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Polyunsaturated fatty acid metabolism in cultured fish cells: Incorporation and metabolism of (n-3) and (n-6) series acids by Atlantic salmon (*Salmo salar*) cells

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Abstract

The incorporation and metabolism of (n-3) and (n-6) polyunsaturated fatty acids (PUFA) supplemented to growing cultures were studied in Atlantic salmon (AS) cells. A fatty acid concentration of 25 μM considerably altered the fatty acid composition of AS cells without increasing the neutral lipid content of the cells or inducing the production of cytoplasmic lipid droplets. Whereas Δ6 and Δ5 desaturase activities were significantly expressed in AS cells, Δ4 desaturase activity was very low. Both the Δ6 desaturase activity and the Δ5 desaturase activity showed some preference for (n-3) PUFA.

Abbreviations: EFA, essential fatty acid; FCS, fetal calf serum; FFA, free fatty acid; PA/CL, phosphatidic acid/cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid(s); SE, sterol ester; SM, sphingomyelin; TAG, triacylglycerol.

Introduction

The only lipid available to cells in culture is that present in the almost ubiquitous serum supplement to the medium. Consequently, cultured cells have a fatty acid composition reflecting that of the serum supplement (Bailey 1967; Spector *et al.* 1981). Fetal calf serum (FCS) is usually the serum of choice due to its superior growth-enhancing properties for most cells and its ready commercial availability. Previously, we have shown that fish cells cultured in FCS are deficient in (n-3)PUFA, and often have increased levels of (n-6)PUFA (Tocher *et al.* 1988). This imbalance can be of major significance when the cells are used in lipid metabolism studies.

We investigated the effects of supplementing the media with various pure (n-6) and (n-3)PUFA and (n-3)PUFA concentrates prepared from marine sources on the fatty acid composition of fish cells in culture (Tocher *et al.* 1989). In addition to indicating the particular PUFA and their levels required to maintain normal fatty acid compositions in fish cells in culture, these studies also suggested that the cultured cells reflected, at least in part, the metabolism of the whole fish (Tocher *et al.* 1989). Nutritional studies have shown that 18:3(n-3) alone satisfied the essential activity (EFA) requirement for rainbow trout (*Salmo gairdneri*) (Castell *et al.* 1972), whereas turbot (*Scophthalmus maximus*) required the longer chain PUFA, 20:5(n-3) and 22:6(n-3), to satisfy their EFA requirement (Owen *et al.* 1975). The results we obtained from cell cultures were consistant with these conclusions. Specifically, rainbow trout cells (RTG-2) expressed Δ6

and Δ5 desaturase activities and so could elongate and desaturate 18:2(n-6) and 18:3(n-3) to 20:4(n-6) and 20:5(n-3), respectively (Tocher *et al.* 1989). However, turbot cells (TF) were deficient in the C18–20 elongase and/or Δ5 desaturase and could not produce the C20 PUFA from the C18 precursors (Tocher *et al.* 1989).

The most commercially important fish in aquaculture in the United Kingdom today is the Atlantic salmon. Therefore, the effects on Atlantic salmon cell (AS) fatty acid composition of supplementing medium with (n-3) and (n-6)PUFA were studied. PUFA incorporation and metabolism to longer chain, more unsaturated species was determined by analysis of the total lipid fatty acid composition. We aimed to determine a) the extent of desaturation/elongation pathways in AS cells and, b) whether the metabolism of (n-3)PUFA by AS cells was substantially different from their metabolism of (n-6)PUFA. We have discussed the results in relation to the data from the other fish species investigated in this way and to the nutrition of salmon.

Materials and methods

Cell line and medium

The AS cell line was a de-differentiated line with epithelial morphology (Nicholson and Byrne 1973) obtained from Flow Laboratories, Rickmansworth, U.K. and maintained in Eagle's minimal essential medium (EMEM) containing 0.1% sodium bicarbonate, 1% non-essential amino acids, antibiotics (50 I.U. ml^{-1} penicillin and 50 $\mu g.ml^{-1}$ streptomycin) and either 10% or 2% FCS.

PUFA supplements

Pure (n-3) and (n-6)PUFA were added to the cell cultures in the form of bovine serum albumin (BSA) complexes, prepared essentially according to the method of Spector and Hoak (1969). All incubations were performed under a steady stream of N_2 at room temperature to protect the PUFA from oxidative damage. The solutions were sterilized by filtration through 0.2 μm filters (Flowpore D26, Flow Laboratories) prior to assay and use. The fatty acid concentration was assayed by the addition of an equal volume of an accurate 1 $mg.ml^{-1}$ 17:0 fatty acid standard to an aliquot of the PUFA/BSA complex followed by lipid extraction, transmethylation and GC analysis. Protein concentration was measured by the method of Lowry *et al.* (1951). The PUFA concentrations were generally in the 2.0–2.8 mM range giving PUFA:BSA ratios of approximately 8–11:1. The PUFA/BSA mixtures were kept in brown vials, gassed with N_2 before sealing and stored at −25°C between procedures.

Cell growth and incubation conditions

The AS cells were grown at 22°C in sealed 75 cm^2 plastic flasks (Gibco-Nunc Ltd., Paisley, U.K.) in 20 ml of medium. For routine cultivation of the cells for provision of experimental material, the cultures were grown in media supplemented with 10% FCS and were harvested for analysis or further subculture within 24 h of reaching confluence, usually about 5–7 days after a 1 to 4 split. For incubation with PUFA, cells were subcultured into media containing 2% FCS and supplemented with the appropriate amount of PUFA/BSA mixture (for 25 μM PUFA this volume did not exceed 2.5% of the total incubation volume). Control flasks received 0.25 mM BSA solution alone.

Cell harvesting and lipid extraction

The cells were harvested and washed as described in detail previously (Tocher *et al.* 1988). After washing the cell numbers were determined by counting in a haemocytometer. Total lipid was then extracted from the cells essentially according to Folch *et al.* (1957) and as described in detail previously (Tocher *et al.* 1988). All solvents contained 0.05% butylated hydroxytoluene (BHT) as an antioxidant.

Lipid class and fatty acid analyses

Lipid class analysis was performed using a one-dimensional, double development high-performance thin-layer chromatographic (HPTLC)/densitometric method described previously (Tocher and Harvie 1988). Fatty acid methyl esters were prepared by acid-catalysed transmethylation overnight at 50°C according to Christie (1982). After extraction, the methyl esters were purified by TLC using hexane/diethyl ether/acetic acid (85:15:1.5, v/v/v) containing 0.05% BHT as developing solvent. The methyl esters were dissolved in hexane/BHT and analysed in a Packard 436 gas chromatograph (Chrompack U.K., Ltd., London) equipped with a chemically bonded CP Wax 52CB fused silica capillary column (50 m × 0.34 mm i.d.) (Chrompack U.K. Ltd., London), using on-column injection and H_2 as carrier gas with a biphasic thermal gradient from 50°C to 225°C. Individual methyl esters were identified as described previously (Tocher and Harvie 1988) and were quantified using a Shimadzu CR-3A recording integrator. Data are means of at least three experiments ± 1 SD. Where indicated statistical analysis was performed using the Student t-test.

Materials

EMEM, sodium bicarbonate, non-essential amino acids, antibiotics and FCS were obtained from Flow Laboratories, Rickmansworth, U.K. Heptadecanoic acid (17:0, approx. 99%), all PUFA (approx. 99% pure), fatty acid-free BSA, lipid class standards and BHT were from Sigma Chemical Co. Ltd., Poole, U.K. TLC (20 × 20 cm × 0.25 mm) and HPTLC (10 × 10 cm × 0.25 mm) plates precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck, Darmstadt, FGR. All solvents were HPLC grade and were obtained from Rathburn Chemicals, Walkerburn, U.K.

Results

The AS cells contained approximately 66 μg lipid/10^6 cells when routinely cultured in 10% FCS (Table 1). This value decreased to about 50 μg/10^6 cells when the cells were cultured in 2% FCS, and was increased to 56 μg/10^6 cells when the 2% FCS was supplemented with 25 μM 18:2(n-6) or 18:3(n-3) (Table 1). However, the errors involved meant that none of these differences were statistically significant ($p > 0.05$). Reducing the serum concentration resulted in reduced neutral lipids due to decreased percentages of cholesterol and TAG, with all the phospholipid classes increasing reciprocally (Table 1). The supplementation of 2% FCS with 25 μM 18:2(n-6) or 18:3(n-3) did not alter the lipid class composition of the cells from that obtained with 2% FCS alone (Table 1). Microscopic examination of the cultures indicated that lipid droplets were present only in small amounts in AS cells in all experiments, except when the concentration of supplemented acid was 50 μM or greater.

After culture in 10% FCS, the fatty acid composition of AS cells showed 9.8% (n-6) and 5.8% (n-3)PUFA, respectively, giving an (n-3)/(n-6) ratio of 0.6 (Table 2). These totals were comprised mainly of 20:4, 20:3 and 18:2 in the case of (n-6)PUFA and 22:5 and 22:6 in the case of (n-3)PUFA (Table 2). The (n-9)PUFA were not major constituents of AS cells under these conditions. Although 20:3(n-9) could not be separated from 20:2(n-6) under the GC conditions used in this study, the combined level of these acids was low. Monoenes, primarily 18:1(n-9), and saturates, primarily 16:0 and 18:0, formed the main bulk of the fatty acids in AS cells cultured in 10% FCS (Table 2).

Reducing the FCS to 2% resulted in significant decreases in (n-6)PUFA ($p < 0.05$), (n-3)PUFA ($p < 0.001$), total PUFA ($p < 0.02$) and a non-significant decrease in saturates (Table 2). There was a reciprocal and significant ($p < 0.05$) increase in total monoenes, primarily due to increased 18:1(n-9) and 16:1. When 25 μM 18:2(n-6) was supplemented, the (n-6)PUFA and total PUFA levels were increased significantly ($p < 0.05$) over those obtained with 10% FCS with the percentage of monoenes reduced to that obtained with 10% FCS (Table 2). The percentages of saturates and (n-3)PUFA were unchanged from those obtained with 2% FCS alone. In a similar manner, supplementation with 25 μM 18:3(n-3) resulted in sig-

Table 1. Lipid content and class compositions of AS cells cultured in 10% or 2% fetal calf serum (FCS) or 2% FCS supplemented with 18:2(n-6) or 18:3(n-3).

	10% FCS	2% FCS	18:2(n-6)	18:3(n-3)
Lipid content ($\mu g/10^6$ cells)	65.9 ± 2.9	50.4 ± 10.0	56.2 ± 12.9	56.4 ± 13.0
Lipid class comp. (% total lipid)				
Total polar lipids	51.9 ± 5.3	62.8 ± 6.0	66.1 ± 5.7	67.5 ± 4.2
Phosphatidylcholine	21.1 ± 2.9	23.9 ± 2.6	23.8 ± 2.8	23.2 ± 3.7
Phosphatidylethanolamine	17.4 ± 1.8	21.2 ± 1.9	22.7 ± 1.6	23.0 ± 2.2
Phosphatidylserine	4.4 ± 0.8	5.2 ± 0.5	5.6 ± 1.1	6.7 ± 0.5
Phosphatidylinositol	4.4 ± 0.6	6.7 ± 1.0	7.4 ± 1.5	8.3 ± 0.3
Phosphatidic acid/cardiolipin	1.1 ± 0.5	2.4 ± 0.7	2.7 ± 0.8	3.4 ± 0.4
Sphingomyelin	3.5 ± 0.8	3.4 ± 0.8	3.9 ± 0.3	3.0 ± 1.4
Total neutral lipids	48.1 ± 5.3	37.2 ± 6.0	33.9 ± 5.7	32.5 ± 4.2
Cholesterol	25.0 ± 3.7	20.9 ± 3.6	21.7 ± 5.7	21.1 ± 1.6
Free fatty acids	1.0 ± 0.3	1.5 ± 0.4	1.3 ± 0.2	1.0 ± 0.6
Triacylglycerols	18.8 ± 6.3	11.5 ± 8.9	8.7 ± 2.9	8.1 ± 3.9
Sterol esters	3.3 ± 1.4	3.2 ± 2.1	2.2 ± 0.8	2.4 ± 1.5

Cells were cultured for 7 days at 22°C with the supplements indicated above. PUFA were added as BSA complexes at a concentration of 25 μM. Cells were harvested, counted, lipid extracted and lipid classes analysed as described in the Materials and Methods. Results are means ± SD (n = 3).

nificant increases in (n-3)PUFA ($p < 0.001$) and total PUFA ($p < 0.01$) over those obtained with 10% FCS, with the monoenes reduced to the same percentage as in 10% FCS (Table 2). Saturates and (n-6)PUFA remained at the same percentage obtained in 2% FCS alone.

Increasing the concentration of 18:3(n-3) or 18:2(n-6) resulted in graded increases in the content of total (n-3) or (n-6)PUFA respectively (Table 3). In general, there was no significant difference ($p > 0.05$) in the respective totals obtained when equivalent concentrations of 18:3(n-3) and 18:2(n-6) were supplemented. However, the rsultant cellular concentration of 18:2(n-6) itself was always significantly greater than the cellular concentration of 18:3(n-3) ($p < 0.05$ or less) (Table 3). Graded increases in 18:4(n-3), 20:3(n-3), 20:4(n-3) and 20:5 (n-3) (up to 25 μM) were obtained with 18:3(n-3) supplementation, as were increases in 18:3(n-6), 20:2(n-6) and 20:3(n-6) with 18:2(n-6) supplementation (Table 3).

The percentage of 20:5(n-3) was also increased by supplementation with 18:4(n-3), 22:6(n-3) and 20:5(n-3) itself (Table 4). Supplementation with 20:5(n-3) also greatly increased the percentage of 22:5(n-3), but only slightly increased 22:6(n-3), which was only greatly increased in AS cells by supplementation with 22:6(n-3) itself (Table 4).

The percentage of 20:3(n-6) was increased in AS cells by supplementation with 18:3(n-6) and 20:3 (n-6) itself, as well as with 18:2(n-6) noted earlier (Table 5). Although supplementation with 18:2 (n-6) did not increase the percentage of cellular 20:4(n-6), this was increased by supplementation with 18:3(n-6), 20:3(n-6) and 20:4(n-6) itself (Table 5). The total C22(n-6) PUFA in AS cells were increased slightly by all supplements, but only 20:4 (n-6) supplementation greatly increased 22:4(n-6).

Discussion

The results of the present study were expressed in relative percentage terms to enable simple comparison with previous, related studies (Ferguson *et al.* 1975; Maeda *et al.* 1978; Spector and Yorek 1985; Lynch *et al.* 1986; Delplanque and Jacotot 1987; Tocher *et al.* 1988, 1989). However, supplementa-

Table 2. Fatty acid compositions of FCS and of total lipid from AS cells cultured in 10% or 2% FCS alone or 2% FCS supplemented with 25 μM 18:2(n-6) or 18:3(n-3).

Fatty acid	FCS	10% FCS	2% FCS	18:2(n-6)	18:3(n-3)
14:0	2.4±0.9	1.6±0.2	1.7±0.3	1.7±0.3	1.3±0.1
15:0	1.3±0.6	0.4±0.2	0.2±0.1	0.1±0.1	0.2±0.1
16:0	21.8±0.5	14.2±0.9	13.3±1.5	12.3±1.2	12.5±1.3
17:0	1.3±0.3	0.5±0.4	0.4±0.4	0.2±0.1	0.1±0.1
18:0	10.4±0.8	8.2±2.0	5.5±0.8	7.9±1.7	8.9±0.3
20:0	0.3±0.1	0.2±0.1	0.1±0.1	0.1±0.1	0.1±0.1
Total saturates	37.5±1.6	25.1±4.1	21.2±2.6	22.2±0.9	23.1±0.6
16:1[a]	5.9±1.2	5.9±0.4	7.8±2.0	6.3±1.0	4.1±0.3
18:1(n-9)	18.7±1.1	37.7±9.9	48.0±2.3	34.7±2.0	36.0±1.0
18:1(n-7)	6.1±0.6	3.5±0.7	3.8±1.0	3.5±1.2	3.0±0.4
20:1(n-9)	1.1±0.2	0.7±0.4	1.6±0.4	1.1±0.4	0.5±0.2
22:1	0.6±0.1	1.0±1.0	0.3±0.1	0.2±0.2	0.1±0.1
24:1	0.5±0.1	0.9±0.3	0.7±0.1	0.5±0.3	0.5±0.1
Total monoenes	34.0±2.5	49.7±6.7	62.2±3.2	46.3±4.3	44.2±1.0
18:2(n-9)	0.9±0.1	1.0±0.3	1.2±0.3	1.5±0.9	0.6±0.4
20:2(n-9)	0.2±0.1	0.2±0.2	0.6±0.1	0.4±0.1	0.2±0.1
Total (n-9)PUFA	1.1±0.1	1.2±0.6	1.8±0.5	1.9±0.9	0.8±0.6
18:2(n-6)	5.0±0.6	1.8±0.7	1.0±0.2	11.6±2.4	0.8±0.2
18:3(n-6)	0.2±0.1	0.2±0.1	0.1±0.1	1.6±0.3	0.2±0.1
20:2(n-6)[b]	0.5±0.2	0.2±0.2	0.2±0.1	1.1±0.3	0.2±0.1
20:3(n-6)	1.7±0.2	1.3±0.3	0.7±0.1	2.1±0.8	0.6±0.1
20:4(n-6)	7.7±1.0	5.7±2.8	2.6±0.5	2.3±0.2	2.2±0.5
22:4(n-6)	0.6±0.1	0.6±0.6	0.2±0.1	0.3±0.1	0.1±0.1
Total (n-6)PUFA	15.7±1.0	9.8±2.9	4.8±0.8	19.0±4.2	4.1±0.8
18:3(n-3)	0.4±0.1	0.2±0.1	0.1±0.1	0.2±0.1	5.1±1.3
18:4(n-3)	0.2±0.2	0.1±0.1	0.1±0.1	0.1±0.1	4.6±0.2
20:3(n-3)	–	–	–	–	1.0±0.1
20:4(n-3)	–	–	–	–	2.5±0.2
20:5(n-3)	0.8±0.3	0.8±0.2	0.4±0.1	0.3±0.1	5.3±0.9
22:5(n-3)	2.8±0.9	2.0±0.2	1.0±0.2	0.9±0.2	1.0±0.3
22:6(n-3)	2.9±0.3	2.7±0.1	1.3±0.1	1.3±0.2	1.1±0.3
Total (n-3)PUFA	7.1±0.7	5.8±0.3	2.9±0.2	2.8±0.4	20.6±0.3
Total PUFA	23.9±1.1	16.8±3.0	9.5±0.4	23.7±2.8	25.5±0.5
(n-3)/(n-6)	0.5±0.1	0.6±0.3	0.6±0.1	0.1±0.1	5.0±1.0
Total dimethylacetals	–	5.1±0.1	5.1±0.6	4.7±0.4	4.5±1.3
Total unidentified	–	3.3±0.7	2.0±1.2	3.1±1.0	2.7±1.0

FCS = Fetal calf serum; PUFA = polyunsaturated fatty acids; [a] predominantly (n-7) isomer; [b] contains 20:3(n-9); – = none detected; incubation conditions are as described in the legend to Table 1. The fatty acid compositions are presented as a % of weight and are means ± SD (n=3).

tion of AS cells with PUFA at 25 μM increased the total lipid content, and so, although the differences were not statistically significant, an increased percentage of a fatty acid after supplementation implies that, in absolute terms, the amount of that acid has increased. A concentration of 25 μM was used in the comparative studies with various PUFA because this concentration gave major significant changes in the PUFA composition of the cells without adversely affecting the lipid class composition or inducing cytoplasmic lipid droplets (Geyer 1967; Moskowitz 1967; Rosenthal 1981; Stubbs and Smith 1984).

The polar and neutral lipid contents of the cells

Table 3. Effect of concentration of supplemented 18:3(n-3) and 18:2(n-6) on the (n-3) and (n-6) polyunsaturated fatty acid (PUFA) composition, respectively, of total lipid from AS cells.

	Concentration of Supplement (μM)						
PUFA	0	5	10	15	25	50	100
+ 18:3(n-3)							
18:3	0.1±0.1	1.2±0.2	2.5±0.1	3.6±0.1	5.9±0.1	10.5±0.2	20.4±2.8
18:4	0.1±0.1	0.8±0.1	1.8±0.2	2.7±0.2	4.7±0.2	7.5±0.1	8.5±0.1
20:3	–	0.2±0.1	0.4±0.1	0.8±0.2	1.0±0.1	1.6±0.1	2.8±0.4
20:4	–	0.7±0.1	1.3±0.3	1.9±0.2	2.5±0.2	2.8±0.1	2.5±0.1
20:5	0.4±0.1	1.4±0.3	2.6±0.3	3.6±0.1	4.7±0.1	4.4±0.1	3.1±0.1
22:5	1.0±0.2	0.8±0.3	0.8±0.1	0.8±0.2	0.9±0.1	0.9±0.1	0.8±0.1
22:6	1.3±0.1	1.1±0.3	1.0±0.2	1.0±0.1	1.0±0.2	1.0±0.1	1.0±0.1
Total (n-3)	2.9±0.2	6.2±1.3	10.4±0.6	14.4±0.6	20.7±0.4	28.7±0.8	39.1±3.1
+ 18:2(n-6)							
18:2	1.0±0.2	4.2±1.4	6.7±0.2	8.4±0.6	12.8±1.0	19.9±1.1	29.4±2.0
18:3	0.1±0.1	0.4±0.2	0.7±0.1	0.9±0.1	1.8±0.1	3.6±0.5	5.3±0.1
20:2	0.2±0.1	0.3±0.2	0.5±0.1	0.7±0.1	1.2±0.3	1.6±0.1	2.5±0.5
20:3	0.7±0.1	0.9±0.4	1.0±0.1	1.5±0.1	2.7±0.7	4.2±0.1	4.5±0.3
20:4	2.6±0.5	2.5±0.5	2.3±0.4	2.3±0.1	2.3±0.3	2.6±0.4	2.3±0.3
22:4	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.1
Total (n-6)[a]	4.8±0.8	8.5±2.5	11.5±0.1	14.2±0.4	21.5±2.5	32.5±1.4	45.3±3.2

[a] Totals include 22:2, 22:3 and 22:5; Cells were cultured for 7 days at 22°C in the presence of 18:3(n-3) or 18:2(n-6) at the concentrations shown. Results are presented as described in Table 2.

Table 4. Effect of supplementation with various (n-3)polyunsaturated fatty acids (PUFA) on the (n-3)PUFA composition of total lipid from AS cells.

(n-3)PUFA	Supplement				
(% of total fatty acids)	None	18:3	18:4	20:5	22:6
18:3	0.1±0.1	5.9±0.1	0.1±0.1	0.2±0.1	0.1±0.1
18:4	0.1±0.1	4.7±0.2	9.7±0.9	0.3±0.1	0.1±0.1
20:3	–	1.0±0.1	–	–	–
20:4	–	2.5±0.2	3.7±0.6	0.6±0.1	0.2±0.2
20:5	0.4±0.1	4.7±0.1	6.0±1.9	15.8±0.1	3.7±0.7
22:5	1.0±0.2	0.9±0.1	1.2±0.2	8.0±1.8	1.3±0.1
22:6	1.3±0.1	1.0±0.2	1.1±0.2	2.0±0.2	24.5±1.4
Total	2.9±0.2	20.7±0.4	21.8±2.1	26.9±2.1	29.9±1.4

Cells were incubated for 7 days at 22°C with the PUFA shown at a concentration of 25 μM. Results are expressed as described in Table 2.

in absolute terms were not measured directly in this study, but can be derived from the data in Table 1. This shows that in addition to decreased neutral lipid (31.7 reduced to 18.8 $\mu g/10^6$ cells), reducing the serum to 2% also reduced total polar lipid from 34.2 to 31.7 $\mu g/10^6$ cells. Supplementation with 18:2(n-6) and 18:3(n-3) increased the total polar lipid to 37.2 and 38.1 $\mu g/10^6$ cells respectively without affecting the neutral lipid (19.1 and 18.3 $\mu g/10^6$ cells). This pattern was also observed with

Table 5. Effect of supplementation with various (n-6) polyunsaturated fatty acids (PUFA) on the (n-6)PUFA composition of total lipid from AS cells.

(n-6)PUFA	Supplement				
(% of total fatty acids)	None	18:2	18:3	20:3	20:4
18:2	1.0±0.2	12.8±1.0	0.9±0.1	0.7±0.1	0.8±0.1
18:3	0.1±0.1	1.8±0.1	10.2±1.4	0.7±0.1	0.1±0.1
20:2	0.2±0.1	1.2±0.3	0.2±0.1	0.2±0.1	0.2±0.1
20:3	0.7±0.1	2.7±0.7	7.1±1.4	13.4±2.3	0.9±0.1
20:4	2.6±0.1	2.3±0.3	3.3±0.6	4.5±1.0	16.9±3.0
22:3	–	0.2±0.1	0.2±0.1	0.8±0.2	–
22:4	0.2±0.1	0.3±0.1	0.4±0.1	0.5±0.1	4.4±0.8
22:5	–	0.1±0.1	0.1±0.1	–	–
Total	4.8±0.8	21.5±2.5	22.4±4.0	20.8±3.6	23.3±3.8

Cells were incubated for 7 days at 22°C with the PUFA shown at a concentration of 25 μM. Results are expressed as described in Table 2.

supplementation of the medium with the other PUFA. This emphasises that it is the metabolism of structural, membrane lipid that is being primarily affected by supplementation with PUFA at this concentration.

The increased percentages of 18:4(n-3) and 20:4 (n-3), and 18:3(n-6) and 20:3(n-6) obtained upon supplementation with 18:3(n-3) and 18:2(n-6), respectively, clearly indicated that AS cells possess Δ6 desaturase activity. Similarly, the increased percentages of 20:5(n-3) obtained after supplementation with 18:3(n-3) and 18:4(n-3) indicate the presence in AS cells of Δ5 desaturase activity. However, the inability of these cultured cells to increase the level of 22:6(n-3) upon supplementation with 18:3(n-3) and 18:4(n-3) indicates that they lack Δ4 desaturase activity. This deficiency may not be total as supplementation with 20:5(n-3), which greatly increased the level of 22:5(n-3), the direct substrate for the Δ4 desaturase, did slightly, but significantly ($p < 0.01$), increase the percentage of 22:6(n-3).

These data indicate that the AS cells from Atlantic salmon have a similar desaturase complement to that found previously in the RTG-2 cells from rainbow trout which also possess both Δ6 and Δ5 desaturase activities, whereas Δ4 desaturase activity is absent (Tocher *et al.* 1989). As with the RTG-2 cells, it is not known whether AS cells when cultured expressed a full complement of desaturase activities and have subsequently lost the Δ4 desaturase, or whether the original cells never expressed this activity. The former is a likely possibility as desaturase activities, including Δ4, are often no longer expressed in cell lines after long-term culture (Maeda *et al.* 1978; Robert *et al.* 1978).

The data also show that there are major differences in the metabolism of (n-3) and (n-6)PUFA by AS cells. It is apparent that (n-3)PUFA are favoured in the desaturase pathway under the culture conditions used in the present study. This is indicated by lower percentages of unmetabolised 18:3(n-3) in comparison to 18:2(n-6) when the respective acids were supplemented. The specificity did not appear to be associated with any specific part of the desaturation/elongation pathway with both Δ6 and Δ5 desaturases showing some preference for (n-3) PUFA based on the percentages of the intermediates and products resulting from the various supplementations. In contrast, the Δ6 desaturase activities of both RTG-2 and TF cells appeared equally active with both 18:2(n-6) and 18:3(n-3) (Tocher *et al.* 1989). However the Δ5 desaturase from RTG-2 cells and the Δ4 desaturase from TF cells preferred (n-3) substrates (Tocher *et al.* 1989). Although a preference for (n-3)PUFA is generally consistent with the respective levels of the (n-3) and (n-6) C20 and C22 PUFA found in fish tissues, it may not indicate any specific adaptation to (n-3)

PUFA as the order of preference for substrates of desaturase activities in mammalian systems is also (n-3) > (n-6) > (n-9) (Stubbs and Smith 1984). Furthermore, the (n-3)PUFA deficiency of fish cells cultured in mammalian sera may predispose the cells to increased (n-3)PUFA metabolism.

Significant retroconversion of 22:6(n-3) to 20:5 (n-3) was detected in AS cells after supplementation with 22:6(n-3). There also appeared to be some limited chain shortening of supplemented 20:3(n-6) to 18:3(n-6) and some saturation of 20:5(n-3) to 20:4(n-3) but these pathways were of little significance. Retroconversion of 22:6(n-3) was not detected in either RTG-2 or TF cells, but significant chain shortening of 20:3(n-6) to 18:3(n-6) was found in TF cells (Tocher *et al.* 1989).

In nutritional studies, specialized tissues or whole body lipids are analysed. However, the data obtained with the AS cells in culture may be partly related to the situation in whole fish. No nutritional data for Atlantic salmon are available (Henderson and Tocher 1987), but work is in progress to optimise PUFA content and quality of artificial diets (Hardy *et al.* 1987). Coho salmon (*Oncorhynchus kisutch*) grew optimally on diets containing 1.0–2.5% 18:3(n-3) (Yu and Sinnhuber 1979). With chum salmon (*O. keta*), optimum growth was obtained with diets containing 1% 18:3(n-3) and 1% 18:2(n-6) (Takeuchi *et al.* 1979; Takeuchi and Watanabe 1982). These data suggest that these species can fully desaturate and chain elongate C18 PUFA and it is probable that Atlantic salmon are similar. However, as with RTG-2 cells, the AS cells do not possess. Δ4 desaturase activity and so, unfortunately, will probably not be able to completely reproduce data obtained from nutritional studies.

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