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Incorporation and metabolism of (n-3) and (n-6) polyunsaturated fatty acids in phospholipid classes in cultured turbot (*Scophthalmus maximus*) cells

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Abstract

The incorporation and metabolism of various (n-3) and (n-6) polyunsaturated fatty acids (PUFA) supplemented to the culture medium was investigated in a turbot cell line (TF). The distribution, and the occurrence and extent of further metabolism of incorporated PUFA via desaturation/elongation mechanisms in specific phospholipid classes was determined from the different fatty acid compositions. The cells contained Δ6 and Δ4 desaturase activities but were generally deficient in C18–20 elongase activity. Δ5 Desaturase activity was generally masked by this deficiency but was present. The compositional data indicated that there was a high degree of specificity between individual phospholipid classes and particular fatty acids probably driven by the specificities of the acylating enzymes. The highest percentages of the supplemented acids were generally observed in the phosphatidic acid/cardiolipin fraction (PA/CL), suggesting a role for PA in the incorporation of the supplemented acids into the phospholipid pool. PI had a characteristic composition consistent with a putative role as a pool of precursor fatty acid for eicosanoid synthesis. Mechanisms were evident for generating and/or maintaining this composition.

Abbreviations: CL, cardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid(s); SM, sphingomyelin.

Introduction

In marine animals, (n-3) polyunsaturated fatty acids (PUFA) predominate rather than the (n-6) series which predominate in terrestrial animals (Sargent 1976; Sargent and Whittle 1981). Thus, fish have a strict dietary requirement for (n-3) PUFA whereas the dietary requirement for 18:2 (n-6) is less well defined (Watanabe 1982; Henderson and Tocher 1987). However, with marine fish, 18:3(n-3) alone has generally not been able to satisfy the fishes' EFA requirement, and longer chain (n-3)PUFA have been required (Yamada *et al.* 1980). More specifically, turbot (*Scophthalmus maximus*) grow optimally on diets containing C20 and C22 (n-3)PUFA rather than diets containing simply 18:3(n-3) (Gatesoupe *et al.* 1977). Nutritional studies showed that turbot could not convert dietary 18:2(n-6) to 20:4(n-6) when fed corn oil or endogenous 18:1(n-9) to 20:3(n-9) when fed on EFA-deficient diet (Cowey *et al.* 1976). Other studies showed that only 3–15% of orally administered [1-^{14}C] 18:1(n-9), 18:2(n-6) or 18:3(n-3) were further desaturated and chain elongated (Owen *et*

al. 1975). Thus, turbot are known to have some deficiency in the fatty acid desaturase/elongase system.

Recently we have shown that cultured cells from turbot (TF) expressed considerable Δ6 desaturase activity and also significant Δ4 desaturase activity (Tocher *et al.* 1989). However, the elongation of C18 to C20 PUFA was deficient and so the cells could not convert supplemented 18:3(n-3) or 18:2(n-6) through to 20:5(n-3) and 22:6(n-3) or 20:4(n-6), respectively (Tocher *et al.* 1989). Δ5 Desaturase activity was present although masked by the lack of the elongase. However, 20:5(n-3) could be incorporated into the cells and metabolized to 22:5(n-3) and 22:6(n-3). Clearly, the TF cell line displayed aspects of lipid metabolim consistent with the situation previously encountered in the various nutritional studies (Owen *et al.* 1975; Cowey *et al.* 1976; Gatesoupe *et al.* 1977).

In our previous study, the incorporation and subsequent metabolism by TF cells of a variety of (n-3) and (n-6)PUFA was investigated in total lipid (Tocher *et al.* 1989). However, total phospholipids accounted for 70% of the total lipid from TF cells and there is currently considerable interest in the precise distribution of different fatty acids between the individual phospholipid classes in normally (n-3)PUFA-rich marine systems (Bell *et al.* 1983, 1985; Tocher and Sargent 1984, 1986; Tocher *et al.* 1985; Tocher and Harvie 1988). The mechanisms of generation of the observed phospholipid class fatty acid compositional data are also being studied (Voss and Sprecher 1988).

TF cells grown in medium supplemented with fetal calf serum (FCS) as the only lipid source have relatively low levels of PUFA, and in particular (n-3)PUFA (Tocher *et al.* 1988), and so have been amenable to alteration by PUFA supplementation (Tocher *et al.* 1989). The present paper describes the incorporation of specific (n-3) and (n-6)PUFA into individual phospholipid classes in TF cells. The occurrence and extent of further metabolism of the incorporated PUFA *via* desaturation and chain elongation was determined from the compositional data. The results were discussed in relation to the particular desaturase activity pattern in TF cells and the involvement of individual phospholipid classes in the generation of specific fatty acid distributions.

In general, all fish cells cultured in mammalian sera have a fatty acid composition that is altered grossly (Tocher *et al.* 1988). For some purposes, *e.g.* eicosanoid metabolism, this would limit their use as an experimental model for fish *in vivo*. Therefore, as a practical application of the incorporation studies, two fish oil concentrates were evaluated as to their use as routine supplements for fish cell culture *via* their ability to restore the fatty acids of the TF cells to a normal composition.

Materials and methods

Cells and medium

The turbot (*Scophthalmus maximus*) fin cell line (TF) was obtained from Dr B Hill, MAFF Fish Diseases Laboratory, Weymouth, U.K. and was maintained in Leibovitz L-15 medium containing 0.35% sodium chloride and supplemented with antibiotics (50 I.U.ml^{-1} penicillin and 50 μg.ml^{-1} streptomycin) and either 10% or 2% FCS.

(n-3) and (n-6)PUFA supplements

PUFA supplements were added to the TF cultures as bovine serum albumin (BSA) complexes prepared essentially according to the method of Spector and Hoak (1969). However, to minimise autoxidation, all procedures were carried out at room temperature and under a stream of N_2. The resultant solutions were filter sterilized through 0.2 μm filters (Flowpore D26, Flow Laboratories) prior to assay and use.

Fatty acid concentrations were determined by addition of an internal standard (17:0) to an aliquot and GC analyses as described previously (Tocher *et al.* 1989). Protein concentrations were measured by the method of Lowry *et al.* (1951). The PUFA concentrations were generally in the range 2.0–2.8 mM (BSA = 0.25 mM) giving PUFA:BSA ratios of approximately 8–11:1. The PUFA/BSA mixtures were stored in brown vials, under N_2 at −25°C between procedures.

Preparation of fish oil concentrates (FOC)

See Tocher (1989), this issue.

Cell growth and incubation conditions

See Tocher (1989), this issue.

Cell harvesting and lipid extraction

See Tocher (1989), this issue.

Phospholipid class separation

See Tocher (1989), this issue.

Fatty acid analysis

See Tocher (1989), this issue.

Materials

Leibovitz L-15 medium, antibiotics and FCS were obtained from Flow Laboratories, Rickmansworth, U.K. All PUFA (approx. 99% pure), fatty acid-free BSA and BHT were from Sigma Chemical Co. Ltd., Poole, U.K. TLC plates (20 × 20 cm × 0.25 mm) precoated with silica gel 60 were obtained from Merck, Darmstadt, FRG. All solvents were HPLC grade and were obtained from Rathburn Chemicals, Walkerburn, U.K.

Results

Fatty acid composition of phospholipid classes from TF cells cultured in medium containing 10% FCS

TF cells grown in 10% FCS are relatively deficient in (n-3) and (n-6)PUFA and accumulate (n-9)-PUFA in total lipid (Tocher *et al.* 1989). Due to the overall deficiency in C18 to C20 elongase activity, 20:3(n-9), the normal PUFA to accumulate in EFA deficiency is not produced and so the product of Δ6 desaturase activity 18:2(n-9), accumulates. Further elongation to 20:2(n-9) is minimal. The present results show that 18:2(n-9) accumulates especially in PC, PE and PS, and that total (n-9)PUFA accounts for over 78%, 53% and 55% of the total PUFA in these phospholipid classes (Table 1). PC also contained high percentages 16:0, 18:1(n-9) and 16:1 with very low (n-3) and (n-6)PUFA. As well as the highest (n-9)PUFA content, PE also had higher (n-6)PUFA, predominantly 20:4(n-6), and higher (n-3)PUFA, predominantly 22:6(n-3), than either PC or PS giving it the highest total PUFA content. PS had very high 18:0, the lowest percentage of 18:1 isomers and the highest percentages of C22 PUFA. PI showed the classic mammalian-type fatty acid distribution with high 18:0 and (n-6)PUFA, especially 20:4(n-6). The lowest percentage of saturates and highest percentage of monoenes, especially 16:1 and 18:1 isomers, were found in PA/CL which also had the highest percentages of C18 PUFA and 22:6(n-3). The highest percentage of saturates including 14:0 and long-chain saturates was found in SM which also had high monoenes, especially 24:1, and virtually no PUFA other than some 18:2(n-9). Plasmalogens are components of the PC and PE classes as shown by the presence of dimethyl acetals in the GC traces (Table 2). In all the fatty acid compositional data presented, the dimethyl acetal components were excluded from the final analyses.

Incorporation and metabolism of 18:3(n-3)

Supplementation with all the (n-3) PUFA reduced 18:2 (n-9) and also 18:1(n-9) levels in all phospholipid classes. Supplementing the cells with 20 μM 18:3 (n-3) for 3 days greatly increased the total (n-3)-PUFA content of each phospholipid class (Table 3). Other than 18:3(n-3), only the product of the Δ6 desaturase, 18:4(n-3), was substantially increased in all phospholipid classes, with the elongation products 20:3(n-3) and 20:4(n-3), only slightly increased. Incubation at the same concentration for 7 days generally increased the percentages of 18:4

Table 1. Fatty acid composition[a] of phospholipid classes from TF cells cultured in medium containing 10% FCS.

Fatty acid	PC	PE	PS	PI	PA/CL	SM
14:0	2.3	1.5	0.2	0.7	1.1	3.6
15:0	0.2	0.2	T	T	0.3	0.4
16:0	13.9	6.7	4.2	4.2	4.2	21.2
17:0	0.2	0.3	0.2	0.2	0.1	0.3
18:0	3.5	12.6	33.4	21.2	4.2	10.1
20:0	0.1	0.4	0.8	0.2	0.2	0.7
22:0	0.1	0.3	0.2	0.1	–	3.6
24:0	–	–	–	–	–	1.8
Total saturates	20.3	22.0	39.0	26.6	10.1	41.7
16:1[b]	15.2	4.9	4.1	2.9	18.7	5.4
18:1(n-9)	29.3	15.9	11.2	21.4	23.9	15.3
18:1(n-7)	4.1	4.5	2.6	2.6	8.6	4.2
20:1(n-9)	0.8	2.6	1.2	1.7	1.6	1.0
22:1	0.1	0.5	0.1	T	0.2	3.2
24:1	0.4	0.2	0.2	0.1	0.2	21.3
Total monoenes	49.9	28.6	19.4	28.7	53.2	50.4
18:2(n-9)	21.1	23.3	20.9	6.0	5.1	3.6
20:2(n-9)	0.4	1.0	0.4	1.8	0.7	–
Total (n-9)PUFA	21.5	24.3	21.3	7.8	5.8	3.6
18:2(n-6)	0.7	1.1	0.6	1.1	4.6	0.3
18:3(n-6)	0.7	0.8	1.4	0.8	3.4	1.3
20:2(n-6)[c]	0.2	0.5	0.2	5.1	0.6	–
20:3(n-6)	0.4	0.4	0.6	3.5	2.7	–
20:4(n-6)	1.2	5.8	0.8	16.2	2.7	0.3
22:4(n-6)	0.4	1.4	3.3	0.7	1.1	–
22:5(n-6)	0.1	0.6	0.6	0.3	0.7	–
Total (n-6)PUFA	4.0	11.3	8.0	28.2	16.3	1.9
18:3(n-3)	0.1	0.1	0.1	0.3	0.2	–
18:4(n-3)	0.2	0.2	0.1	0.3	0.4	–
20:5(n-3)	0.2	0.6	T	0.9	0.2	–
22:5(n-3)	0.3	1.6	3.4	1.4	1.4	–
22:6(n-3)	1.1	7.3	5.5	3.1	9.7	T
Total (n-3)PUFA	1.9	9.8	9.1	6.0	12.0	0
Total PUFA	27.4	45.4	38.4	42.0	34.1	5.5
(n-3)/(n-6)	0.5	0.9	1.1	0.2	0.7	0
Total unidentified	2.4	4.0	3.2	2.7	2.6	2.4

[a]Results are expressed as a percentage of weight; [b]predominantly (n-7) isomer; [c]includes traces of 20:3(n-9); T, trace; –, not detected.

(n-3) and decreased the percentages of 18:3(n-3). However, the elongation products were not increased (Table 3). The largest percentages of 18:3 (n-3) were observed in PA/CL, a feature very prominent when the concentration was increased to 50 μM, whereas PI always showed the lowest percentages.

Incorporation and metabolism of 20:5(n-3) and 22:6(n-3)

The incorporation of 20:5(n-3) was greatest in PE and PA/CL and lowest in PC (Table 4). PC was also characterized by low percentages of the chain elongation and Δ4 desaturase products, 22:5(n-3) and 22:6(n-3), whereas all the other classes had sig-

Table 2. Dimethyl acetal content[a] of PC and PE.

Phospholipid class	Dimethyl acetals			
	16:0	18:0	18:1	Total
PC	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.5
PE	1.1 ± 0.3	2.0 ± 0.6	5.4 ± 1.7	8.5

[a]Percent of total peak area from GC; results are means ± SD (n = 12).

Table 3. (n-3)PUFA composition[a] of phospholipid classes from TF cells cultured in medium supplemented with 18:3(n-3).

	PC			PE			PS			PI			PA/CL		
Fatty acid	20/3	20/7	50/7	20/3	20/7	50/7	20/3	20/7	50/7	20/3	20/7	50/7	20/3	20/7	50/7
18:3	13.5	6.4	13.5	9.3	7.4	13.8	4.1	4.3	8.4	2.5	1.7	3.8	27.8	24.9	41.5
18:4	8.2	8.4	10.2	5.2	7.3	9.7	5.5	8.8	13.5	3.2	3.1	5.9	7.5	9.0	12.7
20:3	0.4	0.4	0.8	0.6	0.5	0.9	0.1	T	T	0.2	0.1	0.3	0.7	0.6	1.1
20:4	0.3	0.3	0.4	0.3	0.3	0.5	0.2	0.2	0.3	0.4	0.4	0.9	0.3	0.4	0.4
20:5	0.7	0.9	1.0	1.2	2.5	2.7	0.2	0.5	0.2	0.8	1.5	2.3	0.3	0.4	0.6
22:5	0.4	0.2	0.3	1.3	0.9	1.1	2.3	1.1	1.6	0.9	0.6	0.7	0.8	0.5	0.5
22:6	0.9	0.8	0.7	6.5	4.6	4.8	4.5	2.7	3.3	1.8	2.0	1.8	5.3	4.3	4.4
Total	24.4	17.4	26.9	24.4	23.5	33.5	16.9	17.6	27.3	9.8	9.4	15.7	42.7	40.1	61.2

[a]Results are expressed as a percentage of the total fatty acids; 20/3, incubation with 20 μM 18:3(n-3) for 3 days; 20/7, incubation with 20 μM 18:3(n-3) for 7 days; 50/7, incubation with 50 μM 18:3(n-3) for 7 days; T, trace.

Table 4. (n-3)PUFA composition[a] of phospholipid classes from TF cells cultured in medium supplemented with 20:5(n-3).

Fatty acid	PC	PE	PS	PI	PA/CL
18:3	T	0.1	T	T	T
18:4	0.9	0.7	0.8	0.4	2.3
20:3	–	–	–	–	–
20:4	–	–	–	–	–
20:5	9.0	16.9	2.1	11.5	12.5
22:5	1.4	4.2	11.0	4.1	4.7
22:6	1.8	8.8	7.9	4.1	10.1
Total	13.1	30.7	21.8	20.1	29.6

[a]Results expressed as a percentage of total fatty acids. Cells were incubated with 20 μM acid supplement for 6 days; T, trace; –, not detected.

nificant percentages of these acids. PS was of particular interest as almost 87% of its total (n-3)PUFA was present as C22 species, in particular 22:5(n-3). Supplementation with 22:6(n-3) resulted in very high incorporation into PE, PA/CL and PS but lesser amounts into PC and PI (Table 5). It was notable that 22:6(n-3) supplementation generated higher total (n-3)PUFA contents than 20:5(n-3) in all phospholipid classes except PI where 20:5(n-3) supplementation was more effective. Increased 18:4(n-3), particularly in PA/CL, and increased 20:5(n-3) in PC and PE suggested that some retroconversion of 20:5(n-3) and 22:6 (n-3), respectively, occurred.

Incorporation and metabolism of 18:2(n-6)

Supplementation with all the (n-6)PUFA reduced 18:2(n-9) and also 18:1(n-9) levels in all phospholipid classes. Supplementing the cells with 20 μM 18:2(n-6) for 3 days greatly increased the total (n-6)PUFA content of each phospholipid class (Table 6). Other than 18:2(n-6), only the product of the Δ6 desaturase, 18:3(n-6), was substantially in-

creased in all phospholipid classes. The elongation products 20:2(n-6) and 20:3(n-6), only slightly increased in all phospholipid classes except for PI which showed a substantial increase in 20:3(n-6). Incubation at the same concentration for 7 days generally increased the percentages of 18:3(n-6) and decreased the percentages of 18:2(n-6). However, the elongation products increased only very slightly (Table 6). As with 18:3(n-3), the highest percentages of 18:2(n-6) were observed in PA/CL, again particularly prominent when the concentration was increased to 50 μM.

Incorporation and metabolism of 18:3(n-6), 20:3 (n-6) and 20:4(n-6)

Supplementation with 18:3(n-6) generated higher total (n-6)PUFA percentages in all phospholipid classes than supplementation with 18:2(n-6) (Table 7). However, a generally similar phospholipid distribution was observed, with PA/CL showing the highest incorporation and PI showing the only significant level of chain elongation resulting in the greatest increase in the percentage of 20:3(n-6). Supplementation with this acid itself was not as effective as 18:3(n-6) and generated total (n-6)PUFA levels similar to 18:2(n-6) (Table 8). The incorporation of 20:3(n-6) was greatest into PA/CL and PI, but there were no increased percentages of 20:4(n-6) in these classes, although this was observed with PC, PE and PS (Table 8). All classes showed increased percentages of 18:3(n-6), indicating a significant degree of chain shortening occurred. Supplemented 20:4(n-6) was incorporated primarily into PI and PE and generated total (n-6) PUFA levels similar to 20:3(n-6) except that the level in PA/CL was lower (Table 9). Chain elongation of 20:4(n-6) and Δ4 desaturation was observed in all classes with C22 PUFA, especially 22:4(n-6), comprising over 73% of the total (n-6)PUFA in PS. Increased percentages of 18:3(n-6) in all classes, and in particular PA/CL, indicated some chain

Table 5. (n-3)PUFA composition of phospholipid classes from TF cells cultured in medium supplemented with 22:6(n-3).

Fatty acid	PC	PE	PS	PI	PA/CL
18:3	0.1	0.1	0.1	T	0.3
18:4	0.7	0.3	0.2	0.3	1.2
20:3	–	–	–	–	–
20:4	0.1	0.1	–	0.1	0.2
20:5	3.5	2.6	0.3	2.4	1.5
22:5	0.4	0.6	1.0	0.9	0.6
22:6	13.6	35.8	29.2	14.4	32.0
Total	18.4	39.5	30.8	18.1	35.8

Incubation conditions and data presented as described in Table 4; T, trace; –, not detected.

Table 6. (n-6)PUFA composition of phospholipid classes from TF cells cultured in medium supplemented with 18:2(n-6).

	PC			PE			PS			PI			PA/CL		
Fatty acid	20/3	20/7	50/7	20/3	20/7	50/7	20/3	20/7	50/7	20/3	20/7	50/7	20/3	20/7	50/7
18:2	17.5	10.7	17.0	10.9	11.2	16.8	6.5	7.1	13.2	5.3	3.5	7.5	32.8	24.8	51.2
18:3	7.4	6.4	7.3	5.9	7.1	9.7	7.1	8.6	11.8	4.4	3.5	6.0	5.4	3.7	6.9
20:2	0.7	0.7	0.8	0.8	0.9	1.0	0.2	0.4	0.2	4.4	5.6	5.3	1.4	0.9	1.8
20:3	0.7	0.9	0.7	0.6	1.3	0.7	0.8	1.2	0.6	7.6	7.4	9.2	1.6	1.1	1.3
20:4	0.9	1.3	1.0	5.3	5.4	4.7	1.1	0.9	0.7	16.5	12.4	12.8	2.5	1.3	2.3
22:2	0.4	0.3	0.4	0.9	0.9	1.1	0.5	0.5	0.5	0.4	0.4	0.4	0.3	0.1	0.3
22:3	0.4	0.3	0.2	0.5	0.5	0.5	1.7	–	–	0.1	0.1	0.1	0.3	0.6	–
22:4	0.3	0.4	0.3	1.1	1.5	1.2	1.5	1.5	1.2	0.8	0.8	0.6	0.5	0.6	0.4
22:5	–	0.2	0.1	0.6	0.6	0.5	0.6	0.5	0.4	0.3	0.3	0.2	0.4	0.3	0.3
Total	28.3	21.2	27.8	26.6	29.4	36.2	20.0	20.7	28.6	39.6	34.0	42.1	45.2	33.4	64.5

Data presented as described in Table 3; –, not detected.

Table 7. (n-6)PUFA composition of phospholipid classes from TF cells cultured in medium supplemented with 18:3(n-6).

Fatty acid	PC	PE	PS	PI	PA/CL
18:2	1.4	0.6	0.5	0.4	3.2
18:3	25.8	22.7	25.5	9.2	38.7
20:2	0.1	0.2	–	1.1	T
20:3	2.5	2.2	1.3	15.9	4.7
20:4	1.1	6.5	0.6	13.7	2.1
22:2	–	0.2	0.2	0.2	–
22:3	1.4	1.3	0.2	0.3	1.2
22:4	0.8	1.7	3.2	1.6	1.5
22:5	0.2	0.7	0.6	0.3	0.4
Total	33.3	36.1	32.1	42.6	51.8

Incubation conditions and data presented as described in Table 4; T, trace; –, not detected.

Table 8. (n-6)PUFA composition of phospholipid classes from TF cells cultured in medium supplemented with 20:3(n-6).

Fatty acid	PC	PE	PS	PI	PA/CL
18:2	0.6	0.5	0.5	0.3	1.4
18:3	4.8	4.9	7.2	1.2	9.0
20:2	0.2	0.4	T	3.0	T
20:3	7.4	7.4	7.7	18.3	21.3
20:4	2.3	11.5	1.2	14.7	2.3
22:2	–	0.4	0.3	0.2	1.1
22:3	1.9	2.6	0.4	0.4	1.1
22:4	0.7	2.0	2.5	1.3	0.8
22:5	0.2	0.8	0.8	0.4	0.6
Total	18.1	30.5	20.6	39.8	37.6

Incubation conditions and data presented as described in Table 4; T, trace; –, not detected.

shortening or retroconversion occurred when 20:3(n-6) and 20:4(n-6) were supplemented.

Supplementation with fish oil concentrates

The fish oil concentrates (FOC) were prepared as a cheaper alternative to pure PUFA as a routine supplement for fish cell cultures and were both added at 25 μM total (n-3)PUFA. The results show that FOC 2 increased the percentage of total (n-3)PUFA to a greater extent, primarily due to increased

Table 9. (n-6)PUFA composition of phospholipid classes from TF cells cultured in medium supplemented with 20:4(n-6).

Fatty acid	PC	PE	PS	PI	PA/CL
18:2	0.6	0.4	0.3	0.5	1.5
18:3	1.8	2.0	3.0	1.4	7.0
20:2	0.2	0.2	T	1.9	T
20:3	0.5	0.4	0.3	1.3	1.5
20:4	7.7	20.2	2.3	27.6	9.6
22:2	–	–	–	–	–
22:3	0.1	0.1	–	–	–
22:4	2.3	5.8	12.1	4.3	4.8
22:5	1.2	3.6	4.2	1.6	3.2
Total	14.4	32.7	22.2	38.6	27.6

Incubation conditions and data presented as described in Table 4; T, trace; –, not detected.

22:6(n-3) Table 10). However, FOC 1 increased 20:5(n-3) and its elongation product 22:5(n-3) more than FOC 2.

Discussion

The phospholipid class distributions of fatty acids in wild turbot have not been reported in the literature. However, in one study young turbot were caught and raised on a standard maintenance diet containing fish oil (Bell *et al.* 1985). Only 20:5(n-3), 22:6(n-3), 20:4(n-6), total saturated and monounsaturated fatty acids for PC, PE, PS, PI and SM were reported in that study. In these fish 22:6(n-3) was highest in PE and PS, 20:4(n-6) was highest in PI, and SM contained almost 70% total saturates and monoenes. These trends were also observed with the TF cells cultured in 10% FCS albeit superimposed upon the general PUFA deficiency. Furthermore, supplemented 22:6(n-3) was preferentially incorporated into PE and PS (and PA/CL) and supplemented 20:4(n-6) was preferentially incorporated into PI. Similarly, 20:5(n-3) was elongated and desaturated to 22:6(n-3) to a greater extent in PE and PS (and PA/CL). These results are consistent with the data for turbot tissues (Bell *et al.* 1985).

The effect of Δ6 desaturase activity was observed in all lipid classes and was greater after 7 days of in-

Table 10. (n-3)PUFA composition of phospholipid classes from cells cultured in medium supplemented with FOC 1 and FOC 2

	FOC 1					FOC 2				
Fatty acid	PC	PE	PS	PI	PA/CL	PC	PE	PS	PI	PA/CL
18:3	0.2	0.1	0.1	0.1	0.6	T	0.1	0.1	–	0.2
18:4	0.1	0.8	1.0	0.3	2.8	0.4	0.4	0.2	0.2	1.0
20:3	–	T	–	–	T	–	–	–	–	–
20:4	0.1	0.1	T	–	0.4	–	0.1	T	–	T
20:5	4.6	8.3	0.9	2.9	3.9	3.7	3.8	1.0	1.7	2.1
22:5	1.0	4.1	6.4	3.5	3.4	0.5	1.2	2.1	1.2	0.9
22:6	3.2	15.8	11.0	5.4	14.2	8.3	28.4	21.1	10.0	24.5
Total	10.1	29.2	19.4	12.2	25.3	12.9	34.0	24.5	13.1	28.7

Data presented as for Table 4. Cells were incubated with oils for 6 days at a concentration of 25 μM total (n-3)PUFA; T, trace; –, not detected.

cubation. The concentration of the main elongation/desaturation products after Δ6 desaturase activity, 20:4(n-6), 20:5(n-3) and 22:6(n-3), tended to decrease slightly after C18 PUFA supplementation.

The results suggest that PA/CL may play an important role in the incorporation of supplemented PUFA. In particular all the C18 PUFA supplemented to the cells were found in the highest percentages in PA/CL, and this class also contained high percentages of 20:5(n-3), 22:6(n-3) and 20:3(n-6) when those PUFA were supplemented. PA/CL constitutes less than 5% of the total phospholipids in TF cells (Tocher *et al.* 1989) and so, in mass terms, these high percentages do not account for a large portion of the incorporated PUFA. However, it is possible that after uptake a significant proportion of the PUFA is incorporated into phospholipids via PA, consistent with its central and well known role in phosphoglyceride biosynthesis (Harwood *et al.* 1986).

Study of the individual phospholipid classes has shown that limited C18–20 elongase activity is present in the cells. This was not apparent when total lipid fatty acids were analysed (Tocher *et al.* 1989). Residual activity was indicated by the increased levels of 20:3(n-6) in PI when 18:2(n-6) and, particularly, 18:3(n-6) were supplemented. Surprisingly, however, the Δ5 desaturase activity product was not observed in PI but an increased percentage of 20:4(n-6) was observed in PE, and also PC to a lesser extent, when the immediate precursor for Δ5 desaturase, 20:3(n-6), was supplemented. Chain shortening to 18:3(n-6) was a significant fate of 20:3(n-6), probably via partial oxidation (β-oxidation). This would also account for the limited retroconversion that was observed with 20:4(n-6), 20:5(n-3) and 22:6(n-3). The Δ4 desaturase activity was evident from the percentages of 22:6(n-3) and 22:5(n-6) in all phospholipids after supplementation with 20:5(n-3) and 20:4(n-6) respectively. Furthermore, the results indicate that there is a high degree of specificity between individual phospholipid classes and particular fatty acids. Other than PA/CL, discussed above, PE always showed the highest percentages of 20:5(n-3) and 22:6(n-3), PI the highest percentages of 20:3(n-6) and 20:4(n-6) and PS 22:5(n-3) and 22:4(n-6). Clearly, the phospholipid and fatty acid specificities of the acylating enzymes are very important in this respect. Interestingly, PC did not appear to incorporate or accumulate any particular PUFA with any specificity.

In marine systems, PI has a unique composition with high 18:0 and 20:4(n-6) as the main PUFA, similar to mammalian PI (Bell *et al.* 1983, 1985; Tocher and Sargent 1984, 1986; Tocher *et al.* 1985; Tocher and Harvie 1988), which has prompted speculation that it has a role as a source of precursor acid for eicosanoid synthesis in these (n-3)

PUFA-rich tissues (Bell *et al.* 1983; Tocher and Sargent 1984). This hypothesis was supported by studies comparing the conversion of 20:4(n-6) and 20:5(n-3) to eicosanoids which showed that 20:4 (n-6) was the preferred substrate in plaice (*Pleuronectes platessa*) skin (Anderson *et al.* 1981) and neutrophils (Tocher and Sargent 1987) and various turbot tissues (Henderson *et al.* 1985). The data from the present study show that PI in the TF cells have the same composition described previously and that mechanisms exist for maintaining that composition. When 18:3(n-3) and 22:6(n-3) were supplemented, the percentages of (n-3)PUFA in PI were lower than any other phospholipid. With 20:5(n-3) the pattern was slightly different, with PI showing a higher percentage of (n-3)PUFA than PC and although the other classes had higher total (n-3)PUFA levels than PI, a greater proportion of that total remained as 20:5(n-3) in PI. In contrast, the percentage of (n-6)PUFA was higher in PI than any other phospholipid except PA/CL when 18:2 (n-6) and 18:3(n-6) were supplemented, and higher than all classes including PA/CL when 20:3(n-6) and 20:4(n-6) were supplemented. Furthermore, PI was unique in that a significant portion of the supplemented C18 (n-6) PUFA were chain elongated to 20:3(n-6) (and the dead-end product 20:2(n-6)) in PI. Obviously, there are highly specific mechanisms for generating the observed composition of PI in animal tissues, clearly demonstrated in these cells from normally (n-3)PUFA-rich tissue.

Both fish oil concentrates favourably altered the fatty acid composition of the TF cells. The precise (n-3)PUFA composition reflected that of the oils themselves. Hence FOC 2, which was predominantly 22:6(n-3), increased the percentage of 22:6(n-3) greater than FOC 1 which increased 20:5(n-3) to a greater extent. As the oils were added to the same concentration of total (n-3)PUFA the results indicate that FOC 2 uptake and/or incorporation was greater than FOC 1, consistent with the data obtained with pure 20:5(n-3) and 22:6(n-3). The overall phospholipid class distribution of 20:5(n-3) and its elongation product 22:5(n-3), and 22:6(n-3) were similar with these mixed substrates to those obtained with the pure acids. This included PI which maintained its normally high 20:4(n-6) content, being 19.7% and 20.5% with FOC 1 and FOC 2, respectively. Although FOC 2 did give rise to higher total (n-3)PUFA levels, a higher 20:5(n-3) content is normally found in fish tissues and so FOC 1 is possibly preferable as a routine supplement for this reason, although a blend of both oils may be optimal.

Overall, this study has shown that supplementation of PUFA to cell cultures and analyses of the resultant fatty acid composition of individual phospholipid classes can give useful information on the possible involvement of individual phospholipid classes with specific intermediates and products of the desaturation/elongation pathway. The phospholipid and fatty acid specificities of the acylating enzymes are central to this, and would appear to be the driving mechanism behind the distributions observed. How the specificities are generated at a molecular level has yet to be elucidated.

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