Effect of Diets Rich in Linoleic or α-Linolenic Acid on Phospholipid Fatty Acid Composition and Eicosanoid Production in Atlantic Salmon (*Salmo salar*)

J. Gordon Bell*, James R. Dick and **John R. Sargent**

N.E.R.C. Unit of Aquatic Biochemistry, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, United Kingdom

Atlantic salmon post-smolts were fed diets rich in linoleic acid (sunflower oil, SO), α-linolenic acid (linseed oil, LO) or long-chain polyunsaturated fatty acids (fish oil, FO) for a period of 12 wk. In the liver phospholipids of fish fed SO, the levels of 18:2n-6, 20:2n-6, 20:3n-6 and 20:4n-6 were significantly elevated compared to both other treatments. In choline phospholipids (CPL), ethanolamine phospholipids (EPL) and phosphatidylserine (PS) the levels of 22:4n-6 and 22:5n-6 were significantly elevated in fish fed SO. In liver phospholipids from fish fed LO, 18:2n-6, 20:2n-6 and 20:3n-6 were significantly elevated but 20:4n-6, 22:4n-6 and 22:5n-6 were similar or significantly decreased compared to fish fed FO. Liver phospholipids from fish fed LO had increased 18:3n-3 and 20:4n-3 compared to both other treatments while EPL and phosphatidylinositol (PI) also had increased 20:5n-3. In fish fed LO, 22:6n-3 was significantly reduced in CPL, PS and PI compared to fish fed FO. Broadly similar changes occurred in gill phospholipids. Production of 12-lipoxygenase metabolites in isolated gill cells stimulated with the Ca^{2+}-ionophore A23187 were significantly reduced in fish fed either SO or LO compared to those fed FO. However, the ratio 12-hydroxy-5, 8, 10, 14-eicosatetraenoic acid (12-HETE)/12-hydroxy-5, 8, 10, 14, 17-eicosapentaenoic acid (12-HEPE) was significantly elevated in stimulated gill cells from SO-fed fish. Although mean values of thromboxane B_2 (TXB_2) and prostaglandin E_2 (PGE_2) were increased in fish fed SO, they were not significantly different from those of the other two treatments. *Lipids 28,* 819–826 (1993).

The so-called essential fatty acids, linoleic acid (18:2n-6) and linolenic acid (18:3n-3), are the precursors of the long-chain n-6 and n-3 species which are important components of the phospholipid bilayer of cell membranes. It is generally accepted that both the n-6 and n-3 fatty acids are metabolized by the same sequence of desaturating and elongating enzymes (1). In these pathways the desaturation steps are generally rate-limiting while elongation is rapid (2), and thus competition between substrate fatty acids at desaturase binding sites will determine the nature of the resulting polyunsaturated fatty acids (PUFA) and ultimately the composition of cellular membranes. In competitive terms, the n-3 PUFA are much more potent inhibitors of n-6 PUFA metabolism than *vice versa* although the relative concentrations of the two substrates will also determine the products that result (3,4). Many species of freshwater fish, including Atlantic salmon (*Salmo salar*), possess the enzymes necessary to elongate and desaturate 18:3n-3 to docosahexaenoic acid (DHA, 22:6n-3) (5) and are capable of metabolizing 18:2n-6 similarly, resulting in increased arachidonic acid (AA; 20:4n-6) in membrane phospholipids (6).

In mammals, AA is the major precursor for biologically active eicosanoids and in humans consuming a typical "Western-type" diet, overproduction of AA-derived eicosanoids may explain the prevalence of the many inflammatory conditions occurring in the developed world (7). Fish provide a useful model system for the study of eicosanoid metabolism since, although AA may be the preferred eicosanoid substrate (8,9), fish contain considerable amounts of eicosapentaenoic acid (EPA, 20:5n-3) and DHA, which can attenuate the production and efficacy of AA-derived eicosanoids (10). EPA can be metabolized by both cyclooxygenase and lipoxygenase enzymes resulting in products of lower bioactivity when compared to their AA homologues (11,12). EPA can also competitively inhibit the prostaglandin synthetase enzyme complex, thereby reducing production of AA-derived prostanoids (13). A number of recent studies have suggested that feeding oils rich in 18:3n-3 can reduce production of AA-derived eicosanoids by increasing levels of EPA in membrane phospholipids (14,15). In addition 18:3n-3 can directly inhibit cyclooxygenase activity (16). In previous studies with salmon we have shown that a high level of dietary 18:2n-6 can result in development of a severe cardiomyopathy involving active necrosis of both atrium and ventricle (17) and altered eicosanoid metabolism in blood leucocytes and gill cells (18).

The objective of the present study was to investigate the metabolism of 18:2n-6 and 18:3n-3 in salmon fed diets containing either sunflower oil (SO), linseed oil (LO) or fish oil (FO) by measuring phospholipid fatty acid compositions in liver and gill. The 12-lipoxygenase products and the cyclooxygenase products prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2) were measured in isolated gill cells stimulated with the calcium ionophore A23187.

MATERIALS AND METHODS

Animals and diets. Three hundred and thirty Atlantic salmon S1 smolts (salmon undergoing transition to seawater in one year) were obtained from the S.O.A.F.D. Fish Cultivation Unit (Aultbea, Wester Ross, Scotland) and distributed randomly into three tanks of 2000 L capacity each, which were supplied with seawater at a rate of 26 L/min. The fish (mean weight *ca.* 86 g) were subject to natural photoperiod, and the water temperature during the experimental period (August–November) varied from 15–10°C. Diets were supplied by automatic feeders which were adjusted to provide 20 g/kg biomass per day. Fish were weighed every 28 d and the ration adjusted accordingly.

*To whom correspondence should be addressed.

Abbreviations: AA, arachidonic acid; BHT, butylated hydroxytoluene; CPL, choline phospholipids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EPL, ethanolamine phospholipids; FO, fish oil; HDHE, hydroxy-4, 7, 10, 13, 16, 19-docosahexaenoic acid; HEPE, hydroxy-5, 8, 10, 14, 17-eicosapentaenoic acid; HETE, hydroxy-5, 8, 10, 14-eicosatetraenoic acid; HPLC, high-performance liquid chromatography; LO, linseed oil; PGE_2, prostaglandin E_2; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SO, sunflower oil; TLC, thin-layer chromatography; TXB_2, thromboxane B_2; UV, ultraviolet.

The diets were formulated to meet the nutritional requirements of salmonid fish (19) and contained 47% protein and 16% lipid. The composition of the basal diet has been described in detail previously (20) and contained fishmeal (650 g/kg) (LT94, Ewos Ltd., Westfield, Lothian, Scotland), pre-cooked starch (150 g/kg), vitamin mix (10 g/kg), mineral mix (24 g/kg), α-cellulose (65.5 g/kg) and choline chloride (4 g/kg). The dietary lipid (100 g/kg) was supplied either as fish oil (Fosol, Seven Seas Ltd., Hull, United Kingdom), sunflower oil (Tesco, Cheshunt, United Kingdom) or linseed oil (ICN Biomedical Ltd., High Wycombe, United Kingdom). An antioxidant mix (0.4 g/kg) was mixed with the oil before adding to the other diet components (20). The fatty acid compositions of the diets are shown in Table 1.

Lipid extraction and fatty acid analysis. Samples were collected after 12 wk of the dietary trial and stored at −80°C until analyzed. Lipids were extracted from liver and gill tissue by the method of Folch *et al.* (21). Total lipid extracts were separated into choline phospholipids (CPL), ethanolamine phospholipids (EPL), phosphatidylserine (PS) and phosphatidylinositol (PI) fractions by thin-layer chromatography (TLC) as described by Vitiello and Zanetta (22). The plates were sprayed with 0.1% 2′,7′-dichlorofluorescein in 97% methanol containing 0.05% butylated hydroxytoluene (BHT), and the lipid bands were visualized under ultraviolet (UV) light. Acid-catalyzed transmethylation was carried out overnight at 50°C as described by Christie (23). The fatty acid methyl esters were separated and quantified by gas–liquid chromatography (Carlo Erba Vega 6000, Fisons Ltd., Crawley, United Kingdom) on a 50 m × 0.32 mm capillary column (CP-Wax 51, Chrompak Ltd., London, United Kingdom). Hydrogen was used as carrier gas, and temperature programming was from 50°C at 35°C/min to 150°C and then to 225°C at 2.5°C/min. Individual methyl esters were identified by comparison with known standards and by reference to published data (24).

Preparation of isolated gill cells. The procedure used for isolating gill cells has been described in detail previously (18). Minced gill filaments were incubated in a Hanks' medium specially formulated for use with salmonid fish (25) containing 0.1% collagenase (type IV, Sigma Chemical Co., Poole, United Kingdom) for 45 min at room temperature with constant stirring. After filtering through nylon gauze, the cells were collected by centrifugation at 400 × *g* for 2 min, washed twice in Hanks' medium and finally resuspended in 1 mL of the same medium containing 1 mM $CaCl_2$.

Ionophore challenge and eicosanoid extraction. Isolated gill cells prepared as described above were placed in glass tubes pre-coated with Sigmacote and incubated in a shaking water bath at 18°C for 10 min. Calcium ionophore was added in 2 μL of dimethyl sulfoxide at a final concentration of 10 μM and the incubation continued for a further 20 min. The cells were sedimented by centrifugation

TABLE 1

Fatty Acid Composition of Diets

Fatty acids	Fish oil diet	Sunflower oil diet (wt%)	Linseed oil diet
14:0	6.6	1.8	1.8
16:0	14.8	9.5	9.1
18:0	2.4	4.3	3.5
Total saturated[a]	24.6	15.9	14.9
16:1n-7	5.1	2.0	2.0
18:1n-9	9.7	17.8	17.4
18:1n-7	2.0	1.0	1.2
20:1n-9	10.5	3.1	3.1
22:1n-11	16.6	4.8	4.4
24:1	1.0	0.6	0.5
Total monoenoic[b]	45.2	29.5	28.7
18:2n-6	1.4	40.3	12.2
18:3n-6	0.2	0.1	t[d]
20:2n-6	0.3	0.1	0.1
20:3n-6	0.1	t	t
20:4n-6	0.6	0.5	0.5
Total n-6[c]	2.8	41.1	12.9
18:3n-3	1.3	0.5	31.8
18:4n-3	3.1	1.0	0.9
20:4n-3	0.7	0.2	0.2
20:5n-3	6.5	3.6	3.4
22:5n-3	0.9	0.5	0.5
22:6n-3	8.8	4.9	4.5
Total n-3	21.3	10.7	41.3
Total PUFA[e]	25.0	51.8	54.2
n-3/n-6 ratio	7.6	0.3	3.2

[a]Includes 15:0, 17:0, 20:0 and 22:0.
[b]Includes 20:1n-11, 20:1n-7 and 22:1n-9.
[c]Includes 22:4n-6 and 22:5n-6.
[d]t = Trace value <0.05%.
[e]PUFA, polyunsaturated fatty acids.

(12000 × *g*, 2 min) and the eicosanoids extracted from the supernatant using C_{18} Sep-Pak minicolumns (Millipore Ltd., Watford, United Kingdom) according to Powell (26)

High-performance liquid chromatography (HPLC). The hydroxy acids 12-hydroxy-5, 8, 10, 14-eicosatetraenoic acid (12-HETE), 12-hydroxy-5, 8, 10, 14, 17-eicosapentaenoic acid (12-HEPE) and 14-hydroxy-4, 7, 10, 13, 16, 19-docosahexaenoic acid (14-HDHE) were separated and quantified by reverse-phase HPLC using a Spherisorb 5 μm octadecyl silane (ODS 2) column (25 cm × 4.6 mm, Anachem, Luton, United Kingdom). The chromatographic system was equipped with Waters Model M-45 pumps (Waters Chromatography, Warford, United Kingdom) and the effluent was monitored at 235 nm using a Pye-Unicam LC-UV detector (Pye-Unicam, Cambridge, United Kingdom). An isocratic solvent system containing acetonitrile/methanol/water/acetic acid (40:29:30:0.5, by vol) was used at a flow rate of 1 mL/min. Quantification was based on use of external standards of 12-HETE and 12-HEPE. Identification of 14-HDHE was done as described previously (18).

Measurement of PGE_2 and TXB_2. PGE_2 and TXB_2, the stable metabolite of thromboxane A_2, were measured by enzyme immunoassay using kits supplied by Cascade Biochemicals Ltd. (Reading, United Kingdom).

Materials. All solvents were of HPLC grade and were obtained from Rathburn Chemicals Ltd. (Walkerburn, Scotland, United Kingdom). TLC plates (20 cm × 20 cm × 0.25 mm), pre-coated with silica gel 60 were obtained from Merck (Darmstadt, Germany). 12(*R,S*)-HETE and 12(*S*)-HEPE were obtained from Cascade Biochemicals Ltd. (Reading, United Kingdom). Sigmacote and A23187 were obtained from Sigma Chemical Co. Ltd. (Poole, Dorset, United Kingdom).

Statistical analysis. Significance of difference ($P < 0.05$) between dietary treatments was determined by analysis of variance using a Statgraphics (system 3.0) computer package. Data which were identified as nonhomogeneous were subjected to either arcsine square root or log transformation before analysis. Differences between means were determined by Tukey's test.

RESULTS

The fatty acid compositions of liver CPL are shown in Table 2. Total monoenoic fatty acids were significantly reduced in SO-fed fish compared to FO-fed fish. In SO-fed fish 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6 were all significantly increased compared to both other dietary treatments. Fish fed LO had significantly increased 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6 and total n-6 PUFA but significantly reduced 22:5n-6 compared to fish fed FO. LO fish had significantly increased 18:3n-3, 18:4n-3, 20:3n-3 and 20:4n-3 compared to both other dietary treatments but had significantly reduced DHA compared to FO-fed fish. In fish fed SO 18:3n-3, 20:4n-3, EPA, 22:5n-3 and total n-3 PUFA were significantly reduced compared with both other dietary treatments while DHA was significantly reduced compared to FO-fed fish. The n-3/n-6 PUFA ratio was significantly different in the three dietary treatments with the highest value in FO-fed fish and the lowest in SO-fed fish.

The fatty acid compositions of liver EPL are shown in Table 3. Total monoenoic fatty acids were significantly different for each dietary treatment with the highest levels in FO-fed fish and the lowest levels in SO-fed fish. Also, 18:2n-6, 20:2n-6, 20:3n-6 and total n-6 PUFA were

TABLE 2

Fatty Acid Compositions of Choline Phospholipids from Salmon Liver[a]

Fatty acids	Fish oil diet	Sunflower oil diet (wt%)	Linseed oil diet
Total saturated	29.3 ± 1.6	29.9 ± 3.5	31.7 ± 3.7
Total monoenoic	16.0 ± 1.6^{b}	13.2 ± 0.8^{c}	14.6 ± 1.1^{b,c}
18:2n-6	1.1 ± 0.3^{d}	10.6 ± 1.7^{b}	4.0 ± 0.5^{c}
18:3n-6	t^{d}	0.5 ± 0.3^{b}	0.2 ± 0.0^{c}
20:2n-6	0.3 ± 0.1^{d}	1.7 ± 0.5^{b}	0.6 ± 0.1^{c}
20:3n-6	0.3 ± 0.1^{d}	6.4 ± 1.0^{b}	2.0 ± 0.5^{c}
20:4n-6	0.7 ± 0.1^{c}	5.5 ± 2.2^{b}	0.6 ± 0.1^{c}
22:4n-6	0.1 ± 0.0^{c}	0.7 ± 0.4^{b}	0.1 ± 0.0^{c}
22:5n-6	0.3 ± 0.1^{c}	0.7 ± 0.3^{b}	0.1 ± 0.0^{c}
Total n-6	2.8 ± 0.3^{d}	26.0 ± 0.4^{b}	7.4 ± 0.4^{c}
18:3n-3	0.3 ± 0.1^{c}	0.1 ± 0.0^{d}	3.6 ± 1.2^{b}
18:4n-3	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.7 ± 0.2^{b}
20:3n-3	t^{c}	t^{c}	0.4 ± 0.1^{b}
20:4n-3	0.8 ± 0.2^{c}	0.2 ± 0.1^{d}	2.9 ± 0.8^{b}
20:5n-3	9.8 ± 1.0^{b}	3.8 ± 0.4^{c}	9.5 ± 1.2^{b}
22:5n-3	3.0 ± 0.6^{b}	2.0 ± 0.5^{c}	3.3 ± 0.4^{b}
22:6n-3	35.9 ± 2.6^{b}	23.8 ± 3.2^{c}	25.5 ± 1.7^{c}
Total n-3	49.6 ± 1.8^{b}	29.7 ± 3.1^{c}	45.8 ± 2.2^{b}
Total PUFA	52.4 ± 1.8	55.7 ± 3.2	53.1 ± 2.1
n-3/n-6	18.0 ± 1.6^{b}	1.2 ± 0.1^{d}	6.2 ± 0.5^{c}
20:4/20:5	0.1 ± 0.0^{c}	1.5 ± 0.6^{b}	0.1 ± 0.0^{c}

[a]Results are % by weight ± SD from four fish per treatment; t = trace value <0.05%. SD <0.05 are recorded as 0.0. PUFA, polyunsaturated fatty acids. Values in the same row with different superscript letters b, c, d are significantly different ($P < 0.05$).

TABLE 3

Fatty Acid Compositions of Ethanolamine Phospholipids from Salmon Liver[a]

Fatty acids	Fish oil diet	Sunflower oil diet (wt%)	Linseed oil diet
Total saturated	12.6 ± 0.7	12.7 ± 0.6	13.6 ± 1.4
Total monoenoic	25.2 ± 1.8[b]	15.5 ± 2.5[d]	21.1 ± 0.4[c]
18:2n-6	1.7 ± 0.4[d]	12.0 ± 2.1[b]	4.7 ± 1.1[c]
18:3n-6	0.1 ± 0.0	0.2 ± 0.1	t
20:2n-6	0.4 ± 0.1[d]	2.3 ± 0.5[b]	0.7 ± 0.1[c]
20:3n-6	0.3 ± 0.1[d]	5.1 ± 0.6[b]	1.5 ± 0.2[c]
20:4n-6	1.3 ± 0.4[c]	9.6 ± 2.7[b]	1.1 ± 0.1[c]
22:4n-6	t[c]	0.8 ± 0.4[b]	t[c]
22:5n-6	0.5 ± 0.0[c]	1.0 ± 0.3[b]	0.3 ± 0.1[d]
Total n-6	4.2 ± 0.4[d]	30.9 ± 2.5[b]	8.1 ± 1.1[c]
18:3n-3	0.8 ± 0.2[c]	0.3 ± 0.1[d]	3.0 ± 0.6[b]
20:3n-3	t[c]	t[c]	0.7 ± 0.1[b]
20:4n-3	0.5 ± 0.1[c]	t[d]	1.7 ± 0.4[b]
20:5n-3	8.8 ± 1.2[c]	3.6 ± 1.0	11.1 ± 1.1[b]
22:5n-3	2.1 ± 0.4[b,c]	1.8 ± 0.4[c]	2.8 ± 0.4[b]
22:6n-3	41.9 ± 1.9[b]	32.5 ± 2.7[c]	36.0 ± 0.9[c]
Total n-3	54.1 ± 0.8[b]	38.2 ± 2.2[c]	55.4 ± 0.3[b]
Total PUFA	58.3 ± 1.2[d]	69.2 ± 2.7[b]	63.5 ± 1.4[c]
n-3/n-6	12.9 ± 1.2[b]	1.3 ± 0.1[d]	6.9 ± 0.9[c]
20:4/20:5	0.2 ± 0.1[c]	2.9 ± 1.4[b]	0.1 ± 0.0[c]

[a]Results are % by weight ± SD from four fish per treatment; t = trace value <0.05%. SD <0.05 are recorded as 0.0. PUFA, polyunsaturated fatty acids. Values in the same row with different superscript letters b, c, d are significantly different ($P < 0.05$).

significantly affected by dietary treatment with the highest levels in SO-fed fish and the lowest levels in FO-fed fish. Levels of 22:5n-6 were also significantly different in each dietary group with highest values in SO-fed fish and lowest in LO-fed fish. AA and 22:4n-6 were significantly increased in fish fed SO compared to both other dietary treatments. Also, 18:3n-3, 20:4n-3 and EPA were significantly different in each dietary treatment with highest levels in LO-fed fish and lowest in SO-fed fish. 22:5n-3 was significantly increased in LO-fed fish compared to SO-fed fish whereas DHA was significantly greater in FO-fed fish compared to both other treatments. Total n-3 PUFA were significantly lower in SO-fed fish while total PUFA were significantly different in each dietary treatment with the highest levels in SO-fed fish and the lowest in FO-fed fish. The ratio of n-3/n-6 PUFA was significantly different in each dietary treatment with the highest value in FO-fed fish and the lowest in SO-fed fish. The AA/EPA ratio of eicosanoid precursors was significantly increased in SO-fed fish compared to both other treatments.

The fatty acid compositions of liver PS are shown in Table 4. Total saturated fatty acids were significantly increased whereas total monoenoic fatty acids were significantly decreased in fish fed SO and LO compared to those fed FO. The level of 20:3n-6 was significantly different in each dietary treatment with the highest levels in SO-fed fish and the lowest in FO-fed fish. AA and 22:5n-6 were also significantly different in each treatment with the highest levels in SO-fed fish and the lowest in LO-fed fish. 18:2n-6, 20:2n-6, 22:4n-6 and total n-6 PUFA were all significantly increased in fish fed SO compared to the other dietary treatments. Levels of 18:3n-3 and 20:4n-3 were significantly greater in fish fed LO than in the two other treatments while EPA was significantly reduced in SO-fed fish compared to LO-fed fish. Total n-3 PUFA were significantly reduced in SO-fed fish compared to both other treatments while DHA was reduced in fish fed SO compared to those fed FO. Total PUFA were significantly greater in SO-fed fish compared to LO-fed fish. The n-3/n-6 PUFA ratio was significantly lower and the AA/EPA ratio significantly greater in SO-fed fish compared to both other treatments.

The fatty acid compositions of liver PI are shown in Table 5. Total saturated fatty acids were significantly elevated in fish fed SO and LO compared with those fed FO. Total monoenoic fatty acids were significantly different in all treatments with the highest levels in fish fed FO and the lowest in those fed SO. Levels of 18:2n-6 and total n-6 PUFA were significantly increased in SO-fed fish compared to the other two treatments whereas 20:3n-6 was significantly greater in LO-fed fish compared to the other treatments. AA was significantly different in all dietary treatments with the highest level in SO-fed fish and the lowest level in LO-fed fish. Also, 18:3n-3, 20:4n-3 and total n-3 PUFA were significantly reduced in fish fed SO compared to the other two treatments. EPA was significantly different in all treatments with the highest level in LO-fed fish and the lowest in SO-fed fish. Levels of 22:5n-3 and DHA were significantly different in all treatments with the highest levels in FO-fed fish and the lowest in SO-fed fish. Total PUFA were significantly greater in SO-fed fish compared to LO-fed fish. The n-3/n-6 PUFA ratio was significantly lower and the AA/EPA ratio significantly greater in fish fed SO compared to both other treatments.

The major PUFA in gill CPL and EPL are shown in Figure 1. in CPL 18:2n-6 and 20:3n-6 were significantly

TABLE 4

Fatty Acid Compositions of Phosphatidylserine from Salmon Liver[a]

Fatty acids	Fish oil diet	Sunflower oil diet (wt%)	Linseed oil diet
Total saturated	31.7 ± 1.5[c]	35.5 ± 1.4[b]	35.8 ± 1.8[b]
Total monoenes	10.3 ± 0.8[b]	6.2 ± 0.5[c]	6.7 ± 1.0[c]
18:2n-6	0.5 ± 0.1[c]	1.5 ± 0.2[b]	0.6 ± 0.1[c]
18:3n-6	0.2 ± 0.2	0.2 ± 0.1	t
20:2n-6	0.2 ± 0.0[c]	1.0 ± 0.3[b]	0.3 ± 0.1[c]
20:3n-6	0.2 ± 0.1[d]	1.6 ± 0.5[b]	0.6 ± 0.1[c]
20:4n-6	0.5 ± 0.1[c]	1.7 ± 0.3[b]	0.2 ± 0.1[d]
22:4n-6	t[c]	0.8 ± 0.5[b]	t[c]
22:5n-6	0.6 ± 0.1[c]	1.1 ± 0.4[b]	0.3 ± 0.0[d]
Total n-6	2.0 ± 0.2[c]	8.0 ± 0.7[b]	2.0 ± 0.1[c]
18:3n-3	0.4 ± 0.1[b,c]	0.2 ± 0.1[c]	0.6 ± 0.2[b]
18:4n-3	0.4 ± 0.1[b]	t[c]	t[c]
20:4n-3	t[c]	t[c]	0.5 ± 0.1[b]
20:5n-3	1.4 ± 0.5[b,c]	0.7 ± 0.3[c]	1.4 ± 0.3[b]
22:5n-3	2.7 ± 0.4	3.2 ± 0.8	3.8 ± 0.6
22:6n-3	47.3 ± 1.6[b]	43.2 ± 2.3[c]	44.6 ± 0.7[b,c]
Total n-3	52.3 ± 0.3[b]	47.2 ± 1.7[c]	50.9 ± 0.7[b]
Total PUFA	54.3 ± 1.0[b,c]	55.2 ± 1.3[b]	52.9 ± 0.7[c]
n-3/n-6	25.9 ± 2.1[b]	5.9 ± 0.6[c]	25.8 ± 1.3[c]
20:4/20:5	0.4 ± 0.2[c]	3.1 ± 1.6[b]	0.2 ± 0.1[c]

[a]Results are % by weight ± SD from four fish per treatment; t = trace value <0.05%. SD <0.05 are recorded as 0.0. PUFA, polyunsaturated fatty acids. Values in the same row with different superscript letters b, c, d are significantly different ($P < 0.05$).

different in all dietary treatments with the highest levels in SO-fed fish and the lowest in FO-fed fish. AA was significantly increased and EPA significantly decreased in SO-fed fish compared to the other treatments. Consequently, the AA/EPA ratio was significantly greater in SO fish compared to the other two treatments. DHA and total n-3 PUFA were significantly decreased in SO-fed fish compared to both other treatments. Largely similar effects occurred in gill EPL except that 20:3n-6 was significantly greater in SO-fed fish compared to both other treatments and DHA was significantly reduced in SO-fed fish compared to FO-fed fish.

The major PUFA of gill PS and PI are shown in Figure 2. In gill PS 18:2n-6 and 20:3n-6 were significantly

TABLE 5

Fatty Acid Compositions of Phosphatidylinositol from Salmon Liver[a]

Fatty acids	Fish oil diet	Sunflower oil diet (wt%)	Linseed oil diet
Total saturated	33.3 ± 1.6[c]	38.1 ± 0.5[b]	38.4 ± 1.1[b]
Total monoenoic	12.5 ± 1.4[b]	6.7 ± 0.7[d]	9.2 ± 0.6[c]
18:2n-6	0.5 ± 0.1[c]	1.4 ± 0.3[b]	0.6 ± 0.1[b]
20:2n-6	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
20:3n-6	2.0 ± 0.3[c]	1.8 ± 0.7[c]	5.8 ± 1.2[b]
20:4n-6	28.4 ± 0.3[c]	41.8 ± 0.4[b]	21.0 ± 1.2[d]
Total n-6	31.5 ± 0.2[c]	46.1 ± 0.5[b]	28.3 ± 1.7[c]
18:3n-3	0.4 ± 0.1[b]	t[c]	0.5 ± 0.1[b]
20:4n-3	0.3 ± 0.1[b]	t[c]	0.5 ± 0.1[b]
20:5n-3	4.9 ± 0.9[c]	0.7 ± 0.3[d]	10.1 ± 2.5[b]
22:5n-3	2.2 ± 0.3[b]	0.6 ± 0.1[d]	1.7 ± 0.2[c]
22:6n-3	12.4 ± 1.2[b]	4.7 ± 0.5[d]	8.1 ± 0.4[c]
Total n-3	19.7 ± 0.4[b]	6.2 ± 0.6[c]	21.5 ± 2.9[b]
Total PUFA	51.4 ± 0.7[b,c]	52.2 ± 1.1[b]	49.7 ± 1.1[c]
n-3/n-6	0.6 ± 0.0[b]	0.1 ± 0.0[c]	0.7 ± 0.2[b]
20:4/20:5	7.0 ± 2.2[c]	76.1 ± 28.2[b]	3.2 ± 2.2[c]

[a]Results are % by weight ± SD from four fish per treatment; t = trace value <0.05%. SD <0.05 are recorded as 0.0. PUFA, polyunsaturated fatty acids. Values in the same row with different superscript letters b, c, d are significantly different ($P < 0.05$).

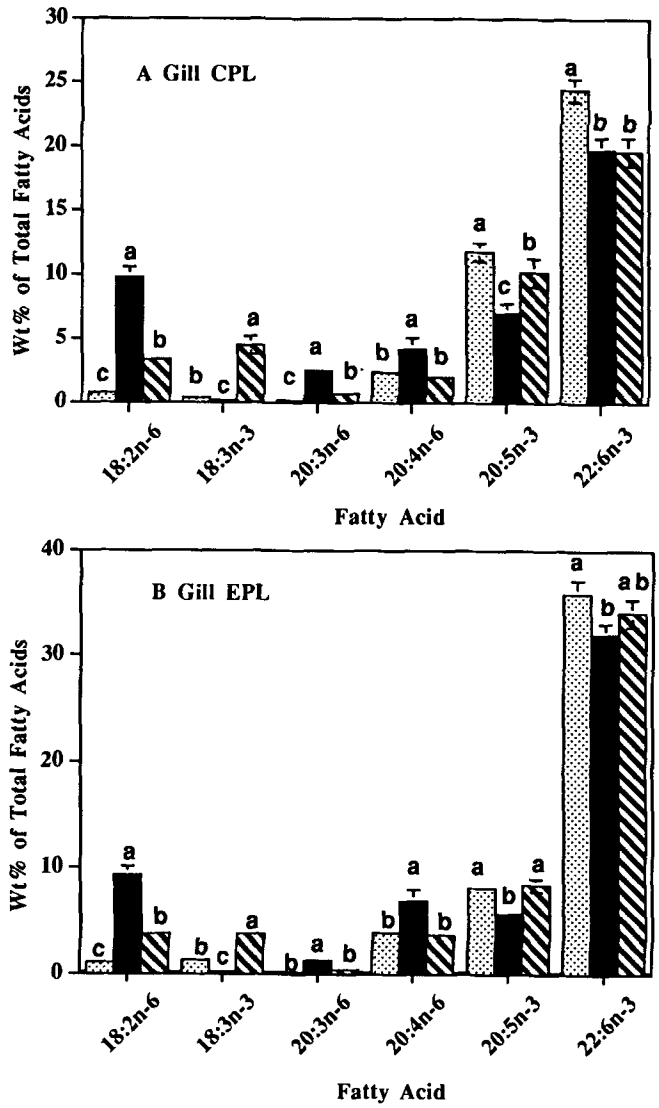

FIG. 1. The levels of the major polyunsaturated fatty acids of gill choline phospholipids (CPL) (A) and ethanolamine phospholipids (EPL) (B) from salmon fed diets containing either fish oil (dotted bars), sunflower oil (filled bars) or linseed oil (hatched bars). Values are means ± SD for four fish per treatment. Values for each fatty acid having a different column letter are significantly different ($P < 0.05$).

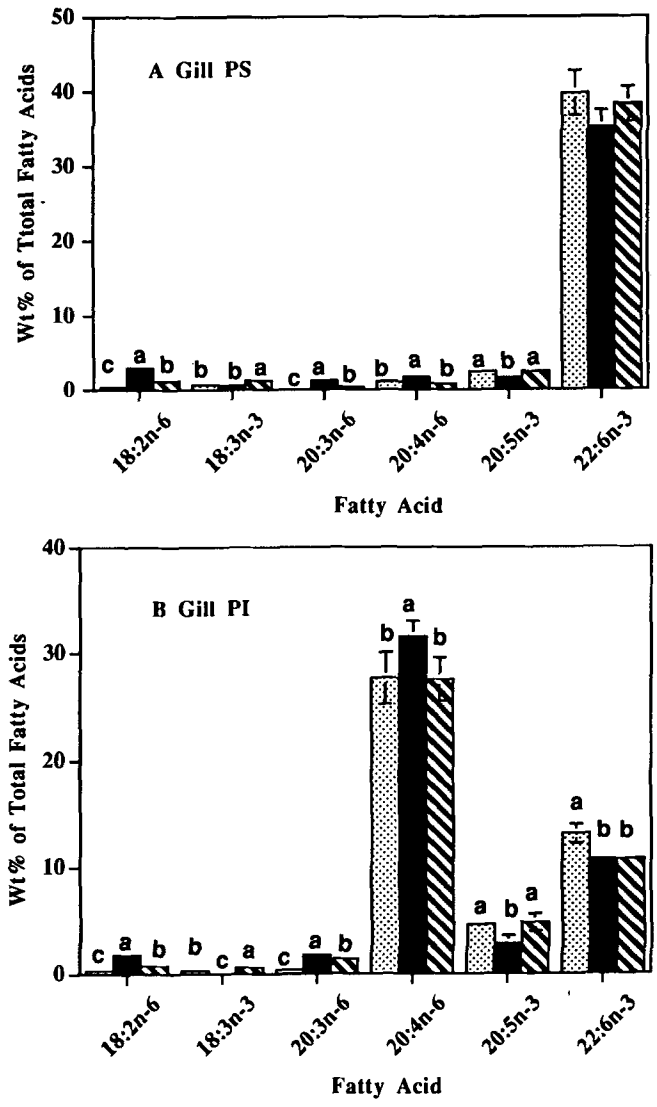

FIG. 2. Levels of the major polyunsaturated fatty acids of gill phosphatidylserine (PS) (A) and phosphatidylinositol (PI) (B) from salmon fed diets containing either fish oil (dotted bars), sunflower oil (filled bars) or linseed oil (hatched bars). Values are means ± SD for four fish per treatment. Values for each fatty acid having a different column letter are significantly different ($P < 0.05$).

different in all dietary treatments with the highest levels in SO-fed fish and the lowest levels in FO-fed fish. AA was significantly increased and EPA significantly reduced in SO-fed fish, resulting in an increased AA/EPA ratio when compared to fish fed either FO or LO. In gill PI the results were largely similar to PS except that DHA was significantly greater in fish fed FO than in both other dietary treatments.

The production of eicosanoids by isolated gill cells stimulated by A23187 is shown in Table 6. The production of 12-HETE, 12-HEPE and 14-HDHE were all significantly reduced in fish fed both LO and SO compared to those fed FO. However, the ratio of 12-HETE/12-HEPE was significantly increased in fish fed SO compared to both other treatments. While mean values of both TXB_2 and PGE_2 were greatest in fish fed SO, they were not significantly different from either of the other dietary treatments.

DISCUSSION

Feeding SO results in increased levels of 18:2n-6, 20:2n-6, 20:3n-6 and AA in all liver phospholipids while 22:4n-6 and 22:5n-6 were increased in CPL, EPL and PS compared to both other treatments. 20:3n-6, the product of Δ6 desaturation and elongation of 18:2n-6, was also increased in fish fed LO, whereas AA, the product of Δ5 desaturation, was generally decreased in those fish compared to fish fed FO. Similarly 22:5n-6, the product of Δ4 desaturation and elongation of AA was decreased in fish fed LO compared to those fed FO. Therefore it appears that feeding LO, which contains 18:2n-6 and 18:3n-3 in a ratio

TABLE 6

Eicosanoids from Isolated Salmon Gill Cells Stimulated with the Ca^{2+}-Ionophore A23187

Eicosanoid[a]	Fish oil diet	Sunflower oil diet	Linseed oil diet
12-HETE	17.7 ± 9.6[b]	5.7 ± 2.9[c]	5.8 ± 0.5[c]
12-HEPE	45.2 ± 12.4[b]	7.8 ± 3.2[c]	21.2 ± 8.2[c]
12-HETE/12-HEPE	0.354 ± 0.094[c]	0.791 ± 0.189[b]	0.327 ± 0.138[c]
14-HDHE	34.1 ± 11.8[b]	6.8 ± 3.8[c]	10.4 ± 1.9[c]
TXB_2	142.8 ± 80.4	229.0 ± 120.9	172.0 ± 59.6
PGE_2	192.3 ± 54.0	807.3 ± 562.3	510.3 ± 177.9

[a]Values are mean ± SD from four fish per treatment. Values for 12-HETE, 12-HEPE and 14-HDHE are ng/mg protein, whereas those for TXB_2 and PGE_2 are pg/mg protein. [b,c]Values in the same row with different superscript letters are significantly different ($P < 0.05$). HETE, hydroxy-5, 8, 10, 14-eicosatetraenoic acid; l HEPE, hydroxy-5, 8, 10, 14, 17-eicosapentaenoic acid; HDHE, hydroxy-4, 7, 10, 13, 16, 19-docosahexaenoic acid; TXB_2, thromboxane B_2; PGE_2, prostaglandin E_2.

of 1:3, has an inhibitory effect on Δ5 (and possibly Δ4) desaturase which is responsible for AA production. The ability of 18:3n-3 to reduce the conversion of 18:2n-6 to AA, by inhibition of Δ6 desaturase, has been recorded previously in mammals (27,28). The differences in 20:3n-6 and AA production by fish fed either SO or LO can be explained by considering the competitive influences of the different dietary fatty acid compositions. In SO-fed fish the high 18:2n-6 level makes AA production inevitable whereas the presence of 18:3n-3 in LO-fed fish results in competition which reduces 20:3n-6 production from 18:2n-6. At the same time Δ6 desaturation and elongation of 18:3n-3 results in increased 20:4n-3 in LO-fed fish, and this, coupled with reduced 20:3n-6, results in reduced AA production and an apparent inhibition of Δ5 desaturase. Similar competitive effects presumably operate to increase or reduce 22:5n-6 levels in SO- and LO-fed fish, respectively.

In a previous study using the related salmonid, the rainbow trout, no increase in elongated and desaturated products of 18:3n-3 were observed in the polar lipid fraction of fish fed 18:3n-3 enriched diets (29). However, in the present study with Atlantic salmon, although dietary EPA levels were similar in both SO and LO diets, the fatty acid compositions of phospholipid classes of both liver and gill showed decreased EPA in SO-fed fish, but LO-fed fish had EPA values greater than or equal to those of FO-fed fish. These results suggest that salmon are capable of EPA production from 18:3n-3 utilizing the pathways of Δ6 and Δ5 desaturation and elongation. Although some phospholipid classes from liver of LO-fed fish had elevated 22:5n-3, there was no apparent increase in DHA compared to fish fed FO. Previous studies with Atlantic salmon parr have demonstrated that they are capable of converting 18:3n-3 to DHA (5) but in the larger post-smolts used in the present study Δ4 desaturation is either very low or, more likely, the requirement for DHA is met by dietary input.

Both AA and EPA can be metabolized by cyclooxygenase to yield prostaglandins of the 2 and 3 series and by lipoxygenase to yield leukotrienes of the 4 and 5 series and a number of HETE and HEPE isomers (14,30). It is generally regarded that EPA is a poorer substrate for cyclooxygenase than AA and thus acts as a competitive inhibitor (16). The biological activity of the EPA-derived prostanoids and leukotrienes is considerably less than that of their AA-derived equivalents (12,31) which explains the ability of so-called 'MaxEPA' preparations to attenuate many of the pathophysiological conditions which occur in humans (7). In the present study feeding LO decreases AA and increases EPA in tissue phospholipids of Atlantic salmon and might therefore be expected to alter the spectrum of eicosanoids produced by these fish.

Gill cells were chosen since they are known to possess a highly active 12-lipoxygenase (18,32) and also to contain cyclooxygenase activity (5). While overall production of 12-lipoxygenase products was greatest in fish fed FO, the ratio of 12-HETE/12-HEPE was increased in SO-fed fish and was similar in both FO- and LO-fed fish. Although no specific physiological role has been identified for products of 12-lipoxygenase, a number of recent studies have implicated 12-HETE as a modulator of ion channels (33,34). Clearly this activity would be particularly important in gills which, along with posterior kidney, are vital in controlling osmoregulation in fish. Diet-induced changes in gill lipoxygenase products might therefore affect the ability of salmon to adapt to varying salinity.

In a previous study Atlantic salmon given increasing dietary linoleic acid produced decreasing amounts of gill 12-lipoxygenase products (18). In the present study salmon fed LO also produced significantly less 12-lipoxygenase products compared to fish fed FO. A similar decrease was also observed in platelet 12-lipoxygenase products from rats fed 18:3n-3 compared to those fed FO (14). One possible explanation is that changes in membrane phospholipid fatty acid composition might affect the activity of phospholipase A_2 which provides the precursors for eicosanoid production. Recent studies have established that phospholipase A_2 activity can be either increased or decreased in different tissues as a result of decreasing the membrane n-3/n-6 PUFA ratio (35,36).

The tendency for the PI fraction to accumulate 20-carbon fatty acids in fish has resulted in the hypothesis that this phospholipid might be the source of precursor fatty acids for eicosanoid production (5,37). However, the ratio of 12-HETE/12-HEPE produced by stimulated gill cells in the current experiment was most similar to the AA/EPA ratio present in gill CPL (0.20, 0.61 and 0.20 for FO, SO and LO diets, respectively). Comparison of the precursor fatty acid ratios of both CPL and PI would make the former class the more likely source of lipoxygenase substrate fatty acids. A similar result has been recorded in mammalian platelets (38) and cultured umbilical cells (39).

The present study demonstrated that while diets rich in 18:2n-6 result in increased AA in membrane lipids of

salmon, the fatty acid compositions can be 'normalized' to the position in fish fed FO, by feeding 18:3n-3. In this context linseed oil could be useful, when used in conjunction with marine oils, as an inhibitor of inflammatory activity in fish.

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