The effect of culture on morphology, lipid and fatty acid composition, and polyunsaturated fatty acid metabolism of rainbow trout (*Oncorhynchus mykiss*) skin cells

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Keywords: rainbow trout, skin , cell culture, lipid composition, polyunsaturated fatty acids, essential fatty acids; metabolism.

Abstract

Rainbow trout (*Oncorhynchus mykiss*) skin cell cultures were obtained by trypsinisation of the tissue and grown in Leibovitz L-15 medium. Lipid class compositions, and fatty acid profiles of total lipids and individual phospholipid classes were determined at different times of culture. The metabolism of polyunsaturated fatty acids (PUFA) was investigated by incubating primary cultures after 7 and 14 days with [1-^{14}C]18:2n-6 and [1-^{14}C-]18:3n-3. The change in morphology between epithelial-like primary cultures and fibroblastic-like secondary subcultures was accompanied by alterations in the lipid composition. Polar lipids became predominant by 14 days in culture. The relative proportions of phosphatidylcholine (PC), the most abundant phospholipid, phosphatidylinositol and cholesterol increased significantly, while sphingomyelin decreased. Saturated fatty acids, 18:1n-9, n-6 and n-9PUFA were more abundant in total lipid in cultures at 14 days and 4 months than in cells initially isolated which contained higher percentages of longer chain monoenes and n-3PUFA. The changes in fatty acid composition with time in culture were observed in all the major phospholipid classes. Rainbow trout skin cells in culture desaturated and elongated both 18:2n-6 and 18:3n-3, with 20:4n-6 and 20:5n-3 being the most abundant products, respectively. PC presented the highest incorporation of radioactivity, especially following incubation with 18:3n-3. Lipid metabolism in general increased with the age of primary cultures, with both the amount of C18 PUFA incorporated and metabolised by desaturation/elongation significantly increased in 14 day cultures compared to 7 day cultures. Product/precursor ratios calculated for both n-6 and n-3 fatty acids showed that, while Δ6 desaturase activity was increased significantly with cell age, Δ5 desaturase activity was more affected by the fatty acid series, with 18:3n-3 being more readily transformed to 20:5n-3 than 18:2n-6 to 20:4n-6. Further desaturation of 20:5n-3 to hexaenes was low. Overall, the data suggested that the trout skin cell cultures were more similar to mammalian skin fibroblasts than mammalian epidermal/keratinocyte cultures.

Introduction

Cultures of fish cells represent, as for mammals, an *in vitro* model to support *in vivo* studies. In particular, cultured fish cells have proved useful in biochemical studies of lipid and polyunsaturated fatty acid (PUFA) metabolism (Tocher and Sargent 1990a,b; Tocher 1993; Tocher *et al*. 1995a,b), with PUFA metabolism observed in studies *in vivo* in intact fish generally being reflected in cell cultures derived from the same species (Tocher and Sargent 1990a). For example, the ability and inability of trout and turbot, respectively, to convert dietary C18 PUFA to longer chain more unsaturated PUFA (Cowey *et al*. 1976) was confirmed by metabolic studies in rainbow trout (RTG-2) and turbot fin (TF) cell lines (Tocher and Sargent 1990a). In addition, the use of cell cultures permitted definition of the metabolic block in turbot as a deficiency in $\Delta 5$ desaturase activity (Tocher *et al*. 1989).

Burr and Burr (1929) first demonstrated the essentiality of linoleic acid (LA;18:2n-6) in the diet of mammals and, more recently, the essentiality of α-linolenic acid (LNA; 18:3n-3) for mammals, particularly during early development, has been appreciated (Neuringer *et al*. 1994). Scaly dermatoses and alteration in the permeability of skin were among the symptoms firstly associated with essential fatty acid (EFA) deficiency in mammals (Burr and Burr 1929, 1930). The importance of EFA in mammalian skin, particularly in relation to maintenance of the water barrier, is mainly due to the presence in the epidermis of large amounts of LA-rich lipids, especially ceramides, although eicosanoids derived from arachidonic acid (AA;20:4n-6) may also be important (Hansen 1986). The nutritional EFA requirement of fish varies between species both qualitatively and quantitatively and is generally different from mammals (Sargent *et al*. 1995). For rainbow trout, LNA alone is sufficient to satisfy the EFA requirement although LA is also necessary for optimal grow (Castell *et al*. 1972). EFA deficiency symptoms in the trout include fin erosion which may be result from loss of

skin integrity conducive to bacterial infection, similar to EFA deficiency leading to tail erosion in the rat (March 1993).

The lipid and fatty acid composition of fish skin is poorly documented. However, we recently demonstrated that rainbow trout skin does not contain large amounts of LA-rich ceramides as in mammalian skin but, as with fish tissues in general, n-3PUFA were abundant with, presumably, an important physiological role (Ghioni *et al*. 1997), However, little or nothing is known about PUFA metabolism in fish skin. Although desaturation and elongation metabolism of PUFA occurs mainly in liver, the relevant enzymes are also found in other tissues (Brenner 1974,1981; Maeda *et al*. 1978; Jimenez *et al*. 1993) and in cell cultures, included skin fibroblasts (Aeberhard *et al*. 1978; Rosenthal 1987). However, in mammals, epidermal cells apparently cannot metabolise LA to AA, an important prostaglandin precursor, as they lack both Δ6 and Δ5 desaturases, so that both LA and AA in the epidermis must be derived from systemic sources (Chapkin and Ziboh 1984). Evidence of conversion of LA to AA in mammalian keratinocytes *in vitro* (Isseroff *et al*. 1987) was not confirmed (Marcelo and Duell 1989).

In the present study, we aimed to elucidate the metabolism of PUFA in fish skin through the utilisation of cell cultures prepared from rainbow trout skin. Our primary objective was to determine if long-chain PUFA, which are abundant in this tissue (Ghioni *et al*. 1997) can be produced in situ from EFA precursors. It was observed that with increasing time in culture, the morphology of the rainbow trout skin cells changed. Given the importance of continuous cellular differentiation in skin from lower to more superficial layers, there may be a link between morphological, metabolic and functional changes in skin cells. Therefore, in addition, the effects of time in primary culture on PUFA metabolism were also determined.

Materials and methods

Experimental animals

Rainbow trout (*Oncorhynchus mykiss*) (0.5 - 1 kg) were obtained from a commercial fish farm (College Mill Trout Farm, Almondbank, Scotland, U.K.). Fish were maintained in 1m circular tanks supplied with freshwater (temperature 6 ± 2 ˚C) in the aquarium at Stirling University during which time they were fed a commercial pelleted feed (Vextra; Ewos Ltd., Westfield, Scotland). The experiment was performed in accordance with the British Home Office guidelines regarding research on experimental animals.

Cell cultures

Fish were killed by a blow to the head, sprayed with 70% ethanol, descaled and mucus removed as much as possible. Lateral skin was removed in long strips 1 cm wide avoiding contamination by muscle tissue and soaked for 1h in Hanks balanced salt solution (HBSS) containing 2 mM L-glutamine, 100 $U.ml^{-1}$ penicillin, 0.1 $mg.ml^{-1}$ streptomycin, 1 $mg.ml^{-1}$ amphotericin B and 5 $mg.ml^{-1}$ kanamycin. Skin was then finely chopped, transferred into sterile trypsinization flasks containing 40 ml of trypsin solution (2.5 $mg.ml^{-1}$in HBSS as above) and stirred for 10 minutes at room temperature. This solution was decanted and discarded, replaced with 80 ml fresh trypsin solution and stirred for 2h. The trypsinized suspension was collected and centrifuged at 300g for 5 min at 4˚C to harvest the cells. Cells were resuspended in a small volume of Leibovitz L-15 medium containing 10 mM HEPES, 2 mM L-glutamine, 100 $U.ml^{-1}$ penicillin, 0.1 $mg.ml^{-1}$ streptomycin, 1 $mg.ml^{-1}$ amphotericin B and 10% fetal bovine serum (FBS), counted using a haemocytometer and seeded at a concentration of approximately 1-1.5 million cells per 25 cm^2 Falcon "Primaria" tissue culture flasks (Becton Dickinson, Oxford, U.K.) in 5 ml of medium. A further 80 ml trypsin solution was added to the remaining skin tissue and the incubation and cell harvesting procedures repeated. Approximately 30 flasks of primary culture were obtained per fish.

Flasks were incubated at 22°C and the medium changed after 48h, after washing the cells with 4 ml Dulbecco's phosphate buffered saline (PBS) per flask. The cell monolayers became confluent after 1-2 weeks. Cells could be subcultured using trypsin/EDTA (0.05% trypsin/0.02% EDTA solution) for dissociation and could be maintained in secondary culture for up to 10 passages.

Lipid extraction

Cells were harvested by trypsinization as above, centrifuged and 1 ml ice-cold chloroform/methanol (2:1, v/v) containing 0.01% BHT as antioxidant added, and total lipid extracted at 0°C according to Folch *et al*. (1957). The lipid content was determined gravimetrically, total lipid resuspended at a concentration of 10 $mg.ml^{-1}$ in chloroform/methanol (2:1, v/v) containing 0.01% BHT and the samples stored at -20 °C until required.

Lipid class analysis

Separation and quantification of lipid classes was performed by high-performance thin-layer chromatography (HPTLC), followed by calibrated scanning densitometry (Bell *et al*. 1993). Approximately 10 μg (1 μl) of lipid extract was loaded as a 2 mm streak and the plate developed to two-thirds distance with methyl acetate/isopropanol/chloroform /methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.) (Vitiello and Zanetta 1978). After desiccation, the plate was fully developed with hexane/diethyl ether/acetic acid (80:20:2, by vol.). Plates were charred at 160°C for 15 min after spraying with 3% aqueous cupric acetate containing 8% phosphoric acid. Densitometric analysis was performed on a Shimadzu CS-9000 dual wavelength scanner and DR-13 recorder.

Fatty acid analysis

To determine fatty acid composition of total lipids, approximately 1 mg of lipid extract was transmethylated overnight in 2 ml of 1% sulphuric acid in methanol and 1 ml of

toluene at 50°C and under nitrogen (Christie 1982). Fatty acid methyl esters (FAME) were extracted and purified as described previously (Tocher and Harvie 1988). Purified FAME were dissolved in hexane containing 0.05% BHT to a final concentration of 1-2 $mg.ml^{-1}$.

Phospholipid class separation was performed as described by Tocher *et al*. (1995a) on lipid extracts from three pooled samples. 60 μl of a lipid extract 100 $mg.ml^{-1}$ (6 mg) were loaded as a 6 cm streak at 2 cm from lower edge of a 20 x 20 cm TLC plate, eluted with methyl acetate/isopropanol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.). Phospholipids were visualized with 0.1% 2',7'-dichlorofluorescein (DCF) in 97% methanol containing 0.05% BHT and the corresponding silica scraped. Transmethylation with methanolic sulphuric acid as above, was performed directly on the silica without prior elution of the lipids as described by Christie (1982) and FAME prepared as described above.

FAME analysis was performed using a BP20 fused silica capillary column (30m x 0.32 mm i.d., S.G.E. Ltd., Milton Keynes, U.K.) in a Canberra Packard 436 gas chromatograph (Chrompack (UK) Ltd., London, U.K.) utilizing hydrogen as carrier gas. Temperature programming was from 50 to 150°C at $35°C.min^{-1}$ and to 225°C at $2.5°C.min^{-1}$. Individual FAME were identified by comparison with known standards or published data (Ackman *et al*. 1980; Tocher and Harvie 1988).

Incubation of cells with ^{14}C-PUFA

Primary cultures only were used for the metabolic studies and routinely were about 95% confluent and 14 days old, unless stated otherwise.

The medium was aspirated from the flasks, the cells washed with PBS and 5 ml of fresh medium, as above except that the FBS was omitted, added. Each flask received 0.25 μCi (50 μl) of either [1-^{14}C]18:2 (n-6) or [1-^{14}C]18:3 (n-3) and incubation was continued for 6 days at 22°C. For each acid in an individual experiment, between 9 and 12 flasks of cells were used for a single data point. This experiment was performed in

triplicate. [1-^{14}C]PUFA were added as bovine serum albumin (BSA) complexes. Briefly, 25 μCi of fatty acid (0.5 μmol) were placed in a reaction vial, solvent evaporated under a stream of nitrogen and 100 μl of 0.1M KOH were added. The mixture was stirred for 10 minutes at room temperature before 5 ml of 50 mg.ml^{-1} fatty acid-free-BSA in HBSS were added and the reaction stirred for a further 45 min at 25°C. Isotope solutions were filter-sterilized (0.2 μm) prior to use.

After 6 days, the medium was aspirated, cells were washed with 4 ml PBS, harvested as described above and washed twice with 5 ml HBSS containing 1% fatty acid-free-BSA. Cell number was determined using a haemocytometer and total lipid extracted as described above. Aliquots of the lipid extract were placed into scintillation vials with 2.5 ml scintillation fluid (Ecoscint A, National Diagnostics, Atlanta, Georgia) and radioactivity determined in a 2000CA TRI-CARB scintillation counter (United Technologies Packard, U.K.). Results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions.

Incorporation of radioactivity in lipid classes

Lipid classes were separated by HPTLC as described above and lipid classes visualized by brief exposure to iodine vapour. Silica was scraped and placed into scintillation vials containing 2.5 ml scintillation fluid and radioactivity determined as above.

Metabolism via desaturation/elongation

Total lipid was transmethylated and FAME extracted and purified as described above. FAME were resuspended in 100 μl hexane containing 0.01% BHT and loaded as a 2.5 cm streak on TLC plates impregnated by spraying each with 2 g silver nitrate in 20 ml acetonitrile and activated at 110°C for 30 min. Plates were fully developed with toluene/ acetonitrile (95:5, v/v) (Wilson and Sargent 1992). Autoradiography was performed using Konica A2 film for 4-7 days at room temperature. Silica corresponding to individual FAME was scraped into scintillation vials containing 2.5 ml scintillation

fluid and radioactivity determined as described above.

Materials

[1-^{14}C]18:2 (n-6) and [1-^{14}C] 18:3 (n-3) (both 50-55 mCi.mmol^{-1}) were from NEN (DuPont (U.K.) Ltd., Stevenage, U.K.). Leibovitz L-15 Medium, HBSS, PBS, glutamine, antibiotics, HEPES buffer, fatty acid free-BSA, trypsin and trypsin/EDTA were obtained from Sigma Chemical Co. Ltd. (Poole, UK). Amphotericin B was purchased from ICN Biomedicals Ltd. (Thame, U.K.). TLC plates (20 cm x 20 cm x 0.25 mm) and HPTLC plates (10 cm x 10 cm x 0.15 mm), pre-coated with silica gel 60 were obtained from Merck (Darmstadt, Germany). All solvents were of HPLC grade (Rathburn Chemicals, Walkerburn, Scotland). Ecoscint A was purchased from National Diagnostic (Atlanta, Georgia).

Statistical analysis

Results are reported as mean ± SD (n = 3) unless otherwise stated. All statistical analyses were performed using a statistical computer package (Statgraphics system 3.0). Differences between means were reported as significant if $p<0.05$. Tukey's test was used to identify differences between means in ANOVA. Variance homogeneity was checked with Bartlett's test and non-homogeneous data were arcsin transformed prior to further statistical analysis.

Results

Morphology

In primary cultures the rainbow trout skin cells appeared predominantly polygonal and had a morphology similar to the epithelial-type cell phenotype (Fig. 1a,b). However,

the primary cultures contained patches of tightly packed, elongated thin cells more closely resembling the fibroblastic-type morphology. With increasing time in culture, and particularly after subculture, the fibroblast-type morphology became predominant (Fig. 1c,d).

Lipid class composition

Neutral lipids, predominantly triacylglycerol (TAG), accounted for almost two-thirds of the total lipid in rainbow trout skin cells upon initial isolation by trypsinization (Table 1). After 14 days in primary culture, the cells had significantly decreased proportions of neutral lipids due to significantly decreased TAG, with a concomitant increased proportion of total polar lipids which was due to generally increased proportions of all phospholipid classes, but especially phosphatidylcholine (PC). However, sphingomyelin (SM) significantly decreased relative to the other polar lipid classes. After 4 months and 9 passages there was a further increase in the relative proportion on total polar lipids mainly due to increased phosphatidylethanolamine (PE) and further reduced neutral lipids, mainly due to a decreased percentage of steryl ester (SE) (Table 1).

Fatty acid composition

Total PUFA, predominantly n-3PUFA (25%), accounted for over 35% of fatty acids in total lipid in rainbow trout skin cells upon initial isolation (Table 2). After 14 days in primary culture, there was a significant decrease in total n-3PUFA to 16%, mainly due to decreased percentages of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), whereas total n-6PUFA increased due to increased AA. These significant effects of decreasing n-3 and increasing n-6PUFA continued in 4 month cultures which were also characterized by significant amounts of n-9PUFA including 20:3n-9, whereas only 18:2n-9 was observed in 14 day primary cultures (Table 2). These changes resulted in the n-3/n-6 ratio decreasing 3.6-fold by 4 months in culture.

In contrast to these general trends, 18:2n-6 was significantly decreased and 22:5n-3 increased by culture. The proportion of total saturated fatty acids was increased by culture, due to increased 18:0, as was the predominant monounsaturated fatty acid, 18:1n-9, although total monounsaturated fatty acids were not greatly affected by culture, due to decreased proportions of 20:1 and 22:1.

The individual glycerophospholipids in trout skin cells upon isolation showed very characteristic fatty acid compositions; PC presenting high percentages of 16:0, 18:1n-9 and the lowest total PUFA; phosphatidylserine (PS) having high percentages of 18:0 and C22 PUFA; phosphatidylinositol (PI) having high 18:0 and AA and PE having low saturated fatty acids and relatively high percentages of all PUFA (Tables 3,4). The decrease in n-3PUFA with time in culture was observed in all the major glycerophospholipid classes, as was the increase in n-6 and n-9PUFA, although the importance of individual PUFA was dependent upon the specific class. However, the increased 18:0 with culture observed in total lipid was mainly restricted to PS, whereas the increase in 18:1n-9 was mainly associated with PC and the decreased percentages of 20:1 and 22:1 were associated primarily with PE/PS and PI, respectively (Tables 3,4).

Polyunsaturated fatty acid metabolism

To maximise the *in vivo* significance of the data, the metabolism of PUFA was only determined in primary cultures. However, as the morphology and lipid composition of the cells changed with time in culture, the experiments were carried out at two time points, 7 and 14 days.

Multifactorial ANOVA showed that the total lipid content of the cells was not dependent on either culture age or the fatty acid used in the incubation (Table 5). However, the uptake (pmol per 10^6 cells) and incorporation (pmol.mg^{-1} total lipid) of both radioactive LA and LNA was significantly greater in 14 day-old cultures compared to 7 day-old cultures, but there was no selectivity in incorporation between LA and LNA (Table 5).

Most of the radioactivity from both LA and LNA was incorporated in polar lipids (97-98%) and this was not affected by either cell age or fatty acid precursor (Table 6). With both PUFA, the greatest incorporation was into PC, followed by PE but a significantly lower percentage of LA was incorporated into PC, and more into PE, compared to LNA. There was little difference between cultures at 7- and 14-days in incorporation of LNA, but with LA there was significantly lower incorporation into PE at 14-days with significantly higher percentages of radioactivity associated with PC, PS and, especially, PI (Table 6). Presenting the data for incorporation into phospholipids relative to the amount of each class (% incorporation/% mass of phospholipid) showed that there was specific incorporation of both PUFA into phosphatidic acid/cardiolipin (PA/CL) and PC, whereas the incorporation into the other phospholipid classes was less than would be expected if incorporation was purely on a mass basis (Table 7).

There was greater conversion of 18:3n-3 *via* the desaturation/elongation pathway compared to 18:2n-6, as evinced by the lower percentage of [1-^{14}C]18:3n-3 that remained unmetabolized (Table 8). [1-^{14}C]18:3n-3 was primarily metabolized to pentaenes (20:5 and 22:5; totalling approx. 35 - 40%) and tetraenes (18:4 and 20:4; totalling 16 - 20%), with only 1.2 - 2.6% being converted to hexaene products. In contrast, [1-^{14}C]18:2n-6 was mainly metabolised to trienes (18:3 and 20:3; 19 - 31%) and tetraenes (20:4 and 22:4; 9 - 16%). The activity of the desaturase/elongase pathway was increased in older cultures with significantly lower percentages of both [1-^{14}C]18:2n-6 and [1-^{14}C]18:3n-3 remaining unmetabolized (Table 8). With LA, there were concomitant significant increases in the percentages of radioactivity associated with 18:3, 20:3 and AA. With LNA, there were similarly increased percentages of radioactivity associated with 18:4n-3, 20:4n-3 and EPA although they were not statistically significant.

Analysis of product/precursor ratios calculated for both n-6 and n-3 fatty acids showed that, the conversion of LA and LNA to AA and EPA, respectively, was significantly affected by both the precursor acid and the age of the culture (ratio a,

Table 9). Analysis of the two desaturase steps in the pathway showed that while Δ6 desaturase activity was increased significantly with cell age (ratio b), Δ5 desaturase activity was more affected by the fatty acid series (ratio c).

Discussion

The present study aimed to develop simple methodology for the preparation of cell cultures from rainbow trout skin and to utilize these cultures as a convenient *in vitro* model system in which to study the lipid and fatty acid metabolism of that tissue. The methods employed for sterilization of the tissue, dissociation of the skin cells and their aseptic culture were highly successful and a consistent high yield of cells in relatively homogeneous monolayers, ideal for metabolic studies, was obtained.

The morphological changes, from more epithelial-like to more classically fibroblast-like cells, observed from early primary cultures to older cultures, both primary and secondary, indicated time in culture was an important variable to control in the metabolic studies. Morphological changes in early passages of cultures are common, although the precise reason for the changes observed in the present study are unknown. However, culture conditions may be a factor as the morphology of the cells at the same time in culture varied with different basal media, *e.g.*, Leibovitz L-15, Eagles minimum essential medium (MEM), Dulbeccos modification of MEM (data not shown). The slight differences in osmolarity between the media will be a factor that can affect the cell morphology. Optimum culture conditions for fish cells have not been well studied and most of the commercially available media and supplements are optimized for mammalian cell cultures. Although Leibovitz L-15 medium supplemented with 10% FCS may not represent an optimized situation for maintaining fish skin cells in culture, it was the medium in which the rainbow trout skin cells were most stable and so was the basal medium of choice in the present study. It was not

clear if the transition to more fibroblast-like morphology observed in Leibovitz L-15 medium occurred as a result of the selection of fibroblasts already present in culture at time zero or was a process of de-differentiation of phenotype by epithelial-like cells. The progressive loss of differentiating potential of cells in culture has long been known (Freshney 1994) and has recently been reported in epidermal cell cultures obtained from explants of rainbow trout skin (Mothersill *et al*. 1995). Whatever the case, all the metabolic experiments in the present study were, therefore, performed in primary cultures before changes in cell morphology occurred.

Differences in the lipid and fatty acid profiles of the cells were also noted with time in culture although these were not necessarily related to morphology changes. The lipid composition of cells in culture can be very different from that of the tissues *in vivo* and reflects the lipid supplement in the media when that lipid supplement is non-limiting and *de novo* synthesis is inhibited (Bailey 1967; Spector *et al*. 1981). Therefore, as the serum supplement usually represents the only source of lipids available to cells cultured in most basal media, it is possible that differentiation state may also be influenced by the supply of lipids. The fact that the lipid available to cells in culture is derived almost exclusively from the serum supplement, which is invariably of mammalian origin, is particularly important when culturing fish cells, as this results in the cells being depleted in n-3 PUFA (Tocher *et al*. 1988). In mammalian cell cultures, serum-free formulations proved to be effective in selecting cell types from mixed cultures and in maintaining specialised cells in a differentiated state (Barnes and Sato 1980; Freshney 1994). Relatively few attempts of this kind have been made for fish cells, as cell growth cannot be supported for long periods under serum-free conditions. However, Collodi and Barnes (1980) successfully stimulated the growth of several fish cell lines in the absence of serum by supplementing the cultures with an extract derived from 21-day-old rainbow trout embryos. The growth of chinook salmon cell line, CHSE-214, in serum-free medium was supported by the inclusion of bovine serum albumin (Barlian and Bols 1991; Barlian *et al*. 1993). Recently, Mothersill *et al*.

(1995), succeeded in maintaining differentiated epidermal cells from rainbow trout skin explants using serum-free media.

The lipid and fatty acid compositions of the newly dissociated cells just after trypsinization (time zero) were very similar to the composition of skin (Ghioni *et al*. 1997). Therefore it is likely that many of the differences observed at 14 days and 4 months were due to the process of culture in FBS (Tocher *et al*. 1988, 1989, 1995a,b; Tocher and Sargent 1990a,b). For fish cells in culture, the routine culture conditions actually represent a situation of borderline EFA deficiency, as demonstrated by the changes in total lipid fatty acid compositions (Tocher *et al*. 1988). PUFA, especially EPA and DHA, were greatly reduced while saturates and short chain monoenes were increased. The same was also found in the skin *in vivo* when rainbow trout where fed an EFA-deficient diet (Ghioni *et al*. 1997). The lipid compositions of the individual phospholipids showed that the variations were not homogeneous in all the classes, although depletion of EPA and DHA was observed in all phospholipid classes. Retention of DHA by PS, which presented a high content of this fatty acid at time zero, was not observed, a finding different from that reported *in vivo* in trout fed an EFA deficient diet (Ghioni *et al*. 1997). The presence of n-9PUFA, particularly 20:2n-9, was another obvious sign of partial EFA deficiency in the cells, and it greatly increased with time in culture, especially from 14 days to 4 months.

It was particularly interesting that the cultures of rainbow trout skin cells were able to desaturate and elongate 18:2n-6 to 20:4n-6 and 18:3n-3 to 20:5n-3, a situation different from that reported for rat and guinea pig epidermal preparations incubated with either 18:2n-6 and 18:3n-6 (Chapkin and Ziboh 1984). Only fatty acyl elongase activity was found in a microsomal preparation of human epidermis (Chapkin and Ziboh 1984). In contrast, mammalian skin fibroblasts expressed an active desaturase/elongase pathway (Aeberhard *et al*. 1978; Rosenthal 1987; Raederstorff *et al*. 1995). The differentiation state of the rainbow trout skin cells used in the present study was not known precisely, but based on the the fatty acid metabolism data it is

likely that the fish skin cells are similar to mammalian skin fibroblasts and may not be directly comparable with mammalian epidermal cell preparations. However, the primary cultures used in the present study formed confluent monolayers rapidly, indicating that the cells cultured had originally constituted a significant portion of the skin tissue used and, therefore, it is reasonable to assume that they are representative of the metabolism of fish skin.

The present data showed that overall lipid metabolism of the fish skin cells increased with culture age, with a greater incorporation of radioactive EFA substrates, in terms of both per cell and unit total lipid, at 14 days, compared to 7 days. As discussed above, this effect may also be induced by the culture conditions, as the medium contained relatively low n-3 PUFA (Tocher *et al*. 1988). However, no preferential incorporation of 18:3n-3 compared to 18:2n-6 was observed with the trout skin cells, with two-way ANOVA showing that significant differences due to culture age only, and not fatty acid substrate. The overall recovery of radioactivity in individual phospholipid classes was little affected by the age of the culture when expressed relative to composition. However in percentage terms, the age of the culture affected the pattern of recovery of radioactivity in individual lipid classes in cells incubated with ^{14}C-LA, with greater recovery in PC and PI and lower recovery in PE. This reflected the increased desaturation and elongation of LA to 20:3n-6 and 20:4n-6 in 14 day compared to 7 day cultures, combined with the fact that the highest concentrations of 20:3n-6 and 20:4n-6 in 14-day old cultures were found in PC and PI, respectively. There was also increased desaturation and elongation of ^{14}C-LNA in 14-day old compared to 7-day old cultures, although only the decreased recovery of radioactivity in 18:3 was statistically significant, the increased recovery being spread between almost all the metabolites of 18:3.

Variations in PUFA metabolism with time in culture have been reported previously in studies on mammalian skin cells. In human keratinocytes, the incorporation of linoleic acid varied with time in culture but this was related to

differentiation state with increased incorporation of 18:2n-6 in differentiated cells (Schürer *et al*. 1995). In human skin fibroblasts, AA production and the overall activity of the desaturase/elongase pathway decreased with the number of passages in culture (Raederstorff *et al*. 1995). Although this is the opposite effect to that observed in the present study with trout skin cells, the time scale in the human skin fibroblast study was considerably greater and was an indication of cellular aging rather than an adaptation to environmental conditions.

High levels of DHA were present in newly dissociated trout skin cells, especially esterified to PS and PE, as already demonstrated for trout skin *in vivo* (Ghioni *et al*. 1997). However, the metabolism data revealed that very little DHA was formed from 18:3n-3, despite the abundant transformation of the EFA precursor to EPA. The low rate of DHA production in the trout skin cells suggests that the high DHA levels usually found in the tissue may be derived predominantly from the uptake of preformed DHA of exogenous origin. The retention of DHA by phospholipids like PS in skin of trout in EFA deficient conditions and the possible mobilisation of this fatty acid from other tissues (Ghioni *et al*. 1997) are in accord with the metabolic data at the cellular level. However, low levels of "Δ4 desaturation" are not an unusual observation in cultured fish cells, with only relatively low levels of DHA produced in rainbow trout gonad (RTG-2; fibroblast), Atlantic salmon (AS; epithelial-type) and turbot fin (TF; fibroblast) cell lines (Tocher and Sargent 1990a). Similarly, only low levels of DHA were produced from [1-^{14}C]18:3n-3 in primary cultures of trout and turbot brain astrocytes and, although greater percentages of DHA were produced from [1-^{14}C]20:5n-3, the conversions were still low (Tocher and Sargent 1990b; Tocher 1993). Therefore, the present study has shown that the primary cells from trout skin display an EFA metabolism very similar to that observed in the previous studies on both an established trout fibroblast line and primary astrocytes, with all the cells expressing relatively high levels of Δ6 and Δ5 desaturase activities but only low "Δ4 desaturation". This is interesting as there is good evidence that the production of DHA in trout

hepatocytes proceeds via elongation of EPA to 24:5n-3, followed by Δ6 desaturation to 24:6n-3 and peroxisomal chain shortening to 22:6n-3 (Buzzi *et al*. 1996) as initially proposed in rat liver by Voss *et al*. (1991). The cultured cell data suggests that different Δ6 desaturase enzymes may be involved in the pathway of DHA production from 18:3n-3.

Comparison between the metabolism of n-6 and n-3PUFA showed that the Δ6 desaturase activity was not significantly influenced by the series of PUFA but was affected by culture age. As lipid composition of cells was also influenced by culture age, a different lipid environment in the membranes could be responsible for the changes in Δ6 enzyme activity (Brenner 1981). In contrast, Δ5 desaturase activity was mostly dependent on the EFA substrate, being higher for n-3, as previously observed in many other cultured cells from fish (Tocher and Sargent 1990a,b; Tocher 1993). The metabolism of n-3 PUFA has not been extensively studied in mammalian skin cell preparations and so comparisons with the data obtained in the present study cannot be drawn although, in general, n-3PUFA are better substrates for the desaturase/elongase pathways in mammals too (Brenner 1974, 1981). However, n-3PUFA may play a more prominent role in fish skin than in mammalian skin. Certainly eicosanoids derived from EPA have been detected in fish skin tissue as well as the AA-derived eicosanoids usually reported in mammalian skin (Knight *et al*. 1995; Ghioni *et al*. 1997).

In the present study a simple method for the preparation of cell cultures from rainbow trout skin was developed and the cultures obtained were used to study the lipid and fatty acid metabolism of that tissue. During the process of culture there was a change in the morphology of the cells from more epithelial-like to fibroblastic-like which was accompanied by alterations in the lipid composition and metabolism. However, it was unclear if these changes were directly linked. The cells obtained incorporated and metabolized [1-^{14}C]18:2n-6 and [1-^{14}C-]18:3n-3 by desaturation/elongation pathways with 20:4n-6 and 20:5n-3 being the major end

products, with further desaturation of 20:5n-3 to 22:6n-3 being low. Whereas Δ6 desaturase activity was increased significantly with cell age, Δ5 desaturase activity was more influenced by the fatty acid series, with 18:3n-3 being more readily transformed to 20:5n-3 than 18:2n-6 to 20:4n-6. Overall, the data suggested that the trout skin cell cultures were more similar to mammalian skin fibroblasts rather than mammalian epidermal/keratinocyte cultures.

Acknowledgement

CG was supported by a European Community Human Capital and Mobility Grant, EABBIOTECH, EU (N. BIO2-CT-94-6390).

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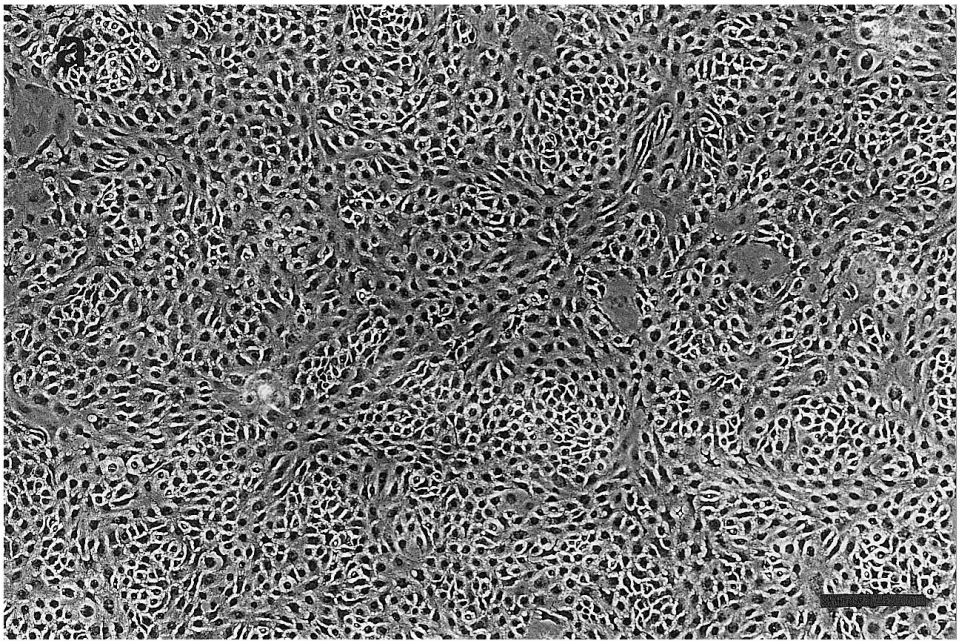
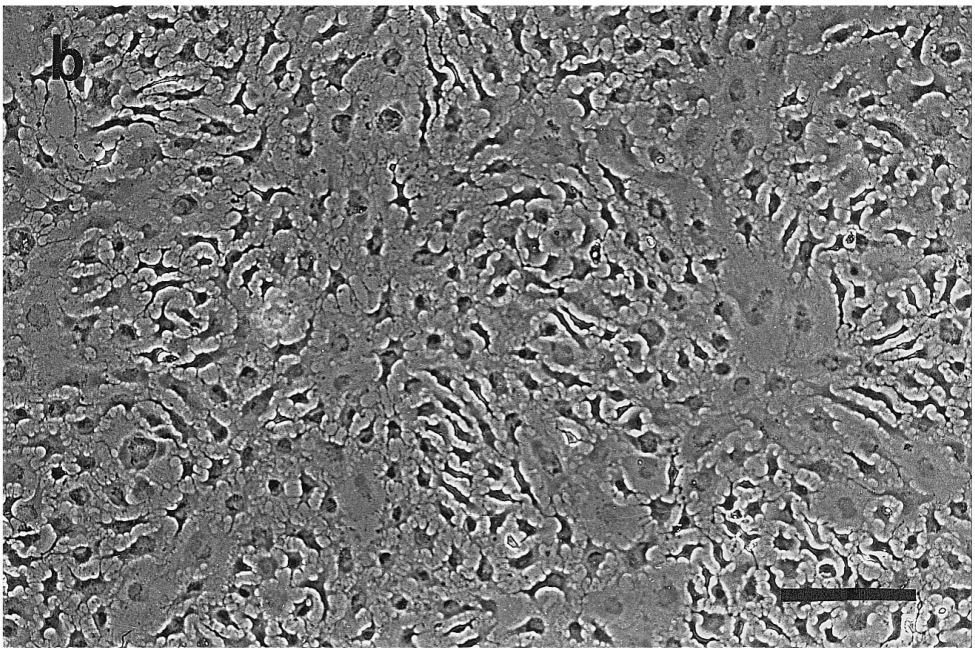
Legend to Figure

Fig. 1. Morphology of rainbow trout skin cells in primary culture. Cells were prepared and maintained as described in the Materials and Methods section. Frames a & b show cells after 7 days in culture that displayed predominantly epithelial-type morphology. Frames c & d show cells after 14 days in culture that displayed predominantly fibroblast-type morphology. Phase-contrast photomicrography was performed with an Olympus IMT-2 inverted microscope and Ilford FP4 (125ASA) film. Frames a and c, x 40 (bar = 100 μm) ; frames b & d, x 100 (bar = 50 μm).

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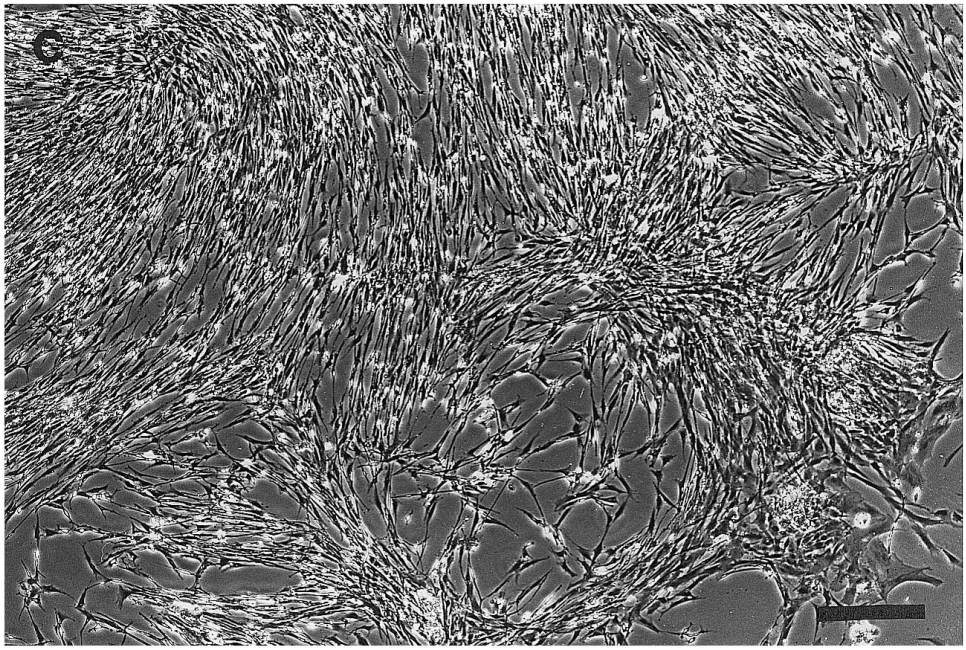
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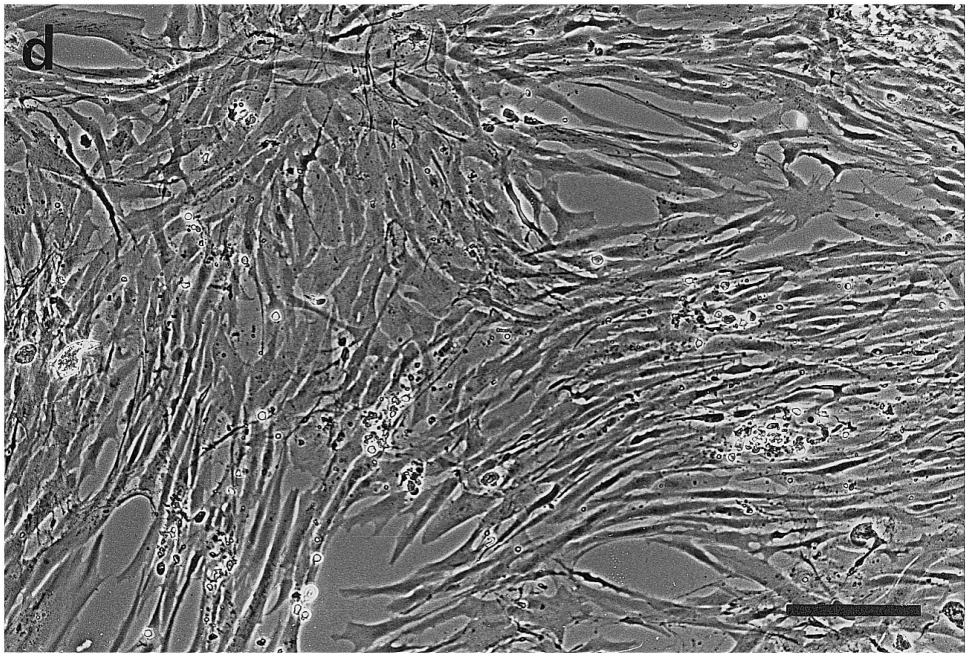
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Table 1. Lipid class compositions of skin cells upon isolation, after 14 days in primary culture and after 4 months (passage 9) in secondary culture.

	Time in culture											
Lipid class	Initially isolated				14 days				4 months			
% of Total Lipids												
SM	4.9	±	2.4		4.6	±	1.0		3.7	±	0.4	
PC	14.2	±	5.0	b	30.3	±	1.0	a	31.2	±	1.1	a
PS	4.5	±	1.8	b	7.6	±	0.1	a	7.2	±	0.4	ab
PI	1.6	±	0.5	b	5.0	±	0.2	a	5.4	±	0.2	a
PA/CL	1.0	±	0.4	b	1.6	±	0.1	b	2.5	±	0.1	a
PE	10.1	±	3.2	b	13.6	±	0.3	ab	19.4	±	0.2	a
Total polar lipids	36.3	±	13.1	b	62.6	±	0.8	a	69.5	±	1.0	a
Cholesterol	16.7	±	4.2	b	24.6	±	0.5	a	23.3	±	0.8	a
FFA	3.4	±	0.7		3.0	±	0.5		2.1	±	0.2	
TAG	38.0	±	16.1	a	3.4	±	1.5	b	2.7	±	0.5	b
Sterol esters	5.6	±	1.8	ab	6.3	±	0.2	a	2.4	±	1.3	b
Total neutral lipids	63.7	±	13.1	a	37.4	±	0.8	b	30.5	±	1.0	b
% of Polar lipids												
SM	14.1	±	2.0	a	7.3	±	1.5	b	5.4	±	0.6	b
PC	38.5	±	0.5	c	48.4	±	1.2	a	45.0	±	0.9	b
PS	12.2	±	1.0	a	12.1	±	0.3	a	10.3	±	0.4	b
PI	4.4	±	0.7	b	8.0	±	0.4	a	7.8	±	0.2	a
PA/CL	2.8	±	0.2	a	2.5	±	0.1	a	3.6	±	0.2	b
PE	28.0	±	1.9	a	21.7	±	0.7	b	27.9	±	0.6	a

Results are means ± SD (n=3). Values in the same row with different superscript letters are significantly different ($p < 0.05$).

FFA, free fatty acids; PA/CL, phosphatidic acid/cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerol.

Table 2. Total lipid fatty acid compositions of rainbow trout skin cells at different times of culture.

	Time in culture											
Fatty acids	Initially isolated				14 days				4 months			
14:0	2.5	±	1.0		1.0	±	0.4		1.2	±	0.3	
16:0	14.2	±	0.4		13.9	±	0.4		15.4	±	2.1	
18:0	4.3	±	1.0	b	10.1	±	0.6	a	10.0	±	0.5	a
Total saturates	21.7	±	0.7	b	26.1	±	0.1	a	27.5	±	2.8	a
16:1	6.1	±	0.2	b	5.0	±	0.4	c	6.9	±	0.2	a
18:1 n-9	15.7	±	1.3	b	23.3	±	0.8	a	26.3	±	1.4	a
18:1 n-7	2.7	±	0.3	b	3.2	±	0.2	b	4.2	±	0.3	a
20:1	6.0	±	1.8	a	0.9	±	0.3	b	0.5	±	0.4	b
22:1	5.5	±	1.5	a	0.3	±	0.2	b	0.1	±	0.1	b
24:1	0.5	±	0.2		0.7	±	0.2		0.6	±	0.4	
Total monoenes	36.6	±	4.4		33.4	±	0.7		38.7	±	1.6	
18:2 n-6	4.2	±	0.9	a	1.5	±	0.0	b	1.3	±	0.0	b
18:3 n-6	0.2	±	0.0	b	0.5	±	0.0	a	0.6	±	0.0	a
20:2 n-6	1.0	±	0.5		0.8	±	0.1		1.1	±	0.1	
20:3 n-6	0.4	±	0.2	c	1.5	±	0.1	b	2.0	±	0.1	a
20:4 n-6	2.2	±	1.1	c	8.6	±	0.5	a	6.5	±	0.3	b
22:4 n-6	n.d.				n.d.				0.4	±	0.0	
22:5 n-6	0.8	±	0.0	a	0.8	±	0.1	a	n.d.			b
Total n-6 PUFA	8.6	±	1.2	b	13.6	±	0.7	a	11.8	±	0.3	a
18:3 n-3	0.8	±	0.3	a	0.2	±	0.1	b	n.d.			c
18:4 n-3	1.1	±	0.5	a	0.5	±	0.1	a	n.d.			b
20:4 n-3	1.4	±	0.1	a	0.4	±	0.4	b	0.1	±	0.1	b
20:5 n-3	5.8	±	1.4	a	2.5	±	0.3	b	1.2	±	0.1	c
22:5 n-3	1.4	±	0.0	b	3.7	±	0.3	a	3.3	±	0.2	a
22:6 n-3	14.4	±	0.7	a	8.6	±	0.5	b	4.7	±	0.0	c
Total n-3 PUFA	25.0	±	1.5	a	16.0	±	0.4	b	9.2	±	0.2	c
18:2 n-9	t			c	1.1	±	0.3	b	3.4	±	0.2	a
20:2 n-9	n.d.			b	n.d.			b	1.0	±	0.1	a
20:3 n-9	n.d.			b	n.d.			b	0.6	±	0.1	a
Total n-9 PUFA	t			c	1.1	±	0.3	b	5.0	±	0.2	a
C16 PUFA	1.6	±	0.3	a	0.5	±	0.0	b	n.d.			c
Total PUFA	35.2	±	2.8	a	31.2	±	1.2	a	26.1	±	0.7	b
Dimethylacetals	1.0	±	0.6		3.5	±	2.0		3.8	±	2.6	
n-3/n-6	2.9	±	0.3	a	1.2	±	0.0	b	0.8	±	0.0	c

Results are percentages of weight (means ± SD; n=3); values in the same row with different superscript letters are significantly different ($p<0.05$). Total saturates includes 15:0, 17:0 and 20:0; 16:1 predominantly 16:1n-7; 20:1 includes 20:1 n-11, 20:1 n-9 and 20:1 n-7; 22:1 includes 22:1n-11, 22:1n-9 and 22:1n-7. PUFA, polyunsaturated fatty acids: n.d., not detected; t, trace (< 0.05%).

Table 3. Fatty acid compositions of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) from rainbow trout skin cells at different times of culture.

	PC			PE		
Fatty acid	ii	14d	4m	ii	14d	4m
14:0	2.8	1.0	0.3	0.4	0.5	0.3
16:0	25.6	17.2	15.6	5.3	5.3	3.9
18:0	2.8	4.8	5.9	4.3	6.7	6.0
Total saturates	32.1	23.7	22.1	10.3	13.1	10.2
16:1	10.2	5.7	8.4	2.2	3.9	4.6
18:1 n-9	15.9	31.2	31.6	16.1	13.0	23.4
18:1 n-7	2.0	3.5	4.0	4.2	4.2	4.9
20:1	1.5	0.4	1.2	4.6	0.6	0.7
22:1	0.5	0.4	n.d.	0.5	n.d.	n.d.
24:1	0.2	0.1	0.5	0.1	0.2	n.d.
Total monoenes	30.2	41.3	45.7	27.7	21.9	33.6
18:2 n-6	2.0	1.5	1.7	2.7	1.5	1.4
18:3 n-6	0.1	0.2	0.8	0.1	0.1	0.5
20:2 n-6	0.3	0.6	0.8	0.6	0.3	0.7
20:3 n-6	0.2	1.8	2.5	0.2	1.0	1.4
20:4 n-6	2.4	8.1	6.0	3.2	10.5	10.8
22:4 n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
22:5 n-6	1.4	1.3	n.d.	0.8	0.5	n.d.
Total n-6 PUFA	6.4	13.5	11.7	7.6	13.8	14.8
18:3 n-3	0.2	0.1	n.d.	0.2	n.d.	n.d.
18:4 n-3	0.2	0.1	n.d.	0.1	0.6	n.d.
20:4 n-3	0.5	0.2	n.d.	0.3	0.3	n.d.
20:5 n-3	10.0	4.4	2.5	7.7	1.7	1.3
22:5 n-3	1.2	3.0	2.9	1.1	5.4	4.7
22:6 n-3	12.8	5.4	3.1	27.6	14.4	7.3
Total n-3 PUFA	25.0	13.2	8.4	37.2	22.4	13.2
C16 PUFA	0.1	0.1	n.d.	0.3	0.4	n.d.
18:2 n-9	0.9	1.6	5.0	2.8	1.9	5.2
20:2 n-9	n.d.	n.d.	1.0	n.d.	n.d.	0.9
20:3 n-9	n.d.	n.d.	0.5	n.d.	n.d.	n.d.
Total n-9 PUFA	0.9	1.6	6.5	2.8	1.9	6.1
Total PUFA	32.5	28.4	26.9	47.9	38.5	34.1
Dimethylacetals	0.6	1.8	1.7	8.6	21.7	17.4
n-3/n-6	3.9	1.0	0.7	4.9	1.6	0.9

Results are percentages of weight. Total saturates includes 15:0, 17:0 and 20:0; 16:1 predominantly 16:1n-7; 20:1 includes 20:1n-11, 20:1 n-9 and 20:1 n-7; 22:1 includes 22:1n-11, 22:1n-9 and 22:1n-7.

ii, initially isolated; 14d, 14 days; 4m, 4 months.

PUFA, polyunsaturated fatty acids; n.d., not detected.

Table 4. Fatty acid compositions of phosphatidylserine (PS) and phosphatidylinositol (PI) from rainbow trout skin cells at different times of culture.

	PS			PI		
Fatty acid	ii	14d	4m	ii	14d	4m
14:0	0.6	0.4	0.3	0.9	0.6	0.5
16:0	7.7	6.3	4.5	9.2	5.1	4.4
18:0	15.9	35.0	35.7	24.7	27.2	24.2
Total saturates	25.4	42.4	41.4	35.8	33.5	29.9
16:1	1.0	1.0	1.8	1.9	1.5	3.7
18:1 n-9	8.8	9.6	11.9	8.6	10.4	13.0
18:1 n-7	2.8	2.0	2.7	1.9	3.2	2.6
20:1	4.9	0.8	1.1	1.2	1.0	2.5
22:1	0.3	n.d.	n.d.	4.1	0.6	n.d.
24:1	n.d.	0.3	0.4	1.1	0.2	n.d.
Total monoenes	17.9	13.7	17.9	18.7	16.9	21.8
18:2 n-6	1.0	0.8	0.8	1.0	0.6	0.7
18:3 n-6	0.1	0.7	0.8	0.3	0.2	n.d.
20:2 n-6	0.6	0.6	0.5	1.4	1.9	2.2
20:3 n-6	0.1	0.3	3.4	0.4	1.7	2.0
20:4 n-6	0.5	3.1	4.8	20.2	31.5	28.8
22:4 n-6	n.d.	n.d.	0.8	n.d.	n.d.	n.d.
22:5 n-6	1.6	0.6	n.d.	1.3	1.6	n.d.
Total n-6 PUFA	4.0	6.1	11.1	24.5	37.6	33.6
18:3 n-3	0.2	0.3	n.d.	0.4	0.1	n.d.
18:4 n-3	0.4	0.5	n.d.	0.2	n.d.	n.d.
20:4 n-3	0.1	0.3	n.d.	0.3	1.4	n.d.
20:5 n-3	0.9	0.5	0.2	6.0	0.4	0.3
22:5 n-3	1.7	8.1	8.9	0.6	1.9	2.6
22:6 n-3	41.8	21.3	13.3	4.8	1.7	2.1
Total n-3 PUFA	45.1	31.1	22.5	12.3	5.5	5.0
C16 PUFA	0.3	0.2	n.d.	n.d.	0.2	n.d.
18:2 n-9	1.0	0.6	3.6	n.d.	0.5	3.1
20:2 n-9	n.d.	n.d.	1.4	n.d.	n.d.	1.2
20:3 n-9	n.d.	n.d.	0.2	n.d.	n.d.	0.5
Total n-9 PUFA	1.0	0.6	5.2	n.d.	0.5	4.7
Total PUFA	50.3	38.0	38.7	36.8	43.7	43.4
Dimethylacetals	1.3	1.6	n.d.	3.3	1.5	n.d.
n-3/n-6	11.4	5.1	2.0	0.5	0.1	0.1

Results are percentages of weight. Total saturates includes 15:0, 17:0 and 20:0; 16:1 predominantly 16:1n-7; 20:1 includes 20:1n-11, 20:1n-9 and 20:1n-7; 22:1 includes 22:1n-11, 22:1n-9 and 322:1n-7. PUFA, polyunsaturated fatty acids; n.d., not detected; ii, initially isolated; 14d, 14 days; 4m, 4 months.

Table 5. Lipid content and incorporation of radioactivity into total lipids of rainbow trout skin cells incubated with [1-14C]18:2n-6 and [1-14C]18:3n-3 at different times in primary culture.

	[1-14C]18:2 n-6				[1-14C]18:3 n-3				Multifactorial ANOVA		
	7 days		14 days		7 days		14 days		FA	time	inter.
Total lipid content											
ug lipid per million cells	103.0 ± 2.8		108.8 ± 4.1		143.4 ± 30.9		132.3 ± 23.6				
Incorporation of radioactivity											
pmol per million cells	1.9 ± 0.1		3.6 ± 0.3		2.1 ± 0.1		3.9 ± 0.5			*	
pmol per mg total lipid	18.5 ± 0.5		32.7 ± 3.8		14.6 ± 2.6		29.4 ± 1.1			*	
% total activity applied	37.8 ± 1.2		49.9 ± 9.1		27.3 ± 0.8		48.0 ± 5.3			*	

Results are means ± SD (n=3); *, significantly different values ($p<0.05$). ANOVA, analysis of variance;

FA, fatty acid effects; time, effects of time in culture; inter., interaction of FA and time effects.

Table 6. Incorporation of radioactivity into lipid classes from rainbow trout skin cells incubated with radiolabeled 18:2n-6 and 18:3n-3 at different times in culture.

	18:2 n-6				18:3 n-3				Multifactorial ANOVA		
Lipid class	7 days		14 days		7 days		14 days		FA	time	inter.
SM	3.9	± 0.1	3.3	± 0.3	1.4	± 0.0	1.8	± 0.3	*		
PC	53.9	± 0.0	57.3	± 0.1	75.0	± 0.2	75.0	± 1.1	*		
PS	2.4	± 0.1	3.6	± 0.4	2.2	± 0.1	2.2	± 0.3	*	*	*
PI	3.7	± 0.1	7.0	± 1.5	1.2	± 0.1	1.2	± 0.1	*	*	*
PA/CL	5.2	± 0.3	4.8	± 0.3	4.0	± 0.3	3.1	± 0.0	*	*	
PE	28.3	± 0.6	22.0	± 2.0	14.4	± 0.4	13.8	± 0.2	*	*	*
TPL	97.4	± 0.2	98.0	± 0.4	98.2	± 0.2	97.1	± 0.2			*
TAG-CE	2.6	± 0.2	2.0	± 0.4	1.8	± 0.2	2.9	± 0.2			*

Results are expressed as percentages of total radioactivity recovered and are means ± SD (n=3).

PA/CL, phosphatidic acid/cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; TAG-CE, triacylglycerol and cholesteryl ester; TPL, total polar lipids. *, significantly different values ($p < 0.05$).

ANOVA, analysis of variance; FA, effects of fatty acids; time, effects of time in culture; inter., interactions between effects of fatty acids and time in culture.

Table 7. Incorporation/composition ratios in rainbow trout skin cells incubated with radiolabeled 18:2n-6 and 18:3n-3 at different times of culture.

	18:2 n-6				18:3 n-3				Multifactorial ANOVA		
Lipid class	7 days		14 days		7 days		14 days		FA	time	inter.
SM	0.4	± 0.02	0.5	± 0.07	0.2	± 0.00	0.2	± 0.02	*		
PC	1.3	± 0.01	1.2	± 0.05	1.9	± 0.08	1.7	± 0.04	*		
PS	0.2	± 0.02	0.4	± 0.12	0.2	± 0.01	0.2	± 0.03			
PI	0.6	± 0.01	1.2	± 0.45	0.2	± 0.01	0.2	± 0.01	*		
PA/CL	2.1	± 0.01	4.1	± 1.39	1.8	± 0.16	2.3	± 0.22			
PE	1.1	± 0.03	0.9	± 0.07	0.6	± 0.02	0.6	± 0.07	*	*	*

Results are expressed as a ratio of the % of radioactivity incorporated/% composition for each class and are means ± SD (n=3). PA/CL, phosphatidic acid/cardiolipin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; *, significantly different values ($p < 0.05$). ANOVA, analysis of variance; FA, effects of fatty acids; time, effects of time in culture; inter., interactions between effects of fatty acids and time in culture.

Table 8. Desaturation and elongation of [1-14C]18:2n-6 and [1-14C]18:3n-3 by rainbow trout skin cells at different times of culture.

	[1-14C] 18:2 n-6								[1-14C] 18:3 n-3							
Fatty acid	7 days			14 days					Fatty acid	7 days			14 days			
18:2	65.3	±	0.3	47.8	±	5.0	*		18:3	41.4	±	0.9	32.2	±	3.2	*
18:3	7.6	±	0.2	9.7	±	0.0	*		18:4	8.1	±	0.2	11.0	±	1.3	
20:2	3.2	±	0.1	3.3	±	0.2			20:3	4.5	±	0.6	3.9	±	0.3	
20:3	11.3	±	0.0	21.0	±	1.6	*		20:4	8.1	±	0.5	9.0	±	0.1	
20:4	7.1	±	0.0	14.2	±	2.2	*		20:5	29.5	±	1.2	33.6	±	3.7	
22:2	2.9	±	0.1	1.5	±	0.1	*		22:4	0.8	±	0.4	0.8	±	0.0	
22:3	1.1	±	0.5	1.0	±	0.2			22:5	6.4	±	0.3	6.9	±	0.8	
22:4	1.6	±	0.1	1.5	±	0.7			24:6+22:6	1.2	±	0.4	2.6	±	0.7	

Results are expressed as percentages of total radioactivity recovered and are means ± SD (n=3). *, significantly different values ($p<0.05$).

Table 9. PUFA metabolism ratios in rainbow trout skin cells incubated with radiolabeled 18:2n-6 and 18:3n-3 at different times in culture.

		n-6			n-3			Multifactorial ANOVA		
Ratio		7 days	14 days		7 days	14 days		FA	time	inter.
a	20:4/18:2	0.1 ± 0.0	0.3 ± 0.1	20:5/18:3	0.7 ± 0.0	1.1 ± 0.2		*	*	
b	20:3/18:2	0.2 ± 0.0	0.4 ± 0.1	20:4/18:3	0.2 ± 0.0	0.3 ± 0.0			*	*
c	20:4/20:3	0.6 ± 0.0	0.7 ± 0.1	20:5/20:4	3.6 ± 0.4	3.7 ± 0.4		*		*

Data are expressed as the ratio of percentages of radioactivity recovered in the various fatty acids.

Values are means ± SD (n=3). *, significantly different values ($p<0.05$). ANOVA, analysis of variance; FA, effects of fatty acids; time; effects of time in culture; inter., interaction between effects of fatty acids and time in culture.