Effects of diets containing linseed oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes in Atlantic salmon (*Salmo salar*).

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Abbreviations: BHT - butylated hydroxytoluene; FAF-BSA - fatty acid-free bovine serum albumin; FO - fish oil; HBSS - Hanks balanced salt solution; HUFA - highly unsaturated fatty acids (carbon chain length ≥ C_{20} with ≥ 3 double bonds); LO - linseed oil.

Abstract

We hypothesized that replacing fish oil with 18:3n-3-rich linseed oil may enable salmon to maintain the levels of tissue n-3HUFA levels through a combination of increased desaturation activity and increased substrate fatty acid provision. To this end we investigated desaturation/elongation of [1-^{14}C18:3n-3 in hepatocytes and intestinal enterocytes, and determined the extent to which 18:3n-3 was oxidized and desaturated by measuring both simultaneously in a combined assay. Salmon smolts were stocked randomly into five seawater pens and fed for 40 weeks on diets in which the fish oil was replaced in a graded manner by linseed oil. At the end of the trial, fatty acyl desaturation/elongation and oxidation activities were determined in isolated hepatocytes and intestinal enterocytes using [1-^{14}C]18:3n-3 as substrate, and samples of liver and intestinal tissue were collected for analysis of lipid and fatty acid composition. The results showed that, despite increased desaturation of [1-^{14}C]18:3n-3 in hepatocytes, provision of dietary 18:3n-3 did not prevent the decrease in tissue n-3HUFA in fish fed linseed oil. Intestinal enterocytes were a site of significant fatty acid desaturation but, in contrast to hepatocytes, the activity was not increased by feeding linseed oil and was generally lower in fish fed linseed oil compared to fish fed only fish oil. In contrast, oxidation of [1-^{14}C]18:3n-3 in enterocytes was generally increased in fish fed linseed oil compared to fish fed the diet containing only fish oil. However, oxidation of [1-^{14}C]18:3n-3 in hepatocytes was 4- to 8-fold lower than in enterocytes and was not affected by diet. Furthermore, oxidation of [1-^{14}C]18:3n-3 in enterocytes exceeded desaturation irrespective of dietary treatment, whereas similar amounts of [1-^{14}C]18:3n-3 were desaturated and oxidized in hepatocytes from fish fed only fish oil and desaturation exceeded oxidation by 3-fold in fish fed the diet containing 100% linseed oil. The molecular mechanisms underpinning these results were discussed.

Introduction

The polyunsaturated fatty acids (PUFA), linoleate (18:2n-6) and linolenate, cannot be synthesized *de novo* by animals, including fish and, therefore, are termed essential fatty acids (EFA) (Holman 1986). Qualitative and quantitative EFA requirements vary between species and, although salmonids require both 18:3n-3 and 18:2n-6 at a combined level of around 1% of the diet, their essentiality actually derives from their desaturation and elongation to the functionally active highly unsaturated fatty acids (HUFA) eicosapentaenoate (20:5n-3), docosahexaenoate (22:6n-3) and arachidonate (20:4n-6) (Sargent et al. 1995, 1999). The fatty acyl desaturase enzyme activities are known to be under nutritional regulation in mammals (Brenner 1981) and this has also been demonstrated in fish (Tocher et al. 1996; Sargent et al. 2002).

Exploitation and, indeed, over-exploitation of wild fisheries has meant that an increasing proportion of fish for human consumption is provided by aquaculture which is expanding at over 10% per year (Tidwell and Allan 2002). Paradoxically, diets for aquaculture, including salmonid (salmon, trout and char) culture, have been based traditionally on fish meals and oils as the predominant protein and lipid sources (Sargent and Tacon 1999). As a consequence, demand for fish oils is rapidly increasing and current estimates suggest aquaculture feeds will consume more than 85% of world fish oil supplies by 2010 and so, if aquaculture is to continue to expand and supply more of the global demand for fish, alternatives to fish oil must be found (Barlow 2000). The only sustainable alternative to fish oils are plant (vegetable) oils which are rich in C_{18} PUFA but devoid of the n-3HUFA abundant in fish oils (Sargent et al. 2002). Therefore, there is currently considerable interest in the regulation of the HUFA biosynthetic pathways in fish to determine the effectiveness with which the fatty acids in vegetable oils can be utilized by commercially important cultured fish species including salmon (Sargent et al. 2002).

In previous trials we have shown that replacing fish oil with rapeseed oil (rich in 18:2n-6 and 18:1n-9) or palm oil (rich in 16:0 and 18:1n-9) in the diets of Atlantic salmon resulted in increased activity of the fatty acyl desaturation/elongation pathway in isolated hepatocytes (Bell et al. 2001a, 2002). Although the diets in both these trials were based on fish meal, which supplied sufficient 20:5n-3 and 22:6n-3 to satisfy the EFA requirements, the fatty acid compositions of tissues, including liver and muscle, were characterized by significantly decreased levels of n-3HUFA, particularly when vegetable oil replaced 100% of the fish oil, which compromised the nutritional quality of the flesh for the human consumer (Bell et al. 2001a, 2002). However, palm oil contains virtually no 18:3n-3 and rapeseed oil contains only moderate levels (Padley et al. 1986). It appeared, therefore, that despite

increased desaturation/elongation activity in the liver, the levels of dietary 18:3n-3 provided may have limited the ability of salmon to maintain levels of tissue n-3HUFA in the flesh. Few plant oils contain high levels of 18:3n-3, a notable exception being linseed oil which can contain up to 56% 18:3n-3 with an 18:3n-3:18:2n-6 ratio of over 3 in selected strains (Padley et al. 1986). The primary hypothesis we aimed to test in the present study was that replacing fish oil with 18:3n-3-rich linseed oil in salmon diets would enable the fish to maintain tissue n-3HUFA levels through a combination of increased desaturation/elongation activity and increased substrate fatty acid provision. Other *in vivo* studies using stable isotopes (deuterium-labeled 18:3n3) had shown that dietary 18:3n-3 was readily and substantially oxidized (Bell et al. 2001b) and that intestine was a tissue with hitherto unappreciated fatty acyl desaturation activity (Bell et al. 2003). Therefore, in addition, we aimed to investigate desaturation/elongation of 18:3n-3 in intestinal enterocytes. Thirdly, we aimed to determine the extent to which the 18:3n-3 tracer was oxidized and compare that with the amount desaturated/elongated by measuring both simultaneously in a combined assay in both hepatocytes and enterocytes.

Salmon smolts were stocked randomly into five seawater pens and, after acclimatization for three weeks, were fed for 40 weeks on diets in which the fish oil (FO) was replaced in a graded manner by linseed oil (LO). At the end of the trial, fatty acyl desaturation/elongation and oxidation activities were determined in isolated hepatocytes and intestinal enterocytes using [1-^{14}C]18:3n-3 as substrate, and samples of liver and intestinal tissue were collected for analysis of lipid and fatty acid composition.

Materials and methods

Animals and diets

In June 2001, Atlantic salmon post-smolts were randomly assigned to 5 cages (5 m x 5 m; 600 fish per cage), with the mean initial weight across the five cages being 127 ± 3 g (range 123 to 130g). The smolts were fed one of five diets, consisting of a control diet containing FO alone and four diets in which the FO was replaced in a graded manner by linseed oil (LO). Specifically, the five diets were 100% FO (FO), 100%LO (LO100) and FO/LO in ratios of 3:1 (LO25), 1:1 (LO50) and 1:3 (LO75). The experimental diets were prepared by the Nutreco Aquaculture Research Centre, Stavanger, Norway and were fed by hand. The formulation, proximate analyses and fatty acid compositions of the diets (6 mm pellet) are shown in Tables 1 and 2. All diets were formulated to satisfy the nutritional requirements of salmonid fish (US National Research Council 1993). The fish were fed the experimental diets for 40 weeks with the experiment terminating in March 2002. The final weights of

the fish were 1.78 ± 0.40 kg (FO), 1.89 ± 0.34 kg (LO25), 1.90 ± 0.33 kg (LO50), 1.87 ± 0.35 kg (LO75) and 1.87 ± 0.33 kg (LO100) and there were no significant differences between different dietary treatments (ANOVA, $p = 0.1555$, $n = 87$). Fish were not fed during the 24 h prior to sampling.

Lipid extraction and lipid class composition

Intact livers were dissected from 18 fish (pooled into 6 samples of 3 livers each) per dietary treatment at each sampling point and immediately frozen in liquid nitrogen. For intestinal samples, four fish were sampled per dietary treatment. Pyloric caecae and portions of the midgut immediately posterior to the caecal regions were dissected from the intestinal tract, adhering adipose tissue removed and any residual lumenal contents gently and carefully extruded before the cleaned caecae and midgut sections were rapidly frozen in liquid nitrogen. Total lipid contents of livers, intestinal tissues and diet samples were determined gravimetrically after extraction by homogenization in chloroform/methanol (2:1, v/v) containing 0.01 % butylated hydroxytoluene (BHT) as antioxidant, basically according to Folch et al. (1957). Separation of lipid classes was performed by high-performance thin-layer chromatography (HPTLC). Approximately 10 μg 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.). After desiccation, the plate was fully developed with isohexane/diethyl ether/acetic acid (85:15:1, by vol.). The classes were quantified by charring at 160 ºC for 15 min after spraying with 3 % (w/v) aqueous cupric acetate containing 8 % (v/v) phosphoric acid, followed by calibrated densitometry using a Shimadzu CS-9000 dual-wavelength flying spot scanner and DR-13 recorder (Henderson and Tocher 1992).

Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared from total lipid by acid-catalyzed transesterification using 2 ml of 1% H_2SO_4 in methanol plus 1 ml toluene as described by Christie (1982) and FAME extracted and purified as described previously (Tocher and Harvie 1988). FAME were separated and quantified by gas-liquid chromatography (Fisons GC8600, Fisons Ltd., Crawley, U.K.) using a 30m x 0.32 mm capillary column (CP wax 52CB; Chrompak Ltd., London, U.K). Hydrogen was used as carrier gas and temperature programming was from 50ºC to 180ºC at 40ºC/min and then to 225ºC at 2ºC/min. Individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman 1980).

Preparation of isolated hepatocytes

Fish were killed by a blow to the head and the livers dissected immediately. The gall bladder was removed carefully and the main blood vessels trimmed. The liver was perfused via the hepatic vein with solution A (calcium and magnesium-free Hanks balanced salt solution (HBSS) + 10 mM HEPES + 1 mM EDTA) to clear blood from the tissue using a syringe fitted with a 23g needle. The liver was chopped finely with scissors and about 1 g of chopped liver was taken and incubated with 20 ml of solution A containing 0.1% (w/v) collagenase in a 25 ml "Reacti-flask" in a shaking water bath at 20ºC for 45 min. The digested liver tissue was filtered through 100 μm nylon gauze and the cells collected by centrifugation at 300 x g for 2 min. The cell pellet was washed with 20 ml of solution A containing 1% w/v fatty acid-free bovine serum albumin (FAF-BSA) and re-centrifuged. The washing was repeated with a further 20 ml of solution A without FAF-BSA. The hepatocytes were resuspended in 10 ml of Medium 199 containing 10 mM HEPES and 2 mM glutamine. One hundred μl of cell suspension was mixed with 400 μl of Trypan Blue and hepatocytes were counted and their viability assessed using a haemocytometer. One hundred μl of the cell suspension was retained for protein determination.

Preparation of isolated enterocytes

The methodology for preparing hepatocytes as above was specifically developed for use in the field and has been successfully used in several previous trials (Bell et al. 1997, 2001a, 2002a: Tocher et al. 1997, 2000, 2001). Preliminary laboratory-based trials had shown that this method, with minor modifications, could be used successfully to isolate enterocyte-enriched preparations from various regions of the intestine including pyloric caecae. Briefly, the intestinal tract was removed and pyloric caecae dissected, cleaned of adhering adipose tissue, slit open and lumenal contents rinsed away with solution A. Similarly, a 1-2 cm portion of the mid gut, immediately posterior to the caecal-containing region, was dissected, cleaned and rinsed as for the caecae. The caecae and midgut samples were chopped finely with scissors and incubated with 20 ml of solution A containing 0.1% (w/v) collagenase in a 25 ml "Reacti-flask" in a shaking water bath at 20ºC for 45 min. The digested intestinal tissues were filtered through 100 μm nylon gauze and the cells collected and washed exactly as described for hepatocytes. The enterocytes were resuspended in 10 ml of Medium 199 containing 10 mM HEPES, 2 mM glutamine and viability checked as for hepatocytes. The enriched enterocyte preparation was very predominantly enterocytes although there were some secretory cells present. The viability was

routinely > 95% at isolation and generally decreased by only 5 – 10% over the period of the incubation. One hundred μl of the cell suspension was retained for protein determination.

Incubation of hepatocyte and enterocyte preparations with [1-^{14}C]18:3n-3

Six ml of each hepatocyte or enterocyte suspension were dispensed into a 25 cm^2 tissue culture flask. Hepatocytes and enterocytes were incubated with 0.3 μCi (~ 1 μM) [1-^{14}C] 18:3n-3, added as a complex with FAF-BSA in phosphate buffered saline prepared as described previously (Ghioni et al., 1997). After addition of isotope the flasks were incubated at 20 ºC for 2 h. After incubation, the cell layer was dislodged by gentle rocking and the cell suspension transferred to glass conical test tubes and 1 ml of each suspension withdrawn into a 2 ml microcentrifuge tube for β-oxidation assay as described below. The cell suspensions remaining in the glass conical centrifuge tubes were used for the desaturation/elongation assay as described below.

Assay of hepatocyte and enterocyte fatty acyl desaturation/elongation activities

The cell suspensions were centrifuged at 500 g for 2 min, the supernatant discarded and the hepatocyte or enterocyte cell pellets washed with 5 ml of ice-cold HBSS/FAF-BSA. The supernatant was discarded and the tubes placed upside down on paper towels to blot for 15 sec before extraction of total lipid using ice-cold chloroform/methanol (2:1, v/v) containing 0.01% (w/v) BHT essentially as described by Folch et al. (1957) and as described in detail previously (Tocher et al. 1988). Total lipid was transmethylated and FAME prepared as described above. The methyl esters were redissolved in 100 μl isohexane containing 0.01% BHT and applied as 2.5 cm streaks to TLC plates impregnated by spraying with 2 g silver nitrate in 20 ml acetonitrile and pre-activated at 110 ºC for 30 min. Plates were fully developed in toluene/acetonitrile (95:5, v/v) (Wilson and Sargent 1992). Autoradiography was performed with Kodak MR2 film for 6 days at room temperature. Areas of silica containing individual PUFA were scraped into scintillation mini-vials containing 2.5 ml of scintillation fluid (Ecoscint A, National Diagnostics, Atlanta, Georgia) and radioactivity determined in a TRI-CARB 2000CA scintillation counter (United Technologies Packard, U.K.). Results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions.

Assay of hepatocyte and enterocyte fatty acyl oxidation activities

The assay of fatty acid oxidation in intact hepatocytes has been demonstrated in rats (Frøyland et al.,

1996; Madsen et al., 1998) and requires the determination of acid-soluble radioactivity as described in detail previously (Frøyland et al., 2000; Torstensen et al., 2000). Briefly, the 1 ml of hepatocyte or enterocyte suspension was homogenized with a hand-held tissue disrupter (Ultra-Turrax T8/S8N-5G probe, IKA-Werke GmbH & Co., Slaufen, Germany) and centrifuged at 2000 x g for 10 min in a microcentrifuge. Five hundred μl of the supernatant was taken into a clean 2 ml microcentrifuge and 100 μl of ice-cold 6% FAF-BSA solution in water was added. After mixing thoroughly, the protein was precipitated by the addition of 1.0 ml of ice-cold 4M perchloric acid ($HClO_4$). After vortexing, the tubes were centrifuged at 5000 g for 10 min in a microcentrifuge. Five hundred μl of the supernatant was carefully transferred to a scintillation vial, 4 ml of scintillant added and radioactivity in the acid-soluble fraction determined as described above for desaturation/elongation assay.

Protein determination

Protein concentration in isolated hepatocyte and enterocyte suspensions was determined according to the method of Lowry et al. (1951) after incubation with 0.4 ml of 0.25% (w/v) SDS/1M NaOH for 45 min at 60ºC.

Materials

[1-^{14}C]18:3n-3 (50-55 mCi/mmol) was obtained from NEN (DuPont (U.K.) Ltd., Stevenage, U.K.). HBSS, Medium 199, HEPES buffer, glutamine, collagenase (type IV), FAF-BSA, BHT, silver nitrate and perchloric acid were obtained from Sigma Chemical Co. (Poole, U.K.). Thin-layer chromatography (TLC) plates, precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific UK, Loughborough, England.

Statistical analysis

All the data are presented as means ± SD (n = 3, 4 or 6 as stated). The relationships between dietary fatty acid contents and growth, liver total lipid and neutral lipid contents and liver fatty acid compositions, and between hepatocyte fatty acyl desaturation activity and both dietary and liver fatty acid compositions were determined by regression analyses (Prism 3, Graphpad Software, Inc., San Diego, USA). Some data such as growth (final weight), and the effects of diet on fatty acid desaturation and oxidation were also analyzed by one-way ANOVA followed where appropriate by Tukey's post

test (Figs. 1 & 3) or the student's t-test (Fig. 2) to determine significant differences between individual treatments. Percentage data and data which were identified as non-homogeneous (Bartlett's test) were subjected to either arcsine or log transformation before analysis. Differences were regarded as significant when $P < 0.05$ (Zar 1984).

Results

Dietary fatty acid compositions

The control diet (FO), formulated with 100% FO, contained approximately 20% total saturates, mainly 16:0, almost 60% total monounsaturated fatty acids over half of which were the long chain monoenes, 20:1 and 22:1, 5% n-6 fatty acids predominantly 18:2n-6, and 16% n-3 fatty acids, predominantly the n-3HUFA, 20:5n-3 and 22:6n-3 in approximately equal amounts, and less than 1% 18:3n-3 (Table 2). Inclusion of graded amounts of LO resulted in graded increased percentages of 18:3n-3, 18:2n-6 and 18:1n-9 with concomitant decreased proportions of n-3HUFA, 16:0, total saturates, 20:1, 22:1 and total monoenes. Thus, in the diet formulated with 100% LO (LO100), the levels of 18:3n-3 and 18:2n-6 had risen to 50% and 15% of total fatty acids, respectively, whereas 20:5n-3 and 22:6n-3 totalled only 2.5%, and total saturates and monoenes were reduced to 10% and 21%, respectively (Table 2).

Effects of diet on liver and pyloric caecal total and neutral lipid contents

The total lipid contents of both liver and pyloric caecae increased with increasing dietary inclusion of LO (Table 3). Regression analyses showed significant positive correlations (slopes) between dietary 18:3n-3 content and total lipid content of the tissues with r^2 values greater than 0.8 (Table 6). The proportion of neutral lipids also generally increased in liver lipids with increasing dietary LO and regression analyses showed there was a significant positive correlation with a degree of fit similar to total lipid (Tables 3 and 6). The proportion of neutral lipids in pyloric caecal lipids was higher than in liver and did not show a significant positive correlation with dietary LO (18:3n-3) although there was an upward trend (Tables 3 and 6). The midgut total and neutral lipid contents were not significantly different to the caecal compositions and so were not presented.

Effects of diet on liver and pyloric caecal fatty acid compositions

The principal fatty acids of liver total lipid from fish fed diet FO were, in rank order, 22:6n-3 (24%), 16:0 (18%), 18:1n-9 (12%), 20:5n-3 (10%) and 20:1n-9 (7%) (Table 4). The graded change in fatty acid compositions of the diets in response to increasing LO content, described above, was reflected in the liver fatty acid compositions with graded increased percentages of 18:3n-3, 18:2n-6 and 18:1n-9 and decreased proportions of n-3HUFA, 16:0, total saturates, 20:1, 22:1 and total monoenes. In addition, however, there were increased proportions of 18:4n-3, 20:3n-3, 20:4n-3 and 20:2n-6, and decreased proportions of 20:4n-6 and 22:5n-3 (Table 4). In the livers of fish fed diet LO100, the levels of 18:3n-3 and 18:2n-6 had risen to almost 33% and 12% of total fatty acids, respectively, whereas 20:5n-3 and 22:6n-3 were reduced to around 3% and 6%, respectively. These effects were highly significant as shown by regression analyses with positive correlations (slopes) between dietary 18:3n-3 and liver 18:3n-3 and negative correlations between dietary 18:3n-3 and liver 20:5n-3 and 22:6n-3 levels (Table 6). Similar highly significant correlations between dietary level and liver level can be derived for any of the major fatty acids.

In comparison with liver, the fatty acid composition of total lipid from pyloric caecae of fish fed the FO diet showed higher levels of monoenes, particularly 20:1 and 22:1 and lower levels of n-3HUFA, with levels of 20:5n-3 and 22:6n-3 only about 50% the levels observed in liver (Table 5). However, the effects of increased inclusion of dietary LO were identical to those described for liver with the levels of 18:3n-3 and 18:2n-6 increased to almost 35% and 13% of total fatty acids, respectively, and 20:5n-3 and 22:6n-3 reduced to around 3% and 5%, respectively, in fish fed the LO100 diet. As with liver, regression analyses showed these effects were highly significant (Table 6). The fatty acid compositions of the midgut samples were virtually identical to those for the caecae (data not shown).

Effects of diet on hepatocyte and pyloric caecal fatty acid desaturation/elongation activities.

The total desaturation of [1-^{14}C]18:3n-3, measured as the summed radioactivity recovered as desaturated fatty acid products (18:4, 20:4, 20:5, 22:5 and 22:6), was 2-fold higher in enterocytes than hepatocytes in fish fed the diet containing FO (Fig.1). However, the desaturation of [1-^{14}C]18:3n-3 in hepatocytes was increased in a graded manner by increasing dietary LO, and regression analyses confirmed a significant positive correlation with a very high degree of confidence ($p = 0.001$ and $r^2 = 0.98$) between dietary 18:3n-3 levels and hepatocyte fatty acid desaturation (Fig.1. and Table 6). In contrast, total desaturation of [1-^{14}C]18:3n-3 in pyloric caecal (PC) enterocytes was significantly decreased in fish fed dietary LO at levels of 50% or more (Fig.1), but there was no direct correlation between dietary 18:3n-3 levels and caecal fatty acid desaturation activity (Table 6). In enterocytes

isolated from the midgut (MG) region just posterior to the caecae, desaturation of [1-^{14}C]18:3n-3 tended to decrease with increasing dietary LO with the activity being significantly lower in fish fed all levels of LO compared to fish fed the diet containing 100% FO (Fig.1).

In both hepatocytes and enterocytes, the main products of 18:3n-3 desaturation were the Δ6 desaturated products, 18:4n-3 and 20:4n-3 (Fig.2). Furthermore, the major effect of feeding diets containing LO on the desaturation of 18:3n-3 in hepatocytes was to increase the amount of Δ6 desaturated products whereas recovery of radioactivity in 20:5n-3 was only slightly, but significantly, increased, and recoveries in 22:5n-3 and 22:6n-3 were unaffected. In MG enterocytes, the recovery of radioactivity in all products of 18:3n-3 desaturation was significantly reduced in fish fed the LO100 diet compared to fish fed the FO diet (Fig.2).

Effects of diet on hepatocyte and pyloric caecal fatty acid oxidation activities.

The production of acid soluble products from [1-^{14}C]18:3n-3 was higher in enterocytes than in hepatocytes, and highest in MG enterocytes, irrespective of dietary treatment (Fig.3). In enterocytes, the production of acid-soluble products from [1-^{14}C]18:3n-3 was generally higher, significantly so in PC enterocytes, in fish fed diets containing LO compared to fish fed the FO diet (Fig.3), but there was no significant correlation with dietary 18:3n-3 levels (Table 6). There was no significant effect of dietary treatment on the production of acid-soluble products from [1-^{14}C]18:3n-3 in hepatocytes (Fig.3, Table 6).

Discussion

The primary hypothesis we aimed to test in the present study was that replacing FO with 18:3n-3-rich LO in salmon diets would enable the fish to maintain tissue n-3HUFA levels through a combination of increased desaturation/elongation activity and increased substrate fatty acid provision. The results show that this was not the case. Increasing dietary LO increased lipid accumulation, through increased neutral lipids, particularly in the liver. This also occurred in intestinal tissue although it was already higher in neutral lipid than liver. In previous trials, increasing inclusion of rapeseed oil or palm oil in the diet also increased the liver lipid levels in Atlantic salmon in seawater (Bell et al. 2001a, 2002). More importantly though, both liver and intestinal tissue showed very large increases in the proportions of 18:3n-3, and also 18:2n-6, with n-3HUFA decreasing significantly with increasing dietary LO, particularly at inclusion of > 50%. The greater effect on n-3HUFA was in liver where 20:5n-3

decreased over 3-fold and 22:6n-3 over 4–fold in fish fed the diet containing 100% LO. Previously, we observed that the level of 22:6n-3 in liver was halved in fish fed a diet containing 100% rapeseed oil compared to fish fed a 100% FO diet (Bell et al. 2001a). Therefore, supplying high amounts of 18:3n-3 in the diet in the form of LO did not alleviate the decrease in tissue n-3HUFA.

The decreased levels of tissue n-3HUFA occurred despite the fatty acid desaturation/elongation activity in liver being increased over 2.6-fold in fish fed the 100% LO diet compared to the control diet. Increasing hepatocyte fatty acid desaturation with increasing inclusion of vegetable oil in the diet has been observed in several previous studies. Similar experiments on salmon in seawater using graded levels of rapeseed oil and palm oil gave similar graded increases in hepatocyte fatty acid desaturation activity, with 100% replacement of FO giving increases of 2.7- (Bell et al. 2001a) and 10-fold (Bell et al. 2002), respectively. In freshwater, salmonid hepatocyte desaturation activities were up to 2.5-fold (Bell et al. 1997), 2.4-fold (Tocher et al. 2000) and 2.8-fold (Tocher et al. 2001) greater in fish fed vegetable oils compared to fish fed FO. It is unclear whether the increased desaturation activity in fish fed vegetable oils is the result of decreasing levels of 20:5n-3 and 22:6n-3 in liver membrane lipids, and, thus, mediated by decreased product inhibition, or whether it is a result of increased levels of membrane 18:2n-6 and/or 18:3n-3 and, thus, mediated by increased substrate provision. In this respect, it is important to note that the correlations reported in Table 6 were between dietary 18:3n-3 levels and the various outcomes, including hepatocyte fatty acyl desaturation/elongation. However, similar correlations are obtained when you compare any of the other dietary fatty acids that vary in a graded manner across the diets, which includes the major fatty acids 18:2n-6, 20:5n-3, 22:6n-3, 18:1n-9, 16:0, 20:1n-9 and 22:1. Therefore, regression analyses of dietary 20:5n-3 or 22:6n-3 with hepatocyte desaturation also shows a significant correlation, albeit negative compared to the correlation with dietary 18:3n-3, but with a similar significance and r^2 value. Therefore, the present study cannot elucidate the mechanism, but it does demonstrate clearly that, irrespective of mechanism, the increased hepatic fatty acyl desaturation/elongation induced by feeding vegetable oils to salmon is ineffective in maintaining tissue levels of either 20:5n-3 or 22:6n-3, even in the presence of high levels of dietary 18:3n-3.

It is perhaps noteworthy that desaturation/elongation activity was measured by incubating cells with [1-^{14}C]18:3n-3 and determining the amount of radioactivity recovered as desaturated and elongated fatty acid products. Thus, desaturation of [1-^{14}C]18:3n-3 in hepatocytes and enterocytes was observed through the levels of radioactivity recovered mainly as 18:4n-3 and 20:4n-3 whereas recovery of radioactivity as 20:5n-3 and 22:6n-3 was much lower. Similarly, the effects of feeding LO are mainly manifested in changes in the recovery of radioactivity in the two main products 18:4n-3 and 20:4n-3.

These data suggest that production of 20:5n-3 and, especially 22:6n-3, is low within the time-scale of the assay. That desaturation of 18:3n-3 was not sufficient to maintain tissue levels of 20:5n-3 and 22:6n-3 in fish fed increasing LO suggests that these steps are also low *in vivo*. Therefore, the reduced level of dietary n-3HUFA as dietary LO inclusion increased was the biggest factor in determining the tissue levels of 20:5n-3 and 22:6n-3. Consistent with this, the intermediates 18:4n-3 and 20:4n-3 were increased in both liver and intestinal tissue lipids with LO feeding. Interestingly, there was no evidence for desaturation of dietary 18:2n-6 as, in addition to declining 20:4n-6, there was no increase in the levels of the intermediates 18:3n-6 or 20:3n-6 in either liver or intestinal tissue. This is probably due to the excess of 18:3n-3 effectively blocking the Δ6 desaturase and preventing desaturation of 18:2n-6, an effect has been observed previously (Bell et al. 1993). The only indication of metabolism of 18:2n-6 in fish fed LO was the increased levels of the so-called "dead end" products, 20:2n-6 (and 20:3n-3 from 18:3n-3) in both liver and intestinal tissue.

A secondary aim in the present study was to determine if intestinal enterocytes were a site of significant fatty acid desaturation/elongation in salmon and this was proved to be the case. Indeed, enterocytes were more active than hepatocytes in desaturating and elongating the exogenously added [1-^{14}C]18:3n-3 substrate in fish fed 100% FO. However, in fish fed dietary LO, the intestinal enterocytes did not appear to have the capacity to increase their desaturation activity as with hepatocytes. In fact, fatty acid desaturation/elongation activity decreased in MG enterocytes proportionally with increasing dietary LO, and initially decreased (up to 50% LO inclusion) in PC enterocytes. This was surprising and different to the data obtained with hepatocytes in the present study and all previous studies on hepatocytes (Bell et al. 1997, 2001a, 2002: Tocher et al. 1997, 2000, 2001). One possible explanation is that fatty acyl desaturation and elongation in the two tissues serves different purposes with different regulatory mechanisms. Thus, the primary purpose of hepatic fatty acid desaturation may be to provide the body with sufficient long-chain HUFA for membrane biosynthesis and as such it is nutritionally induced when membrane HUFA declines as it does when feeding vegetable oil diets. In contrast, the fatty acyl desaturation activity in enterocytes may be simply to supply its own needs for HUFA for biomembrane synthesis. Being a tissue with a high turnover of cells/membranes, fatty acid desaturation/elongation may be relatively high and unaffected by diet. However, the activities in enterocytes were not maintained in the present study suggesting this is not a full explanation. A full explanation of the apparent difference in fatty acyl desaturation and elongation activities in hepatocytes and enterocytes and their nutritional regulation in liver and intestine *in vivo* will require considerably more research.

Our third aim in the present study was to determine the extent to which 18:3n-3 was oxidized in comparison with the amount desaturated/elongated simultaneously, in a combined assay, in both hepatocytes and enterocytes. Measurement of fatty acid oxidation in Atlantic salmon using isolated hepatocytes and [1-^{14}C]16:0, rather than labeled acyl CoA substrates as used in mitochondrial assays, has been described previously (Ji et al. 1996). In the present study we modified existing methods (Frøyland et al. 1995, 2000; Ji et al. 1996) slightly in order to determine fatty acid oxidation and the desaturation pathway in a single, combined assay. Our results showed that in hepatocytes from fish fed the FO diet, there was a similar amount of radioactivity recovered in acid soluble products as there was in desaturated products indicating that approximately equal amounts of the exogenously added [1-^{14}C]18:3n-3 were being oxidized and desaturated. In contrast, fatty acid oxidation activity was about 1.5- fold and 3-fold higher than desaturation activity in PC and MG enterocytes, respectively, in fish fed the FO diet. In addition, fatty acid oxidation activity was around 5- to 8-fold greater in intestinal enterocytes compared to hepatocytes in fish fed the FO diet. There are few data in the literature with which to compare the present data. Relatively high levels of fatty acid oxidation have been reported for red muscle and heart in trout, whereas liver, kidney and white muscle have only limited capacity to oxidize fatty acids (Henderson and Tocher 1987). Recently though, white muscle was shown to be a quantitatively important site of fatty acid catabolism in salmon, particularly juveniles, when expressed on a per tissue basis (Frøyland et al. 1998, 2000). However, liver was not a major site of mitochondrial or peroxisomal β-oxidation irrespective of how the data were presented (Frøyland et al. 2000). Certainly, the level of fatty acid oxidation appeared relatively low in the hepatocytes, but there are few data on the fatty acid oxidative capacity of fish intestinal tissues (Small and Connock 1981).

It terms of dietary effects, again there was a difference between hepatocytes and enterocytes in fatty acid oxidation in response to dietary LO. In hepatocytes, and in contrast to the desaturation pathway, there was no dietary effect on the oxidation of [1-^{14}C]18:3n-3. However, in enterocytes, fatty acid oxidation activity was generally increased in fish fed diets containing LO whereas desaturation activity generally decreased although there was no correlation with the dietary level of 18:3n-3. This was interesting in relation to the discussion above on the effects of diet on desaturation/elongation activities, because the apparent reciprocal manner in which the oxidation and desaturation assays varied in enterocytes suggested that there was the possibility of competition between the two pathways for available labeled fatty acid substrate. Whether this is the case and whether such competition between the two pathways for fatty acid could occur *in vivo* is unclear.

In rats, supplementation of the diet with 20:5n-3 and 22:6n-3 increased β-oxidation of PUFA, especially 18:2n-6, 18:3n-3 and 20:5n-3 (Grønn et al. 1992a). The results in rats suggested both

mitochondrial and peroxisomal oxidation were increased but particularly peroxisomal with respect to n-3 PUFA. In Atlantic salmon, fatty acid oxidation is predominantly mitochondrial β-oxidation with peroxisomal β-oxidation accounting for less than 10% of total oxidation (Frøyland et al. 2000). Mitochondrial β-oxidation of [1-^{14}C]palmitoyl-CoA in red muscle of Atlantic salmon was not significantly affected by feeding vegetable oils namely, high oleic acid sunflower oil or palm oil, as 100% replacement of FO (capelin oil) for 21 weeks (Torstensen et al. 2000). In that study, the β-oxidation activity in liver homogenates, using [1-^{14}C]palmitoyl-CoA as substrate, was about 100-fold lower than in red muscle and 7-fold lower than in white muscle in fish fed capelin oil at the start of the trial, and no β-oxidation activity was detected in liver after feeding capelin oil or the vegetable oils (Torstensen et al. 2000). Studies in rat liver parenchymal cells and subcellular fractions suggested that 20:5n-3 can increase mitochondrial fatty acid oxidation, possibly by increasing transport of fatty acids into mitochondria as it increased the level of carnitine palmitoyltransferase-I mRNA (Madsen et al. 1999). The effect of 20:5n-3 on gene transcription may have been mediated through activation of peroxisome proliferator activated receptor α (PPARα) (Berge et al. 1999).

Although oxidation in intestinal enterocytes increased rather than decreased as the level of dietary 20:5n-3 decreased in the present study, the above suggests a possible molecular mechanism for the effects of diet on fatty acid desaturation and oxidation. Dietary fatty acids are known to affect the expression of fatty acid desaturase genes in both mammals (Cho et al. 1999a,b) and trout (Seiliez et al. 2001). PUFA can potentially affect gene transcription by a number of direct and indirect mechanisms (Jump et al. 1999) and are known to bind and directly influence the activities of a variety of transcription factors including PPARs, which in turn have been shown to be regulators of many genes involved in lipid homeostatic processes (Jump 2002). In rodents, peroxisomal proliferators, which also activate PPARs, are known to up-regulate fatty acyl desaturation and oxidation (Grønn et al. 1992b). PPAR genes have been identified in Atlantic salmon and plaice (Ruyter et al. 1997; Leaver et al. 1998) and the peroxisomal proliferator, clofibrate, increased the desaturation of 20:5n-3 in rainbow trout (Tocher and Sargent 1993). However, these data do not exclude the possibility that fatty acids may also influence desaturation and oxidation more directly at a membrane level through alterations in fluidity or membrane microenvironments.

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Legends to Figures:

FIG. 1. Total fatty acid desaturation/elongation activity in hepatocytes and enterocytes isolated from pyloric caecae (PC) and mid gut (MG) after feeding the experimental diets for 40 weeks. Results are means ± S.D. (n = 3) and represent the rate of conversion (pmol.h^{-1}.mg $protein^{-1}$) of [1-^{14}C]18:3n-3 to all desaturated products (sum of radioactivity recovered as 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3). Columns for a specific a tissue with different superscript letters are significantly different as determined by one-way ANOVA followed by the Tukey post test ($P < 0.05$). FO, fish oil; LO, linseed oil.

FIG. 2. Individual fatty acid products of the desaturation of [1-^{14}C]18:3n-3 in hepatocytes and enterocytes isolated from the mid gut (MG) from fish fed the FO and LO100 diets for 40 weeks. Results are means ± S.D. (n = 3) and represent the rate production (pmol.h^{-1}.mg $protein^{-1}$) of individual fatty acids as determined by the recovery of radioactivity in each fatty acid fraction. Mean values for radioactivity recovered as individual fatty acids in a specific tissue in fish fed the LO100 diets were significantly different to those for fish fed the FO diet where indicated (*) as determined by the student t-test ($P < 0.05$). FO, fish oil; LO, linseed oil.

FIG. 3. Fatty acid β-oxidation activity in hepatocytes and enterocytes isolated from pyloric caecae (PC) and mid gut (MG) after feeding the experimental diets for 40 weeks. Results are means ± S.D. (n = 3) and represent the rate of oxidation (pmol.h^{-1}.mg $protein^{-1}$) of [1-^{14}C]18:3n-3 to acid soluble products. Columns for a specific a tissue with different superscript letters are significantly different as determined by one-way ANOVA followed by the Tukey post test ($P < 0.05$). FO, fish oil; LO, linseed oil.

Table 1. Formulation (g/kg) of feed.

Component	FO	LO25	LO50	LO75	LO100
Fishmeal[1]	338	338	338	338	338
Maize gluten[2]	200	200	200	200	200
Soya (Hi Pro)[3]	100	100	100	100	100
Fish oil[4]	258	193.5	129	64.5	0
Linseed oil[5]	0	64.5	129	193.5	258
Micronutrients[6]	25	25	25	25	25

[1]Scandinavian LT-fish meal (Nordsildmel, Norway).

[2]Cargill/ADM, Decatur, Illinois.

[3]Soybean meal (Denofa, Fredrikstad, Norway).

[4]Capelin oil (Nordsildmel, Norway) supplemented with 200 ppm BHT.

[5]Crude E.C.C. linseed oil (N.V. Oliefabriek Lictervelde, Belgium) supplemented with 500ppm Ronoxan A (Roche, Switzerland).

[6]Vitamin, mineral and carotenoid pigment premix formulated to Nutreco specification (Farmix, Trouw Nutrition, TheNetherlands).

Table 2. Proximate (% of weight of feed) and fatty acid compositions (% of total fatty acids by weight) of the experimental diets.

	FO	LO25	LO50	LO75	LO100
Protein	44.1	44.1	44.2	44.4	43.6
Lipid	28.9	28.9	30.2	29.5	31.1
Ash	6.5	6.9	7.2	7.5	7.6
Moisture	5.9	6.3	5.7	6.1	5.6
Fatty acid					
14:0	6.3	4.7	3.4	2.0	0.4
16:0	12.1	10.6	9.3	8.1	6.9
18:0	1.1	1.7	2.1	2.7	3.1
Total saturates	19.9	17.2	15.1	13.0	10.5
16:1n-7	8.1	6.1	4.2	2.3	0.5
18:1n-9	11.9	13.6	15.1	16.0	17.0
18:1n-7	3.3	2.6	2.2	1.6	1.0
20:1n-9	17.9	13.1	9.0	5.0	1.1
22:1n-11	13.3	10.1	7.1	4.3	1.1
22:1n-9	2.1	1.5	1.0	0.5	0.1
Total monoenes	58.4	48.4	39.6	30.5	21.1
18:2n-6	4.2	7.4	9.8	12.3	15.1
20:4n-6	0.2	0.2	0.1	0.1	0.1
Total n-6PUFA	5.0	8.0	10.2	12.6	15.2
18:3n-3	0.9	14.0	25.6	37.8	50.4
18:4n-3	2.9	2.1	1.6	0.9	0.2
20:5n-3	5.9	4.6	3.5	2.2	1.0
22:6n-3	5.0	4.0	3.4	2.4	1.5
Total n-3PUFA	15.7	25.6	34.6	43.7	53.3
Total PUFA	21.7	34.4	45.3	56.5	68.5

[1]Totals include 15:0, present at up to 0.3%;[2]Totals include 16:1n-7, 20:1n-11, 20:1n-7 and 24:1, present at up to 0.5%; [3]Totals include 18:3n-6, 20:2n-6 and 20:3n-6, present at up to 0.2%; [4]Totals include 20:3n-3, 20:4n-3 and 22:5n-3, present at up to 0.4%. PUFA, polyunsaturated fatty acids.

Table 3. Effects of dietary oil on lipid content (percentage of wet weight) and neutral (storage lipid cotent (percentage of total lipid of liver and pyloric caecae from Atlantic salmo (*Salmo salar* L.)

	Liver		Pyloric caecae	
Treatment	lipid content	neutral lipids	lipid content	neutral lipids
FO	3.8 ± 0.3	53.6 ± 2.9	4.0 ± 0.4	79.3 ± 3.7
LO25	4.4 ± 0.3	52.9 ± 2.0	4.8 ± 1.0	81.6 ± 1.4
LO50	4.5 ± 0.5	59.7 ± 2.4	4.6 ± 0.9	81.0 ± 2.3
LO75	5.2 ± 0.5	59.3 ± 3.0	5.4 ± 1.0	84.1 ± 3.0
LO100	7.3 ± 1.5	73.1 ± 5.2	5.5 ± 1.2	82.9 ± 1.7

Results are means ± S.D. (n = 6 for liver and 4 for caecae). FO, fish oil; LO, linseed oil.

Table 4. Fatty acid compositions (percentage of total fatty acids by weight) of total lipid of liver from Atlantic salmon (*Salmo salar* L.) fed the experimental diets for 40 weeks.

	FO	LO25	LO50	LO75	LO100
14:0	2.8 ± 0.2	2.2 ± 0.2	1.7 ± 0.1	1.1 ± 0.0	0.5 ± 0.0
16:0	17.9 ± 1.1	15.0 ± 1.6	13.3 ± 1.0	11.3 ± 1.1	7.3 ± 0.8
18:0	3.1 ± 0.2	3.8 ± 0.2	4.1 ± 0.2	4.6 ± 0.1	4.9 ± 0.2
Total saturated[1]	24.1 ± 1.2	21.3 ± 1.8	19.3 ± 1.0	17.0 ± 1.1	12.7 ± 1.0
16:1n-7	3.5 ± 0.2	3.0 ± 0.2	2.4 ± 0.2	1.5 ± 0.1	0.7 ± 0.1
18:1n-9	11.9 ± 1.1	14.7 ± 0.8	14.1 ± 1.5	15.7 ± 1.3	19.1 ± 2.4
18:1n-7	3.5 ± 0.1	3.0 ± 0.1	2.3 ± 0.1	1.7 ± 0.1	1.2 ± 0.1
20:1n-9	6.8 ± 1.2	6.1 ± 0.5	4.2 ± 0.7	3.0 ± 0.2	1.4 ± 0.3
22:1	2.1 ± 0.4	1.9 ± 0.3	1.6 ± 0.5	1.0 ± 0.1	0.5 ± 0.1
24:1n-9	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.4 ± 0.1
Total monoenes[2]	30.2 ± 3.1	31.1 ± 1.9	26.4 ± 3.0	24.5 ± 1.9	23.9 ± 2.9
18:2n-6	2.6 ± 0.1	4.4 ± 0.1	6.0 ± 0.3	8.0 ± 0.2	11.6 ± 0.6
20:2n-6	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	1.1 ± 0.1
20:4n-6	1.4 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.3 ± 0.1
Total n-6 PUFA[3]	4.9 ± 0.2	6.6 ± 0.2	8.0 ± 0.3	10.0 ± 0.2	13.3 ± 0.7
18:3n-3	0.5 ± 0.2	6.8 ± 0.5	14.4 ± 0.6	20.3 ± 1.1	32.6 ± 1.2
18:4n-3	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	1.1 ± 0.2
20:3n-3	0.1 ± 0.0	0.8 ± 0.1	1.6 ± 0.2	2.5 ± 0.2	3.8 ± 0.4
20:4n-3	1.6 ± 0.1	1.9 ± 0.1	2.4 ± 0.2	3.0 ± 0.1	3.1 ± 0.4
20:5n-3	10.0 ± 0.4	8.5 ± 0.5	7.6 ± 0.7	6.4 ± 0.8	3.1 ± 1.1
22:5n-3	3.7 ± 0.4	2.8 ± 0.2	2.3 ± 0.3	1.6 ± 0.1	0.8 ± 0.3
22:6n-3	24.4 ± 1.6	19.7 ± 1.5	17.2 ± 1.7	13.7 ± 1.4	5.6 ± 1.6
Total n-3 PUFA	40.6 ± 2.1	40.8 ± 2.0	46.0 ± 2.4	48.3 ± 1.1	50.0 ± 3.3
Total PUFA	45.7 ± 2.0	47.6 ± 1.9	54.3 ± 2.2	58.5 ± 0.9	63.4 ± 2.6

Results are means ± SD (n = 6). [1] Includes 15:0 present at up to 0.3%; [2] Includes 16:1n-9, 20:1n-7, 20:1n-11 and 22:1n-9 each present at up to 0.4%; [3]Includes 18:3n-6, 20:3n-6 and 22:5n-6 present at up to 0.4%; FO, fish oil; LO, linseed oil; PUFA, polyunsaturated fatty acids.

Table 5. Fatty acid compositions (percentage of total fatty acids by weight) of total lipid of pyloric caeca from Atlantic salmon (*Salmo salar* L.) fed the experimental diets for 40 weeks.

	FO	LO25	LO50	LO75	LO100
14:0	4.0 ± 0.5	3.4 ± 0.2	2.5 ± 0.2	1.5 ± 0.1	0.5 ± 0.0
16:0	16.1 ± 0.6	13.6 ± 0.8	13.0 ± 1.0	10.7 ± 0.6	9.7 ± 0.5
18:0	3.5 ± 0.3	3.5 ± 0.3	4.4 ± 0.4	4.4 ± 0.2	5.0 ± 0.3
Total saturated[1]	24.0 ± 0.5	20.8 ± 1.0	20.1 ± 1.4	16.7 ± 0.7	15.3 ± 0.8
16:1n-7	5.5 ± 0.5	4.5 ± 0.2	3.0 ± 0.2	1.9 ± 0.1	0.6 ± 0.0
18:1n-9	14.4 ± 1.0	15.2 ± 0.4	15.8 ± 0.6	16.2 ± 0.6	17.3 ± 0.2
18:1n-7	3.9 ± 0.2	3.3 ± 0.1	2.6 ± 0.1	1.8 ± 0.0	1.2 ± 0.0
20:1n-9	13.8 ± 1.5	11.3 ± 0.9	7.9 ± 0.8	4.6 ± 0.5	1.4 ± 0.1
22:1	7.9 ± 1.3	7.4 ± 0.6	4.9 ± 0.7	3.2 ± 0.5	1.1 ± 0.1
24:1n-9	0.9 ± 0.0	0.8 ± 0.0	0.7 ± 0.1	0.4 ± 0.0	0.3 ± 0.0
Total monoenes[2]	47.1 ± 4.0	43.0 ± 1.8	35.4 ± 2.2	28.4 ± 1.8	22.0 ± 0.4
18:2n-6	3.3 ± 0.2	5.9 ± 0.2	7.3 ± 0.6	10.5 ± 0.4	12.7 ± 0.4
20:2n-6	0.5 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
20:4n-6	1.1 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
Total n-6 PUFA[3]	5.2 ± 0.2	7.8 ± 0.2	9.5 ± 0.5	12.1 ± 0.4	14.1 ± 0.3
18:3n-3	0.7 ± 0.2	8.9 ± 0.6	13.5 ± 1.5	27.1 ± 1.5	34.6 ± 1.9
18:4n-3	0.8 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	1.3 ± 0.1
20:3n-3	0.1 ± 0.1	0.8 ± 0.1	1.7 ± 0.2	2.5 ± 0.5	2.4 ± 0.2
20:4n-3	1.0 ± 0.1	1.4 ± 0.2	1.9 ± 0.0	1.7 ± 0.3	1.9 ± 0.2
20:5n-3	5.3 ± 1.2	4.9 ± 0.3	5.1 ± 0.9	3.3 ± 0.3	2.7 ± 0.5
22:5n-3	2.1 ± 0.5	1.8 ± 0.2	1.9 ± 0.3	1.0 ± 0.1	0.7 ± 0.1
22:6n-3	13.4 ± 2.5	9.4 ± 1.1	9.6 ± 1.9	6.2 ± 1.2	4.9 ± 0.7
Total n-3 PUFA	23.5 ± 3.6	28.2 ± 1.8	34.7 ± 1.7	42.7 ± 1.4	48.4 ± 1.0
Total PUFA	28.8 ± 3.5	36.0 ± 1.7	44.3 ± 1.6	54.7 ± 1.2	62.5 ± 0.9

Results are means ± SD (n = 6).[1] Includes 15:0 present at up to 0.3%; [2] Includes 16:1n-9, 20:1n-7, 20:1n-11 and 22:1n-9 each present at up to 0.4%; [3]Includes 18:3n-6, 20:3n-6 and 22:5n-6 present in at up to 0.4%. FO, fish oil; LO, linseed oil; PUFA, polyunsaturated fatty acids.

Table 6. Correlation (regression) analyses (r^2, slope values and significance) for dietary 18:3n-3 and liver and caecal total and neutral lipid contents, percentages of liver and caecal 18:3n-3, 20:5n-3 and 22:6n-3, and fatty acyl desaturation and β-oxidation activities in hepatocytes and enterocyte from caecae and mid gut.

	Liver			Caecae		
Fatty acid	r^2	slope	significance	r^2	slope	significance
Total lipid content	0.83	0.064 ± 0.017	0.0315	0.86	0.029 ± 0.007	0.0222
Neutral lipid content	0.78	0.369 ± 0.113	0.0466	0.70	0.079 ± 0.030	0.0756
18:3n-3	0.98	0.633 ± 0.051	0.0011	0.98	0.700 ± 0.059	0.0013
20:5n-3	0.94	-0.130 ± 0.080	0.0070	0.83	-0.055 ± 0.014	0.0306
22:6n-3	0.96	-0.356 ± 0.043	0.0036	0.93	-0.165 ± 0.026	0.0077
Total desaturation	0.98	0.051 ± 0.004	0.0010	0.43	-0.030 ± 0.020	0.2265
(mid-gut)				0.74	-0.038 ± 0.013	0.0630
β-Oxidation	0.06	-0.003 ± 0.006	0.6946	0.41	0.058 ± 0.040	0.2433
(mid-gut)				0.3	0.038 ± 0.034	0.3387

Fig.1

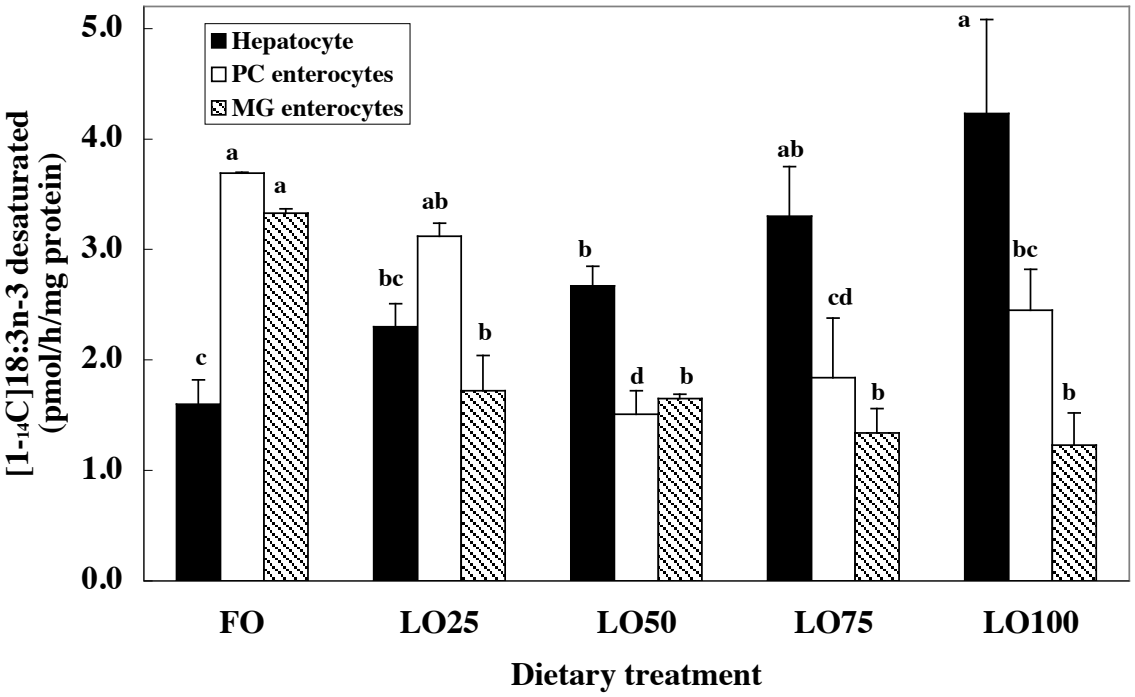

Hepatocyte
PC enterocytes
MG enterocytes
[1-14C]18:3n-3 desaturated (pmol/h/mg protein)
5.0
4.0
3.0
2.0
1.0
0.0
a
a
c
ab
bc
b
b
d
b
ab
cd
b
a
bc
b
FO
LO25
LO50
LO75
LO100
Dietary treatment

Fig.2.

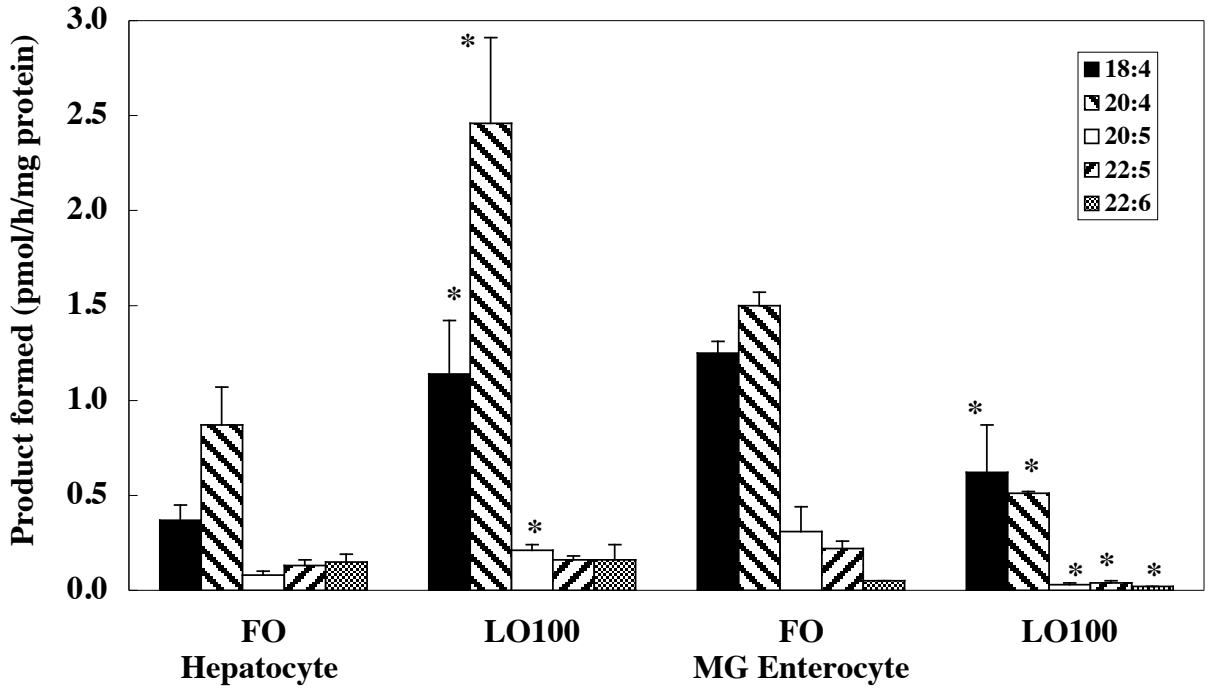
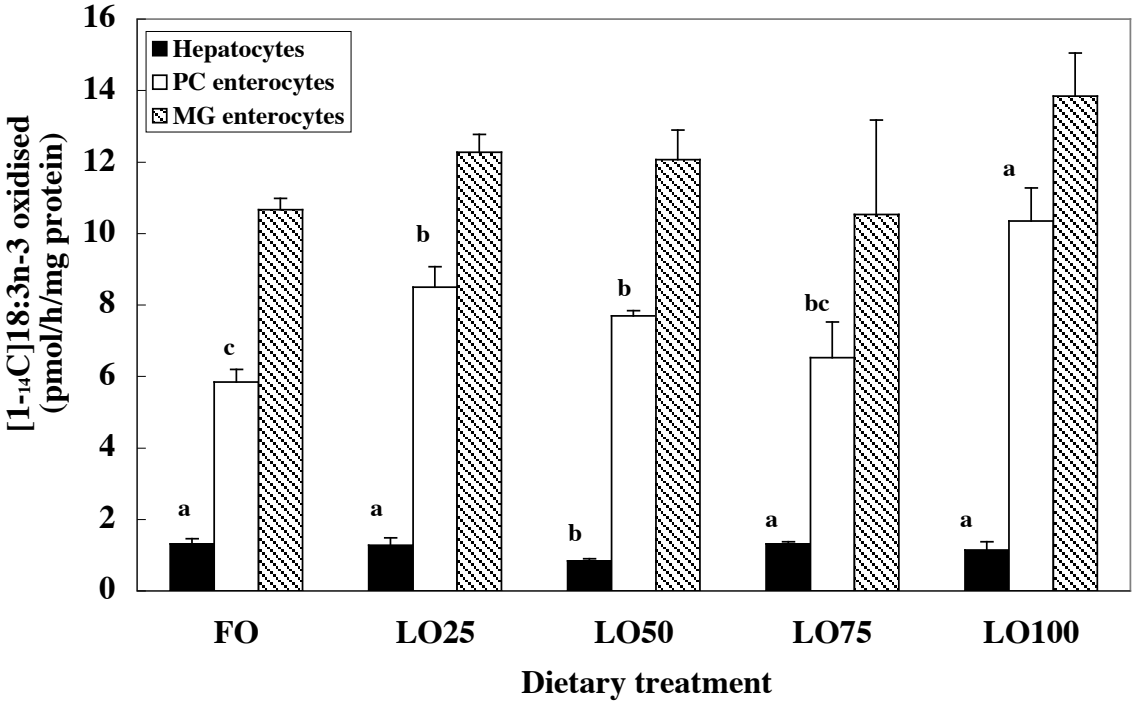

Product formed (pmol/h/mg protein)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
18:4
20:4
20:5
22:5
22:6
FO
LO100
FO
LO100
Hepatocyte
MG Enterocyte

Fig.3


Hepatocytes
PC enterocytes
MG enterocytes
[1-14C]18:3n-3 oxidised (pmol/h/mg protein)
16
14
12
10
8
6
4
2
0
a
c
a
b
b
b
a
bc
a
a
FO
LO25
LO50
LO75
LO100
Dietary treatment