | 0.8 | 0.8 | 1.0 | 1.0 | 0.5 | 1.3 |
| 20:5(n-3) | 18.5 | 20.7 | 17.3 | 14.1 | 2.1 | 20.3 |
| 22:1(n-9) | 0.9 | 0.9 | 1.3 | 0.6 | 0.0 | 0.8 |
| 22:5(n-3) | 1.5 | 3.0 | 1.1 | 5.7 | 0.7 | 8.7 |
| 22:6(n-3) | 16.0 | 25.0 | 8.5 | 16.8 | 1.7 | 20.9 |

contained (g/kg diet) cornstarch 367, casein 200, α-cornstarch 132, sucrose 100, fat 100, cellulose 50, mineral mixture (AIN-93G-MX) 35, vitamin mixture (AIN-93G-VX) 10, L-cystine 3, choline bitartrate 2.5, and *tert*-butylhydroquinone 0.014. The mineral and vitamin mixtures were purchased from Nihon Nosan Kogyo, Tokyo. Dietary fats were designed to have a constant polyunsaturated/monounsaturated/saturated fatty acid ratio of 1/1/1. Dietary fats in the control group were a mixture of palm, high oleic safflower, and safflower oils (56.3/7.7/36.0, by weight). Dietary fats in the groups of seal and fish oils were a mixture of seal, palm, high oleic safflower, and safflower oils (20.9/54.4/0.9/23.8) and fish, palm, high oleic safflower, and safflower oils (21.8/48.7/6.4/23.1), respectively. The fatty acid compositions of the three dietary fats are shown in Table II. Seal and fish oils contained cholesterol at less than 0.7%. Therefore, cholesterol contents in the diets containing seal or fish oils were less than 0.015%. Only a freshly prepared experimental diet was given to rats in every evening for 3 weeks. After they were starved for 7 h (7:00–14:00), the rats were killed by withdrawing blood from abdominal aorta in a syringe containing 3.8% trisodium citrate under diethyl ether anesthesia.

*Analysis of lipids.* Triglyceride structures of seal and fish oils and lymph triglyceride were analyzed by a Grignard degradation method.[19-21] Ethyl gallate (25 mg/liter in solution) was added in solvents as an antioxidant.[22] Oil (10 mg as triglyceride) was dissolved in 0.6 ml of diethyl ether. Under continuous mixing with a magnetic stirrer, 0.25 ml of 1 M ethylmagnesium bromide in diethyl ether (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) was added. After 25 s, 0.25 ml of acetic acid–diethyl ether (1:9, v/v) was added to stop the reaction and 5 ml of 10% boric acid solution were added while mixing. The reaction mixture was extracted by 5 ml of diethyl ether saturated with 10% boric acid. The diethyl ether phase was washed twice with 1 ml of 2% aqueous $NaHCO_3$ and twice with 1 ml water. The solution was dried by a small amount of anhydrous sodium sulfate and dried up under nitrogen at room temperature. The lipids were applied to a silica gel G thin layer chromatography (TLC) plate containing 5% boric acid and the plate was developed with chloroform–acetone (96:4, v/v). 2-Monoglyceride and 1,3-diglyceride fractions were transmethylated with sulfuric acid–methanol (1:115, v/v). Fatty acid methyl esters extracted with hexane were analyzed by gas-liquid chromatography (GLC) on an Omegawax 320 capillary column (Supelco Japan, Tokyo; 30 meters length, 0.25 mm film, 0.32 mm i.d., helium as a carrier gas, and split ratio of 1:100). Column, injector, and detector temperatures were 200°C, 250°C, and 250°C, respectively.

Lipids were extracted by the method of Folch *et al.*[23] Cholesterol,[24] triglyceride,[25] and phospholipid[26] in liver were analyzed as previously described. Plasma cholesterol, triglyceride, and phospholipid were measured using a cholesterol C-test Wako, triglyceride G-test Wako, and phospholipid B-test Wako (Wako Pure Chemical Industries, Osaka), respectively. Plasma high density lipoprotein (HDL-cholesterol) was analyzed with HDL-C·DAIICHI (Daiichi Pure Chemicals, Tokyo). Plasma and tissue lipids were separated into triglyceride and phospholipids by TLC and the fatty acid composition was analyzed by GLC as described. Pentadecanoic acid (Sigma Chemical Co., St. Louis, MO, U.S.A.) was used as an internal standard for measurement of fatty acids in lymph lipids. The fatty acid recovery rate was calculated by subtracting the fatty acid amount in the blank lymph from that in the lymph collected after the administration of the test emulsion.

**Table II.** Fatty Acid Composition of Dietary Fats

| Fatty acids | Groups | | |
|---|---|---|---|
| | Control | Fish oil | Seal oil |
| | wt% | | |
| 14:0 | 0.7 | 1.1 | 1.0 |
| 16:0 | 28.9 | 26.6 | 26.9 |
| 16:1 | 0.2 | 1.8 | 2.8 |
| 18:0 | 3.3 | 3.1 | 3.1 |
| 18:1(n-9) | 32.3 | 29.7 | 28.6 |
| 18:2(n-6) | 32.1 | 22.8 | 23.5 |
| 18:4(n-3) | 0.0 | 0.8 | 1.0 |
| 20:5(n-3) | 0.0 | 3.6 | 2.7 |
| 22:5(n-3) | 0.0 | 0.3 | 1.2 |
| 22:6(n-3) | 0.0 | 3.5 | 3.6 |
| SFA | 34.5 | 34.5 | 34.1 |
| MUFA | 33.0 | 33.3 | 33.3 |
| PUFA(n-6) | 32.1 | 23.3 | 23.5 |
| PUFA(n-3) | 0.5 | 9.0 | 9.1 |

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Fatty acids less than 1% were not shown.

*Analysis of prostaglandin and platelet aggregation.* 6-Keto-prostaglandin $F_{1\alpha}$ and thromboxane $B_2$ ($TXB_2$) were analyzed as stable metabolites of prostacyclin ($PGI_2$) and thromboxane $A_2$ ($TXA_2$), respectively. Approximately 28 mg of thoracic aorta was incubated in Krebs–Henseleit bicarbonate buffer (pH 7.4) at 25°C for 30 min,[27] and the concentration of 6-keto-prostaglandin $F_{1\alpha}$ was measured by using a 6-keto-prostaglandin $F_{1\alpha}$ enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, U.S.A.). Citrated plasma was centrifuged at $160 \times g$ for 10 min to obtain platelet-rich plasma and platelet-poor plasma was obtained from centrifugation of platelet-rich plasma at $1000 \times g$ for 15 min. $TXB_2$ concentration in platelet-rich plasma, platelet-rich plasma stimulated by ADP (final concentration of ADP was 5 μM), and citrated blood stimulated by thrombin (final concentration of thrombin was 1 U/200 μl citrated blood) was measured by using a $TXB_2$ enzyme immunoassay kit (Cayman Chemical Co.). Platelet aggregation induced by 5 μM ADP was measured with an automated platelet aggregation analyzer (Aggrecorder II, Kyoto Daiichi Kagaku, Kyoto).[28]

*Statistical analysis.* Data were analyzed by Student's *t*-test[29] in Expt. 1 and Duncan's new multiple-range test[30] in Expt. 2. Differences were considered significant at $p < 0.05$.

## Results

### *Experiment 1*

*Lymphatic recovery of fatty acids.* There was no difference in lymph flow rate between rats given seal and fish oils (Table III). Lymphatic recoveries for 24 h of EPA and DHA in rats given fish oil were significantly higher than those in rats given seal oil, although the recoveries until 9 h were the same between the two fats (Fig.). There was no significant difference in the recovery of fatty acids other than EPA and DHA.

*Structural distribution of fatty acids in lymph triglyceride.* EPA and DHA and 22:5n-3 in seal oil were mainly located

at the 1 and 3 positions, in contrast, 16:1 and 18:1 were at the 2 position (Table I). These positional distribution was highly conserved in lymph collected from 0 to 3 h after the administration of seal oil (Table IV). Fish oil triglyceride predominantly contained DHA at the 2 position and 16:1 and 18:1 at the 1 and 3 positions. DHA was conserved at the 2 position in the 0–3 h lymph after the administration of fish oil, but 16:1 and 18:1 were evenly distributed at the 2 and 1,3 positions. EPA was almost evenly distributed at the 2 and 1,3 positions in fish oil. However, it tended to be rich in the 1,3 positions of lymph triglyceride of rats given fish oil.

*Experiment 2*

*Growth, food intake, and liver weight.* Body weight, food intake, and relative liver weight are shown in Table V. There were no significant differences in these parameters among the three groups.

*Plasma and liver lipid concentration.* The concentrations of plasma and liver lipids are shown in Table V. The concentrations of plasma and liver triglyceride were significantly lower in the seal oil group than in the control group, and those in the fish oil group was intermediate between the seal oil and the control groups. Plasma phospholipid concentration was significantly lower in the fish oil and seal oil groups compared to the control group. There were no significant differences in the concentration of total and HDL-cholesterol in plasma and cholesterol and phospholipid in liver.

*Fatty acid composition in various tissues.* Fatty acid compositions in phospholipids of plasma, liver, heart, splenocyte, platelet, and aorta were measured. Percentage of 20:4n-6 in the phospholipids of these tissues, except heart cardiolipin, was significantly lower in the fish oil and seal oil groups than in the control group (Tables VI–VIII). In contrast, the percentage of 18:2n-6 was higher in the former groups. Seal oil was more effective to lower 20:4n-6 in hepatic phosphatidylcholine and phosphatidylethanolamine (Table VI) and plasma phosphatidylcholine (data not shown) than fish oil. The same tendency was also observed in heart phosphatidylcholine (data not shown). There were no significant difference in the percentage of 20:4n-6 in platelet phospholipid and aortic phosphatidylcholine between the rats fed seal and fish oils (Table VII). Total n-6 PUFAs was significantly lower in plasma, liver, and heart phosphatidylcholines of the seal oil group than in those of the fish oil group. In contrast, total n-3 PUFAs was higher in plasma and heart phosphatidylcholine and liver phosphatidylethanolamine of the seal oil group. In heart cardiolipin, more than 85% was occupied with linoleic acid (Table VIII). Fish and seal oils did not significantly affect the linoleic acid content. In plasma and liver triglycerides, percentage of n-3 PUFA increased and that of n-6 PUFA decreased in the seal oil and fish oil groups compared to the control group (data not shown). There was no difference on fatty acid composition of these triglycerides between the rats fed seal and fish oils.

*Production of eicosanoids and platelet aggregation.* Production of aortic $PGI_2$ and platelet $TXA_2$ and platelet aggregation are shown in Table IX. There were no differences in aortic $PGI_2$ production among the three groups. Production of $TXA_2$ in platelet-rich plasma stimulated by 5 $\mu$M ADP and in citrated blood stimulated by thrombin (1 U/200 $\mu$l) were significantly lower in the seal oil and fish oil groups compared to the control group. The production tended to be lower in the seal oil group than in the fish oil group when stimulated by ADP and thrombin. As a result, the ratio of the productions of aortic $PGI_2$ and platelet $TXA_2$ stimulated by thrombin was significantly higher in the seal oil group than in the control and fish oil groups ($0.65 \pm 0.15$, $0.32 \pm 0.07$, and $0.33 \pm 0.08$ in the seal oil, control, and fish oil groups, respectively). There were no difference among the three groups in platelet aggregation induced by 5 $\mu$M ADP.

**Table III.** Body Weight and Lymph Flow in Expt. 1

| Groups | Body wt. (g) | Lymph flow (ml) | | | | |
|---|---|---|---|---|---|---|
| | | 0–3 h | 3–6 h | 6–9 h | 9–24 h | 0–24 h |
| Fish oil | 305±4 | 20.8±1.3 | 16.8±2.0 | 20.5±2.3 | 103±10 | 161±13 |
| Seal oil | 310±5 | 19.6±2.3 | 19.5±2.7 | 21.0±2.7 | 101±10 | 161±16 |

Mean ± SE of 5 and 8 rats in fish oil and seal oil groups, respectively.

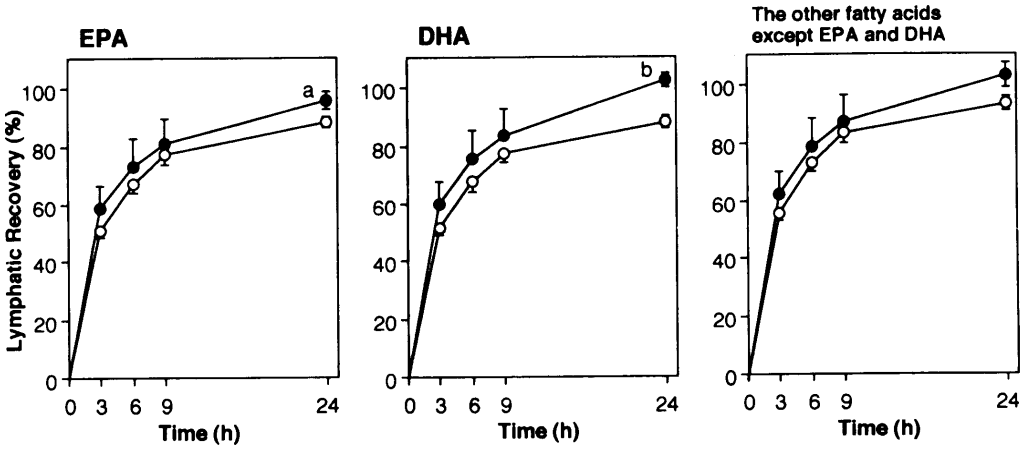


**Fig.** Recovery of EPA, DHA, and the Fatty Acids except EPA and DHA in Thoracic Duct Lymph of Rats after Intragastric Administration of a Fat Emulsion Containing Sodium Taurocholate and Albumin.

●, fish oil ($n=5$); ○, seal oil ($n=8$). Data are mean ± SE.

[a,b] Significantly different at $p<0.05$ and $p<0.01$, respectively.

**Table IV.** Fatty Acid Composition of Lymph Triglyceride in Rats Given Seal or Fish Oils in Expt. 1

| Fatty acids | Fish oil | | | Seal oil | | |
|---|---|---|---|---|---|---|
| | Total | sn-2 | sn-1,3 | Total | sn-2 | sn-1,3 |
| | mol% | | | | | |
| 14:0 | 3.4±0.1 | 3.0±0.4 | 2.8±0.2 | 3.3±0.1 | 4.0±0.5 | 1.7±0.1 |
| 14:1 | 0.2±0.1 | 0.3±0.1 | 0.1±0.1 | 2.7±0.1 | 3.6±0.4 | 1.2±0.1 |
| 16:0 | 11.6±0.2 | 12.6±0.3 | 12.6±0.4 | 5.0±0.1 | 7.5±0.3 | 5.7±0.2 |
| 16:1 | 8.7±0.2 | 8.0±0.4 | 8.9±0.3 | 14.9±0.2 | 21.3±0.7 | 10.4±0.2 |
| 16:2 | 1.7±0.0 | 2.4±0.1 | 2.1±0.1 | 1.2±0.0 | 1.8±0.1 | 0.8±0.0 |
| 16:3 | 1.1±0.0 | 1.5±0.2 | 1.2±0.1 | 0.8±0.0 | 1.5±0.3 | 0.8±0.1 |
| 16:4 | 1.4±0.0 | 1.4±0.1 | 1.1±0.1 | 1.1±0.0 | 1.2±0.1 | 0.9±0.0 |
| 18:0 | 3.1±0.1 | 1.3±0.2 | 4.9±0.2 | 1.9±0.1 | 1.3±0.2 | 3.4±0.1 |
| 18:1(n-9) | 12.5±0.1 | 15.0±0.4 | 14.2±0.1 | 13.3±0.1 | 20.8±0.5 | 12.6±0.1 |
| 18:1(n-7) | 3.6±0.0 | 2.1±0.1 | 5.0±0.0 | 2.2±0.0 | 1.9±0.1 | 2.9±0.1 |
| 18:2(n-6) | 6.8±0.6 | 11.8±1.0 | 5.2±0.5 | 8.0±0.4 | 15.0±0.6 | 5.4±0.3 |
| 18:3(n-3) | 1.1±0.0 | 1.3±0.0 | 1.0±0.0 | 1.5±0.0 | 2.6±0.1 | 1.0±0.0 |
| 18:4(n-3) | 3.5±0.1 | 3.7±0.1 | 3.4±0.1 | 4.9±0.1 | 4.3±0.2 | 5.1±0.1 |
| 20:1 | 2.2±0.1 | 1.6±0.1 | 3.1±0.1 | 2.8±0.0 | 1.0±0.0 | 4.6±0.1 |
| 20:4(n-6) | 2.7±0.1 | 3.0±0.1 | 2.3±0.0 | 1.9±0.1 | 1.9±0.1 | 1.8±0.1 |
| 20:4(n-3) | 0.8±0.0 | 0.8±0.1 | 1.0±0.0 | 0.9±0.0 | 0.9±0.1 | 1.3±0.0 |
| 20:5(n-3) | 16.9±0.3 | 11.0±0.3 | 18.1±0.4 | 11.8±0.2 | 3.5±0.2 | 15.4±0.3 |
| 22:1(n-9) | 0.7±0.1 | 0.5±0.0 | 1.4±0.1 | 0.5±0.0 | 0.0±0.0 | 1.0±0.0 |
| 22:5(n-3) | 1.6±0.0 | 2.7±0.6 | 1.5±0.0 | 5.4±0.1 | 1.7±0.1 | 7.7±0.1 |
| 22:6(n-3) | 16.6±0.1 | 16.2±0.8 | 10.3±0.2 | 16.2±0.2 | 4.3±0.3 | 16.4±0.3 |

Mean±SE of 5 and 8 rats in fish oil and seal oil groups, respectively. Lymph samples collected at 0–3 h were analyzed.

**Table V.** Growth Parameters, and Plasma and Liver Lipid Concentrations

| | Groups | | |
|---|---|---|---|
| | Control | Fish oil | Seal oil |
| Growth parameters | | | |
| Initial body weight (g) | 130±2 | 129±2 | 127±1 |
| Body weight gain (g) | 147±8 | 156±4 | 152±5 |
| Food intake (g/day) | 20.1±0.6 | 19.6±0.5 | 20.5±0.5 |
| Relative liver weight (g/100 g body weight) | 4.41±0.05 | 4.39±0.26 | 4.34±0.11 |
| Plasma lipid concentration (mg/dl) | | | |
| Total cholesterol | 75.0±6.3 | 62.4±4.1 | 65.3±4.2 |
| HDL-cholesterol | 55.4±5.6 | 48.1±2.1 | 53.7±3.9 |
| Triglyceride | 159±24[a] | 111±29[ab] | 72.3±8.8[b] |
| Phospholipid | 193±13[a] | 154±12[b] | 144±9[b] |
| Liver lipid concentration (mg/g liver) | | | |
| Total cholesterol | 2.69±0.18 | 3.04±0.24 | 2.59±0.06 |
| Triglyceride | 19.0±2.1[a] | 16.0±1.4[ab] | 11.6±1.1[b] |
| Phospholipid | 29.3±0.3 | 31.2±1.2 | 31.6±0.7 |

Mean±SE of 5–6 rats. [ab] Values with different superscript letters are significantly different at $p<0.05$. HDL, high density lipoprotein.

## Discussion

It is known that fatty acids at the 2 position of dietary triglyceride are retained during absorption processes.[16] This may be caused by a positional specificity of pancreatic lipase, which hydrolyzes fatty acids presented at the 1 and 3 positions of triglyceride and produces 2-monoglyceride and free fatty acids. After incorporation of these hydrolysis products into intestinal mucosal cells, most of the free fatty acids are reesterified to 2-monoglyceride and resynthesized triglyceride is incorporated into chylomicrons, which are secreted into lymph.[31] Metabolic difference between fatty acids at the 2 position and 1 and 3 positions of triglyceride after absorption has been thought to be a cause of the different physiological functions.[7,8] Although there was no

**Table VI.** Fatty Acid Composition of Liver Phospholipids

| Fatty acids | Groups | | |
|---|---|---|---|
| | Control | Fish oil | Seal oil |
| | wt% | | |
| Phosphatidylcholine | | | |
| 18:2(n-6) | 7.5±0.4[a] | 10.6±1.1[b] | 11.5±0.4[b] |
| 20:3(n-6) | 0.7±0.1[a] | 1.5±0.1[b] | 1.8±0.1[b] |
| 20:4(n-6) | 32.0±0.4[a] | 20.6±0.9[b] | 17.4±0.8[c] |
| 20:5(n-3) | 0.7±0.2[a] | 3.4±0.6[b] | 4.3±0.6[b] |
| 22:5(n-6) | 1.1±0.1[a] | 0.0±0.0[b] | 0.0±0.0[b] |
| 22:5(n-3) | 0.8±0.1[a] | 1.4±0.2[b] | 1.7±0.1[b] |
| 22:6(n-3) | 7.0±0.2[a] | 11.2±0.7[b] | 12.1±0.3[b] |
| (n-6) PUFA | 41.2±0.4[a] | 32.8±0.7[b] | 30.7±0.6[c] |
| (n-3) PUFA | 8.4±0.4[a] | 16.0±1.1[b] | 18.1±0.6[b] |
| Phosphatidylethanolamine | | | |
| 18:2(n-6) | 12.3±0.6 | 12.6±0.9 | 12.3±0.6 |
| 20:3(n-6) | 0.5±0.1[a] | 0.9±0.1[b] | 0.9±0.1[b] |
| 20:4(n-6) | 22.9±0.5[a] | 15.3±0.9[b] | 12.7±0.6[c] |
| 20:5(n-3) | 0.9±0.2[a] | 3.3±0.6[b] | 5.3±0.5[c] |
| 22:5(n-6) | 1.6±0.2[a] | 0.0±0.0[b] | 0.0±0.0[b] |
| 22:5(n-3) | 1.3±0.1[a] | 2.2±0.3[b] | 2.3±0.1[b] |
| 22:6(n-3) | 14.6±0.8[a] | 20.6±0.8[b] | 21.8±0.4[b] |
| (n-6) PUFA | 37.2±0.7[a] | 28.8±1.3[b] | 25.8±1.0[b] |
| (n-3) PUFA | 16.7±0.6[a] | 26.1±1.2[b] | 29.4±0.7[c] |

Mean±SE of 5–6 rats. [abc] Values with different superscript letters are significantly different at $p<0.05$. PUFA, polyunsaturated fatty acid.

**Table VII.** Fatty Acid Composition of Platelet Phospholipid and Aortic Phosphatidylcholine

| Fatty acids | Groups | | |
|---|---|---|---|
| | Control | Fish oil | Seal oil |
| | wt% | | |
| Platelet phospholipid | | | |
| 18:2(n-6) | 6.3±0.2[a] | 10.2±0.3[b] | 10.8±0.4[b] |
| 20:4(n-6) | 24.7±0.4[a] | 16.9±0.6[b] | 15.6±0.7[b] |
| 20:5(n-3) | 0.9±0.1[a] | 4.8±0.4[b] | 5.8±0.4[c] |
| 22:4(n-6) | 4.4±0.2[a] | 1.0±0.1[b] | 1.1±0.3[b] |
| 22:5(n-3) | 0.3±0.1[a] | 2.2±0.2[b] | 2.2±0.3[b] |
| 22:6(n-3) | 0.5±0.1[a] | 1.3±0.0[b] | 1.4±0.1[b] |
| (n-6) PUFA | 35.3±0.4[a] | 28.1±0.5[b] | 27.4±0.7[b] |
| (n-3) PUFA | 1.8±0.2[a] | 8.3±0.5[b] | 9.5±0.6[b] |
| Aortic phosphatidylcholine | | | |
| 18:2(n-6) | 10.1±1.4 | 10.8±0.5 | 12.6±1.2 |
| 20:3(n-6) | 0.7±0.0[a] | 1.4±0.1[b] | 1.5±0.1[b] |
| 20:4(n-6) | 17.9±0.6[a] | 11.7±0.4[b] | 10.7±0.3[b] |
| 20:5(n-3) | 0.4±0.1[a] | 2.0±0.2[b] | 2.4±0.2[b] |
| 22:5(n-6) | 0.9±0.1[a] | 0.4±0.0[b] | 0.3±0.0[b] |
| 22:5(n-3) | 0.3±0.0[a] | 0.9±0.0[b] | 1.0±0.1[b] |
| 22:6(n-3) | 1.1±0.0[a] | 2.3±0.1[b] | 2.4±0.1[b] |
| (n-6) PUFA | 29.6±1.2[a] | 24.3±0.5[b] | 25.0±1.0[b] |
| (n-3) PUFA | 1.8±0.1[a] | 5.2±0.2[b] | 5.7±0.3[b] |

Mean±SE of 5–6 rats. [abc] Values with different superscript letters are significantly different at $p<0.05$. PUFA, polyunsaturated fatty acid.

**Table VIII.** Fatty Acid Composition of Heart Cardiolipin

| Fatty acids | Groups | | |
|---|---|---|---|
| | Control | Fish oil | Seal oil |
| | | wt% | |
| 18:2(n-6) | 88.0±0.6 | 85.7±2.2 | 85.1±1.7 |
| 20:3(n-6) | 0.7±0.0[a] | 0.9±0.1[ab] | 1.1±0.1[b] |
| 20:4(n-6) | 1.3±0.1 | 1.1±0.2 | 1.2±0.2 |
| 20:5(n-3) | 0.0±0.0[a] | 0.1±0.0[b] | 0.1±0.0[b] |
| 22:6(n-3) | 0.9±0.1[a] | 3.4±1.0[b] | 3.5±0.9[b] |
| (n-6) PUFA | 90.1±0.5 | 87.7±1.9 | 87.4±1.4 |
| (n-3) PUFA | 0.9±0.1[a] | 3.5±1.0[b] | 3.6±0.9[b] |

Mean±SE of 5–6 rats. [ab] Values with different superscript letters are significantly different at $p<0.05$. PUFA, polyunsaturated fatty acid.

**Table IX.** Production of Aortic Prostacyclin and Platelet Thromboxane $A_2$ and Platelet Aggregation

| Groups | $PGI_2$ | $TXA_2$ | | | Maximal aggregation |
|---|---|---|---|---|---|
| | | PPP | ADP | Thrombin | |
| | pg/mg | | ng/ml | | % |
| Control | 240±53 | 0.543±0.118 | 3.58±0.99[a] | 692±68[a] | 49.0±2.3 |
| Fish oil | 137±30 | 0.371±0.059 | 1.70±0.45[b] | 418±22[b] | 47.7±3.4 |
| Seal oil | 189±33 | 0.312±0.017 | 1.02±0.21[b] | 316±60[b] | 46.1±4.5 |

Mean±SE of 5–6 rats. [ab] Values with different superscript letters are significantly different at $p<0.05$. Prostacyclin ($PGI_2$) and thromboxane $A_2$ ($TXA_2$) were measured as 6-keto-$PGF_{1\alpha}$ and $TXB_2$, respectively. PPP, platelet-poor plasma; ADP, adenosine diphosphate. Final concentration of ADP was 5 μM. Thrombin, final concentration of thrombin was 1 U/200 μl citrated blood; Maximal aggregation, platelet was stimulated by 5 μM ADP.

information whether the triglyceride structure of fish and seal oils rich in DHA and EPA is conserved during absorption, our results in Expt. 1 showed that the positional distribution of fatty acids in lymph triglyceride was similar to the distribution in seal and fish oils.

In Expt. 2, to compare quantitatively the effects of intramolecular structure of EPA and DHA in dietary triglyceride given as fish and seal oils, we adjusted the content of n-3 and n-6 PUFAs and also the content of polyunsaturated, monounsaturated, and saturated fatty acids in dietary fats to the same. It is known that the difference of these parameters significantly affects lipid metabolism and eicosanoid production.[32,33] Under these experimental conditions, seal and fish oils had different influences on lipid metabolism. Since the intramolecular structure of seal and fish oils was the sole different factor in the dietary fats, the effects observed can be ascribed to the differences in the triglyceride structures.

Under these experimental conditions, dietary seal oil more effectively lowered plasma and liver triglyceride concentrations than fish oil. It has been reported that dietary fish oil lowers plasma triglyceride concentration in experimental animals and humans.[1-3] The hypotriglyceridemic effect is attributed to EPA and DHA in fish oil. Hepatic triglyceride concentration is also reduced by feeding fish oil to experimental animals.[34] These n-3 PUFAs suppress hepatic fatty acid synthesis and formation of very low density lipoprotein (VLDL) and hence, decrease secretion of VLDL from liver to the bloodstream.[35,36] Although there is a possibility that clearance of plasma VLDL-triglyceride in peripheral tissues may be accelerated by dietary fish oil, the decrease of VLDL secretion from liver may be a major cause of the hypotriglyceridemic activity of EPA and DHA.[37] Although lymphatic recovery of EPA and DHA given as seal oil was slightly but significantly lower than that given as fish oil in Expt. 1, fatty acid composition in liver phospholipids showed that more n-3 PUFAs were incorporated into hepatic phospholipids in rats fed seal oil than in those fed fish oil. The results suggest that the availability of n-3 PUFAs in the liver increased upon the feeding of seal oil. There is a possibility that the increased availability of n-3 PUFAs caused the reduced synthesis of fatty acids and hence, the decreased synthesis of triglyceride and secretion of VLDL.

Analysis of fatty acid composition in various tissue lipids showed that the different intramolecular structures of EPA and DHA in dietary triglyceride altered fatty acid composition of phospholipids, but not of triglyceride. Although both seal and fish oils reduced the percentage of arachidonic acid and increased linoleic acid in tissue phospholipids, seal oil was more effective to reduce the arachidonic acid content than fish oil, in particular in plasma and liver phospholipids. Significant reduction of total n-6 PUFAs and significant increase of total n-3 PUFAs in dietary seal oil was observed in phosphatidylcholine in plasma and heart and in phosphatidylethanolamine in liver. These observations suggest that incorporation of n-3 PUFAs into various tissues, particularly into liver, was more preferential in rats fed seal oil than in those fed fish oil.

Ackman[38] suggested a possibility that when EPA and DHA are present at the 2 position of triglyceride, these n-3 PUFAs are transported to liver and in contrast, EPA and DHA at the 1 and 3 positions are incorporated into peripheral tissues. His speculation was based on the specificity of lipoprotein lipase, which preferentially hydrolyzes the 1 and 3 positions of triglyceride. He thought that after hydrolysis of triglyceride by lipoprotein lipase, fatty acids released from the 1 and 3 positions are incorporated into peripheral tissues and remained 2-monoglyceride are transported to liver as a member of chylomicron remnants. However, our results did not support this view, because fish oil rich in n-3 PUFAs at the 2 position did not increase n-3 PUFAs in liver phospholipids compared to seal oil. Recently, Christensen *et al.* reported that plasma clearance of labeled cholesterol in chylomicrons collected from rats fed seal oil was faster than that from those fed fish oil.[11] However, there was no difference in the clearance of labeled palmitic acid between chylomicrons originated from seal and fish oils. The result suggests a possibility that incorporation of chylomicron remnants into liver, which was measured by the clearance of labeled cholesterol, was faster in rats fed seal oil than in those fed fish oil. This may be a reason why n-3 PUFAs were enriched in hepatic phospholipids in rats fed seal oil compared to fish oil. A series of studies by Redgrave *et al.*[39,40] showed that metabolism of chylomicron remnants may be altered by a variety of fatty acids bound to 2-monoglyceride released after lipolysis by lipoprotein lipase and remained in chylomicron remnants. 2-Monoglyceride rich in EPA or DHA might

slow down the clearance of chylomicron remnants from the bloodstream.

Platelet $TXA_2$ production was reduced by feeding fish oil as previously reported.[41] Seal oil tended to be more effective to reduce $TXA_2$ production than fish oil. The ratio of aortic $PGI_2$ and $TXA_2$ produced by the stimulation of thrombin was significantly higher in rats fed seal oil than in rats fed fish oil. The results indicate that seal oil has a more potent antithrombotic activity than fish oil. It was reported that platelet $TXA_2$ production was highly dependent on the content of arachidonic acid in tissue phospholipids.[41] Although there was no difference in the percentage of arachidonic acid in platelet phospholipid between the seal oil and fish oil groups, platelet $TXA_2$ production in rats fed seal oil tended to be lower in platelet-poor plasma and platelet-rich plasma stimulated by ADP or thrombin. These results suggest a possibility that other factors than arachidonic acid content in phospholipids are responsible for the different effect of these oils. In this context, it was reported that fatty acid composition of cellular nonesterified fatty acid, which may be the precursor for eicosanoid synthesis, might influence platelet $TXA_2$ production.[42]

Our results showed that different intramolecular distribution of n-3 PUFAs had different effects on triglyceride metabolism, distribution of PUFA in tissue phospholipids, and eicosanoids production. However Schrijver *et al.* reported that when rats were fed on fish oil or randomized fish oil added to a nonpurified diet, no difference was observed in plasma lipid parameters and fatty acid profiles in plasma and liver phospholipids.[43] It is difficult to explain the reasons for these contradictory results at present. More studies are necessary to confirm the physiological effects of structured triglyceride.

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