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The incorporation and metabolism of polyunsaturated fatty acids in phospholipids of cultured cells from chum salmon (*Oncorhynchus keta*)

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Abstract

The incorporation and metabolism of (n − 3) and (n − 6) polyunsaturated fatty acids were studied in a cell line derived from chum salmon heart (CHH-1). Supplementing media with 25 μM fatty acid considerably altered the cellular fatty acid composition but did not affect the lipid class composition or cause the appearance of cytoplasmic lipid droplets. CHH-1 cells exhibited considerable Δ-6-desaturase activity but showed no preference between (n − 3) and (n − 6)PUFA substrates. CHH-1 cells also possess Δ-5-desaturase activity which showed preference towards (n − 3)PUFA, but Δ-4-desaturase activity was totally absent. Elongation of 20-carbon PUFA was especially active in CHH-1 cells with 22-carbon PUFA being specifically incorporated into PE and PS lipid classes. The fatty acid composition of PI indicated specific incorporation of 20-carbon PUFA into this lipid class. Supplementation with 22:6(n − 3) generated fatty acid compositions more closely resembling those of intact salmonid hearts. Substantial chain shortening of 22:6(n − 3) to 20:5(n − 3) occurred.

Introduction

Fish tissues are characterized by high levels of polyunsaturated fatty acids (PUFA) which are predominantly of the (n − 3) series as opposed to the (n − 6) series PUFA which predominate in terrestrial animals (Padley *et al.* 1986; Henderson and Tocher 1987). In general, freshwater fish can metabolize linolenic acid, 18:3(n − 3), by Δ-6-desaturation to 18:4(n − 3) and after subsequent elongation and Δ-5- and Δ-4-desaturation, produce docosahexaenoic acid, 22:6(n − 3) (Henderson and Tocher 1987). Marine fish, such as the turbot (*Scophthalmus maximus*), apparently lack Δ-5-desaturase and require the long chain PUFA eicosapentaenoic acid, 20:5(n − 3), and 22:6(n − 3) to be supplied by the diet (Owen *et al.* 1975). These metabolic pathways have recently been confirmed in cultured cells from turbot and rainbow trout (*Oncorhynchus mykiss*) (Tocher 1990; Tocher and MacKinlay 1990). In an anadramous species, Atlantic salmon (*Salmo salar*), the ability to elongate and desaturate 18:2(n − 6) was apparently lost during smoltification (Ackman and Takeuchi 1986) but

Abbreviations: BHT, butylated hydroxytoluene; BSA, bovine serum albumin; CL, cardiolipin; FCS, fetal calf serum; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SM, sphingomyelin.

was regained in post-smolts (Bell *et al.* 1989).

In cultured cells, the only added lipid tends to be derived from the serum supplement which is usually of mammalian origin. This results in cultured cells having a fatty acid composition which largely reflects that of the serum supplement (Spector *et al.* 1981). Fetal calf serum (FCS) is normally the serum of choice but fish cells cultured in FCS are generally deficient in (n − 3)PUFA and usually contain elevated concentrations of (n − 6)PUFA (Tocher *et al.* 1988).

In a recent study with Atlantic salmon, we observed the development of a severe cardiomyopathy in fish given diets containing sunflower oil (high in 18:2(n − 6)) whereas no lesion was seen in fish given diets containing fish oil (Bell *et al.* 1991). A number of other cardiac abnormalities including epicarditis and haemopericarditis have also been observed in farmed Atlantic salmon (Ferguson *et al.* 1986; Raynard and Houghton 1991).

Feeding diets with varying levels of (n − 3) and (n − 6)PUFA to rats resulted in modification of cardiac membrane lipids (Swanson and Kinsella 1986) and subsequent alteration of membrane bound enzyme activities (Leonardi *et al.* 1987; Swanson *et al.* 1989). In the present study we investigated the incorporation and metabolism of (n − 3) and (n − 6)PUFA in an established cell line (CHH-1) from chum salmon (*Oncorhynchus keta*) (Lannan *et al.* 1984). These cells had been derived from primary cultures of heart cells from juvenile salmon prepared as described by Wolf and Quimby (1976). Our aim was to determine the pathways of desaturation and elongation present in these cells and to observe whether there was preferential metabolism of (n − 3)PUFA compared to (n − 6) PUFA.

Materials and methods

Materials

Eagle's Minimum Essential Medium (EMEM), trypsin-EDTA and fetal calf serum (FCS) were obtained from Northumbria Biologicals Ltd., Cramlington, U.K. Tryptose phosphate broth, 10× concentrated non-essential amino acids, sodium bicarbonate, antibiotics and HCl were obtained from Flow Laboratories, Rickmansworth, U.K. All PUFA (approx. 99% pure), fatty acid-free bovine serum albumin (BSA), butylated hydroxy toluene (BHT), and Trizma were obtained from Sigma Chemical Co. Ltd., Poole, U.K. All solvents were HPLC grade and were obtained from Rathburn Chemicals, Walkerburn, U.K.

Cells and medium

The chum salmon heart cell line (CHH-1) was obtained from Dr. D. Smail, S.O.A.F.D. Marine Laboratory, Aberdeen, U.K., and was maintained in EMEM (with Earle's salts) containing 1% tryptose phosphate broth, 4 mM Tris-HCl buffer (Trizma, pH 7.4), 1% non-essential amino acids, 0.275% sodium bicarbonate, 2 mM HCl, antibiotics (50 I.U.ml^{-1} penicillin and 50 μg.ml^{-1} streptomycin) and either 10% or 1% FCS.

PUFA supplements

PUFA supplements were added to the CHH-1 cultures as BSA complexes prepared largely by the method of Spector and Hoak (1969). To reduce oxidation all procedures were carried out at room temperature under a stream of nitrogen. The PUFA-BSA complexes were filter sterilized through 0.2 μm filters (Flowpore, Flow Laboratories) prior to use. The fatty acid concentrations were assayed after addition of an internal standard (17:0) to an aliquot of the complex. The lipids were then extracted, transmethylated and quantitated by gas-liquid chromatography. PUFA concentrations were in the range 1.5–2.0 mM (BSA = 0.25 mM) giving a PUFA:BSA ratio of 6–8. The PUFA:BSA mixtures were stored under nitrogen at −20°C in darkened vials.

Incubation conditions

For routine cell cultivation the cultures were grown in sealed 75 cm^2 flasks (Northumbria Biological

Ltd.) in 15 ml medium containing 10% or 2% FCS. Cells were harvested for analysis or subculture within 24h of achieving confluence (usually after 5–7 days). Cells incubated with PUFA were subcultured into 225 cm^2 flasks with 50 ml medium containing 1% FCS and an appropriate volume of PUFA:BSA complex to give a final fatty acid concentration of 25 μM. FCS contains approximately 0.37% lipid of which around 50% is fatty acid. The lipid class composition and fatty acid composition of FCS has been given in detail previously (Tocher *et al.* 1988). Previous studies with cultured fish cells (Tocher *et al.* 1988, 1989) indicated that this fatty acid concentration resulted in considerable incorporation and metabolism into phospholipids without causing increased deposition of neutral lipid. Control flasks received the same volume of fatty acid free BSA solution. All incubations were performed at 22°C.

Cell harvesting and lipid extraction

Cells were harvested within 24h of reaching confluence. The media were removed by aspiration and the cells were washed with 20 ml phosphate-buffered saline before addition of 3 ml trypsin-EDTA (0.05% and 0.02% respectively). Once the cells became dissociated 6 ml of basal medium were added to inhibit trypsin activity. Cells from two 225 cm^2 flasks were pooled and centrifuged at 600 × g for 10 min at 4°C, the supernatant poured off and the cell pellet washed twice with 25 ml of phosphate-buffered saline to ensure removal of serum. Total lipid was extracted from the cells largely by the method of Folch *et al.* (1957). Cells were homogenised in 5 ml of ice-cold chloroform: methanol (2:1, v/v), 1 ml of 0.88% KCl added, followed by mixing on a vortex mixer. The phases were separated by centrifugation, the chloroform layer removed and dried under nitrogen and the residue dissolved in chloroform:methanol (2:1, v/v) containing 0.05% BHT at a final concentration of 50 mg/ml.

Lipid class analysis

Approximately 10 μg of total lipid were applied to a 10 × 10 cm high-performance thin-layer chromatography (HPTLC) plate that had been pre-run in hexane:diethyl ether (1:1, v/v) and activated at 110°C for 30 min. The plates were developed to 6 cm in methylacetate:isopropanol:chloroform:methanol:0.25% aqueous KCl (25:25:25:10:9 by volume) to separate phospholipid classes with neutral lipids running at the solvent front (Vitiello and Zanetta 1978). After drying, the plates were developed fully in hexane:diethyl ether:acetic acid (85:15:1.5, v/v/v) to separate neutral lipids and cholesterol. Lipid classes were visualised by charring at 160°C for 15 min after spraying with 3% copper acetate (w/v) in 8% phosphoric acid (v/v) and identified by comparison with commercially available standards. Lipid classes were quantified by scanning densitometry using a Shimadzu CS-930 dual wavelength TLC scanner and a DR-2 recording integrator. Data are means of triplicate analyses; standard deviations are omitted for clarity but were generally less than 5% of the mean.

Lipid class separation and fatty acid analysis

Total lipid (approx. 1 mg) was applied in a 1 cm streak to a 10 × 10 cm HPTLC plate and separated into phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) fractions as decribed by Vitiello and Zanetta (1978). The plates were sprayed with 0.1% 2′,7′-dichlorofluorescein in 97% methanol containing 0.05% BHT, and the lipid bands were visualised under UV light. The lipid bands were scraped from the plate and acid-catalysed transmethylation was performed overnight at 50°C by the method of Christie (1982). The fatty acid methyl esters were separated and quantified by gas-liquid chromatography (Carlo Erba GC 6000 Vega series 2, Fisons Ltd., Crawley, U.K.) using a capillary column (CP-Wax 51, 50 m × 0.32 mm, Chrompack (U.K.) Ltd., London) with on-column injection using hydrogen as carrier gas and with a two-stage thermal gradient from 50°C to 225°C.

Table 1. Lipid class composition of CHH-1 cells cultured in 10% fetal calf serum (FCS) or 1% FCS supplemented with various fatty acids. Results are means of triplicate experiments

	Lipid Supplement							
Lipid	10% FCS	18:2 n−6	18:3 n−3	18:3 n−6	20:3 n−6	20:4 n−6	20:5 n−3	22:6 n−3
%Phospholipids	69.0	63.8	57.1	59.7	66.4	69.5	64.8	60.2
%Neutral lipids	29.6	34.6	40.4	38.6	33.6	30.5	34.9	39.8
Phospholipid classes: (% total lipid)								
SM	3.3	3.5	3.5	3.6	3.9	3.9	3.7	3.6
PC	23.0	22.9	20.1	22.1	28.8	27.4	23.6	24.0
PE	16.2	17.6	16.1	16.9	18.1	19.9	18.6	16.7
PS	9.6	6.6	6.2	6.5	4.7	7.1	7.3	5.7
PI	12.3	8.9	7.4	7.2	6.8	7.8	7.8	6.3
PA/CL	4.6	4.4	3.9	3.7	4.3	3.6	4.0	4.0
Neutral classes: (% total lipid)								
Triacylglycerol	5.9	8.8	12.3	11.7	12.6	9.0	6.4	16.3
Free Fatty Acids	0.8	1.3	3.7	3.3	5.0	5.4	6.3	7.6
Diacylglycerol	0.3	1.0	3.2	2.7	1.9	3.3	3.7	3.0
Cholesterol	22.5	23.6	21.3	21.0	14.1	12.8	18.7	13.1

– = not detected.

Individual methyl esters were identified by comparison with known standards and by reference to published data (Ackman 1980; Bell *et al.* 1983).

Statistical analysis

Significance of difference ($p < 0.05$) between data in Table 2 and data in Tables 3–9 was determined by Student's t-test.

Results

The lipid class compositions of CHH-1 cells grown in 10% FCS or 1% FCS plus various fatty acids at 25 μM are shown in Table 1. In all cases, PC was the predominant phospholipid closely followed by PE which together accounted for approximately 40% of the total lipid. PS and PI were present in approximately equal amounts with PI always the dominant species followed by PA/CL and SM. Some accumulation of neutral lipid was observed in cells supplemented with 18:3(n−3) and 22:6(n−3) but microscopic examination of the cells indicated minimal presence of lipid droplets in all of the experimental treatments. Cells grew well and attained confluence in 5–7 days with all fatty acid supplements except 20:3(n−6). Cells supplemented with 20:3(n−6) did not attain confluence, after 4–5 days they appeared pyknotic and after 6–7 days they began to detach from the flask.

The fatty acid compositions of phospholipid classes of cells cultured in 10% FCS are shown in Table 2. The (n−9) series PUFA are dominant in all phospholipid classes, except PI, where (n−6) species predominate. 18:2(n−9) is the predominant (n−9)PUFA in all phospholipid classes except PI where 20:2(n−9) predominates. 20:3(n−9), which could not be separated from 20:2(n−6) in this chromatographic system, did not accumulate in CHH-1 cells. The (n−6)PUFA were generally more abundant than (n−3)PUFA, except in PS, and the major (n−6) species were 20:4, 18:2 and 20:2. The major (n−3)PUFA were 22:5 and 22:6. The greatest amount of PUFA were found in PI with the least being in PC, but in all classes the monoenes, mostly 18:1(n−9), and the saturates, mostly 16:0

Table 2. Fatty acid composition of phospholipid classes of CHH-1 cells cultured in medium containing 10% fetal calf serum

Fatty acid	Phospholipid class			
	PC	PS	PI	PE
14:0	1.5 ± 0.1	0.8 ± 0.1	0.4 ± 0.0	0.3 ± 0.0
16:0	18.8 ± 0.2	9.8 ± 0.1	4.4 ± 0.1	5.0 ± 0.2
18:0	6.5 ± 0.1	26.4 ± 1.4	19.2 ± 1.7	5.1 ± 0.3
Total saturates[a]	27.6 ± 0.3	37.7 ± 1.4	25.0 ± 1.5	10.8 ± 0.4
16:1n−9	5.2 ± 0.2	3.1 ± 0.1	0.8 ± 0.1	3.3 ± 0.0
16:1n−7	4.9 ± 0.0	2.8 ± 0.1	1.1 ± 0.1	3.2 ± 0.2
18:1n−9	44.2 ± 1.0	25.1 ± 0.3	25.8 ± 0.9	36.2 ± 0.4
18:1n−7	3.1 ± 0.1	1.7 ± 0.1	2.2 ± 0.5	2.9 ± 0.1
20:1n−9	0.8 ± 0.4	1.0 ± 0.1	0.4 ± 0.0	0.5 ± 0.4
24:1	0.7 ± 0.2	0.9 ± 0.4	1.0 ± 0.5	0.5 ± 0.1
Total monoenes	58.9 ± 0.5	34.6 ± 0.6	31.3 ± 0.9	46.6 ± 0.4
18:2n−9	4.3 ± 0.2	5.6 ± 0.0	2.6 ± 0.3	5.3 ± 0.1
20:2n−9	3.8 ± 0.1	4.6 ± 0.1	11.1 ± 0.4	4.3 ± 0.1
Total n−9	8.1 ± 0.3	10.2 ± 0.2	13.7 ± 0.7	9.6 ± 0.0
18:2n−6	0.6 ± 0.1	1.0 ± 0.1	0.7 ± 0.0	1.0 ± 0.1
18:3n−6	0.2 ± 0.1	0.7 ± 0.4	0.5 ± 0.3	0.1 ± 0.1
20:2n−6[b]	0.3 ± 0.0	0.3 ± 0.0	3.6 ± 0.1	1.5 ± 0.1
20:3n−6	0.3 ± 0.0	0.6 ± 0.2	2.1 ± 0.2	0.5 ± 0.0
20:4n−6	0.4 ± 0.1	0.6 ± 0.1	7.8 ± 1.0	4.5 ± 0.2
22:2n−6	–	0.4 ± 0.0	0.5 ± 0.2	0.3 ± 0.1
22:4n−6	–	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.0
22:5n−6	0.4 ± 0.3	0.7 ± 0.1	0.4 ± 0.0	0.4 ± 0.2
Total n−6	2.2 ± 0.4	4.9 ± 0.3	16.1 ± 1.5	8.7 ± 0.2
18:3n−3	t	0.1 ± 0.0	0.1 ± 0.0	t
18:4n−3	t	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
20:5n−3	0.1 ± 0.0	0.1 ± 0.0	0.8 ± 0.3	1.4 ± 0.0
22:5n−3	0.3 ± 0.0	3.6 ± 0.5	4.2 ± 0.7	2.8 ± 0.0
22:6n−3	0.2 ± 0.0	2.2 ± 0.3	3.7 ± 0.8	4.2 ± 0.2
Total n−3	0.6 ± 0.0	6.2 ± 0.7	9.0 ± 1.2	8.5 ± 0.2
Total PUFA	10.9 ± 0.6	21.3 ± 0.6	38.8 ± 0.5	26.8 ± 0.4
n−3/n−6	0.3 ± 0.1	1.4 ± 0.4	0.6 ± 0.2	1.0 ± 0.1
Total dimethyl acetals	0.4 ± 0.0	–	–	13.3 ± 0.7
Total unidentified	2.2 ± 0.2	6.4 ± 0.2	4.9 ± 2.0	2.5 ± 0.3

Data are shown as means ± SD of triplicate experiments; results are expressed as % by weight; [a] includes 15:0, 17:0 and 20:0; [b] includes 20:3n−9; t = trace value less than 0.1%; SD < 0.05 are tabulated as 0.0.

and 18:0, formed the main bulk of phospholipid fatty acids.

The data in the following tables (Tables 3–9) are dealt with in comparison to data in Table 2. The (n−6)PUFA composition of phospholipid classes from CHH-1 cells supplemented with 18:2(n−6) is shown in Table 3, where the total (n−6)PUFA content of all phospholipid classes is significantly increased. Apart from 18:2(n−6), there were significant increases in the Δ-6-desaturation product 18:3(n−6) and the elongation product 22:3(n−6) in PC and PE. 20:2(n−6) and 20:3(n−6) were significantly increased in all phospholipid classes. 20:3(n−6) showed the greatest increase, particularly in PI. The highest percentage of 18:2(n−6) was observed in PE.

Table 3. (n – 6)PUFA composition of phospholipid classes from CHH-1 cells cultured in medium supplemented with 18:2(n – 6)

n – 6PUFA	PC	PS	PI	PE
18:2	23.8 ± 1.5*	19.7 ± 1.5*	17.2 ± 1.1*	34.4 ± 0.2*
18:3	0.8 ± 0.1*	1.0 ± 0.1	0.7 ± 0.1	0.9 ± 0.0*
20:2	0.8 ± 0.1*	1.4 ± 0.3*	5.1 ± 0.2*	1.9 ± 0.1*
20:3	1.2 ± 0.2*	3.7 ± 0.5*	11.8 ± 0.7*	1.8 ± 0.1*
20:4	0.2 ± 0.1	0.5 ± 0.2	5.2 ± 0.8*	1.3 ± 0.2*
22:2	0.2 ± 0.0*	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.2
22:3	0.1 ± 0.0*	0.6 ± 0.2	0.2 ± 0.2	0.3 ± 0.1*
22:4	t	0.2 ± 0.0*	0.1 ± 0.1*	0.2 ± 0.0
22:5	0.2 ± 0.1	0.2 ± 0.1*	0.2 ± 0.1*	t
Total	27.3 ± 1.8*	27.7 ± 1.0*	40.9 ± 2.6*	41.4 ± 0.4*

Data are shown as means ± SD of triplicate experiments; results are expressed as a % of total fatty acids; t = trace value less than 0.1%; SD < 0.05 are tabulated as 0.0; * Values are significantly different ($p < 0.05$) compared to cells cultured in 10% FCS.

Table 4. (n – 3)PUFA composition of phospholipid classes from CHH-1 cells cultured in medium supplemented with 18:3(n – 3)

n – 3PUFA	PC	PS	PI	PE
18:3	24.9 ± 2.3*	21.0 ± 0.8*	12.5 ± 1.0*	30.1 ± 0.4*
18:4	1.4 ± 0.1*	1.4 ± 0.1*	0.7 ± 0.0*	1.3 ± 0.1*
20:3	2.0 ± 0.5*	1.8 ± 0.3*	2.4 ± 0.2*	2.8 ± 0.6*
20:4	3.2 ± 0.5*	4.2 ± 0.2*	9.4 ± 0.1*	5.3 ± 0.8*
20:5	0.3 ± 0.1*	0.5 ± 0.1*	4.5 ± 0.8*	2.6 ± 0.0*
22:5	0.2 ± 0.0	1.8 ± 0.3*	1.2 ± 0.3*	1.4 ± 0.1*
22:6	0.1 ± 0.0	1.3 ± 0.2*	0.7 ± 0.3*	1.2 ± 0.1*
Total	32.1 ± 3.4*	32.0 ± 0.9*	31.0 ± 1.3*	44.7 ± 1.9*

Data presented as described in Table 3.

The (n – 3)PUFA composition of phospholipid classes from CHH-1 cells supplemented with 18:3(n – 3) is shown in Table 4. Supplementing the media with 25 μM 18:3(n – 3) significantly increased the total (n – 3)PUFA content of all phospholipid classes with the highest levels again occurring in PE. The Δ-6-desaturase product, 18:4(n – 3) was significantly increased in all phospholipid classes as were the elongation products 20:3(n – 3) and 20:4(n – 3), with the latter being the major metabolite of 18:3(n – 3). The Δ-5-desaturation product 20:5(n – 3) was significantly increased in all phospholipid classes with the biggest increase occurring in PI. The elongation and desaturation products of 20:5(n – 3) were significantly decreased in PS, PI and PE.

The (n – 6)PUFA composition of phospholipid classes from CHH-1 cells supplemented with 18:3(n – 6) is shown in Table 5. The increase in total (n – 6)PUFA resulting from 18:3(n – 6) supplementation was generally greater than that for 18:2 (n – 6), with the exception of PE. PC showed the highest incorporation of 18:3(n – 6) and its metabolites including a significant increase in the Δ-5-desaturase product, 20:4(n – 6). The major metabolite was the elongation product, 20:3(n – 6) which showed greatest incorporation into PI but was also significantly increased in all phospholipids. The incorporation of the elongation product 22:3(n – 6) was significantly increased in all phospholipid classes, especially PE.

The (n – 6)PUFA composition of phospholipid

Table 5. (n – 6)PUFA composition of phospholipid classes from CHH-1 cells cultured in medium supplemented with 18:3(n – 6)

n – 6PUFA	PC	PS	PI	PE
18:2	0.4 ± 0.1	0.5 ± 0.0*	0.5 ± 0.1*	0.5 ± 0.1*
18:3	27.4 ± 0.3*	22.9 ± 0.1*	12.7 ± 0.9*	18.0 ± 2.4*
20:2	0.7 ± 0.0*	0.3 ± 0.0	3.6 ± 0.4	1.4 ± 0.3
20:3	14.3 ± 0.4*	6.1 ± 0.0*	16.3 ± 0.2*	10.4 ± 1.0*
20:4	1.3 ± 0.0*	0.8 ± 0.2	6.5 ± 0.4	2.9 ± 0.4*
22:2	0.2 ± 0.0*	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1*
22:3	1.9 ± 0.4*	0.9 ± 0.0*	1.8 ± 0.2*	3.1 ± 0.5*
22:4	0.2 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.6 ± 0.1*
Total	46.4 ± 0.2*	32.4 ± 0.4*	42.3 ± 1.9*	37.5 ± 4.5*

Data presented as described in Table 3.

Table 6. (n – 6)PUFA composition of phospholipid classes from CHH-1 cells cultured in medium supplemented with 20:3(n – 6)

n – 6PUFA	PC	PS	PI	PE
18:2	0.4 ± 0.0*	0.7 ± 0.1*	0.4 ± 0.0*	0.4 ± 0.1*
18:3	2.7 ± 0.1*	2.0 ± 0.1*	1.4 ± 0.1*	1.2 ± 0.1*
20:2	1.0 ± 0.1*	0.7 ± 0.1*	5.6 ± 0.4*	2.0 ± 0.1*
20:3	30.5 ± 1.9*	19.2 ± 1.1*	26.3 ± 0.4*	22.3 ± 0.5*
20:4	2.0 ± 0.3*	0.8 ± 0.2	7.8 ± 1.3	3.0 ± 0.2*
22:2	0.2 ± 0.0*	0.6 ± 0.1*	0.4 ± 0.1	0.7 ± 0.2*
22:3	4.5 ± 0.3*	4.6 ± 0.3*	3.4 ± 0.2*	9.7 ± 1.1*
22:4	0.3 ± 0.1*	0.7 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
Total	41.6 ± 1.3*	29.3 ± 1.7*	45.7 ± 1.4*	39.8 ± 1.1*

Data presented as described in Table 3.

classes from CHH-1 cells supplemented with 20:3(n – 6) is shown in Table 6. The increase in total (n – 6)PUFA was of a similar magnitude to that achieved with 18:3(n – 6) with the highest incorporation into PC. PC showed a significant increase in the Δ-5-desaturase product 20:4(n – 6) but the same fatty acid was significantly reduced in PE. All classes had significantly increased levels of the elongation product 22:3(n – 6). All phospholipid classes showed significantly increased 18:3(n – 6) demonstrating the presence of chain shortening activity.

The (n – 6)PUFA composition of phospholipid classes from CHH-1 cells supplemented with 20:4 (n – 6) is shown in Table 7. Supplemented 20:4 (n – 6) generated total (n – 6)PUFA levels in all phospholipid classes similar to 20:3(n – 6) but was preferentially incorporated into PI. Chain elongation to 22:4(n – 6) was extensive with maximum incorporation into PS. No Δ-4-desaturation and elongation to 22:5(n – 6) was observed.

The (n – 3)PUFA composition of phospholipid classes from CHH-1 cells supplemented with 20:5(n – 3) is shown in Table 8. Incorporation of 20:5(n – 3) was greatest into PI although, as a result of extensive elongation to 22:5(n – 3), the highest levels of total (n – 3)PUFA were in PE. The incorporation of 22:5(n – 3) was greatest in PE and PS where it comprised 70 and 91% of the total (n – 3)PUFA respectively. There was no evidence of Δ-4-desaturation and elongation to 22:6(n – 3).

The (n – 3)PUFA composition of phospholipid classes from CHH-1 cells supplemented with 22:6(n – 3) is shown in Table 9. Supplementation with 22:6(n – 3) produced levels of total (n – 3)

Table 7. (n – 6)PUFA composition of phospholipid classes from CHH-1 cells cultured in medium supplemented with 20:4(n – 6)

n – 6PUFA	PC	PS	PI	PE
18:2	0.7 ± 0.3	1.0 ± 0.4	0.6 ± 0.0	0.4 ± 0.0*
18:3	0.7 ± 0.1*	0.8 ± 0.3	0.8 ± 0.3	0.5 ± 0.1*
20:2	0.4 ± 0.1	t	1.7 ± 0.4*	0.7 ± 0.1*
20:3	1.2 ± 0.2*	0.4 ± 0.0	0.8 ± 0.1*	0.9 ± 0.3*
20:4	12.5 ± 0.3*	2.2 ± 0.5*	23.6 ± 1.6*	13.3 ± 0.1*
22:2	0.2 ± 0.0*	0.2 ± 0.0*	0.2 ± 0.1	0.2 ± 0.0
22:3	0.2 ± 0.1*	0.2 ± 0.0*	0.2 ± 0.0*	0.2 ± 0.0*
22:4	21.5 ± 1.2*	26.8 ± 2.4*	14.7 ± 0.8*	22.7 ± 1.8*
22:5	0.1 ± 0.0	0.1 ± 0.1*	t*	0.3 ± 0.1
Total	37.5 ± 1.4*	35.3 ± 2.8*	42.6 ± 1.8*	39.2 ± 1.9*

Data presented as described in Table 3.

Table 8. (n – 3)PUFA composition of phospholipid classes from CHH-1 cells cultured in medium supplemented with 20:5(n – 3)

n – 3PUFA	PC	PS	PI	PE
18:3	t	t	–	–
18:4	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	–
20:3	t	t	0.3 ± 0.1*	t
20:4	0.5 ± 0.1*	0.4 ± 0.1*	0.3 ± 0.1*	0.3 ± 0.0*
20:5	11.0 ± 0.3*	1.6 ± 0.3*	14.8 ± 1.1*	9.7 ± 0.1*
22:5	26.3 ± 0.3*	34.6 ± 0.2*	23.4 ± 1.0*	30.7 ± 0.7*
22:6	0.5 ± 0.0*	1.3 ± 0.1*	1.0 ± 0.1*	2.9 ± 0.1*
Total	38.4 ± 0.6*	38.0 ± 0.2*	39.9 ± 0.3*	43.6 ± 0.7*

Data presented as described in Table 3.

Table 9. (n – 3)PUFA composition of phospholipid classes from CHH-1 cells cultured in medium supplemented with 22:6(n – 3)

n – 3PUFA	PC	PS	PI	PE
18:3	–	–	0.1 ± 0.0	–
18:4	–	–	–	–
20:3	–	*0.3 ± 0.1	*0.3 ± 0.1	*0.3 ± 0.1
20:4	*0.1 ± 0.0	t	t	*0.1 ± 0.0
20:5	*5.2 ± 1.0	*1.2 ± 0.0	*3.8 ± 0.2	*2.0 ± 0.2
22:5	*1.9 ± 0.2	*1.8 ± 0.0	*1.8 ± 0.0	*1.1 ± 0.1
22:6	*27.2 ± 5.4	*37.2 ± 2.6	*33.3 ± 1.1	*44.0 ± 1.5
Total	*34.4 ± 6.5	*40.5 ± 2.5	*40.2 ± 1.5	*47.5 ± 1.0

Data presented as described in Table 3.

PUFA similar to those with 20:5(n – 3). Incorporation of 22:6(n – 3) was particularly high in PE and PS (93 and 92% of total (n – 3)PUFA respectively) while increased 20:5(n – 3) in all phospholipid classes suggested that significant retroconversion of 22:6(n – 3) was occurring.

Discussion

A concentration of 25 μM fatty acid was chosen since supplementation at this level produced considerable alterations in PUFA content, without significantly altering lipid class composition or inducing intracellular lipid droplet formation in a number of previous studies utilising cultured cells (Stubbs and Smith 1984; Tocher 1990; Tocher and MacKinlay 1990). All CHH-1 cells grew well at this concentration of fatty acid supplementation except those grown in 20:3(n − 6) which did not achieve confluence and began to detach from the flask after 6–7 days. The experiment was repeated twice, using the same fatty acid concentration, with two different sources of 20:3(n − 6) and the same result was recorded. While the exact cause of the growth inhibition remains unclear, it seems unlikely that it was due to the presence of toxic oxidation products since measurement of TBA-reactive material in the PUFA-BSA complexes indicated greatest levels of oxidation products in the 20:5(n − 3) and 22:6 (n − 3) supplements which demonstrated no growth inhibition (Results not shown). In a recent study with Atlantic salmon, we observed the development of pathophysiological lesions in heart of fish given diets containing elevated 18:2(n − 6) which caused a decreased (n − 3)/(n − 6)PUFA ratio in cardiac tissue (Bell *et al.* 1991). While CHH-1 cells supplemented with 20:3(n − 6) also had a considerably reduced (n − 3)/(n − 6)PUFA ratio it is unlikely that this was solely to blame for the growth inhibition since supplementation with other (n − 6)PUFA at the same concentration did not affect growth.

The long term culture of fish cells in FCS is known to cause a deficiency in essential fatty acids of the (n − 3) series (Tocher *et al.* 1988). Although the presence of considerable amounts of (n − 9)PUFA indicates that CHH-1 cells grown in 10% FCS were deficient in (n − 3)PUFA, the distribution of fatty acids between the phospholipid classes show many of the features occurring in whole fish. For example, PC contains high levels of 16:0 and 18:1(n − 9), PS and PI high 18:0 and PI high 20:4(n − 6). With all fatty acid supplements PI tended to accumulate 20-carbon PUFA which is consistent with a role as a precursor pool for eicosanoid synthesis as postulated previously (Bell *et al.* 1983). However, the extent of the (n − 3)PUFA deficiency is apparent when comparing the CHH-1 cells grown in 10% FCS with cardiac tissue from Atlantic salmon (Bell *et al.* 1991). While CHH-1 PE contained the highest levels of 20:5(n − 3) and 22:6(n − 3) in any phospholipid class (1.4 and 4.2% respectively) the same fatty acids comprise 4 and 42% of the total in salmon heart.

The increase in 18:3(n − 6) and 20:3(n − 6) on supplementation with 18:2(n − 6) and the increase in 18:4(n − 3) and 20:4(n − 3) on supplementation with 18:3(n − 3) clearly indicates the presence of Δ-6-desaturase activity in CHH-1 cells. The relative percentages of the Δ-6-desaturase products of 18:2(n − 6) and 18:3(n − 3) indicated that there was no specificity for either substrate. This result is similar to that observed by Tocher *et al.* (1989) with cells derived from rainbow trout gonad (RTG-2) and turbot fin (TF), but differed from Atlantic salmon cells (AS) which showed a preference towards the (n − 3) substrate (Tocher and Dick 1990). However, the Δ-5-desaturase in CHH-1 cells showed a preference towards the (n − 3) substrate since supplementation with 18:3(n − 3) resulted in increased 20:5(n − 3) incorporation while 18:2 (n − 6) supplementation resulted in minimal 20:4(n − 6) production. This may not reflect a specific adaptation of fish cells since a similar substrate preference has also been observed in mammals (Stubbs and Smith 1984) and may be enhanced by the (n − 3)PUFA deficiency which exists in cells routinely cultured in FCS. In general, however, the accumulation of 20:3(n − 6) and 20:4(n − 3) on supplementation with 18:2(n − 6) and 18:3(n − 3) is compatible with a low Δ-5-desaturase activity in CHH-1 cells which is considerably less than the activity in both RTG-2 and AS cell lines (Tocher 1990; Tocher and Dick 1990).

CHH-1 cells like other salmonid derived cell lines appear to possess both Δ-6- and Δ-5-desaturase activities, while Δ-4-desaturase is apparently absent. Whether the full desaturase complement was lost as a result of continuous culture or whether the original cells were never capable of Δ-4-desaturation is not known. However, a number of cell lines are known to lose their ability to express desaturase ac-

tivities after long term culture (Maeda *et al.* 1978; Robert *et al.* 1978).

The considerable accumulation of 22C PUFA in comparison to other fish cell lines studied (Tocher 1990; Tocher and MacKinlay 1990; Tocher and Dick 1990) suggests appreciable elongase activity is present in CHH-1 cells. The large concentrations of elongation products arising from supplementation with both 20:4(n − 6) and 20:5(n − 3) may suggest some functional role for long-chain PUFA in these cells. 22:6(n − 3) is a major component of both rat cardiac phospholipids (Swanson and Kinsella 1986) and Atlantic salmon cardiac phospholipids (Bell *et al.* 1991). The concentration of 22:6(n − 3) in membrane phospholipids of cardiomyocytes can profoundly influence the activities in a number of membrane associated enzymes including Ca^{2+}-Mg^{2+} ATPase and phospholipase A (Swanson *et al.* 1989; Nalbone *et al.* 1990). Culture of CHH-1 cells in medium supplemented with 1% FCS and 25 μM 22:6(n − 3) resulted in phospholipid fatty acid compositions similar to those in Atlantic salmon heart (Bell *et al.* 1991). The ability to return these cells to a lipid composition approaching that of the salmonid heart makes them a useful model system to study the effects of membrane fatty acid composition on a number of biochemical and physiological functions.

The ability of CHH-1 cells to extensively elongate both 20:4(n − 6) and 20:5(n − 3) is contrary to the situation in rat heart and isolated cardiomyocytes which are unable to perform elongation (Mohammed *et al.* 1990) or desaturation reactions (Hagve and Sprecher 1989). Future studies in this laboratory using isolated and primary cultures of salmonid cardiomyocytes would hope to elucidate whether the high levels of 22:6(n − 3) in heart are a result of the synthetic capacity of the cardiomyocytes themselves, or are a result of hepatic modulation of dietary fatty acids followed by release, and uptake by extrahepatic tissues.

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