Atlantic Salmon (*Salmo salar*) Muscle Lipids and Their Response to Alternative Dietary Fatty Acid Sources†

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Post-smolt Atlantic salmon (*Salmo salar*) were fed one of four diets with 10% of lipid sources differing in fatty acid combinations. Growth over several months provided newly formed lipids, representing dietary fatty acid input, for study. Edible muscle from each diet group, including one fillet frozen for several months at −12 °C, was extracted, and the classes in total lipid were determined. Slight hydrolysis of phospholipids during frozen storage was detected. The fatty acids of phospholipids were readily altered to take up dietary 18:1*n* − 9, 18:2*n* − 6, 18:3*n* − 3, and 20:5*n* − 3. The triglycerides responded in a similar fashion but excluded dietary 18:3*n* − 3 and 20:4*n* − 6. There was no evidence of practical-scale conversion of dietary 18:2*n* − 6 to 20:4*n* − 6 or of 18:3*n* − 3 to 20:5*n* − 3 or 22:6*n* − 3.

INTRODUCTION

The majority of the studies of the metabolism of fatty acids in salmonids have been concerned with growth and health in the fish (Ackman and Takeuchi, 1986; Hardy et al., 1987; Thomassen and Røsjø, 1989; Sowizral et al., 1990) or with the fine details observed in the enzymes and phospholipids of organs such as the gills, brain, and liver and even cultured cell lines and erythrocytes [e.g., Bell and Dick (1990), Tocher and Dick (1990), and Bell et al. (1991)].

The gross fatty acid composition of edible salmon parts, both natural and in response to different fats in the diet, has been the subject of extensive study (Ackman, 1989a), and salmon as a food has lately received extra attention with the emphasis on the health potential of *n* − 3 fatty acids (Hardy and King, 1989; Nelson et al., 1991). These fatty acids are believed by many to play a natural prevention role in cardiovascular disease and to alleviate other adult-onset health problems (Simopoulos, 1989). Well over a thousand biomedical literature references attest to the interest in this field (Van de Kamp, 1990), usually targeting the role of *n* − 3 fatty acids. These fatty acids possibly receive undue emphasis relative to the dietary long-chain (C_{20} + C_{22}) *n* − 6 fatty acids, since the latter have a different geographic distribution in fish food resources (Ackman, 1989a).

Among the objectives of our study of salmon fillet fats as food was an examination of the balance of C_{20} and C_{22} *n* − 6 and *n* − 3 fatty acids which could be achieved in the total edible muscle fat of Atlantic salmon, since this balance is of particular interest in the whole question of human nutrition and health (Weber, 1988; Burr, 1989; Harris, 1989; Simopoulos, 1989). In addition to a fish meal lipid base fat providing to all groups the *n* − 3 fatty acids essential for salmonids, there was a reference group fed a standard herring oil supplement and three experimental groups of fish fed supplemental canola oil or fatty acid ethyl esters differing in at least one important fatty acid and usually different in several respects. The fats in these experimental diets were selected to maximize the effect on flesh phospholipids as well as fillet triglycerides.

MATERIALS AND METHODS

Animals and Feeding Procedures. Atlantic salmon smolts (45–159 g) were obtained from the Mactaquac, NB, Fish Culture Station of the Department of Fisheries and Oceans Canada and were maintained in eight 2.86-m^3 cylindrical fiberglass tanks, with 300 fish per tank, at the Department of Fisheries and Oceans Biological Station in St. Andrews, NB. All fish were gradually acclimated to seawater (30–31% salinity). Fish were fed to satiation on each of the four diets (in pellet form) for 29 weeks beginning on May 6, 1987. Every 6 weeks four fish were removed at random from each diet treatment group for analysis, killed by a blow on the head, and transported to Halifax where the muscle lipids were examined (Polvi, 1989).

In the October sample, fillets from one side of the fish sampled from each diet were frozen for 3 months at −12 °C to mimic commercial handling and then analyzed. The lipids of these fillets were compared to those of matching control fillets frozen briefly at −30 °C.

Preparation and Analysis of Diets. Diets were prepared at the Department of Fisheries and Oceans, Halifax, NS. Table I gives the composition of the diets which were made into pellets with a California pellet mill (Model CL-2) in the pilot plant of Fisheries and Oceans Canada. The lipid sources consisted of two natural triglyceride oils and two mixtures of ethyl esters of fatty acids. The triglycerides were respectively commercial herring (*Clupea harengus*) and canola (*Brassica* sp.) oils. Ethyl ester concentrates of EPA (eicosapentaenoic or 20:5*n* − 3 acid) and DHA (docosahexaenoic or 22:6*n* − 3 acid) from fish oil and of arachidonic acid (20:4*n* − 6) from hen eggs were prepared by urea complexing (Ackman, 1988; Ratnayake et al., 1988). The EPA/DHA concentrate was prepared from redfish (*Sebastes* sp.) oil from the National Sea Products plant in Canso, NS, and hen eggs were obtained from the Agriculture Canada Research Station in Kentville, NS.

Removal of PCBs from Redfish Oil. The redfish oil was stripped of polychlorinated biphenyls (PCBs) using a POPE wiped-wall short-path still (POPE Scientific Inc., Menomonee Falls, WI) (Ackman, 1988). Operating conditions were as follows: vacuum, 0.5 Torr; flow rate, 10 L/h; temperature, 250 °C; wipers, 300 rpm; cold water temperature, 12 °C. The PCBs were analyzed in the original, in the stripped oil, and in the final concentrate using the method described by Waliszewski and Szymczynski (1982). The stripped oil was then converted to a concentrate of EPA and DHA, as ethyl esters.

Hen Egg Lipid Fatty Acids. The second ethyl ester preparation was based on fatty acids of hen egg lipids. Initially the above procedure for saponification, urea crystallization, and

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† This work was supported by a Strategic Grant from the Natural Sciences and Engineering Research Council of Canada to R.G.A. and funding of the Atlantic Salmon Federation through contributions of Henry G. Walter, Jr., of the Monel Corp., to R. L. Saunders and A. P. Farrell.

0021-8561/92/1440-1001$03.00/0

Table I. Composition of Experimental Diets[a]

ingredient	g/kg
herring meal (68% crude protein)	350
feather meal, hydrolyzed (80% crude protein)	60
soybean meal (48% crude protein)	80
brewers' dried yeast (45% protein)	50
wheat gluten (79% crude protein)	80
wheat middlings (17% crude protein)	185
whey, spray dried (12% crude protein)	70
choline chloride (50%)	3
DL-methionine	2
vitamin premix[b]	10
mineral premix[c]	10
lipid source[d]	100

[a] Proximate analysis (percent air-dry basis): dry matter, 95.1; crude protein (% N × 6.25), 45.0; lipid, 14.2; ash, 7.7. [b] Vitamins added to supply the following per kilogram of diet: retinyl acetate, 8000 IU; cholecalciferol, 4000 IU; menadione sodium bisulfite, 30 mg; *dl*-α-tocopheryl acetate, 350 IU; thiamin hydrochloride, 40 mg; riboflavin, 50 mg; *d*-calcium pantothenic acid, 150 mg; biotin, 0.1 mg; folic acid, 15 mg; vitamin B_{12}, 0.1 mg; niacin, 200 mg; pyridoxine hydrochloride, 40 mg; ascorbic acid, 1 g; inositol, 400 mg; ethoxyquin, 200 mg. [c] Minerals were added to supply (milligrams of element per kilogram of diet) the following: 10 mg of I, potassium iodide (76.4% I); 40 mg of Mn, manganous sulfate (32.5% Mn); 50 mg of Fe, ferrous sulfate (20.1% Fe); 100 mg of Zn, zinc sulfate (22.7% Zn). [d] Lipid source: (1) herring oil; (2) canola oil; (3) EPA/DHA ethyl ester concentrate; (4) hen egg fatty acids as ethyl esters.

Table II. Important Fatty Acids of Diets Fed to Salmon

fatty acid	diet 1, herring oil	diet 2, canola oil	diet 3, EPA/DHA concentrate[a]	diet 4, egg lipid[a]
14:0	8.5	2.1	3.5	2.4
16:0	14.3	9.2	8.8	8.7
18:0	1.3	1.8	1.0	1.5
16:1*n* − 7	7.4	1.9	2.7	4.9
18:1*n* − 9	9.9	41.1	6.9	29.7
Σ20:1	12.2	3.7	3.6	3.6
Σ22:1[b]	17.8	4.6	5.3	4.5
Σsatd + mono	71.4	64.4	31.8	55.3
18:2*n* − 6	6.9	19.2	7.6	25.0
18:3*n* − 3	1.2	7.1	1.0	3.5
18:4*n* − 3	1.9	0.5	4.3	0.5
20:4*n* − 6	0.3	0.1	0.5	3.3
20:4*n* − 3	0.3	0.1	0.9	0.1
20:5*n* − 3	5.2	1.7	18.3	1.9
22:5*n* − 6	0.1	0.1	0.5	0.9
22:5*n* − 3	0.6	0.2	0.9	0.4
22:6*n* − 3	5.7	3.2	23.4	4.6
others	6.4	3.4	10.8	4.5

[a] Fed as ethyl esters. [b] Primarily from herring meal; canola oil contains the 22:1*n* − 9 isomer but no 22:1*n* − 11 and *n* − 13 fatty acids.

formation of ethyl esters was also used when arachidonic and other unsaturated fatty acids from egg lipids were concentrated. The very high proportion of cholesterol (500 mg/100 g of egg) created problems and only a small amount of fatty acid product was obtained, indicating the need to further investigate the procedure and proportions of reagents for concentrates of hen egg lipid fatty acids. The complexing procedure with urea was then omitted. The solution of free fatty acids in 95% ethanol was instead chilled to 0 °C and filtered. The filtration removed most of the cholesterol and some of the saturated fatty acids.

General Analysis. Moisture and protein analyses followed AOAC or similar procedures. Lipids of all diets (20-g samples) were extracted according to the method of Bligh and Dyer (1959) and the fatty acids were converted to methyl esters for analysis by gas–liquid chromatography (Table II). Fillets of fish killed in October were treated similarly. The lipid classes in the fish muscle total lipid extracts were determined by TLC/FID (thin-layer chromatography/flame ionization detection) with separation on Chromarods and measurement by an Iatroscan Mark III (Ackman et al., 1990). Results were converted from w/w percent lipid to grams per 100 g of sample. All analyses of fatty acids from lipid samples were done using gas chromatography. A Perkin-Elmer (Norwalk, CT) 900 gas chromatograph fitted with a DB-Wax (bonded polyethylene glycol) fused silica column, dimensions 30 m × 0.25 mm, from J & W Scientific Inc. (Folsom, CA) was used. The helium carrier gas pressure was set at 180 kPa. The oven temperature was 200 °C. The percentage (mole) of each fatty acid was obtained by a computer program (Ackman and Eaton, 1978). Peaks were identified by comparing the retention times with those of a mixture of standard methyl esters and also by a semilogarithmic plot of retention time vs carbon number. "Others" refers to 20:0, 20:3*n* − 6, 20:4*n* − 3 etc., the odd-chain linear and methyl-branched shorter chain (15:0, 17:0) saturated fatty acids and the C_{16} polyunsaturated fatty acids.

RESULTS AND DISCUSSION

Salmon take time to adapt to changes in diet. For this reason the growth data presented in Table III are started from a date in June when all groups were considered to be fully adapted and feeding normally. Salmon also reduce feed intake as the water temperature drops below approximately 5 °C. Growth after the October date when fish were removed for analysis was much reduced compared with the summer period. Table III establishes that the bulk of the triglycerides in all fish would represent newly deposited body fat and not a rearrangement of pre-existing muscle depot fat.

The proportion of lipid classes in salmon, as in many fish, is variable on a wet weight (or edible portion) basis (Ackman, 1989a), and these data are presented in Table IV. The fatty acids of the total lipid (Table V) reflect those of the basic cellular phospholipids (Table VI) plus those of the depot fat triglycerides (Table VII). Although the groups are identified below by type of added dietary fat, it should be noted that the herring meal lipid amounted to an additional supplement of about 4% of diet weight throughout all four diets. This lipid has a fatty acid composition similar to the total diet referred to as "herring oil" (Table II, diet 1).

Large changes in the fatty acids of the phospholipids were not expected, but there were in fact some changes of considerable interest (Table VI). The canola oil-fed group showed, relative to the herring diet-fed group, less 16:0, 18:0, and 16:1 but more 18:1. With 20:1 and 22:1 included, the totals for this group of fatty acids were the same at 49.2% and 48.2%, respectively, for the fresh (or briefly frozen) fish. The balance, all polyunsaturated fatty acids, showed a strong effect of diet with 18:2*n* − 6 and 18:3*n* − 3 from the canola oil replacing 20:5*n* − 3 and 22:6*n* − 3. These two groups of fish were both fed triglyceride oils. The group fed canola oil had the highest total fat (Table IV). The growth rate (Table III) was essentially the same for diets 1 and 2, and the fish were in good health.

The two groups fed ethyl esters as part of their dietary fat had less phospholipid and less triglyceride than those fed triglyceride oils (Table IV). The fatty acids of the muscle phospholipid for the EPA/DHA concentrate-fed group (Table VI) had much less of the saturated/monounsaturated acids (35.8%) and a complementary enrichment in polyunsaturated acids. However, this was directed largely to 22:6*n* − 3, and the 20:5*n* − 3 was relatively much lower than in the dietary fatty acids (Table II). The muscle phospholipids of the group fed egg lipid fatty acid ethyl esters displayed a high proportion of 18:1*n* − 9, in parallel with the diet proportion, confirming the finding for the canola oil group. Despite the very high proportion of 18:2*n* − 6 in the diet, the muscle phospholipids of this group accumulated less 18:2*n* − 6 than the canola group. The point of most interest was, however, the limited incorporation of 20:4*n* − 6. This had been an important reason

Table III. Average Weights and Lengths[a] of Atlantic Salmon, Percentage Weight Increase, and Amount of Triglyceride in Salmon at the Beginning and near Termination of Basic Feeding Trials

parameter	date	diet 1, herring oil	diet 2, canola oil	diet 3, EPA/DHA concentrate[b]	diet 4, egg fatty acids[b]
length, cm	June 11[c]	20.9 ± 0.29a	20.9 ± 0.28a	20.9 ± 0.29a	20.9 ± 0.28a
	Oct 15[d]	32.5 ± 0.85a	33.7 ± 0.69a	31.7 ± 0.75a	32.7 ± 0.54a
wt, g	June 11[c]	88.1 ± 6.15a	87.5 ± 6.37a	86.1 ± 4.06a	87.2 ± 5.23a
	Oct 15[d]	460.9 ± 18.03ab	513.4 ± 15.35b	391.9 ± 13.39a	445.6 ± 9.29ab
g of TG in above wt of fish muscle	June 11	1.96	1.95	1.92	1.94
	Oct 15	22.40	28.19	13.68	15.55
TG wt gain,[e] g		20.44	26.24	11.76	13.61
TG wt gain as % of October muscle TG[e]		91.2	93.0	86.0	87.5

[a] Values in the same row followed by the same letter are not significantly different ($P < 0.05$). On each sampling date, 15–20 fish were taken for length and weight. [b] Ethyl esters. [c] $n = 20$. [d] $n = 15$. [e] These figures take the percent TG and the weight of fish into account. Although the total weight of fish is not all muscle, it is assumed that the proportion of muscle is the same in all samples. This is only a rough estimate.

Table IV. Total Lipid Content and Lipid Classes of Muscle in Atlantic Salmon from October Sampling before and after Frozen Storage (Grams per 100 g of Tissue)

	treatment[a]	total lipid[d]	lipid class[b,c] PL	CH	TG	FFA
herring oil	1	5.88 ± 0.37	0.67 ± 0.03	0.07 ± 0.01	5.14 ± 0.03	tr
	2		0.58 ± 0.05	0.02 ± 0.01	5.09 ± 0.15	0.19 ± 0.1
canola oil	1	6.63 ± 0.86	0.66 ± 0.11	0.02 ± 0.01	5.95 ± 0.20	tr
	2		0.65 ± 0.03	0.02 ± 0.01	5.73 ± 0.12	0.23 ± 0.01
EPA/DHA concentrate	1	4.38 ± 0.77	0.54 ± 0.15	0.05 ± 0.01	3.76 ± 0.13	0.04 ± 0.01
	2		0.43 ± 0.11	0.04 ± 0.01	3.75 ± 0.13	0.16 ± 0.01
egg lipid	1	4.44 ± 0.35	0.56 ± 0.06	0.06 ± 0.01	3.81 ± 0.15	0.01 ± 0.005
	2		0.44 ± 0.01	0.05 ± 0.01	3.72 ± 0.12	0.23 ± 0.05

[a] 1, frozen briefly at −30 °C; 2, frozen briefly at −30 °C and then stored at −12 °C for 3 months. [b] PL, phospholipd; CH, cholesterol; TG, triglyceride; FFA, free fatty acid. [c] tr, trace (≤0.01 g/100 g). [d] Weights after frozen storage differed. To facilitate comparisons, the lipid classes have been converted proportionately to match the total for fresh salmon muscle.

Table V. Important Fatty Acids of Total Lipid in Muscle of Atlantic Salmon from October Sampling, Fed Four Experimental Diets and Analyzed before and after Frozen Storage, with Comparable Literature Data

fatty acid	herring oil		canola oil		EPA/DHA concentrate		egg lipid		literature[b]	
treatment[a]	1	2	1	2	1	2	1	2	A	B
14:0	7.7	9.9	2.5	2.8	3.4	3.4	3.2	3.2	2.4	3.3
16:0	17.3	18.6	13.3	13.5	14.6	13.4	15.3	15.1	11.2	15.9
18:0	2.3	2.2	2.8	2.6	2.4	2.4	2.5	2.2	3.8	3.8
16:1*n* − 7	8.4	9.1	2.7	3.2	4.3	4.0	5.8	7.0	4.5[c]	4.0
18:1*n* − 9	12.9	12.0	40.1	38.4	8.2	8.0	38.5	38.6	24.0	22.5
18:1*n* − 7	2.2	2.0	2.1	2.4	1.3	3.0	3.2	3.2		3.4
Σ20:1	12.4	11.4	4.4	4.5	3.9	4.4	4.6	4.5	4.0	8.1
Σ22:1	12.4	12.3	2.7	3.3	3.6	4.5	2.9	3.4	5.0	7.1
Σsatd + mono	75.6	77.5	70.6	70.7	41.7	43.1	76.0	77.2	54.9	68.1
18:2*n* − 6	7.3	7.1	15.6	16.1	8.2	7.9	12.5	11.9	3.1	6.4
18:3*n* − 3	1.1	1.6	4.5	4.5	1.0	1.0	0.7	0.7	5.2	1.5
18:4*n* − 3	1.6	1.1	1.4	1.1	3.8	3.6	0.7	0.7	1.5[d]	0.4
20:4*n* − 6	0.4	0.4	1.2	0.3	0.6	0.6	1.5	1.2	4.8	0.6
20:5*n* − 3	3.3	3.1	0.4	1.2	15.0	14.2	1.3	1.1	5.7	4.2
22:5*n* − 6	0.1	0.1	0.1	0.1	0.3	0.3	0.4	0.2		
22:5*n* − 3	1.0	1.0	0.4	0.4	2.9	3.1	0.5	0.5	5.1	1.7
22:6*n* − 3	9.2	7.7	5.6	5.0	25.1	24.9	6.1	6.1	19.8	11.0
others	0.4	0.4	0.2	0.6	1.4	1.3	0.3	0.4	0.1	6.1

[a] 1, frozen briefly at −30 °C; 2, frozen briefly at −30 °C and then stored at −12 °C for 3 months. [b] A, Hardy and King (1989). B, Hardy et al. (1987). [c] Listed as 16:1*n* − 9 in Hardy et al. (1987). [d] Listed as 18:4*n* − 6 in Hardy and King (1989).

for including the dietary egg lipid polyunsaturated fatty acids (Table II), but 20:4*n* − 6 seemed to follow proportionately the limited incorporation of 20:5*n* − 3.

The fatty acids of the muscle triglycerides (Table VII) were expected to follow those of the diet more closely than the fatty acids of the "essential" cellular phospholipids. Accepting the triglycerides of the herring oil group as "normal", the triglycerides of the fish fed canola oil had almost the same total for saturated plus monounsaturated acid content, largely due to a heavy deposition of 18:1*n* − 9 from the canola oil replacing the herring oil 20:1 and 22:1.

In the C_{18} polyunsaturated acids the 18:2*n* − 6 also appeared to reflect dietary levels, but 18:3*n* − 3 was not deposited to the same extent. There was no evidence that this 18:3*n* − 3 was converted to either 20:5*n* − 3 or 22:6*n* − 3 or that 18:2*n* − 6 was converted to 20:4*n* − 6.

The triglycerides of the EPA/DHA concentrate-fed fish had much less saturated plus monounsaturated fatty acid. The C_{20} and C_{22} fatty acids were the major features of the polyunsaturated acids with 20:5*n* − 3 and 22:6*n* − 3 deposited in the same ratio as in the diet. Only one of the minor acids differed; 22:5*n* − 3 was much more than in diet. The 18:4*n* − 3 was probably deposited directly. The

Table VI. Fatty Acid Composition of Total Phospholipid in Muscle of Atlantic Salmon from October Sampling, Fed Four Experimental Diets and Analyzed before and after Frozen Storage

	diet and treatment[a]							
	herring oil		canola oil		EPA/DHA concentrate		egg lipid	
fatty acid	1	2	1	2	1	2	1	2
14:0	3.3	3.9	1.6	2.0	2.1	2.3	1.6	1.8
16:0	16.0	24.7	12.1	19.7	13.9	18.3	12.8	10.5
18:0	3.0	4.7	1.9	2.2	2.8	3.1	2.7	2.9
16:1$n-7$	5.4	5.0	1.8	2.2	3.3	2.6	3.4	3.7
18:1$n-9$	10.6	11.9	25.1	23.1	9.2	8.2	25.5	23.9
18:1$n-7$	2.0	2.5	2.8	2.6	1.1	1.7	2.3	2.8
Σ20:1	5.4	3.6	2.1	2.1	2.4	1.9	2.3	2.3
Σ22:1	2.8	1.5	0.7	0.8	1.0	1.0	0.9	0.9
Σsatd + mono	49.2	57.8	48.2	55.1	35.8	39.1	52.5	48.8
18:2$n-6$	4.5	6.7	11.2	10.0	4.9	2.0	7.7	8.1
18:3$n-3$	0.9	0.8	3.5	2.8	0.7	1.1	0.7	0.7
18:4$n-3$	1.3	1.2	1.3	1.4	2.5	0.8	0.5	0.7
20:4$n-6$	0.8	1.1	1.3	1.3	0.7	0.7	3.7	4.2
20:5$n-3$	6.8	7.4	3.5	4.4	8.6	9.4	2.6	4.1
22:5$n-6$	0.3	0.3	0.4	0.3	0.4	0.4	1.7	1.7
22:5$n-3$	1.9	1.7	1.9	1.0	2.8	2.0	1.3	1.3
22:6$n-3$	33.6	22.4	27.2	22.0	42.6	39.7	30.2	30.2
others	0.7	0.3	0.0	0.1	1.0	4.8	0.1	0.2

[a] 1, frozen briefly at −30 °C; 2, frozen at −30 °C and then stored at −12 °C for 3 months.

Table VII. Fatty Acid Content of Triglycerides in Muscle of Atlantic Salmon from October Sampling, Fed Four Experimental Diets and Analyzed before and after Frozen Storage

	diet and treatment[a]							
	herring oil		canola oil		EPA/DHA concentrate		egg lipid	
fatty acid	1	2	1	2	1	2	1	2
14:0	8.7	8.8	2.7	2.5	4.8	4.7	3.3	3.3
16:0	18.6	17.5	13.0	12.7	15.9	15.3	14.3	14.1
18:0	2.8	2.6	2.9	3.0	2.4	2.6	2.4	2.5
16:1$n-7$	9.5	9.6	3.0	3.2	5.7	5.7	5.9	6.5
18:1$n-9$	13.5	13.5	41.1	42.0	9.3	9.3	40.6	41.0
18:1$n-7$	2.7	2.3	2.2	2.1	1.8	1.7	3.2	3.0
Σ20:1	12.4	14.2	4.5	5.1	4.3	4.9	5.0	5.6
Σ22:1	11.9	14.6	2.7	3.3	3.7	4.7	3.6	4.2
Σsatd + mono	80.0	83.1	72.1	73.9	47.9	49.9	78.3	80.2
18:2$n-6$	7.6	7.0	16.8	15.9	9.4	9.1	12.6	11.9
18:3$n-3$	1.0	1.0	4.4	4.0	1.1	1.1	0.7	0.7
18:4$n-3$	1.3	1.2	1.3	1.1	3.2	3.8	0.6	0.6
20:4$n-6$	0.3	0.3	0.3	0.2	0.6	0.6	1.0	0.9
20:5$n-3$	2.5	1.9	0.8	0.7	13.2	12.7	0.9	0.7
22:5$n-6$	<0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.3
22:5$n-3$	1.0	0.7	0.4	0.4	2.7	2.8	0.6	0.4
22:6$n-3$	5.5	4.3	2.9	3.3	19.2	19.4	4.2	4.0
others	0.8	0.4	0.4	0.4	2.4	0.2	0.8	0.3

[a] 1, frozen briefly at −30 °C; 2, frozen at −30 °C and then stored at −12 °C for 3 months.

attempt to increase 20:4$n-6$ in the triglyceride with egg lipid fatty acids was not very successful. The total saturated and monounsaturated fatty acids were qualitatively and quantitatively similar to those of the fish fed canola oil. The 18:2$n-6$ was not deposited in proportion to dietary availability, and 18:3$n-3$ was also reduced in importance. The C_{20} and C_{22} polyunsaturated fatty acids on the other hand were apparently laid down with very little discrimination.

The total lipid fatty acids (Table V) reflect the triglyceride patterns in general, although the very high 22:5$n-3$ and 22:6$n-3$ in the phospholipids of the EPA/DHA-enriched group (Table VI) show up in this total. In most fish phospholipids 22:6$n-3$ exceeds 20:5$n-3$, and this result would be expected for muscle of lean fish where phospholipids exceed triglycerides (Ackman, 1989a).

As part of this study we compared fresh frozen and frozen-stored salmon fillets for lipid content in view of the historical controversy about whether fish phospholipids and not triglycerides, or both, were hydrolyzed in lengthy frozen storage (Polvi, 1989; Polvi et al., 1991). Table IV shows that hydrolysis to liberate free fatty acids did take place to a modest extent, and the results are discussed in detail elsewhere (Polvi et al., 1991). Tables V–VII include the fatty acids of the long-term frozen storage samples as the data confirm the fatty acid compositions for the fresh or briefly frozen fish. Effectively, the second columns are replicates of analyses and validate the main conclusion of lipid response to dietary fatty acids.

It was expected that the muscle phospholipids of Atlantic salmon would be functional even when the proportions of certain fatty acids were altered (Bell et al., 1989; Olsen et al., 1991). Our confirmation of this view follows from an extended period on each single diet. In the wild a more varied input of dietary fatty acids from food sources would be expected, but the intake of 18:2$n-6$ and 18:3$n-3$ from saltwater organisms would probably not be available at more than 1–2% of the total fatty acids

of North Atlantic invertebrates and fish (Ackman, 1989a,b). The canola oil may be regarded as an exotic component, but owing to the vegetable matter (Table I) in the basic herring group oil diet (Table II), the latter contained appreciable 18:2n – 6, although less 18:3n – 3. A close approximation of the canola oil diet is diet B of Sowizral et al. (1990), based on 4% linseed oil and 16% olive oil, in a four-diet study with rainbow trout (formerly *Salmo gairdneri*, now *Oncorhynchus mykiss*). Unfortunately, their phospholipid data are given only for a 20% linseed oil diet fed for 64 days, but the replacement of 20:5n – 3 and especially of 22:6n – 3 by 18:3n – 3 is clearly shown, thus confirming the effect of canola oil on phospholipid fatty acids shown in Table VI. Compared with the herring oil and 20:5n – 3/22:6n – 3 enriched groups, the phospholipids of the canola oil-fed group show a modest enrichment in 20:4n – 6, a view supported by the findings of Olsen et al. (1991) and Raynard et al. (1991). Although this fatty acid is available to a limited extent from the diet, the high dietary intake of 18:2n – 6 is more likely to be responsible for this accumulation in phospholipids. In freshwater fish the n – 6 system may be the more evident in polar lipids (Ackman and Takeuchi, 1986; Greene and Selivonchick, 1987, 1990; Henderson and Tocher, 1987; Tocher and Dick, 1990). Of the three experimental diets (Table II) for fish lipids reported in Table VI, that enriched in EPA and DHA is not too dissimilar from the fatty acid compositions of lean marine invertebrates (Ackman, 1989b) that might form part of the diet of Atlantic salmon in nature. The changes in the fatty acids of phospholipids for this group, compared to those of the herring oil group, are probably, in fact, minimal. There is a possibility of conversion of 20:5n – 3 through 22:5n – 3 to 22:6n – 3, since direct deposition would likely have preserved the proportions of these fatty acids shown in Table II.

The group fed the poultry egg lipid fatty acids confirm that available 18:1n – 9 can be incorporated into phospholipids, as shown for the canola oil group. The 18:2n – 6 is evidently limited in impact relative to the canola oil group, and the 18:3n – 3 is normally not incorporated. The 20:5n – 3 in both the canola and egg lipid diets was low, and clearly this is reflected in the respective salmon muscle phospholipids. The objective of promoting incorporation of 20:4n – 6 into salmon lipids is partially successful in the phospholipids of the egg fatty acid group but is based on preformed 20:4n – 6 and not on extensive conversion of 18:2n – 6 to 20:4n – 6. Olsen et al. (1991) observed similar effects in phospholipids of the Arctic charr, *Selvelinus alpinus* (L.). This direct deposition of preformed 20:4n – 6 is probably also true in the case of lean tropical fish from Australian waters, as discussed elsewhere (Ackman, 1989a). The problem of an "oversupply" of 18:2n – 6 not only is of scientific interest in respect to fish health (Bell et al., 1991; Olsen et al., 1991) but also has practical consequences in aquaculture markets (Jahncke et al., 1988) and consequently in our diets and nutrition (Van Vliet and Katan, 1990).

The triglycerides constitute the dominant lipid class in fillets from our experimental Atlantic salmon (Table IV) and indeed in all retail samples of either canned Pacific salmon or fresh or frozen fillets of either Atlantic or Pacific salmon (Ackman, 1989a; Vanderstoep et al., 1990). A formula based on total lipid is available to rationalize the proportions of triglycerides and phospholipids in lipids of most fish (Exler et al., 1975). Farmed Atlantic salmon with as much as 14% fat have been marketed (Ackman, 1989a). Table IV shows the fatty acid compositions attained by extended feeding on the dietary fatty acids of Table II. The fish of the herring oil diet group have triglycerides with just over 70% saturated and monounsaturated fatty acids. The 22:1 has possibly been chain-shortened to 18:1 (Flatmark et al., 1988). It is surprising to find 18:2n – 6 deposited almost directly in proportion to diet, although this possibility is more or less confirmed by the soybean oil diet fed by Hardy et al. (1987) to salmon, the peanut oil fed to cod by Lie et al. (1986), and the 18:2n – 6 fed to Arctic charr by Olsen et al. (1991). In the longer chain polyunsaturated fatty acids 22:6n – 3 is also representative of diet, but the exact role of the dietary 20:5n – 3, reduced by half, is not known.

The triglycerides of the group fed canola oil also had the same total for saturated and monounsaturated fatty acids as the herring oil-fed group, but as in the phospholipids, 18:1n – 9 was the dominant fatty acid. The 20:1 and 22:1 were much less than in the herring oil diet group. The 18:2n – 6 and, to a lesser extent, the 18:3n – 3 of the diet were barely diminished in proportion among the polyunsaturated fatty acids relative to diet, although 20:5n – 3 was, as in the phospholipids, diminished relative to 22:6n – 3. There was no evidence of conversion of 18:2n – 6 to 20:4n – 6. The 18:3n – 3 may not be available for esterification to glycerol if recent work on rat liver enzymes (Gavino and Gavino, 1991) is applicable to salmon muscle. This same work ranks 22:6n – 3 as the most reactive of several fatty acids, and the high proportions of 22:6n – 3 in salmon triglycerides suggest that there may be parallels.

The ethyl esters of the polyunsaturated fatty acids of the EPA/DHA group displaced total saturated and monounsaturated fatty acids in the totals for these classes. Unlike in the phospholipids of the group fed this diet enriched in ethyl esters of 20:5n – 3 and 22:6n – 3, an elevation 22:5n – 3 was also apparent. Compared to the herring-fed group there is no change of note in 18:2n – 6 or 18:3n – 3. From analysis of mackerel triglycerides, we have some evidence that the chain length in the C_{20}–C_{22} group may be more critical in accumulations in the triglycerides of some species than is the degree of unsaturation (Ackman et al., 1991).

The triglycerides of the hen egg lipid group resembled the canola oil pattern and total in respect to the saturated and unsaturated fatty acids, with elevated 16:0 and 18:1n – 9 being featured. The 18:2n – 6 was not as much enriched in these triglycerides as in those of the canola group, despite a higher dietary input in the hen egg fatty acid group. Neither 20:4n – 6 nor 20:5n – 3 was deposited in proportion to dietary 22:6n – 3, and the enrichment expected for 20:4n – 6 was of minor importance, presumably since n – 3 fatty acids were preferred for triglyceride synthesis.

The fatty acids of total lipids (Table V) do not differ markedly from the triglycerides except, as already remarked, in the 22:6n – 3 arising from the role of that fatty acid in some phospholipids (Table VI). The total fatty acids are presented since this is the format useful to dieticians and others and because literature data can be included for comparison. Hardy et al. (1987) included menhaden oil in their study of dietary fats, with 20:5n – 3 at 9.6% of dietary fatty acids and 22:6n – 3 at 7.3%. However, their total fatty acid contained 6.7% and 15.6% of these acids, respectively.

All of these results indicate that Atlantic salmon muscle triglycerides invariably include 22:6n – 3 > 20:5n – 3. It is also apparent from our work that 20:4n – 6 is not going to be more than slightly enriched in the salmon triglyceride of larger salmon, even if fed in the form of the natural preformed C_{20} acid, and not at all from dietary 18:2n – 6. The latter view is supported by feeding studies with cod

(Lie et al., 1986). Even 37% of 18:2$n-6$ in the diet of rainbow trout failed to elevate 20:4$n-6$ in muscle lipid (Greene and Selivonchick, 1990).

From a health perspective wild salmon have a moderate fat level, usually 5–8% (Ackman, 1989a; Vanderstoep et al., 1990), whereas farmed salmon almost invariably have more fat (Van Vliet and Katan, 1990). This is part of a problem created by dietary changes in the Western world in which higher fat fish such as salmon are suggested as "natural" sources of $n-3$ fatty acids (Burr, 1989; Burr et al., 1989; Hardy and King, 1989) at the same time as reduced fat intake is suggested by health authorities. Retention of fish quality (Hadlett and Raab, 1990) is often more practical for aquaculture fish than for ocean fish since distribution is very rapid compared to fish held for up to 2 weeks on ice before landing and processing (Kramer and Liston, 1987). In one sense fresh, cooked, and smoked salmon, especially the latter, are considered expensive compared with other protein sources. The health benefits of the fatty acids (Ackman and Ratnayake, 1989; Burr, 1989) may be an overriding consideration, since it is not likely to be an obvious quality consideration to lay persons compared with consumer interest in color. Although there are many changes possible in dietary fats for fish (Haumann, 1989), the Atlantic salmon triglyceride seems to have a fairly constant composition as long as basic fish oil fatty acids are part of the diet.

Once assimilated and deposited in muscle, the fatty acids of the ethyl esters had little effect (Polvi et al., 1991) on the activity of lipolytic enzymes in frozen storage (Table IV). The lesser fat content of the fish fed ethyl esters results from less efficient utilization of ethyl esters (Sigurgisladottir et al., 1990), although fish health and growth were reasonably good (Polvi, 1989). The absorption of ethyl esters has also been a questionable aspect of high dosages (>4 g/day) of fish oil fatty acids encapsulated in the form of ethyl esters for humans (Ackman and Ratnayake, 1989). However, it is unlikely that ethyl esters would be of importance in aquaculture except in dietary experiments such as we have conducted.

ACKNOWLEDGMENT

S. P. Lall of the Canada Department of Fisheries and Oceans, Research Branch, Scotia-Fundy Region, Halifax, NS, supervised diet formulations and R. L. Saunders of the Canada Department of Fisheries and Oceans, Biological Sciences Branch, St. Andrews, NB, supervised salmon acquisition and rearing. We thank W. M. N. Ratnayake for his assistance and expertise in analyzing the data. We also thank Derek Knox, who reared the fish and administered the dietary lipid feeding program at the St. Andrews Biological Station.

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Received for review November 27, 1991. Accepted March 3, 1992.

Registry No. EPA ethyl ester, 84494-70-2; DHA ethyl ester, 73310-11-9; 18:1*n*–9, 112-80-1; 18:2*n*–6, 60-33-3; 18:3*n*–3, 463-40-1; 20:5*n*–3, 10417-94-4; 20:4*n*–6, 506-32-1; cholesterol, 57-88-5.