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In vivo metabolism of [1-^{14}C]linolenic acid (18:3($n-3$)) and [1-^{14}C]eicosapentaenoic acid (20:5($n-3$)) in a marine fish: Time-course of the desaturation/elongation pathway

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Abstract

The metabolism (via the desaturation/elongation pathways) of [1-^{14}C]18:3($n-3$) and [1-^{14}C]20:5($n-3$) in a marine fish, gilthead sea bream (*Sparus aurata* L.), were investigated over 8 days to determine the time-courses for the production of Δ^6 and Δ^5-desaturase products and 22:6($n-3$). Fish were starved for 1 week prior to, and during, the period of the experiment. The recovery of radioactivity from [1-^{14}C]20:5($n-3$) in tissue lipids exceeded that of [1-^{14}C]18:3($n-3$) at all time points. The recoveries of both fatty acids decreased by 85–89% between days 2 and 8, indicating that substantial loss of radioactivity due to β-oxidation occurred. Incorporation of 18:3($n-3$) and 20:5($n-3$) was predominantly into triacylglycerol but during the time-course of the experiment there were decreased percentages of radioactivity from both labelled fatty acids recovered in triacylglycerol with concomitant increased percentages recovered in phospholipids indicating preferential oxidation of fatty acids in triacylglycerol and/or redistribution of incorporated fatty acids. Recovery of radioactivity in 22:6($n-3$) was 10-fold greater with [1-^{14}C]20:5($n-3$) than with [1-^{14}C]18:3($n-3$). However, there were few consistently significant trends in the levels of components of the desaturation/elongation pathways during the time-course of the experiment. In particular, the relative recovery of radioactivity in 22:6($n-3$) did not increase during the experiment with either substrate. Substantial amounts of radioactivity were found in 24:5($n-3$) and 24:6($n-3$), particularly after injection with [1-^{14}C]20:5($n-3$), indicating that the conversion of 20:5($n-3$) to 22:6($n-3$) in sea bream may occur by a pathway utilizing Δ^6-desaturase activity rather than by a Δ^4-desaturation.

Key words: Linolenic acid; Eicosapentaenoic acid; Metabolism; Desaturation; Elongation; (Fish)

1. Introduction

Animals do not possess the Δ^{12}- and Δ^{15}-fatty acid desaturase enzymes necessary for the biosynthesis of linoleic (18:2($n-6$)) and linolenic (18:3($n-3$)) acids, respectively, from oleic acid, 18:1($n-9$) [1,2]. The requirement for dietary C_{18} polyunsaturated fatty acid (PUFA) is related to the ability of the species to convert the C_{18} PUFA, via the desaturation/elongation pathways, to the longer-chain more unsaturated C_{20} and C_{22} PUFA. Nutritional and biochemical studies indicated that freshwater fish, or at least salmonids, are capable of converting C_{18} PUFA of both the ($n-3$) and ($n-6$) series, specifically 18:2($n-6$) and 18:3($n-3$), to their highly unsaturated derivatives (HUFA), 20:4($n-6$)/22:5($n-6$) and 20:5($n-3$)/22:6($n-3$), respectively, to a great extent [1,2]. Marine fish, however, are deficient in these conversions and so require preformed HUFA in the diet. There is accumulating evidence that this is due to marine fish having only very low levels of Δ^5-desaturase activity [2–6].

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Abbreviations: 22:6($n-3$), 4,7,10,13,16,19-docosahexaenoic acid; 20:5($n-3$), 5,8,11,14,17-eicosapentaenoic acid; HUFA, highly unsaturated fatty acid(s) ($\geq C_{20}$ with ≥ 3 double bonds); 18:3($n-3$), 9,12,15-octadecatrienoic acid (α-linolenic acid); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acid(s); TAG, triacylglycerol.

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Several recent in vivo studies, using intraperitoneally-injected radioactively-labelled C_{18} PUFA have been carried out in order to determine the ability of fish species to synthesize C_{20} and C_{22} HUFA [7–11]. The results of those experiments appear to confirm that there is a fundamental difference between freshwater fish and marine fish in their abilities to convert C_{18} PUFA to C_{20} and C_{22} HUFA, with the freshwater species studied having a high capacity for desaturation and elongation of C_{18} PUFA, whereas the marine species studied had a low capacity for this conversion.

In mammals, deficiencies in the desaturase/elongase pathways have been associated with dietary habits, with extreme carnivores, such as cats, lacking Δ^6- and Δ^5-desaturases [12]. The marine fish species studied, included turbot *Scophthalmus maximus* L. [8], gilthead sea bream *Sparus aurata* L. [10] and golden grey mullet *Liza aurata* L. [11] (carnivorous esthenohaline, carnivorous eurihaline and herbivorous eurihaline, respectively) had various biological and ecological differences, including different feeding habits, but displayed a similar pattern of PUFA metabolism: a limited ability to convert C_{18} PUFA to C_{20} and C_{22} HUFA. The evidence is consistent with the hypothesis that low Δ^5-desaturase activity is a general characteristic of marine fish which prevails in spite of habitat, behaviour and feeding habits.

It is well known that fatty acid desaturases, particulary Δ^6-desaturase, have relatively low substrate specificity which is similar in mammals [13] and fish [2,14–19]. The low substrate specificity has several consequences, one of which is that $18:3(n-3)$, $18:2(n-6)$ and $18:1(n-9)$ all compete for the Δ^6-desaturase which rate limits the conversion of C_{18} PUFA to C_{20} and C_{22} HUFA. The efficiency of competition either in mammals [20] or fish [18] is $18:3(n-3) > 18:2(n-6) \gg 18:1(n-9)$. In addition, the Δ^6-desaturase is subjected to retro-inhibition by HUFA [2,18–20]. Another consequence of the competitive interactions between dietary PUFA and HUFA and desaturase activities relates to the presumed Δ^4-desaturation step. Quantitatively meaningful conversion of $20:5(n-3)$ to $22:6(n-3)$ has been difficult to establish with certainty in a range of systems from various species including mammals and fish. This has led to some authors to question the existence of a Δ^4-desaturase per se and it has been suggested recently that Δ^6-desaturase may be responsible for the presence of Δ^4 bonds as in $22:6(n-3)$ (4,7,10,13,16,19-docosahexaenoic acid) [23,24]. Briefly, microsomal fatty acid elongase converts $22:5(n-3)$ to $24:5(n-3)$ which is then Δ^6-desaturated to $24:6(n-3)$ [23,24]. The final product $22:6(n-3)$ is produced by limited chain-shortening via peroxisomal β-oxidation. This is not only a longer and more complex process than a direct Δ^4-desaturase activity, but also complicates the desaturase pathway by introducing further substrates and potential competitive inhibitors for Δ^6-desaturase.

The foregoing process is particulary important in marine fish as there is currently great uncertainty about whether $20:5(n-3)$ can be bioconverted to $22:6(n-3)$ at a rate sufficient to meet the requirements of the developing nervous system in larval and juvenile fish, particulary when diets are rich in $20:5(n-3)$ but relatively deficient in $22:6(n-3)$ [21,22]. Studies of PUFA metabolism have established that $20:5(n-3)$ can be bioconverted to $22:6(n-3)$ in marine fish, but only at a relatively slow rate [8,10,11]. However, all previous in vivo experiments were performed over only 2 days [8–11]. The aim of the present study was to investigate the desaturation/elongation pathways in vivo in a marine fish using a substantially longer time-course of 8 days. The metabolism, via these pathways, of [1-^{14}C]$18:3(n-3)$ and [1-^{14}C]$20:5(n-3)$ was investigated to determine the time-courses for the production of fatty acid products with Δ^6-, Δ^5- and Δ^4-unsaturation.

2. Materials and methods

Fish

Juvenile gilthead sea bream, *Sparus aurata* L., of 2–5 g, reared on a commercial diet, were obtained from a commercial marine fish farm (Cultivos Piscícolas Marinos S.A., San Fernando (Cádiz), Spain). The fish were maintained in 50 l rectangular fibre-glass tanks, supplied with underground sea water (salinity 32 g/l and temperature 20 ± 1°C) in an open circuit system with aeration. Prior to experimentation, the fish were fed with a commercial dry pelleted diet in a daily ration of 5% of total body weight.

Experimental protocol

It was previously established that radioactive fatty acids (3–5 μCi), dissolved in a small volume of ethanol (5–20 μl) could be injected into the intraperitoneal cavity of anaesthetized fish without apparent detrimental effects, yielding sufficient levels of radioactivity in the body lipids to permit subsequent analyses [8,9]. In the present study, fourteen groups of three fish were starved for 1 week prior to injection, and during the course of the experiment after injection, to ensure no dietary input of C_{20} and C_{22} HUFA and to maximize the rate of bioconversion of the injected ^{14}C-labelled $18:3(n-3)$ and $20:5(n-3)$. Four groups of three fish were injected with [1-^{14}C]$18:3(n-3)$ and another four groups were injected with [1-^{14}C]$20:5(n-3)$, each fish receiving a dose of 3 μCi of either labelled PUFA suspended in 5 μl of ethanol. The fish in two further groups of three fish were injected with 5 μl of ethanol alone as a sham injection control. Fish were injected

after anaesthetization by hypothermic shock [25], whereby the fish were placed in a beaker containing a 1:1 mixture of sea water and crushed ice for 1–2 min until immobilized, as it had been shown that some commonly used fish anaesthetics may interfere in fatty acid metabolism [26]. No mortalities occurred during starvation or during the incubation period after the fish were injected with radioactive PUFA. After injection, the groups of fish were maintained in cylindrical glass aquaria containing 2 l of sea water with aeration. Fish recovered from anesthesia and injection within 5 min with no apparent ill effects. Fish injected with ^{14}C-labelled PUFA were removed from the aquaria in groups of three after 2, 4, 6 and 8 days, carefully blotted with filter paper, killed by immersion in liquid nitrogen, and the wet weight determined. Three groups of three fish were used to determine lipid class and fatty acid compositions during the time-course experiment. These fish were removed at days 0, 7 and 15, corresponding to the beginning of the starvation period, the injection date and the end of the time-course experiment with day 7 and 15 fish being the sham-injection groups. The remaining three groups of three fish were used for dry weight determination throughout the experiment; wet weight was determined as described above, fish were finely chopped, desiccated at 110°C for 24 h and then cooled in vacuo before dry weight was determined.

Lipid extraction

Lipids were extracted from weighed fish by homogenization in chloroform/methanol (2:1, v/v), containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant, by a modification of the method of Folch et al. [27], as described previously [28]. The lower solvent phase was evaporated under a stream of nitrogen and lipid extracts desiccated overnight in vacuo before weighing. Lipid extracts were redissolved in chloroform/methanol (2:1, v/v) at a concentration of 50 mg/ml and stored under an atmosphere of nitrogen at −20°C until analysis. An aliquot of the total lipid was taken for determination of radioactivity as described below.

Lipid class quantification

Lipid classes were separated by high-performance thin-layer chromatography (HPTLC) using a single-dimension double-development method described previously [28]. The classes were quantified by charring followed by calibrated densitometry using a Shimadzu CS-9000 dual-wavelength flying spot scanner and DR-13 recorder [29].

Fatty acid analysis

Individual polar lipid classes, with total neutral lipids running at the solvent front, were separated by thin-layer chromatography (TLC) essentially according to the method of Vitiello and Zanetta [30], using methyl acetate/isopropanol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.) as developing solvent. For the separation of neutral lipid classes hexane/diethyl ether/acetic acid (80:20:2, v/v) was used [31]. Fatty acid methyl esters, from total lipids, total neutral lipids and individual phosphoglyceride classes were prepared by acid-catalyzed transmethylation for 16 h at 50°C, using nonadecanoic acid (19:0) as internal standard [31]. Fatty acid methyl esters and dimethyl acetals, produced by methylation of the alk-1-enyl chains of plasmalogens, were extracted and purified as described previously [28]. The fatty acid methyl esters were analyzed in a Hewlett-Packard 5890A Series II gas chromatograph equipped with a chemically-bonded (PEG) Omegawax 320 fused-silica wall-coated capillary column (30 m × 0.32 mm i.d.) (Supelco, Bellefonte, USA), an on column injection system, and hydrogen as carrier gas with an oven thermal gradient from 185°C to 235°C. Individual fatty acid methyl esters were identified by reference to authentic standards and to a well characterized fish oil and were quantified using a Hewlett-Packard 3394 recording integrator [28]. The identity of 24:5($n-3$) and 24:6($n-3$) was confirmed by GC-mass spectrometric analysis of picolinyl derivatives. All solvents contained 0.01% BHT as an antioxidant.

Incorporation of radioactivity into total lipids and individual lipid classes

Samples of total lipids (3 mg) from [^{14}C]PUFA-injected fish were applied in 4 cm streaks to TLC plates and polar lipid and neutral lipid classes separated as above. Lipid classes were visualized by brief exposure to iodine vapour, bands marked and the iodine removed under vacuum [28]. Individual classes were scraped into scintillation mini vials, 4 ml of liquid scintillation cocktail (Ready Safe, Beckman) added, and radioactivity determined in a Beckman LS 5000 CE liquid scintillation spectrophotometer. Results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions.

Incorporation of radioactivity into fatty acid methyl esters from total lipids

Fatty acid methyl esters from total lipids were prepared as described above. Methyl esters were separated by argentation-TLC, using 2% silver nitrate-impregnated TLC plates with toluene/acetonitrile (95:5, v/v) as developing solvent [32]. This system resolves the methyl esters into discrete bands based on both degree of unsaturation and chain length [31,32]. Developed TLC plates were subjected to autoradiography for 14 days using Kodak X-OMAT AR-5 X-ray film; the labelled bands were scraped into scintillation vials

and their radioactivity determined as above. Identification of labelled bands was confirmed by using authentic unlabelled standards run on parallel plates, with visualization by charring as above.

Materials

[1-^{14}C]PUFA (all 50–53 mCi · mmol^{-1} and 99% pure) were obtained from NEN Dupont, Investigación Técnica Industrial S.A. (ITISA), Madrid, Spain. BHT, silver nitrate and potassium chloride were from Sigma (Poole, Dorset, UK). TLC (20 × 20 cm × 0.25 mm) and HPTLC (10 × 10 × 0.15 mm) glass plates, precoated with silica-gel 60 (without fluorescent indicator), were purchased from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fluka (Glossop, Derbyshire, UK).

Statistical analysis

Results are presented as means ± S.D. of triplicate experiments. The data were checked for homogeneity of the variances by the Bartlett test and, where necessary, the data were arc-sin transformed before further statistical analysis. Differences between mean values for incorporation of radioactivity into total lipids and individual lipid classes were analyzed by one-way ANOVA followed (where appropriate) by a multiple comparison test (Tukey) [33].

3. Results

Fish weights and lipid class compositions during the starvation period

Both wet weight and dry weights of the fish decreased during the period of the experiment with the weights of the fish after 15 days starvation significantly lower than the initial weights (Table 1). In percentage terms, the fish lost 11% and 15% of their wet and dry weights, respectively, over the 7 days prior to injection, and a total of 32% of wet weight and 39% of dry weight by the end of the experiment. Total lipid, total polar lipids and total neutral lipids showed no significant change during the first 7 days of fasting (Table 1). However, there was a significant decrease in total lipid in the fish during the second week of starvation (Table 1). Total neutral lipid, expressed as a percentage of the dry weight, significantly decreased by almost 57% during the second week of starvation due entirely to a significant decrease in the amount of triacylglycerol (TAG) (Table 1). In contrast, there was a significant increase in total polar lipids and individual polar lipid classes during the same period (Table 1).

Fatty acid compositions during starvation

Total saturated and monounsaturated fatty acid contents in total lipid showed a significant decrease from day 7 to day 15 (Table 2). In contrast, dimethyl acetals significantly increased during the first week and remained higher over the second week of starvation. All the $(n-6)$ and $(n-3)$PUFA in total lipid decreased during the starvation period, significantly so from day 7 to 15 (Table 2).

Table 1
Weights of fish (g), and total lipid and lipid class contents (percentage of dry weight) of sea bream during the 15 day starvation period

	Time of starvation (days)		
	0	7	15
Wet weight (g)	2.86 ± 0.28 ^{a}	2.53 ± 0.36 ^{a,b}	1.95 ± 0.06 ^{b}
Dry weight (g)	0.70 ± 0.08 ^{a}	0.59 ± 0.06 ^{a}	0.43 ± 0.01 ^{b}
Dry weight (%)	24.4 ± 0.4	23.6 ± 0.9	21.9 ± 1.1
Total lipid	22.9 ± 0.3 ^{a}	21.7 ± 1.0 ^{a}	16.7 ± 0.5 ^{b}
Total polar lipids	5.3 ± 0.2 ^{a}	5.1 ± 1.0 ^{a}	9.5 ± 0.4 ^{b}
Sphingomyelin	0.2 ± 0.0 ^{a}	0.3 ± 0.1 ^{a}	0.6 ± 0.0 ^{b}
Phosphatidylcholine	2.4 ± 0.1 ^{a}	2.3 ± 0.6 ^{a}	4.3 ± 0.1 ^{b}
Phosphatidylethanolamine	1.6 ± 0.1 ^{a}	1.6 ± 0.3 ^{a}	2.8 ± 0.1 ^{b}
Phosphatidylserine	0.4 ± 0.1 ^{a}	0.3 ± 0.1 ^{a}	0.7 ± 0.0 ^{b}
Phosphatidylinositol	0.4 ± 0.0	0.3 ± 0.1	0.5 ± 0.1
Phosphatidic acid/cardiolipin	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.0
Glycosylglycerides	tr	tr	tr
Total neutral lipids	17.6 ± 0.2 ^{a}	16.6 ± 1.0 ^{a}	7.2 ± 0.5 ^{b}
Cholesterol	2.0 ± 0.2	2.2 ± 0.4	2.1 ± 0.1
Free fatty acids	0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.4
Triacylglycerol	14.6 ± 0.7 ^{a}	12.9 ± 1.8 ^{a}	4.1 ± 0.1 ^{b}
Sterol esters	0.5 ± 0.2	0.9 ± 0.3	0.4 ± 0.2

Data are means ± S.D. (n = 3). S.D. = 0.0 implies an S.D. of < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different.

Total neutral lipids showed significantly decreased contents of total monounsaturated fatty acids, total $(n-6)$PUFA, 18:3$(n-3)$ and 20:5$(n-3)$ during the second week of starvation (Table 2). Total saturated fatty acids, total $(n-3)$PUFA and all the other individual $(n-3)$ and $(n-6)$PUFA showed substantial decreases in content (Table 2).

The fatty acid compositions of the major phosphoglycerides were less affected by starvation. The composition of phosphatidylcholine (PC) remained relatively constant with the exception of 22:6$(n-3)$ which significantly increased during the starvation period (Table 3). There was also a trend of increasing 22:6$(n-3)$ in phosphatidylethanolamine (PE), although the only significant change was increased dimethyl acetals, indicating an increased percentage of alk-1-enyl chains and hence of ethanolamine plasmalogen in the total ethanolamine glycerophospholipids (Table 3).

Time-course of incorporation of [1-^{14}C]18:3$(n-3)$ into lipid classes

The recovery of radioactivity from injected [1-^{14}C]18:3$(n-3)$ in total lipid, as a percentage of total radioactivity injected, significantly decreased during the

Table 2
Fatty acid contents of total lipid and total neutral lipids (μg fatty acid/mg dry weight) in sea bream during the 15 day starvation period

	Time of starvation (days)		
	0	7	15
Total lipid			
Total saturated	49.8±3.1 [a]	50.7±3.6 [a]	36.7±1.7 [b]
Total monounsaturated	75.2±3.7 [a]	69.5±3.2 [a]	47.3±2.6 [b]
Total dimethyl acetal	0.4±0.0 [a]	0.7±0.0 [b]	0.8±0.1 [b]
18:2($n-6$)	30.3±1.7 [a]	25.8±1.6 [a]	17.7±1.7 [b]
20:4($n-6$)	2.1±0.1 [a]	2.1±0.5 [a]	1.7±0.0 [b]
Total ($n-6$) PUFA	37.3±2.3 [a]	33.3±0.3 [a]	22.8±2.1 [b]
18:3($n-3$)	2.3±0.3 [a]	1.6±0.2 [a,b]	1.1±0.1 [b]
18:4($n-3$)	1.9±0.1 [a]	1.4±0.3 [a]	0.9±0.1 [b]
20:4($n-3$)	1.1±0.0 [a]	0.9±0.1 [a]	0.6±0.0 [b]
20:5($n-3$)	11.1±0.4 [a]	8.4±0.4 [b]	5.3±0.4 [c]
22:5($n-3$)	3.8±0.1 [a]	3.2±0.1 [a]	2.2±0.3 [b]
22:6($n-3$)	20.9±0.8 [a]	19.3±2.9 [ab]	15.1±0.5 [b]
Total ($n-3$) PUFA	44.1±1.7 [a]	37.4±2.2 [a]	27.0±1.6 [b]
Total neutral lipid			
Total saturated	31.1±2.1	29.2±3.7	23.1±3.5
Total monounsaturated	51.9±1.1 [a]	48.4±3.9 [a]	36.9±2.6 [b]
18:2($n-6$)	20.6±0.9	18.3±3.3	13.8±2.2
20:4($n-6$)	0.7±0.0	0.7±0.1	0.6±0.1
Total ($n-6$) PUFA	24.7±1.0 [a]	21.7±3.8 [a]	15.9±2.5 [b]
18:3($n-3$)	1.6±0.1 [a]	1.2±0.3 [a,b]	0.9±0.1 [b]
18:4($n-3$)	1.3±0.1	1.1±0.2	0.8±0.1
20:4($n-3$)	0.7±0.0	0.6±0.1	0.4±0.1
20:5($n-3$)	5.5±0.2 [a]	4.7±1.1 [a,b]	3.0±0.5 [b]
22:5($n-3$)	2.0±0.0	1.8±0.5	1.4±0.2
22:6($n-3$)	8.5±0.1	8.1±1.2	6.2±1.1
Total ($n-3$) PUFA	21.1±0.6	18.7±1.6	13.5±2.1

Data are means±S.D. (n = 3). S.D. = 0.0 implies an S.D. of < 0.05. Totals include some minor components (< 0.1%) not shown. PUFA, polyunsaturated fatty acid. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different.

time-course period (Table 4). The absolute incorporation of radioactivity expressed as dpm · 10^6/fish decreased from 2.1 ± 0.2 at day 2 to only 0.2 ± 0.0 at day 8.

The radioactivity from [1-^{14}C]18:3($n-3$) was initially distributed primarily in neutral lipids, with almost 73% of the total recovered in TAG (Table 4). However, the percentage of radioactivity from [1-^{14}C]18:3($n-3$) increased significantly in polar lipids during the time-course with over 68% being recovered in total polar lipids and only 21.4% recovered in TAG on day 8 (Table 4). The increase in relative incorporation into polar lipids was accounted for by significant increases in the percentages of radioactivity from [1-^{14}C]18:3($n-3$) recovered in all polar lipid classes without specific enrichment in any single polar lipid class.

Time-course of incorporation of [1-^{14}C]20:5($n-3$) into lipid classes

The recoveries of radioactivity from [1-^{14}C]20:5($n-3$) in total lipid, as a percentage of total radioactivity injected, were greater than those for [1-^{14}C]18:3($n-3$), although the differences were only statistically significant at 2 and 4 days (Tables 4 and 5). The recovery of radioactivity from [1-^{14}C]20:5($n-3$) also decreased during the time-course period, particularly over the first 4 days (Table 5). The absolute incorporation of radioactivity expressed as dpm · 10^6/fish decreased from 3.1 ± 0.5 at day 2 to only 0.5 ± 0.2 at day 8.

As with [1-^{14}C]18:3($n-3$), the radioactivity from [1-^{14}C]20:5($n-3$) was initially distributed primarily in neutral lipids, with over 62% of the total recovered in TAG (Table 5). Similarly, the percentage of radioactivity from [1-^{14}C]20:5($n-3$) increased in polar lipids during the time-course so that by 8 days the radioactivity was distributed equally between polar and neutral lipids. These changes were most evident and significant between days 2 and 4 after injection (Table 5). The increase in relative incorporation into polar lipids was observed in all classes but was most significant in PC

Table 3
Fatty acid contents of phosphatidylcholine and phosphatidylethanolamine (μg fatty acid/mg dry weight) in sea bream during the 15 day starvation period

	Time of starvation (days)		
	0	7	15
Phosphatidylcholine			
Total saturated	7.3±0.5	7.3±1.7	7.7±1.2
Total monounsatured	3.4±0.2	4.0±1.0	4.5±0.6
Total dimethyl acetal	nd	nd	nd
18:2($n-6$)	1.3±0.1	1.6±0.5	1.9±0.2
20:4($n-6$)	0.2±0.0	0.3±0.1	0.4±0.1
Total ($n-6$) PUFA	1.9±0.1	2.3±0.5	2.7±0.4
18:3($n-3$)	0.1±0.0	0.1±0.0	0.1±0.0
18:4($n-3$)	tr	tr	tr
20:4($n-3$)	0.1±0.0	tr	tr
20:5($n-3$)	1.1±0.1	1.3±0.2	1.4±0.2
22:5($n-3$)	0.2±0.0	0.3±0.1	0.3±0.0
22:6($n-3$)	1.8±0.2 [a]	3.5±0.8 [a,b]	4.0±0.5 [b]
Total ($n-3$) PUFA	3.5±0.2	5.4±1.4	5.9±0.7
Phosphatidylethanolamine			
Total saturated	3.1±0.1	2.8±0.5	2.6±0.2
Total monounsaturated	1.5±0.1	1.7±0.4	1.7±0.2
Total dimethyl acetal	0.5±0.0 [a]	1.2±0.3 [b]	1.1±0.1 [b]
18:2($n-6$)	0.4±0.1	0.5±0.1	0.5±0.0
20:4($n-6$)	0.2±0.0	0.3±0.1	0.3±0.1
Total ($n-6$) PUFA	0.9±0.1	1.0±0.2	1.0±0.1
18:3($n-3$)	tr	tr	tr
18:4($n-3$)	tr	tr	tr
20:4($n-3$)	tr	tr	tr
20:5($n-3$)	0.4±0.0	0.5±0.1	0.5±0.1
22:5($n-3$)	0.2±0.0	0.3±0.0	0.2±0.0
22:6($n-3$)	2.4±0.1	3.4±0.9	3.2±0.4
Total ($n-3$) PUFA	3.4±0.2	4.4±1.0	4.2±0.4

Data are means±S.D. (n = 3). S.D. = 0.0 implies an S.D. of < 0.05. Totals include some minor components (< 0.1%) not shown. nd, not detected. tr, values < 0.05. PUFA, polyunsaturated fatty acid. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different.

Table 4
Incorporation of radioactivity from intraperitoneally-injected [1-^{14}C]18:3($n-3$) in lipid classes in sea bream

Time after injection (days)	2	4	6	8
Recovery in lipid (%)	31.8 ± 3.7 [a]	12.1 ± 1.7 [b]	8.1 ± 0.7 [b,c]	3.5 ± 0.2 [c]
Incorporation into total lipid (dpm · 10^6/fish)	2.1 ± 0.2 [a]	0.8 ± 0.1 [b]	0.5 ± 0.0 [c]	0.2 ± 0.0 [d]
Distribution in lipid classes (%)				
Total polar lipids	22.6 ± 3.5 [a]	39.2 ± 1.0 [b]	61.2 ± 1.8 [c]	68.5 ± 1.1 [d]
Phosphatidylcholine	17.2 ± 1.1 [a]	25.4 ± 1.1 [b]	43.7 ± 2.0 [c]	40.8 ± 2.6 [c]
Phosphatidyloethanolamine	2.5 ± 0.8 [a]	4.4 ± 1.2 [a,b]	5.9 ± 0.2 [b,c]	7.8 ± 0.5 [c]
Phosphatidylserine	0.5 ± 0.1 [a]	1.2 ± 0.4 [a,b]	2.2 ± 0.2 [b,c]	3.5 ± 1.1 [c]
Phosphatidylinositol	0.6 ± 0.1 [a]	1.0 ± 0.3 [a,b]	1.5 ± 0.0 [b]	2.3 ± 0.5 [c]
Phosphatidic acid/cardiolipin	0.9 ± 0.2 [a]	3.7 ± 1.4 [a,b]	5.7 ± 0.3 [b,c]	8.9 ± 2.1 [c]
Glycosylglycerides	0.6 ± 0.1 [a]	1.2 ± 0.1 [a,b]	1.0 ± 0.1 [a,b]	1.9 ± 0.7 [b]
Sphingomyelin	0.2 ± 0.0 [a]	2.2 ± 1.0 [a,b]	1.2 ± 0.1 [b,c]	3.2 ± 0.5 [c]
Total neutral lipids	77.3 ± 2.4 [a]	60.8 ± 5.5 [b]	38.8 ± 1.2 [c]	31.5 ± 1.8 [d]
Monoacylglycerol	2.0 ± 0.5 [a]	1.6 ± 0.2 [a]	4.3 ± 0.1 [b]	2.9 ± 1.0 [a,b]
Diacylglycerol	1.5 ± 0.1 [a]	2.6 ± 0.6 [a,b]	2.5 ± 0.8 [a,b]	5.7 ± 2.5 [b]
Free fatty acid	0.2 ± 0.0 [a]	0.3 ± 0.0 [a,b]	0.5 ± 0.2 [b,c]	0.6 ± 0.1 [c]
Triacylglycerol	72.7 ± 2.9 [a]	55.4 ± 1.7 [b]	30.8 ± 1.3 [c]	21.4 ± 4.8 [d]
Steryl ester	0.9 ± 0.1	0.9 ± 0.2	0.7 ± 0.2	0.9 ± 0.4

Data are means ± S.D. (n = 3). S.D. = 0.0 implies an S.D. < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different.

and PE, whereas the concomitant decrease in relative incorporation in neutral lipids was exclusively due to a decrease in TAG (Table 5).

Metabolism of injected [1-^{14}C]18:3($n-3$) via desaturation / elongation

The majority of the radioactivity from injected [1-^{14}C]18:3($n-3$) was recovered unmetabolized by the desaturation/elongation pathway (Table 6). This percentage tended to decrease over the time-course but the differences were not statistically significant. The total percentages of [1-^{14}C]18:3($n-3$) desaturated were relatively small (15.6%, 18.1%, 17.3% and 16.3% on days 2, 4, 6 and 8, respectively), with 6.1% recovered in 20:5($n-3$) and 1.3% recovered in 22:6($n-3$) on day 2 (Table 6). There were significant increases in the percentages of radioactivity recovered in 20:3($n-3$) on day 8 and 24:6($n-3$) on days 4 and 8, but otherwise there were no major changes in the relative proportions of radioactivity from [1-^{14}C]18:3($n-3$) recovered in the products of the desaturation/elongation pathway (Table 6).

Metabolism of injected [1-^{14}C]20:5($n-3$) via desaturation / elongation

As with [1-^{14}C]18:3($n-3$), approx. 75% of the radioactivity from injected [1-^{14}C]20:5($n-3$) was recovered unmetabolized by the desaturation/elongation

Table 5
Incorporation of radioactivity from intraperitoneally-injected [1-^{14}C]20:5($n-3$) in lipid classes in sea bream

Time after injection (days)	2	4	6	8
Recovery in lipid (%)	46.5 ± 8.9 [a]	20.1 ± 1.3 [b]	16.1 ± 3.2 [b,c]	7.2 ± 1.6 [c]
Incorporation into total lipid (dpm · 10^6/fish)	3.1 ± 0.5 [a]	1.3 ± 0.1 [b]	1.1 ± 0.5 [b]	0.5 ± 0.2 [b]
Distribution in lipid classes (%)				
Total polar lipids	29.7 ± 1.5 [a]	45.9 ± 4.4 [b]	48.6 ± 7.8 [b]	49.3 ± 2.6 [b]
Phosphatidylcholine	19.7 ± 0.6 [a]	30.4 ± 2.9 [a,b]	30.8 ± 7.6 [a,b]	33.9 ± 3.6 [b]
Phosphatidylethanolamine	4.7 ± 0.1 [a]	8.0 ± 0.5 [b]	8.0 ± 1.1 [b]	9.1 ± 1.2 [b]
Phosphatidylserine	0.8 ± 0.1 [a]	1.3 ± 0.3 [a,b]	1.7 ± 0.5 [a,b]	1.4 ± 0.0 [b]
Phosphatidylinositol	1.4 ± 0.4 [a]	2.5 ± 0.1 [a,b]	3.0 ± 0.5 [b]	2.3 ± 0.7 [a,b]
Phosphatidic acid/cardiolipin	0.6 ± 0.1	1.2 ± 0.3	1.6 ± 0.7	1.1 ± 0.1
Glycosylglycerides	0.8 ± 0.3 [a]	1.5 ± 0.7 [a,b]	2.4 ± 0.9 [b]	0.7 ± 0.0 [a]
Sphingomyelin	0.2 ± 0.0	0.8 ± 0.3	1.0 ± 0.6	0.6 ± 0.1
Total neutral lipids	71.7 ± 0.4 [a]	54.1 ± 3.6 [b]	51.4 ± 8.5 [b]	50.7 ± 4.5 [b]
Monoacylglycerol	2.8 ± 0.6	2.4 ± 0.5	2.1 ± 1.0	2.1 ± 0.2
Diacylglycerol	3.6 ± 0.5	3.2 ± 0.5	3.2 ± 1.1	4.5 ± 1.2
Free fatty acid	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1
Triacylglycerol	62.3 ± 1.4 [a]	46.7 ± 3.4 [b]	44.1 ± 7.5 [b]	41.7 ± 2.0 [b]
Steryl ester	1.2 ± 0.5	1.4 ± 0.3	1.5 ± 0.6	1.9 ± 0.1

Data are means ± S.D. (n = 3). S.D. = 0.0 implies an S.D. < 0.05. Values within a given row not bearing the same superscript letter are significantly different at $P < 0.05$. If nosuperscript appears, values are not different.

Table 6
Metabolism of injected [1-^{14}C]18:3($n-3$) via the desaturase/elongase pathways in sea bream

Fatty acid	Time after injection (days)			
	2	4	6	8
18:3($n-3$)	76.1±3.9	74.5±0.6	74.7±1.7	68.4±3.9
20:3($n-3$)	4.9±3.3 ^{a,b}	4.4±0.1 ^{a}	5.7±1.2 ^{a,b}	11.9±2.5 ^{b}
22:3($n-3$)	2.5±0.3	2.9±0.4	2.2±0.1	3.2±0.7
18:4($n-3$)	1.4±0.5 ^{a}	2.9±0.7 ^{b}	0.9±0.1 ^{a}	1.6±0.3 ^{a,b}
20:4($n-3$)	2.7±1.1	4.5±0.7	2.4±0.4	2.9±0.7
20:5($n-3$)	6.1±1.7	6.3±0.2	6.4±0.5	4.4±0.6
22:5($n-3$)	0.9±0.4	0.6±0.1	2.6±1.4	1.4±0.2
24:5($n-3$)	3.2±0.4 ^{a}	1.0±0.0 ^{b}	3.1±0.2 ^{a}	3.3±0.8 ^{a}
22:6($n-3$)	1.3±0.4	1.3±0.1	1.1±0.1	1.3±0.4
24:6($n-3$)	tr ^{a}	1.5±0.1 ^{b}	0.8±0.1 ^{a,b}	1.4±0.5 ^{b}

Values are the radioactivity recovered in each fatty acid fraction expressed as a percentage of the total radioactivity recovered and represent means ± S.D. (n = 3). S.D. = 0.0 implies an S.D. < 0.05. tr, trace (< 0.05). Values within a given row with different superscript letters are significantly different at $P < 0.05$. If no superscript appears, values are not different.

pathway (Table 7). However, this percentage tended to decrease during the course of the experiment with small, but significant, decreases in the amount of radioactivity recovered unmetabolized on days 4 and 8 (Table 7). Approx. 16% of the radioactivity from [1-^{14}C]20:5($n-3$) was recovered as desaturated products on day 2 with almost 11% and 5% recovered in 22:6($n-3$) and 24:6($n-3$), respectively (Table 7). These percentages were constant over days 2–6, but decreased on day 8 due to significant decreases in the percentages of radioactivity revcovered in both 22:6($n-3$) and 24:6($n-3$). However, an increasing percentage of radioactivity recovered in 24:5($n-3$) was the most significant trend over the entire time-course of the experiment (Table 7).

4. Discussion

The present experiment was designed to determine to what extent essential ($n-3$)HUFA such 22:6($n-3$) were synthesized in fish during a relatively long time-course of 8 days. In vivo studies of this duration have not been reported previously in any species, including fish. Although it is generally accepted that marine fish, including sea bream, have only low Δ^5-desaturase activity, the activity is present and so both [1-^{14}C]18:3($n-3$) and [1-^{14}C]20:5($n-3$) were used as precursor fatty acids for 22:6($n-3$) biosynthesis in this experiment [2,34]. A further aim of the present study was to determine specifically the levels of C_{24} HUFA that are intermediates in the putative pathway for the conversion of 20:5($n-3$) to 22:6($n-3$) without Δ^4-deasaturase activity [23,24], in order to establish if this pathway was operative in fish.

The fish weight data showed that the fish were used significant amounts of endogenous energy reserves during the course of the experiment. The levels of total lipid, triacylglycerol and fatty acids, including PUFA, declined during the second week of starvation, indicating that lipids were a significant portion of the endogenous reserves being used for energy during the period of the time-course experiment. Therefore, the experimental animals were in a condition that should enhance the bioconversion of the injected ^{14}C-labelled PUFA via the desaturation/elongation pathways as dietary fatty acids, especially PUFA, supress de novo fatty acid biosynthesis and conversion [18,35].

The incorporation and recovery of radioactivity in total lipids was generally greater for [1-^{14}C]20:5($n-3$) than for [1-^{14}C]18:3($n-3$), perhaps indicating preferential retention of C_{20} HUFA. Although this was not particularly evident in a previous study on sea bream juveniles [10], it is in agreement with results found in turbot and golden grey mullet [8,11]. Therefore, it is likely that preferential retention of C_{20} and C_{22} ($n-3$)HUFA generally occurs in marine fish [36].

In absolute terms, the amount of radioactivity from [1-^{14}C]18:3($n-3$) incorporated into total polar lipids by 2 days ($0.47 \cdot 10^6$ dpm/fish) was identical to that found previously for the same species ($0.46 \cdot 10^6$ dpm/fish) [10]. This figure is an intermediate value between those found for golden grey mullet ($0.35 \cdot 10^6$ dpm/fish) [11] and turbot ($0.98 \cdot 10^6$ dpm/fish) [8] under identical experimental conditions. However, for [1-^{14}C]20:5($n-3$), the amount of radioactivity incorporated into total polar lipids by day 2 ($0.92 \cdot 10^6$ dpm/fish) was more than double that found ($0.41 \cdot 10^6$ dpm/fish) in a previous experiment with sea bream [10] but similar to values found for golden grey mullet ($1.13 \cdot 10^6$ dpm/fish) and turbot ($1.22 \cdot 10^6$ dpm/fish) [8,11]. Therefore, there is reasonable agreement between the data for different marine species, particularly considering there is clearly significant intra-species variation. The recovery of radioactivity represents the net result of initial incorporation and metabolism via β-oxidation resulting in loss of radioactivity as $^{14}CO_2$. The recovery

Table 7
Metabolism of injected [1-^{14}C]20:5($n-3$) via the desaturase/elongase pathways in juvenile sea bream

Fatty acid	Time after injection (days)			
	2	4	6	8
20:5($n-3$)	75.6±0.7 ^{a}	72.0±0.6 ^{b}	74.3±2.2 ^{a,b}	71.5±0.6 ^{b}
22:5($n-3$)	5.5±0.8	5.4±0.6	4.3±0.9	5.6±0.7
24:5($n-3$)	3.3±0.3 ^{a}	6.1±0.2 ^{b}	6.1±0.3 ^{b}	11.3±0.1 ^{c}
22:6($n-3$)	10.8±0.0 ^{a}	10.9±0.3 ^{a}	11.4±1.0 ^{a}	7.9±0.1 ^{b}
24:6($n-3$)	4.9±0.4 ^{a}	5.6±0.1 ^{b}	3.9±0.1 ^{c}	3.7±0.0 ^{c}

Values are the radioactivity recovered in each fatty acid fraction expressed as a percentage of the total radioactivity recovered and represent means ± S.D. (n = 3). S.D. = 0.0 implies an S.D. < 0.05. Values within a given row bearing different superscript letters are significantly different at $P < 0.05$. If no superscript appears, values are not different.

of radioactivity in sea bream lipids in the present study decreased by between 9-fold (18:3) and over 6-fold (20:5) from day 2 to 8. This indicated that as much as 89% of the [1-^{14}C]18:3($n-3$) and 84% of the [1-^{14}C]20:5($n-3$) incorporated in lipids on day 2 were subsequently oxidized by day 8.

In previous in vivo studies with marine fish, the distribution of radioactivity in lipid classes 2 days after injection showed higher proportions of both [1-^{14}C]18:3($n-3$) and [1-^{14}C]20:5($n-3$) incorporated into polar lipid classes [10,11]. In the present study, incorporation of both fatty acids was initially predominantly into neutral lipid with the percentages incorporated into polar lipid increasing during the time-course concomitant with decreased percentages incorporated into neutral lipids. The decreased percentages of radioactivity in neutral lipid were due solely to decreased percentages in TAG, whereas the changes in polar lipids were across all the major phospholipid classes. Therefore it is possible that incorporation of the PUFA was initially into TAG with subsequent hydrolysis of fatty acids for β-oxidation with selective retention and reacylation of PUFA into phospholipids. However, considering the high level of oxidation of both labelled PUFA, it is possible that oxidation of PUFA in TAG was simply greater than that in phospholipids, as would be expected, resulting in an apparent redistribution of radioactivity. Either possibility is consistent with ($n-3$)PUFA being retained specifically in membrane polar lipids, such as PC and PE, whereas neutral lipids, particularly TAG, are a reservoir of fatty acids for energy production via β-oxidation. The former possibility would appear to demonstrate unusual roles for TAG initially as a receiver of PUFA and subsequently involved in the supply of PUFA to phospholipid moieties during remodelling reactions of deacylation/reacylation or de novo phospholipid synthesis involved in membrane biosynthesis and turnover [2,18–19,33–35]. The fish in the present study were essentially starved fish, and so there were no substantial lipid energy reserves and so protein could be being used for energy. Therefore, surplus cellular membranes may be being recycled and used in the provision of fatty acids, amino acids and glycerol for energy. The apparent redistribution occurred to a greater extent with [1-^{14}C]18:3($n-3$), consistent with it being readily oxidized [37] whereas [1-^{14}C]20:5($n-3$) was initially more specifically incorporated into phospholipids with a significant change in distribution only between days 2 and 4.

Although there was considerable metabolism of the ^{14}C-labelled PUFA via β-oxidation, the remaining PUFA was preferentially retained in phospholipids. Therefore, it was noteworthy that there were few consistent significant trends observed in the relative amounts of radioactivity recovered in the different fatty acid fractions, particularly desaturated products, comprising the desaturation/elongation pathways, especially with [1-^{14}C]18:3($n-3$). It may have been expected that the relative proportion of precursor fatty acid to metabolites would decrease during the time-course due to the combined effects of β-oxidation, which could be expected to preferentially oxidize less unsaturated fatty acids, such as 18:3, and apparent redistribution into phospholipid classes which could be expected to be more specific for longer-chain more unsaturated fatty acids. However, the fact that the percentages of the precursors, intermediates and products of the desaturation/elongation pathways remain relatively constant over the time-course suggests a lack of specificity in these metabolic processes greater than expected but possibly consistent with a process including cellular protein turnover as described above. Interestingly, the pattern observed during the whole time-course in the present study is similar to those found in other marine fish species studied over a short time-course, where about 75% of the precursor remained unmetabolized and only 16% were desaturated to longer more unsaturated fatty acids [8,10,11].

In contrast to the usual components of the 18:3($n-3$) desaturation pathway, which showed few significant changes during the time-course, elongation of 18:3 to 20:3($n-3$) and 22:3($n-3$) increased from 7.4% to 15.1% mainly due to a significant increase in 20:3. These elongated fatty acids are termed 'dead end' products as they cannot be desaturated due to their structure, with the first double bond at Δ^{11} and Δ^{13}, respectively. It has been suggested that these products are stored in the neutral lipids before retroconversion [18,38]. Similarly, with [1-^{14}C]20:5($n-3$) as substrate, it is the product of elongation, 24:5($n-3$) that showed the greatest increase during the time-course, doubling from day 2 to 4 and almost doubling again from day 6 to 8. However, after an initial increase in its desaturated product, 24:6($n-3$), between days 2 and 4, the percentages of radioactivity recovered in hexaenoic fatty acids decreased significantly by day 8. The increased percentage of radioactivity recovered in 24:5($n-3$) combined with the decreased percentages recovered in hexaenes may suggest some (product?) inhibition of Δ^6-desaturase at this time point.

In the freshwater fish, *Pimelodus maculatus*, different enzymes are responsible for the Δ^6- and Δ^5-desaturations [39], and although no studies of this type have been carried out on marine fish, the low level of Δ^5-desaturase activity in marine fish implies different enzymes. Although neither Δ^6- nor Δ^5-desaturase enzymes appear very active with 18:3($n-3$) as substrate in sea bream, it is clear that the production of 22:6($n-3$) is several-fold more efficient with 20:5($n-3$) as substrate compared to 18:3($n-3$). The presence of significant amounts of label in both 24:5($n-3$) and

24:6($n-3$) suggests that the pathway for the production of 22:6($n-3$) from 20:5($n-3$) via a Δ^6 desaturation, as proposed by Voss et al. [23,24], could be operating in sea bream. Consistent with this, the percentages of [1-^{14}C]18:3($n-3$) desaturated on days 2, 4, 6 and 8 were 15.6, 18.1, 17.3 and 16.3%, respectively, which compared well with the percentages of [1-^{14}C]20:5($n-3$) that were desaturated (15.7, 16.5, 15.3 and 11.6) over the time-course. This, therefore, supports a lack of sufficient Δ^5-desaturase activity as the mechanism underpinning low production of 22:6($n-3$) from 18:3($n-3$) in marine fish.

The specificities of enzyme-substrate interactions in fatty acid metabolism are much more dependent on weaker 'hydrophobic' interactions such as Van der Waals and dispersion forces than on strong ionic and hydrogen bond interactions. This implies a low substrate specificity, with 16:1($n-7$), 18:1($n-9$), 18:2($n-6$), 18:3($n-3$) and possibly 24:4($n-6$) and 24:5($n-3$) all competing for a single enzyme, Δ^6 fatty acid desaturase [23,24,34]. This emphasises the complex interactions and multitude of potential rate controls that can occur in the conversions of dietary unsaturated fatty acids that determine final PUFA/HUFA compositions of phospholipids in cellular membranes [34]. It also highlights the importance of chain elongation activity (conversions of dietary C_{18} to C_{24} fatty acids) and chain shortening activity (conversions of C_{24} to C_{22} and C_{20} HUFA) in determining cellular PUFA/HUFA levels, particulary since 22:6($n-3$) and 22:5($n-6$) themselves can readily be chain shortened by peroxisomal β-oxidation to generate 20:5($n-3$) and 20:4($n-6$) respectively [40,41]. There is evidence, that in mammals, the balance of elongation/shortening for the ($n-6$)PUFA is such that 20:4($n-6$) is formed and incorporated into phospholipids in preference to 22:5($n-6$), whereas for the ($n-3$)PUFA, 22:6($n-3$) is formed and incorporated into phospholipids in preference to 20:5($n-3$) [39]. The extent to which this applies to fish is a subject for further research, mainly the elucidation of the complex biochemical conversions and their controls.

In conclusion, a pattern of PUFA metabolism in sea bream consistent with that previously obtained with marine fish, with relatively low Δ^5-desaturase activity, was observed throughout the 8 days of the time-course, with up to 10-fold more 22:6($n-3$) produced from 20:5($n-3$) compared to 18:3($n-3$) as substrate. There was considerable oxidation of the labelled PUFA during the time-course, particularly with [1-^{14}C]18:3($n-3$). Incorporation of both PUFA was initially predominantly into TAG, although the effect of oxidation, possibly preferentially from TAG, resulted in increased percentages of radioactivity recovered in polar lipids, although the possibility of active redistribution of labelled PUFA between TAG and polar lipids exists. There was no increase in the relative amount of radioactivity recovered in 22:6($n-3$) during the experiment. The data were consistent with the pathway for the production of 22:6($n-3$) from 20:5($n-3$) via a Δ^6-desaturation, as proposed by Voss et al. [23,24], operating in sea bream.

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