Fatty Acyl Desaturation in Isolated Hepatocytes from Atlantic Salmon (*Salmo salar*): Stimulation by Dietary Borage Oil Containing γ-Linolenic Acid

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ABSTRACT: The effects of different dietary oils on the fatty acid compositions of liver phospholipids and the desaturation and elongation of [1-^{14}C]18:3n-3 and [1-^{14}C]18:2n-6 were investigated in isolated hepatocytes from Atlantic salmon. Atlantic salmon smolts were fed diets containing either a standard fish oil (FO) as a control diet, a 1:1 blend of Southern Hemisphere marine oil and tuna orbital oil (MO/TO), sunflower oil (SO), borage oil (BO), or olive oil (OO) for 12 wk. The SO and BO diets significantly increased the percentages of 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, and total n-6 polyunsaturated fatty acids (PUFA) in salmon liver lipids in comparison with the FO diet. The BO diet also increased the percentage of 20:4n-6. Both the SO and BO diets significantly reduced the percentages of all n-3 PUFA in comparison with the FO diet. The OO diet significantly increased the percentages of 18:1n-9, 18:2n-6, total monoenes, and total n-6 PUFA in liver lipids compared to the FO diet, and the percentages of all n-3 PUFA were significantly reduced. With [1-^{14}C]18:3n-3, the recovery of radioactivity in the products of Δ6 desaturation was significantly greater in the hepatocytes from salmon fed SO, BO, and OO in comparison with the FO diet. The BO diet also increased the recovery of radioactivity in the products of Δ5 desaturation. Only the BO diet significantly affected the desaturation of [1-^{14}C]18:2n-6, increasing recovery of radioactivity in both Δ6- and Δ5-desaturation products. In conclusion, dietary BO, enriched in γ-linolenic acid (18:3n-6), significantly increased the proportions of both 20:3n-6 and 20:4n-6 in salmon liver phospholipids and also significantly increased the desaturation of both 18:2n-6 and 18:3n-3 in salmon hepatocytes. The possible relationships between dietary fatty acid composition, tissue phospholipid fatty acid composition, and desaturation/elongation activities are discussed.

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The fatty acid compositions of animal tissues are determined both by the type of dietary lipid ingested and the ability of the individual species to modify that dietary input *via* both catabolism and by pathways of desaturation and elongation (1–4). Both the fatty acid-desaturating and -elongating enzyme systems are microsomal, consisting of electron transport chains linked to terminal desaturase and elongase activities (5–7). As such, they represent membrane-bound systems whose activities, like all membrane-bound enzymes and receptors, are highly dependent upon membrane fatty acid compositions (8–11). Therefore, hepatic desaturation and elongation of fatty acids are modulated by nutritional state including the content and composition of dietary lipids and fatty acids (5,6). For instance, fish oil-containing diets, rich in eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA), tended to reduce rat liver microsomal Δ6-desaturase activity, particularly with 18:2n-6 as substrate, in comparison with diets rich in 18:2n-6 or 18:3n-3 (12–14). However, fatty acyl desaturase activities will themselves alter cellular fatty acid compositions, and it is unclear what precise relationship exists between dietary fatty acids, fatty acid desaturation, and tissue fatty acid compositions.

Freshwater fish are able to elongate and desaturate 18:3n-3 to 22:6n-3, whereas marine fish lack or have a very low activity of Δ5 desaturase and so require the long-chain polyunsaturated fatty acids (PUFA), EPA and DHA, to be supplied by the diet (15–17). Dietary specialization is an important factor in determining this difference since many freshwater fish are herbivorous or omnivorous, whereas the limited number of marine fish studied to date are predominantly carnivorous (17,18). Therefore, in terms of desaturase activities, freshwater fish are similar to terrestrial omnivores such as rodents and humans, but marine fish are more similar to terrestrial carnivores such as cats, which have relatively low desaturase activities (19–21). Atlantic salmon (*Salmo salar*) is an anadromous fish which begins life in freshwater and migrates to the sea after 1–2 yr, before returning to freshwater to breed after spending 1–3 yr at sea. Like other salmonids, such as trout, the desaturase profile of salmon appears to be that of freshwater fish (22,23). However, the commercial culture of most fish species, including salmon, is currently highly dependent on diets containing fish oil and fish meal which are increasingly expensive and whose supply is both volatile/variable

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Abbreviations: AA, arachidonic acid; ANOVA, analysis of variance; BHT, butylated hydroxytoluene; BO, borage oil diet; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAF-BSA, fatty acid-free bovine serum albumin; FAME, fatty acid methyl esters; FO, standard Northern Hemisphere fish oil diet; GLA, γ-linolenic acid; HBSS, Hanks' balanced salt solution; MO, marine oil; OO, olive oil diet; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acid(s); SO, sunflower oil diet; TLC, thin-layer chromatography; TO, tuna orbital oil diet.

and becoming increasingly environmentally undesirable owing to the overexploitation of unsustainable marine fish stocks (24). Fish oils are also in increasing demand as direct sources of n-3 PUFA for human nutrition (25). Several studies have investigated the use of other oils in the nutrition of salmon (25–27). Different dietary oils have been shown to significantly alter tissue phospholipid fatty acid compositions in salmon (28,29) and have significant effects on functional activities including phospholipase A activity (30), eicosanoid metabolism (31), and immune function (32). However, despite the importance of understanding the relationship between dietary fatty acids, tissue fatty acid compositions and fatty acyl desaturation, there are few data on the effects of dietary oils and fatty acids in modulating desaturase activities in fish and none on the commercially important salmon (33,34).

In the present study, we investigated the effects of different dietary oils on the desaturation of both [1-^{14}C]18:3n-3 and [1-^{14}C]18:2n-6 in isolated hepatocytes from Atlantic salmon. Because the long-chain n-3 PUFA, EPA, and DHA are known to be inhibitory to fatty acyl desaturases (5,19), oils were chosen to alter tissue EPA and DHA levels. With a standard fish oil diet as the control diet, one diet contained a blend of Southern Hemisphere fish oil (high EPA) and tuna orbital oil (high DHA) to increase EPA and DHA. Other diets were formulated to reduce cellular EPA and DHA by using combinations of 18:1n-9, 18:2n-6, and 18:3n-6. The results showed that dietary borage oil (BO), enriched in γ-linolenic acid (GLA; 18:3n-6), significantly increased the proportions of both 20:3n-6 and 20:4n-6 in salmon liver phospholipids and also significantly increased the desaturation of both 18:2n-6 and 18:3n-3 in salmon hepatocytes.

MATERIALS AND METHODS

Animals and diets. Five hundred fifty Atlantic salmon S1 smolts (initial mean weight 38 g) were obtained from the S.O.A.E.F.D. Fish Cultivation Unit (Aultbea, Wester Ross, Scotland) and distributed randomly into five tanks of 500-L capacity each, which were supplied with seawater at a rate of 10 L/min. The fish were subject to natural photoperiod, and the water temperature during the experimental period (July–October) varied from 9–17°C. Diets were fed for 12 wk and were supplied by automatic feeders which were adjusted to supply 25 g/kg biomass/day. Fish were weighed individually at the start and finish of the experiment and weighed in bulk every 28 d and the ration adjusted accordingly. The experiment was conducted in accordance with the British Home Office guidelines regarding research on experimental animals.

The diets were formulated to satisfy the nutritional requirements of salmonid fish and contained 48% crude protein and 25% lipid (35). The exact formulation of the diets is shown in Table 1. All diets were prepared to order by Ewos Ltd. (Technology Centre, Livingston, United Kingdom). Pellets, containing 3% fish oil, were manufactured by extrusion, and experimental oils (160 g/kg) were then added after addition of an antioxidant mix (0.4 g/kg; composition as described in Table 1) to each oil before mixing with the other ingredients. The dietary oils were a standard Northern Hemisphere fish oil (FO) (Fosol; Seven Seas Ltd., Hull, United Kingdom) as a control diet, a 1:1 mix of a Southern Hemisphere fish oil [marine oil (MO)] (Marinol K; Fishing Industry Research Unit, Rosebank, Republic of South Africa) and DHA-rich tuna orbitol oil (TO) (Croda Universal Ltd., Hull, United Kingdom), sunflower oil (SO) (Tesco Ltd., Cheshunt, United Kingdom), borage oil (BO) (Croda Universal Ltd.), and olive oil (OO) (Tesco Ltd.). The fatty acid compositions of the five diets are shown in Table 2.

TABLE 1
Formulation of Diets[a]

Component	Content (g/kg)
Fish meal[b]	620
Soya meal (full fat)	80
Wheat meal	66
Mineral mix[c]	24
Vitamin mix[d]	10
Choline chloride (40% wt/vol)	10 mL (4 g)
Feeding stimulant[e]	6
Fish Oil[f]	30
Oil coating[g]	160

[a]Diets were manufactured to order by Ewos Ltd., Technology Centre, Livingston, United Kingdom. Base pellets containing 3% fish oil were prepared by extrusion. Experimental oils were then added to the base pellets.
[b]LT94, Low-temperature fish meal (Ewos Ltd., Bathgate, United Kingdom).
[c]Supplied (per kg diet): KH_2PO_4, 22 g; $FeSO_4 \cdot 7H_2O$, 1.0 g; $ZnSO_4 \cdot 7H_2O$, 0.13 g; $MnSO_4 \cdot 4H_2O$, 52.8 mg; $CuSO_4 \cdot 5H_2O$,12 mg; $CoSO_4 \cdot 7H_2O$, 2 mg; and KI, 2 mg.
[d]Supplied (mg/kg diet): ascorbic acid, 1000; myoinositol, 400; nicotinic acid, 150; calcium pantothenate, 44; all-*rac*-α-tocopheryl acetate, 40; riboflavin, 20; pyridoxine hydrochloride, 12; menadione, 10; thiamine hydrochloride, 10; retinyl acetate, 7.3; folic acid, 5; biotin, 1; cholecalciferol, 0.06; cyanocobalamin, 0.02.
[e]Finnstim (Ewos Ltd., Bathgate, United Kingdom).
[f]Norsalmoil (United Fish Products, Aberdeen, United Kingdom).
[g]The experimental oils were: A control diet containing a standard Northern Hemisphere fish oil (Fosol; Seven Seas Ltd., Hull, United Kingdom); a 1:1 mix of a Southern Hemisphere fish oil (Marinol K; Fishing Industry Research Unit, Rosebank, Republic of South Africa) and docosahexaenoic acid-rich tuna orbital oil (Croda Universal Ltd., Hull, United Kingdom); sunflower oil (Tesco Ltd., Cheshunt, United Kingdom); borage oil (Croda Universal Ltd.); olive oil (Tesco Ltd.). All experimental oils had antioxidant mix [dissolved in propylene glycol and containing (g/L): butylated hydroxyanisole, 60; propyl gallate, 60; citric acid, 40] added prior to coating.

Lipid extraction, glycerophospholipid separation, and fatty acid analysis. Fish were killed by a blow to the head and livers were dissected from four fish per dietary treatment and immediately frozen in liquid nitrogen. Samples were stored at −40°C prior to lipid extraction. Total lipid was extracted from liver and diet samples by the method of Folch *et al.* (36). Samples of liver total lipid (3–4 mg) were applied to a 4-cm origin on a thin-layer chromatography (TLC) plate, and the polar lipid classes were separated using methyl acetate/propan-2-ol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol) as developing solvent (37). Lipid

TABLE 2
Fatty Acid Compositions (wt%) of Diets[a]

	Diet				
Fatty acid	Fosol	Tuna oil/Marinol	Sunflower oil	Borage oil	Olive oil
14:0	4.9	4.8	2.0	1.6	1.5
16:0	14.4	16.0	9.8	11.9	12.9
18:0	2.5	3.3	3.6	3.4	3.1
Total saturates	22.9	25.4	16.5	17.5	18.4
16:1n-7	4.9	6.3	1.8	1.5	2.0
18:1n-9	14.4	12.5	18.2	16.3	46.8
18:1n-7	2.5	2.5	1.3	1.2	2.0
20:1n-9	8.7	4.6	3.1	5.2	3.4
22:1n-11	12.4	6.1	3.8	3.7	3.7
22:1n-9	0.3	0.3	0.2	1.6	0.4
24:1	0.9	0.6	0.3	1.1	0.4
Total monoenes	44.2	33.9	28.7	30.6	58.7
18:2n-6	6.0	5.6	46.4	31.7	14.2
18:3n-6	0.1	0.2	trace	12.5	0.2
20:2n-6	0.2	0.2	trace	0.2	0.1
20:3n-6	trace	trace	n.d.	n.d.	n.d.
20:4n-6	0.5	1.1	0.2	0.2	0.1
22:5n-6	0.2	0.5	trace	trace	trace
Total n-6 PUFA	7.0	7.6	46.6	44.6	14.6
18:3n-3	1.6	1.2	0.9	0.9	1.1
18:4n-3	2.6	2.0	0.8	0.8	0.6
20:4n-3	0.7	0.6	0.2	0.2	0.1
20:5n-3	6.0	9.5	2.6	2.1	1.7
22:5n-3	1.1	1.2	0.3	0.2	0.2
22:6n-3	8.8	12.6	3.7	3.2	2.7
Total n-3 PUFA	20.8	27.1	8.3	7.4	6.4
C16 PUFA	0.9	2.0	trace	trace	trace
Total PUFA	27.8	34.7	54.9	52.0	21.0
n-3/n-6	3.0	3.6	0.2	0.2	0.4

[a]n.d., not detected; trace value, < 0.05%; PUFA, polyunsaturated fatty acids; Fosol (Seven Seas Ltd., Hull, United Kingdom); Marinol (Fishing Industry Research Unit, Rosebank, Republic of South Africa).

classes were visualized under ultraviolet light after spraying with 2,7-dichlorofluorescein. Individual glycerophospholipid classes were scraped into stoppered test tubes and transmethylated directly on the silica after addition of 1 mL toluene and 2.5 mL 1% sulfuric acid in methanol, and fatty acid methyl esters (FAME) prepared as described below. FAME were prepared from total lipid and individual glycerophospholipid classes by acid-catalyzed transmethylation at 50°C for 16 h (38) and were extracted and purified as described previously (39). FAME were analyzed in a Packard 436 gas chromatograph (Canberra Packard, Pangbourne, United Kingdom) equipped with a CP Wax 52CB fused-silica capillary column (30 m × 0.32 mm i.d., Chrompack U.K. Ltd., London, England), with on-column injection using hydrogen as carrier gas and a biphasic thermal gradient from 50 to 225°C. FAME were identified by comparison with known standards and a well-characterized fish oil and by reference to published data as described previously (39) and were quantified using a Shimadzu CR6A data processor (Kyoto, Japan). All solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant.

Preparation of isolated hepatocytes. Intact, metabolically viable liver parenchymal cells were prepared by the technique of *in vitro* collagenase dispersion essentially as described in detail previously (23). Briefly, fish were killed by a blow to the head and the liver dissected immediately. The gall bladder was removed carefully from the liver and the main blood vessels removed. The liver was perfused *via* the hepatic vein with solution A [calcium- and magnesium-free Hanks' balanced salt solution (HBSS) + 10 mM HEPES + 1 mM EDTA], using a syringe fitted with a 23-gauge needle, to clear blood from the tissue. The liver was chopped finely with scissors and incubated with 20 mL of solution A containing 0.1% (wt/vol) collagenase in a 25 mL "Reacti-flask" in a shaking water bath at 20°C for 45 min. The digested liver was filtered through 100-μm nylon gauze and the cells collected by centrifugation at 1000 × *g* for 5 min. The cell pellet was washed with 20 mL of solution A containing 1% wt/vol fatty acid-free bovine serum albumin (FAF-BSA) and recentrifuged. The hepatocytes were resuspended in 10 mL of Medium 199 (Sigma Chemical Co., Poole, United Kingdom) containing 10 mM HEPES, 2 mM glutamine, 100 U/mL penicillin, and 0.1

mg/mL streptomycin. One hundred μL of cell suspension was mixed with 400 μL of Trypan Blue, and hepatocytes were counted and their viability assessed using a hemacytometer. One hundred μL of the cell suspension was retained for protein determination.

Preparation of isotopes for addition to cell cultures. The radioactive isotopes, [1-^{14}C]18:3n-3 or [1-^{14}C]18:2n-6, were added to cells as complexes with FAF-BSA. Briefly, 25 μCi of fatty acid (0.5 μmol) in ethanol was placed in a reaction vial, solvent was evaporated under a stream of nitrogen, and 100 μL of 0.1M KOH were added. The mixture was stirred for 10 min at room temperature before 5 mL of 50 mg/mL FAF-BSA in HBSS containing 10 mM HEPES buffer was added and the mixture stirred for 45 min at 20°C.

Assay of hepatocyte fatty acyl desaturation/elongation activities. Five mL of each hepatocyte suspension was dispensed into two 25-cm^2 tissue culture flasks. Hepatocytes were incubated with 0.25 μCi of either [1-^{14}C]18:3n-3 or [1-^{14}C]18:2n-6, added as complexes with FAF-BSA prepared as described above. After addition of isotope, the flasks were incubated at 20°C for 3 h. After incubation, the cell layer was dislodged by gentle rocking, transferred to glass conical test tubes, and the flasks were washed with 1 mL of ice-cold HBSS containing 1% FAF-BSA. The cell suspensions were centrifuged at 300 × *g* for 2 min, the supernatants discarded, and the cell pellets washed with 5 mL of ice-cold HBSS/FAF-BSA. The supernatants were discarded and the tubes placed upside down on paper towels to blot for 15 s before extraction of total lipid using ice-cold chloroform/methanol (2:1, vol/vol) containing 0.01% (wt/vol) BHT essentially as described by Folch *et al.* (36) and as detailed previously (40).

Total lipid was transmethylated and FAME were prepared as described above. The methyl esters were redissolved in 100 μL hexane containing 0.01% BHT and applied as 2.5-cm streaks to TLC plates impregnated by spraying with 2 g silver nitrate in 20 mL acetonitrile and preactivated at 110°C for 30 min. Plates were fully developed in toluene/acetonitrile (95:5, vol/vol) (41). Autoradiography was performed with Kodak Biomax MR-2 film for 4–7 d at room temperature. Silica corresponding to individual PUFA was scraped into scintillation minivials containing 2.5 mL of scintillation fluid (Ecoscint A. National Diagnostics, Atlanta, GA) and radioactivity determined in a TRI-CARB 2000CA scintillation counter (United Technologies Packard, Pangbourne, United Kingdom). Results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions.

Protein determination. Protein concentration in isolated hepatocyte suspensions was determined according to the method of Lowry *et al.* (42) after incubation with 0.25 mL of 0.25% (wt/vol) SDS/1 M NaOH for 45 min at 60°C.

Materials. [1-^{14}C]18:3n-3 and [1-^{14}C]18:2n-6 (both 50–55 mCi/mmol) were from NEN [DuPont (U.K.) Ltd., Stevenage, United Kingdom)]. HBSS, HEPES buffer, glutamine, penicillin, streptomycin, collagenase (type IV), FAF-BSA, BHT, and silver nitrate were obtained from Sigma Chemical Co. TLC plates, precoated with silica gel 60 (without fluorescent indicator), were obtained from Merck (Darmstadt, Germany). All solvents were HPLC-grade and were obtained from BS & S Ltd. (Edinburgh, Scotland).

Statistical analysis. Significance of difference between dietary treatments ($P < 0.05$) was determined by analysis of variance (ANOVA). Analyses were performed using a Statgraphics (system 3.0) computer package (Statistical Graphics Corp., Rockville, MD). Data which were identified as nonhomogeneous (using Bartlett's test) were subjected to either arcsin square root or log transformation before analysis. Where appropriate, differences between means were determined by Tukey's test.

RESULTS

The mean initial weight of all the fish was 37.9 ± 10.8 g, and after 12 wk on the diets the final weights of the fish were 150.0 ± 49.5 g (FO), 159.5 ± 40.0 g (TO/MO), 128.8 ± 48.2 g (SO), 137.9 ± 32.8 g (BO), and 150.5 ± 47.7 g (OO). The only difference in mean final weights that was significant (one-way ANOVA) was between the TO/MO and SO diets.

Replacing the control fish oil (Fosol; FO) with the combination of Southern Hemisphere MO and TO increased the EPA, DHA, total n-3 PUFA, and total PUFA contents of the diet at the expense of saturates (Table 2). The SO and BO both increased the total n-6 PUFA contents of the diets, owing solely to increased 18:2n-6 in SO and 18:2n-6 and 18:3n-6 in BO, at the expense of all other fatty acids compared to the control diet. The OO diet contained increased 18:1n-9, 18:2n-6, total monoenes, and total n-6 PUFA compared to the FO diet and proportions of saturated fatty acids and n-3 PUFA similar to those of the SO and BO diets (Table 2).

The MO/TO diet did not significantly affect the percentages of EPA, DHA, or total n-3 PUFA in salmon liver total lipid in comparison with the control diet (Table 3). However, the MO/TO diet significantly reduced the percentages of 20:1 and 22:1 and the n-3/n-6 ratio and increased the proportion of saturated fatty acids. The SO diet significantly increased the percentages of 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, and total n-6 PUFA in total lipid from salmon liver in comparison with the FO diet (Table 3). The BO diet had similar effects to those of SO except that it also increased the percentage of arachidonic acid (20:4n-6; AA). However, the levels of 18:2n-6 and 20:2n-6 were significantly lower in the BO-fed fish compared to the SO-fed fish, whereas the percentages of 18:3n-6, 20:3n-6, and 20:4n-6 were all significantly higher in the BO diet (Table 3). There was no difference between the SO and BO diets in the percentages of n-3 PUFA in the liver total lipid although both the SO and BO diets significantly reduced the percentages of all n-3 PUFA in comparison with the FO diet. The OO diet significantly increased the percentages of 18:1n-9, 18:2n-6, total monoenes, and total n-6 PUFA in liver total lipid compared to the FO diet, and the percentages of all n-3 PUFA were significantly reduced and similar to the levels observed in the SO- and BO-fed fish (Table 3).

The differences in specific fatty acids observed in liver total

TABLE 3
Effects of Diet on the Fatty Acid Compositions of Total Lipid from the Liver of Atlantic Salmon[a]

	Diet				
Fatty acid	Fosol	Tuna oil/Marinol	Sunflower oil	Borage oil	Olive oil
Total saturates	21.7 ± 1.0[b]	24.2 ± 0.4[a]	20.1 ± 1.7[b]	22.3 ± 1.6[a,b]	20.1 ± 0.5[b]
16:1[1]	2.5 ± 0.2[a,b]	3.0 ± 0.2[a]	1.3 ± 0.2[c]	1.7 ± 0.6[b,c]	1.7 ± 0.1[b,c]
18:1n-9	11.2 ± 0.7[b]	9.6 ± 0.3[b]	12.4 ± 1.5[b]	13.1 ± 4.1[b]	30.5 ± 1.6[a]
18:1n-7	2.2 ± 0.2[a,b]	2.3 ± 0.1[a]	1.1 ± 0.1[c]	1.1 ± 0.2[c]	1.9 ± 0.1[b]
20:1[2]	5.0 ± 0.7[a]	2.8 ± 0.3[b,c]	2.2 ± 0.4[c]	2.6 ± 0.5[c]	3.8 ± 0.2[b]
22:1n-11	2.3 ± 0.6[a]	1.3 ± 0.3[b]	1.2 ± 0.4[b]	1.1 ± 0.3[b]	1.4 ± 0.3[b]
Total monoenes	23.2 ± 2.5[b]	19.0 ± 0.7[b]	18.3 ± 2.1[b]	19.6 ± 4.9[b]	39.3 ± 1.2[a]
18:2n-6	1.9 ± 0.1[d]	2.2 ± 0.2[c,d]	22.7 ± 1.3[a]	10.9 ± 3.2[b]	5.3 ± 0.2[c]
18:3n-6	n.d.[c]	0.2 ± 0.1[c]	0.5 ± 0.1[b]	3.2 ± 0.3[a]	0.1 ± 0.1[c]
20:2n-6	0.6 ± 0.1[b]	0.4 ± 0.1[b]	3.3 ± 1.3[a]	1.0 ± 0.3[b]	0.9 ± 0.1[b]
20:3n-6	0.2 ± 0.0[d]	0.3 ± 0.1[d]	3.3 ± 0.7[b]	7.0 ± 0.6[a]	1.6 ± 0.1[c]
20:4n-6	2.9 ± 0.2[b]	3.3 ± 0.2[b]	3.5 ± 0.4[a,b]	5.9 ± 2.4[a]	1.8 ± 0.1[b]
22:4n-6	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.5	n.d.
22:5n-6	0.5 ± 0.0[a,b]	0.8 ± 0.1[a,b]	0.4 ± 0.1[b]	1.3 ± 0.3[a]	0.3 ± 0.1[b]
Total n-6 PUFA	6.2 ± 0.2[d]	7.3 ± 0.2[c,d]	33.9 ± 2.1[a]	29.8 ± 2.1[b]	10.0 ± 0.2[c]
18:3n-3	0.6 ± 0.1[a]	0.5 ± 0.1[a]	0.2 ± 0.1[b,c]	0.2 ± 0.1[c]	0.3 ± 0.1[b]
18:4n-3	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.1 ± 0.1[b]	0.2 ± 0.0[a,b]	0.2 ± 0.0[a,b]
20:3n-3	0.2 ± 0.1[a]	0.1 ± 0.1[b]	n.d.[c]	n.d.[c]	n.d.[c]
20:4n-3	1.1 ± 0.2[a]	0.7 ± 0.1[b]	0.3 ± 0.1[c,d]	0.2 ± 0.1[d]	0.4 ± 0.1[c]
20:5n-3	6.7 ± 0.3[a]	7.0 ± 0.9[a]	2.6 ± 0.3[c]	2.8 ± 0.8[c]	4.0 ± 0.3[b]
22:5n-3	2.6 ± 0.2[a]	2.5 ± 0.2[a]	1.2 ± 0.2[b]	1.3 ± 0.3[b]	1.3 ± 0.2[b]
22:6n-3	34.4 ± 1.1[a]	35.4 ± 1.2[a]	21.9 ± 2.0[b]	23.6 ± 4.3[b]	23.8 ± 0.8[b]
Total n-3 PUFA	46.0 ± 1.1[a]	46.6 ± 0.6[a]	26.3 ± 2.5[b]	28.3 ± 5.2[b]	30.0 ± 1.0[b]
Total PUFA[3]	53.2 ± 1.3[c]	54.8 ± 0.7[b,c]	60.7 ± 1.6[a]	58.6 ± 3.9[a,b]	40.5 ± 0.9[d]
n-3/n-6	7.4 ± 0.2[a]	6.4 ± 0.2[b]	0.8 ± 0.2[d]	1.0 ± 0.3[d]	3.0 ± 0.2[c]

[a]Results are expressed as percentage of weight and are means ± SD ($n = 4$). The significance of differences between the dietary treatments was analyzed by one-way analysis of variance. Mean values with different superscript letters within a given row are significantly different ($P < 0.05$) as determined by Tukey's multiple range test. [1], Predominantly n-7 isomer; [2], predominantly n-9 isomer; [3], includes C_{16} and n-9 PUFA present in all samples at percentages varying from 0.3 to 0.6 and 0.2 to 0.4, respectively; n.d., not detected; PUFA, polyunsaturated fatty acids. See Table 2 for company sources.

lipid were associated with specific phospholipid classes related to the unique fatty acid compositions of the individual phospholipid classes (Tables 4–7). Therefore, in the fish fed the SO diet which, in particular, significantly increased the percentages of 18:2n-6 and 20:2n-6, the increases were most prominent in phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (Tables 4–6). With the BO diet, which showed the highest 18:3n-6 and 20:3n-6 in liver total lipid, the increased percentage of 18:3n-6 was mainly found in PC, whereas the increased percentages of 20:3n-6 were found in both PC and PE (Tables 4,5). The level of 20:4n-6 was significantly increased by both the SO and BO diets in PC and PE (Tables 4,5). However, the percentages of 20:4n-6 were greatest in phosphatidylinositol which was also the class most resistant to dietary effects with only the OO diet resulting in a significantly lower level of 20:4n-6 in phosphatidylinositol (Table 7).

Two-way ANOVA showed that less radioactivity was recovered unmetabolized with [1-^{14}C]18:3n-3 as substrate compared to [1-^{14}C]18:2n-6, whereas significantly more 18:2n-6 was metabolized by elongation to the so-called "dead-end product," 20:2n-6, compared to the similar product, 20:3n-3, arising by elongation of 18:3n-3 (Table 8). The amount of radioactivity recovered as either 18:3n-3 or 18:2n-6 and, therefore, unmetabolized, as a percentage of the total radioactivity recovered in PUFA was significantly lower in fish fed BO compared to fish on any of the other diets (Table 8). There was no significant difference between the fish on the FO, TO/MO, SO, and OO diets in the amount of radioactivity recovered unmetabolized as either 18:3n-3 or 18:2n-6. The percentage of radioactivity recovered in the elongation products, 20:3n-3 and 20:2n-6, was significantly lower in BO-fed fish compared to fish fed the diets containing fish oils, FO and TO/MO (Table 8). There was no significant difference between the fish on the FO, TO/MO, SO, and OO diets in the amount of radioactivity recovered as either 20:3n-3, but the OO diet significantly reduced the recovery of radioactivity in 20:2n-6 compared to the diets containing fish oil.

The total amount of desaturation of [1-^{14}C]18:3n-3 was significantly greater in the hepatocytes from salmon fed the n-6 PUFA-rich oils and OO in comparison with both the diets containing fish oil (Table 9). Both the n-6 PUFA-rich oils and OO increased the amount of radioactivity recovered in the Δ6 products compared to the fish oil diets, whereas the BO diet also markedly increased the recovery of radioactivity in the

TABLE 4
Effects of Diet on the Fatty Acid Compositions of Phosphatidylcholine from the Liver of Atlantic Salmon[a]

	Diet				
Fatty acid	Fosol	Tuna oil/Marinol	Sunflower oil	Borage oil	Olive oil
Total saturates	31.3 ± 1.4	33.7 ± 1.9	30.6 ± 2.7	32.5 ± 1.1	31.7 ± 0.7
Total monoenes	15.3 ± 0.8^{b}	12.6 ± 0.9^{c}	12.2 ± 0.9^{c}	12.8 ± 0.8^{c}	24.8 ± 1.2^{a}
18:2n-6	1.1 ± 0.1^{d}	0.9 ± 0.2^{d}	15.2 ± 0.9^{a}	8.0 ± 0.7^{b}	4.5 ± 0.4^{c}
18:3n-6	n.d.^{b}	n.d.^{b}	0.2 ± 0.0^{b}	2.6 ± 0.5^{a}	0.1 ± 0.0^{b}
20:2n-6	0.5 ± 0.1^{b,c}	0.3 ± 0.1^{c}	3.4 ± 0.6^{a}	0.8 ± 0.3^{c}	1.1 ± 0.2^{b}
20:3n-6	0.1 ± 0.1^{d}	0.2 ± 0.1^{d}	3.8 ± 0.5^{b}	6.8 ± 0.9^{a}	1.6 ± 0.2^{c}
20:4n-6	1.0 ± 0.0^{b,c}	1.3 ± 0.2^{b,c}	1.8 ± 0.3^{a,b}	2.6 ± 0.9^{a}	0.6 ± 0.1^{c}
22:4n-6	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.2	0.1 ± 0.0
22:5n-6	0.5 ± 0.1^{a,b}	0.8 ± 0.2^{a}	0.4 ± 0.1^{a,b}	0.6 ± 0.2^{a,b}	0.4 ± 0.1^{b}
Total n-6 PUFA	3.5 ± 0.2^{d}	3.6 ± 0.2^{d}	25.0 ± 1.5^{a}	21.6 ± 1.5^{b}	8.4 ± 0.5^{c}
18:3n-3	0.2 ± 0.1^{a}	0.2 ± 0.1^{a,b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}
18:4n-3	0.2 ± 0.0^{a}	0.1 ± 0.1^{a,b}	trace^{b}	0.2 ± 0.1^{a,b}	0.1 ± 0.1^{a,b}
20:3n-3	0.2 ± 0.1^{a}	0.1 ± 0.1^{b}	n.d.^{c}	n.d.^{c}	n.d.^{c}
20:4n-3	0.7 ± 0.1^{a}	0.4 ± 0.1^{b}	0.2 ± 0.0^{c}	0.2 ± 0.0^{c}	0.4 ± 0.1^{b}
20:5n-3	7.0 ± 0.3^{a}	6.7 ± 1.4^{a}	2.8 ± 0.5^{b}	3.3 ± 0.5^{b}	4.3 ± 0.6^{b}
22:5n-3	2.2 ± 0.2^{a}	1.9 ± 0.2^{a,b}	1.2 ± 0.2^{c}	1.3 ± 0.2^{c}	1.4 ± 0.3^{b,c}
22:6n-3	35.6 ± 0.6^{a}	38.0 ± 2.7^{a}	25.6 ± 0.4^{b}	26.2 ± 0.8^{b}	27.1 ± 2.1^{b}
Total n-3 PUFA	45.9 ± 0.4^{a}	47.3 ± 2.0^{a}	29.9 ± 0.6^{b}	31.3 ± 0.9^{b}	33.5 ± 2.0^{b}
Total PUFA	50.2 ± 0.5^{b}	51.6 ± 1.9^{a,b}	55.3 ± 2.1^{a}	53.3 ± 1.6^{a,b}	42.2 ± 1.6^{c}
n-3/n-6	13.7 ± 0.8^{a}	13.5 ± 1.1^{a}	1.2 ± 0.1^{c}	1.5 ± 0.1^{c}	4.0 ± 1.6^{b}

[a]See Table 3 footnote; trace value, < 0.05%. See Table 2 for company sources.

Δ5 products, which was 2.6-fold greater than with any other diet, and the Δ6* products (hexaenes, predominantly 22:6n-3; formerly termed Δ4 products). Two-way ANOVA showed that as well as the significant effects of diet, there was a very significant effect of substrate with significantly greater desaturation activity toward [1-^{14}C]18:3n-3 compared to [1-^{14}C]-18:2n-6 with significantly greater recovery of radioactivity in the Δ6-, Δ5- and total-desaturation products (Table 9). The

TABLE 5
Effects of Diet on the Fatty Acid Compositions of Phosphatidylethanolamine from the Liver of Atlantic Salmon[a]

	Diet				
Fatty acid	Fosol	Tuna oil/Marinol	Sunflower oil	Borage oil	Olive oil
Total saturates	16.2 ± 0.8^{a,b}	18.2 ± 0.5^{a}	16.3 ± 1.9^{a,b}	16.8 ± 0.3^{a,b}	15.2 ± 0.6^{b}
Total monoenes	22.2 ± 0.8^{b}	17.8 ± 0.7^{c}	11.6 ± 0.7^{e}	14.1 ± 0.3^{d}	25.9 ± 0.6^{a}
18:2n-6	1.4 ± 0.2^{c}	2.1 ± 0.3^{c}	13.3 ± 1.4^{a}	9.7 ± 0.3^{b}	3.2 ± 0.2^{c}
18:3n-6	0.2 ± 0.1^{b,c}	0.2 ± 0.0^{b}	n.d.^{c}	0.4 ± 0.2^{a}	n.d.^{c}
20:2n-6	0.9 ± 0.0^{b}	0.7 ± 0.1^{b}	4.3 ± 0.7^{a}	1.2 ± 0.5^{b}	1.0 ± 0.1^{b}
20:3n-6	0.1 ± 0.1^{d}	0.2 ± 0.1^{d}	4.0 ± 0.5^{b}	6.3 ± 0.7^{a}	1.6 ± 0.1^{c}
20:4n-6	1.6 ± 0.1^{b}	2.3 ± 0.2^{b}	5.0 ± 0.7^{a}	5.8 ± 1.4^{a}	1.5 ± 0.1^{b}
22:4n-6	0.1 ± 0.1^{b}	0.1 ± 0.0^{b}	0.4 ± 0.1^{a}	0.3 ± 0.1^{a}	0.1 ± 0.0^{b}
22:5n-6	0.9 ± 0.1^{b,c}	1.3 ± 0.1^{a}	1.2 ± 0.1^{a}	1.1 ± 0.2^{a,b}	0.7 ± 0.1^{c}
Total n-6 PUFA	5.2 ± 0.4^{c}	6.9 ± 0.3^{c}	28.2 ± 1.7^{a}	24.8 ± 1.9^{b}	8.1 ± 0.0^{c}
18:3n-3	0.2 ± 0.1^{a}	0.3 ± 0.1^{a}	n.d.^{c}	0.1 ± 0.0^{b}	n.d.^{c}
20:3n-3	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	n.d.^{b}	n.d.^{b}	n.d.^{b}
20:4n-3	0.7 ± 0.1^{a}	0.5 ± 0.1^{b}	0.1 ± 0.0^{d}	0.1 ± 0.0^{d}	0.2 ± 0.1^{c}
20:5n-3	5.0 ± 0.1^{a}	5.9 ± 0.5^{a}	3.2 ± 0.1^{b}	3.0 ± 0.6^{b}	6.1 ± 0.9^{a}
22:5n-3	2.2 ± 0.3^{a}	1.9 ± 0.1^{a}	1.3 ± 0.1^{b}	1.2 ± 0.2^{b}	1.4 ± 0.1^{b}
22:6n-3	41.5 ± 0.7^{a,b}	42.7 ± 1.2^{a}	36.0 ± 0.4^{c}	36.0 ± 1.1^{c}	39.1 ± 1.1^{b}
Total n-3 PUFA	49.8 ± 0.7^{a}	51.5 ± 0.8^{a}	40.6 ± 0.3^{c}	40.4 ± 1.3^{c}	46.8 ± 0.3^{b}
Total PUFA	56.9 ± 0.8^{d}	60.2 ± 1.0^{c}	69.6 ± 1.5^{a}	66.1 ± 0.9^{b}	55.8 ± 0.3^{d}
n-3/n-6	9.7 ± 0.7^{a}	7.5 ± 0.2^{b}	1.4 ± 0.1^{d}	1.6 ± 0.2^{d}	5.9 ± 0.1^{c}

[a]See Table 3 footnote. See Table 2 for company sources.

TABLE 6
Effects of Diet on the Fatty Acid Compositions of Phosphatidylserine from the Liver of Atlantic Salmon[a]

	Diet				
Fatty acid	Fosol	Tuna oil/Marinol	Sunflower oil	Borage oil	Olive oil
Total saturates	28.4 ± 1.5[b]	32.1 ± 0.8[a,b]	31.0 ± 0.2[a,b]	32.4 ± 2.6[a]	28.4 ± 0.8[b]
Total monoenes	19.1 ± 1.6[a]	11.6 ± 1.1[b]	8.8 ± 0.4[b]	9.4 ± 1.5[b]	19.0 ± 2.3[a]
18:2n-6	1.5 ± 0.1[c]	1.1 ± 0.1[c]	11.0 ± 1.3[a]	5.2 ± 2.0[b]	2.4 ± 0.5[b,c]
18:3n-6	n.d.[b]	n.d.[b]	0.1 ± 0.0[b]	0.3 ± 0.1[a]	0.3 ± 0.1[a]
20:2n-6	0.8 ± 0.1[b]	0.5 ± 0.2[b]	3.3 ± 0.4[a]	0.9 ± 0.4[b]	1.1 ± 0.7[b]
20:3n-6	0.1 ± 0.1[c]	0.1 ± 0.1[c]	2.5 ± 0.3[a]	3.6 ± 0.9[a]	0.8 ± 0.0[b]
20:4n-6	0.6 ± 0.3[b]	1.0 ± 0.4[a,b]	1.7 ± 0.4[a]	1.6 ± 0.4[a]	0.4 ± 0.0[b]
22:4n-6	0.1 ± 0.0[c]	0.1 ± 0.1[b,c]	0.4 ± 0.2[a]	0.3 ± 0.1[a,b]	trace[c]
22:5n-6	0.8 ± 0.0[b,c]	1.4 ± 0.1[a]	0.8 ± 0.2[b,c]	1.0 ± 0.2[b]	0.6 ± 0.1[c]
Total n-6 PUFA	3.9 ± 0.4[c]	4.2 ± 0.3[c]	19.8 ± 1.1[a]	12.9 ± 3.5[b]	5.6 ± 1.0[c]
18:3n-3	0.5 ± 0.2[a]	0.7 ± 0.1[a]	n.d.[b]	0.2 ± 0.2[b]	0.2 ± 0.0[b]
20:3n-3	0.2 ± 0.0[a]	0.1 ± 0.0[b]	n.d.[c]	n.d.[c]	n.d.[c]
20:4n-3	0.5 ± 0.0[a]	0.3 ± 0.1[b]	0.1 ± 0.1[c]	trace[c]	0.2 ± 0.0[b]
20:5n-3	1.3 ± 0.2[a]	1.3 ± 0.2[a]	1.0 ± 0.1[a,b]	0.7 ± 0.2[b]	1.2 ± 0.1[a]
22:5n-3	1.9 ± 0.3	1.9 ± 0.2	1.3 ± 0.2	1.5 ± 0.4	1.4 ± 0.2
22:6n-3	38.9 ± 1.3[a,b]	43.4 ± 1.8[a]	33.2 ± 1.2[b]	38.2 ± 2.4[a,b]	35.6 ± 3.4[b]
Total n-3 PUFA	43.3 ± 1.6[a,b]	47.7 ± 1.6[a]	35.6 ± 1.3[c]	40.6 ± 2.5[b,c]	38.7 ± 3.6[b,c]
Total PUFA	49.2 ± 1.6[b,c]	53.0 ± 1.7[a,b]	56.2 ± 0.4[a]	54.2 ± 1.2[a]	45.3 ± 2.6[c]
n-3/n-6	11.3 ± 1.0[a]	11.5 ± 0.6[a]	1.8 ± 0.2[c]	3.3 ± 1.0[c]	7.2 ± 2.0[b]

[a]See Table 3 footnote; trace value, < 0.05%. See Table 2 for company sources.

desaturation of [1-^{14}C]18:2n-6 was much less affected by diet but, as with the desaturation of [1-^{14}C]18:3n-3, the fish fed BO displayed significantly greater recoveries of radioactivity in Δ6- (over 2.4-fold greater than any other diet), Δ5-, and total-desaturation products (Table 9). There was no significant difference between the two diets containing fish oil, FO and MO/TO, in the desaturation of either [1-^{14}C]18:3n-3 or [1-^{14}C]18:2n-6 although the MO/TO diet consistently gave the lowest percentages of radioactivity recovered in almost all the fatty acid fractions.

TABLE 7
Effects of Diet on the Fatty Acid Compositions of Phosphatidylinositol from the Liver of Atlantic Salmon[a]

	Diet				
Fatty acid	Fosol	Tuna oil/Marinol	Sunflower oil	Borage oil	Olive oil
Total saturates	34.9 ± 1.2[b]	35.3 ± 1.9[a,b]	38.1 ± 0.7[a]	36.9 ± 1.0[a,b]	30.0 ± 0.7[c]
Total monoenes	9.3 ± 1.1[b]	8.1 ± 1.6[b,c]	4.8 ± 0.7[d]	6.2 ± 0.7[c,d]	13.7 ± 0.4[a]
18:2n-6	0.4 ± 0.1[b]	0.4 ± 0.1[b]	2.3 ± 0.5[a]	2.3 ± 0.6[a]	1.4 ± 0.1[c]
18:3n-6	0.6 ± 0.0[a,b]	0.8 ± 0.2[a]	0.1 ± 0.0[c]	0.3 ± 0.3[b,c]	0.2 ± 0.0[c]
20:2n-6	0.2 ± 0.0[b]	0.1 ± 0.1[b]	0.7 ± 0.1[a]	0.3 ± 0.1[b]	0.6 ± 0.2[a]
20:3n-6	0.7 ± 0.1[c]	0.6 ± 0.2[c]	4.2 ± 0.6[b]	5.9 ± 1.1[a]	6.9 ± 0.7[a]
20:4n-6	26.0 ± 1.4[a]	29.7 ± 1.7[a]	31.2 ± 1.0[a]	26.3 ± 4.3[a]	18.6 ± 0.7[b]
22:4n-6	0.1 ± 0.1	trace	0.1 ± 0.1	0.1 ± 0.1	trace
22:5n-6	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Total n-6 PUFA	28.7 ± 1.2[c]	32.0 ± 1.5[b,c]	38.8 ± 1.7[a]	35.5 ± 2.4[a,b]	27.9 ± 0.3[c]
18:3n-3	n.d.	trace	n.d.	0.2 ± 0.1	0.1 ± 0.1
20:4n-3	0.3 ± 0.1[a]	0.3 ± 0.1[a]	trace[b]	trace[b]	0.3 ± 0.1[a]
20:5n-3	3.1 ± 0.8[a]	2.4 ± 0.5[a,b]	1.2 ± 0.1[b,c]	0.9 ± 0.4[c]	3.6 ± 0.2[a]
22:5n-3	1.9 ± 0.1[a]	1.6 ± 0.0[b]	0.9 ± 0.1[d]	1.1 ± 0.1[c,d]	1.4 ± 0.2[b,c]
22:6n-3	18.9 ± 1.4[a]	17.5 ± 0.9[a]	13.3 ± 1.0[b]	16.0 ± 1.4[a,b]	17.6 ± 0.5[a]
Total n-3 PUFA	24.2 ± 1.1[a]	21.8 ± 0.8[a]	15.4 ± 1.2[b]	18.2 ± 1.6[b]	23.0 ± 0.8[a]
Total PUFA	53.1 ± 0.0[a,b]	54.0 ± 0.6[a]	54.3 ± 1.4[a]	53.8 ± 0.9[a,b]	52.0 ± 0.2[b]
n-3/n-6	0.9 ± 0.1[a]	0.7 ± 0.1[b,c]	0.4 ± 0.0[d]	0.5 ± 0.1[c,d]	0.8 ± 0.1[a,b]

[a]See Table 3 footnote. See Table 2 for company sources.

TABLE 8
Effects of Diet on the Recovery of Radioactivity Unmetabolized or in Products Only Elongated by Isolated Hepatocytes from Atlantic Salmon (*Salmo salar*) Incubated with [1-^{14}C]18:3n-3 or [1-^{14}C]18:2n-6[a]

Fatty acid fraction	Diet				
	Fosol	Tuna oil/Marinol	Sunflower oil	Borage oil	Olive oil
[1-^{14}C]18:3n-3					
18:3n-3	67.9 ± 2.8^{a}	72.4 ± 1.6^{a}	65.8 ± 2.1^{a}	54.3 ± 2.2^{b}	65.4 ± 3.3^{a}
20:3n-3	19.5 ± 0.9^{a}	18.7 ± 1.9^{a}	13.4 ± 0.9^{a}	5.2 ± 1.2^{b}	13.3 ± 1.5^{a}
[1-^{14}C]18:2n-6					
18:2n-6	71.1 ± 1.0^{a}	73.1 ± 1.0^{a}	75.1 ± 2.8^{a}	66.8 ± 1.6^{b}	76.4 ± 1.3^{a}
20:2n-6	21.6 ± 0.6^{a}	21.3 ± 1.6^{a}	15.4 ± 3.5^{a,b,c}	13.9 ± 0.8^{c}	14.2 ± 0.6^{b,c}

[a]Hepatocytes were isolated and incubated with either [1-^{14}C]18:3n-3 or [1-^{14}C]18:2n-6 as described in the Materials and Methods section. Results are expressed as the amount of radioactivity recovered in the fractions as percentages of total radioactivity recovered. The significance of differences between the dietary treatments was analyzed by one-way analysis of variance (ANOVA). Mean values with different superscript letters within a given row are significantly different ($P < 0.05$) as determined by Tukey's multiple range test. The results of two-way ANOVA were: substrate—significant for both the C_{18} ($P = 0.0000$; mean values for 18:3 and 18:2 being 65.9 and 72.8) and C_{20} ($P = 0.0000$; mean values for 20:3 and 20:2 being 14.3 and 17.4); diet—significant for both the C_{18} [$P = 0.0000$; mean value for borage oil (BO) being significantly lower than all other diets at 60.7, with other diets varying between 69.6 and 72.8 and no significant difference between them] and C_{20} ($P = 0.0019$; mean value for BO being significantly lower than all other diets at 9.5, with other diets varying between 13.7 and 20.5 with some significant differences). See Table 2 for company sources.

TABLE 9
Effects of Diet on the Desaturation of [1-^{14}C]18:3n-3 and [1-^{14}C]18:2n-6 by Isolated Hepatocytes from Atlantic Salmon (*Salmo salar*)[a]

Desaturation products	Diet				
	Fosol	Tuna oil/Marinol	Sunflower oil	Borage oil	Olive oil
[1-^{14}C]18:3n-3					
Δ6	7.5 ± 0.6^{b}	5.9 ± 0.5^{b}	11.7 ± 0.9^{a}	13.4 ± 0.9^{a}	10.8 ± 0.8^{a}
Δ5	4.8 ± 2.4^{b,c}	2.4 ± 0.5^{c}	7.2 ± 1.8^{b}	22.9 ± 0.9^{a}	8.8 ± 1.0^{b}
Δ6*	0.3 ± 0.1^{c}	0.6 ± 0.1^{c}	1.9 ± 0.5^{b}	4.2 ± 0.8^{a}	1.7 ± 0.0^{b,c}
Total	12.6 ± 1.9^{c}	8.9 ± 0.3^{c}	20.8 ± 2.5^{b}	40.5 ± 1.1^{a}	21.3 ± 1.8^{b}
[1-^{14}C]18:2n-6					
Δ6	4.5 ± 2.0^{b}	3.3 ± 1.4^{b}	6.3 ± 0.6^{b}	15.4 ± 0.5^{a}	6.2 ± 0.9^{b}
Δ5	1.4 ± 0.1^{b}	0.8 ± 0.2^{b}	1.3 ± 0.3^{b}	2.4 ± 0.1^{a}	1.0 ± 0.0^{b}
Δ6*	1.4 ± 0.2	1.5 ± 0.3	1.9 ± 0.4	1.5 ± 0.1	2.2 ± 0.2
Total	7.3 ± 1.6^{b,c}	5.6 ± 1.8^{c}	9.5 ± 0.8^{b}	19.3 ± 0.8^{a}	9.4 ± 0.7^{b,c}

[a]Hepatocytes were isolated and incubated with either [1-^{14}C]18:3n-3 or [1-^{14}C]18:2n-6 as described in the Materials and Methods section. Results are expressed as the amount of radioactivity recovered in the various desaturated products as percentages of total radioactivity recovered. The significance of differences between the dietary treatments was analyzed by one-way analysis of variance (ANOVA). Mean values with different superscript letters within a given row are significantly different ($P < 0.05$) as determined by Tukey's multiple range test. Δ6, products of Δ6 desaturation (18:4 and 20:4 for n-3, and 18:3 and 20:3 for n-6); Δ5, products of Δ5 desaturation (20:5 and 22:5 for n-3, and 20:4 and 22:4 for n-6); Δ6*, hexaene products for n-3 and pentaene products for n-6. The results of two-way ANOVA were: diet—Δ6, Δ5, Δ6* and total all significant ($P = 0.0000$); substrate—Δ6, Δ5 and total all significant ($P = 0.0000$), but Δ6* ns, $P = 0.7342$ with the differences (Tukey's) being Δ6, n-3 > n-6 (9.66 vs. 6.74); Δ5, n-3 > n-6 (8.48 vs. 1.33); total, n-3 > n-6 (19.76 vs. 9.75); interaction—Δ6, Δ5, Δ6* and total all significant $P = 0.0000$ except Δ6 $P = 0.0006$. See Table 2 for company sources.

DISCUSSION

In the present study, the use of intact hepatocytes to determine the metabolism of fatty acids by desaturation and elongation was preferred to the use of microsomes. Therefore, it should be noted that the present data do not measure desaturase enzyme activity as directly as with microsomal preparations. Microsomal preparations, though, are limited, particularly in relation to 18:3n-3 metabolism in fish, in that the production of DHA cannot be determined in microsomes alone because peroxisomes are also required (7). The utilization of intact hepatocytes enables the whole desaturation/elongation pathway to be assayed. Furthermore, dietary influences of membrane fatty acid composition will extend to all cell membranes including plasma membranes. Therefore, altered membrane fatty acid compositions may affect other membrane processes such as fatty acid uptake into the cell (43). Fatty acids also have to be transported intracellularly and activated by acyl-CoA synthetase, and so there are other enzyme activities whose modulation could contribute to the overall level of fatty acyl desaturation observed (44). Nonetheless, assaying fatty acyl desaturation/elongation in intact hepatocytes can

arguably be regarded as more accurately reflecting the effect of diet on all these processes compared to subcellular microsomal preparations. However, diet is known to directly affect desaturase activities in mammals (5,19) and the differential effects of an individual diet on Δ6, Δ5 and Δ6* activities suggest that much of the dietary effect may be on the desaturation/elongation enzyme systems themselves.

The results clearly demonstrated that 18:3n-3 and n-3 PUFA in general were the preferred substrates for desaturation in salmon hepatocytes, irrespective of diet. Less [1-^{14}C]18:3n-3 was recovered unmetabolized with a greater proportion recovered as desaturated products, compared to [1-^{14}C]18:2n-6. This observation is in agreement with previous studies in rats (3), rainbow trout (45), Atlantic salmon parr (23), and established fish cell lines (46). In contrast, it was noteworthy that a greater proportion of [1-^{14}C]18:2n-6 was converted by elongation to 20:2n-6 compared to the production of 20:3n-3 from [1-^{14}C]18:3n-3.

The MO/TO diet was formulated to contain more n-3 PUFA than the FO diet, with less of the long-chain monoenes, such as 20:1 and 22:1, characteristic of Northern Hemisphere fish oil. However, it was clear from the liver lipid data that the MO/TO diet resulted in fatty acid compositions very similar to the FO diet, and this was reflected in the fatty acid metabolism data where there were no differences between the two diets containing fish oil. However, the vegetable oil diets, SO, BO and OO, resulted in very significant effects on the liver phospholipid fatty acid compositions compared to the fish oil diets, and there were also clear differences between the diets containing fish oil and vegetable oils in their effects on PUFA metabolism. Most obvious was the fact that the desaturation of [1-^{14}C]18:3n-3 was considerably greater in hepatocytes from fish fed diets containing vegetable oils. The desaturation of [1-^{14}C]18:2n-6 was less affected but also tended to be increased in fish fed diets containing vegetable oils. It is probably appropriate to interpret such data as inhibition of fatty acyl desaturation by long-chain n-3 PUFA in the fish oil diets since such long-chain n-3 PUFA are known to be inhibitory to the desaturation of both 18:3n-3 and 18:2n-6 in rats (13,47,48) and rainbow trout (45). The present study confirms that in this respect fish are no different from mammals and that diets containing long-chain n-3 PUFA-rich fish oils suppress hepatic fatty acyl desaturation. Similarly, it was noteworthy that there was very little difference between SO and OO in their effects on hepatic fatty acyl desaturation in salmon. Previously it was shown that there was no difference in Δ6-desaturase activity in duodenal mucosa microsomes from dogs fed SO or OO (49).

Although the difference between diets containing fish oil and diets containing vegetable oil was very clear, the most significant finding in the present study was that BO greatly increased hepatic fatty acid desaturation. In hepatocytes from fish fed BO, the total desaturation products were double that found with any other diet and almost fourfold that of the lowest level of desaturation observed in the MO/TO diet. Dietary 18:3n-6 has been reported to have variable and conflicting effects in rats. An early study reported that 18:3n-6 in the form of evening primrose oil did not modify liver Δ6- or Δ5-desaturase activities in rats after 11 wk of feeding (50). However, in young rats fed a diet containing 18:3n-6, Δ5-desaturase activity was depressed (51). In contrast, a diet containing evening primrose oil depressed liver Δ6-desaturase activity in 8-mon-old rats but not in 3-wk-old rats (52). Borage oil (18:3n-6 only) and blackcurrant oil (18:3n-6 and 18:4n-3) both tended to decrease liver microsomal Δ6- and Δ5-desaturase activities in younger rats but tended to increase both activities in older rats (53). Clearly, age of the animal and possibly the different fatty acid compositions of the oils containing 18:3n-6 that were used in these studies have contributed to the conflicting data obtained. Certainly, rat liver Δ6-desaturase activity decreases with age (5), and it was shown that dietary supplementation of GLA in the form of evening primrose oil could reverse this aging effect in rats (54). There are no data on the effects of aging on desaturase activities in fish, but considering the very significant results from the present study, it appears that hepatic fatty acyl desaturation fish may be more affected by dietary GLA than in mammals.

The present study also revealed some other dietary effects that were noteworthy. Two-way ANOVA showed significant interactions indicating that the effects of the diets were dependent upon the substrate fatty acid used. For instance, the greatest effect of BO on the desaturation of 18:3n-3 was at the Δ5 level, whereas it was at the Δ6 level for the desaturation of 18:2n-6. Most previous work in mammals has focused on the effects of diet on Δ6-desaturase activity, regarded as the rate-limiting step of the desaturase pathway (5,19). Although some studies have also measured Δ5 desaturation, few have investigated the effects on 18:3n-3 metabolism or of dietary 18:3n-6 (12).

As the diet containing BO was significantly different from the diets containing SO and OO in its effects on fatty acid desaturation, the question must be what were the major differences in liver fatty acid compositions between the diets containing vegetable oil that may be influencing factors. Liver total lipid of salmon fed the BO, SO, and OO diets all had very similar levels of EPA, DHA, and total n-3 PUFA, and although there were slight differences in the distribution of n-3 PUFA in individual phospholipids between the diets containing vegetable oil, it was not likely that differences in n-3 PUFA inhibition were responsible. Total PUFA levels in liver phospholipids in the BO-fed fish were also very high indicating that a generally low PUFA level was not a factor. Similarly, the levels of 18:2n-6 and 18:3n-3 did not correlate with levels of desaturation activity as the level of 18:2n-6 in BO-fed fish was intermediate between SO and OO, and hepatic 18:3n-3 levels were very low in all diets. However, 20:3n-6, and to a lesser extent AA, were generally significantly higher in liver lipids in the BO-fed fish compared to all the other diets. AA is the preferred precursor substrate PUFA for eicosanoid synthesis in fish, despite EPA being more abundant in tissue phospholipids (15,55), and increasing the levels of 20:3n-6 in cell phospholipids significantly increases the

production of 1-series prostaglandins and inhibits the production of 2- and 3-series prostaglandins in fish (55). There are few data on the effects of eicosanoids on fatty acid desaturation, but it is possible that modulation of eicosanoids may play a role in the effect of GLA on hepatic fatty acid desaturation in the present study.

This was not a nutritional study, and so the direct application of the results in the modification of salmon diets in the future is limited. However, in the present study the vegetable oil diets did not result in significantly lower final weights compared to the control FO diet. There was considerable variation though, and fish on the SO diet were significantly lighter than fish on the TO/MO diet, suggesting that diets with very high 18:2n-6 were potentially detrimental. The study also showed that the vegetable oil diets increased the desaturation of [1-^{14}C]18:3n-3 in salmon liver, consistent with other studies that have shown that salmon can utilize vegetable oils, providing the oils contain sufficient 18:3n-3 to satisfy essential fatty acid requirements (28,30–32). In the previous studies, linseed oil diets were not associated with adverse effects, whereas sunflower oil diets were shown to have potentially detrimental effects in salmon (31). Recently, however, we have shown that a blend of 18:3n-3 and 18:2n-6 fed to salmon parr enhanced hepatic desaturase activities and were beneficial in effecting successful seawater transfer (23). Similarly, the present study has indicated that if vegetable oils are used to replace fish oils in salmon smolt diets, the inclusion of borage oil in the blends may be beneficial.

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