PAPER I

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Dietary Rapeseed Oil Affects the Expression of Genes Involved in Hepatic Lipid Metabolism in Atlantic Salmon (*Salmo salar* L.)[1,2]

Ann-Elise O. Jordal,[3] Bente E. Torstensen, Stephen Tsoi,*[4] Douglas R. Tocher,† Santosh P. Lall,* and Susan E. Douglas*

*National Institute of Nutrition and Seafood Research, N-5817 Bergen, Norway; *Institute for Marine Biosciences, Halifax, Nova Scotia, Canada B3H 3Z1; and †Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK*

ABSTRACT Supplies of marine fish oils (FO) are limited, and sustainable production in aquaculture dictates that alternatives that do not compromise fish health and product quality, such as vegetable oils, must be found. Nutrigenomics will increase our understanding of how nutrition influences metabolic pathways and homeostatic control, and may be used to measure and validate subtle changes in organ-specific, metabolic gene expression signatures. We compared 2 groups of Atlantic salmon fed diets containing 100% FO or 75% rapeseed oil (RO) for 42 wk. A small-scale cDNA microarray was constructed to screen for changes in the expression of lipid metabolism genes in the liver resulting from this partial substitution of RO for FO. Δ5 fatty acid desaturase gene expression was significantly greater in fish fed 75% RO than in fish fed the control diet; this was confirmed by quantitative real time PCR analysis. In addition, several genes, among these mitochondrial proteins, peroxisome proliferator-activated receptor γ, as well as other transcription factors, coactivators, and signal transducers, showed significant differential regulation. This partially validated microarray may be used for further gene expression profiling using other dietary comparisons, and for further characterization of selected genes. J. Nutr. 135: 2355–2361, 2005.

KEY WORDS: • *fatty acid* • *microarray* • *quantitative PCR* • *lipid metabolism* • *Atlantic salmon*

Partial substitution of marine fish oils (FO)[5] by vegetable oils in fish diets has become increasingly common in aquaculture, due to limited supplies of FO (1). These dietary changes are thought to influence lipid metabolic gene expression patterns in liver of Atlantic salmon, as in mammals, where changes in quantity or composition of dietary fat affect hepatic de novo lipogenesis, VLDL synthesis and secretion, fatty acid oxidation, and cholesterol metabolism (2). Several genes involved in these metabolic pathways are regulated in mammals through response elements for nuclear receptors. Hepatic nuclear factor-4α (HNF-4α) positively regulates apolipoprotein (apo) C-II (2) and apo A-I gene expression (3). The (n-3) and (n-6) fatty acids and other peroxisome proliferator-activated receptor (PPAR)α activators induce transcription of liver X receptor (LXR)-α through DR1 elements (4) and regulate the expression of apo C-II (5) and sterol regulatory element binding protein (SREBP)-1c (6). In addition, Δ9-fatty acid desaturase (SCD) (2,7), Δ6-, and Δ5- (8) fatty acid desaturases (FADs) were induced by elevated SREBP-1c and PPARα ligands. PPARα is required for the induction of mitochondrial enzymes such as long-chain acyl CoA synthetase (long-chain ACS) (2) and peroxisomal bifunctional enzyme containing enoyl CoA hydratase (9).

The influences of dietary lipids on hepatic de novo lipogenesis, lipoprotein metabolism, fatty acid oxidation, and cholesterol metabolism (10–12) as well as nutritional effects on fatty acid desaturation and elongation activities (13,14) in Atlantic salmon (*Salmo salar*) have been thoroughly assayed. However, little is known about the expression of hepatic genes involved in lipid metabolism in salmonids. Fatty acids regulate the expression of genes involved in fatty acid desaturation and elongation (15). Leaver and co-workers (16) characterized PPARs from 3 fish species, including Atlantic salmon (unpublished results). All PPARs were detected in all tissues studied, and it was suggested that fish PPARs bind a wide range of fatty acids. PPARα, involved in the induction of mammalian FADs (2,7,8), was particularly responsive to unsaturated fatty acids.

Hepatic mitochondrial β-oxidation exhibits substrate specificity in fish (17–19), and carnitine palmitoyltransferase (CPT)-II, which is induced by 20:5(n-3) and 22:6(n-3) in rat

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[3] To whom correspondence should be addressed.
E-mail: ann-elise.jordal@nifes.no.
[4] Present address: Department of Obstetrics and Gynecology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205.
[5] Abbreviations used: ACS, acyl-CoA synthetase; apo, apolipoprotein; CLA, conjugated linoleic acid; CPT, carnitine palmitoyltransferase; Cy, cyanine; EST, expressed sequence tag; FAD, fatty acid desaturase; FO, fish oil; HNF-4, hepatic nuclear factor-4; HUFA, highly unsaturated fatty acid (carbon chain length ≥ C20 with ≥3 double bonds); Lb-FABP, liver basic fatty acid binding protein; LO, linseed oil; LXR, liver X receptor; MIAME, minimum information about a microarray experiment; OO, olive oil; PPAR, peroxisome proliferator-activated receptor; Q-PCR, quantitative real time-PCR; RO, rapeseed oil; SAM, significance analysis of microarrays; SCD, Δ9 fatty acid desaturase; SREBP, sterol regulatory element binding protein.

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liver (20), has been used as a marker for mitochondrial β-oxidation activity in the liver of brown trout (*Salmo trutta* L.) (21) and Atlantic salmon (22,23). However, to date, no one has studied mitochondrial β-oxidation gene expression patterns in salmon.

In the present study, we examined whether nutrigenomics could be used as a tool for the analysis of nutrient-gene interactions in aquaculture nutrition. Because large-scale fish-specific arrays were not available at the time of the study, a small-scale lipid metabolism cDNA microarray was designed using amplicons from available sequenced cDNAs. This microarray was then used to determine the effects of dietary oil on hepatic gene expression in salmon fed either a 100% FO or a 75% rapeseed oil (RO) diet in a large-scale nutritional trial. Samples were pooled to ensure low variability and a normal distribution within the population. In accordance with this, an experimental design was chosen using a high number of technical replicates to validate the array itself for use in further studies. To further validate some of the result on the array quantitative real time PCR (Q-PCR) analyses were performed to confirm some of the results from the microarray profiling research.

MATERIALS AND METHODS

Dietary experiment

The dietary experiment was performed at Gildeskaal Research Station, Inndyr, Norway as described in more detail in Torstensen et al. (24). In wk 21, 2001, ~600 postsmolt Atlantic salmon, mean body weight 0.142 kg, were randomly distributed to 7 net pens, of 125 m^3 each, and fed a control diet containing 100% FO (2 replicates) or experimental diets in which FO was replaced by 0, 25, 50, 75, and 100% RO or 50% olive oil (OO) (one replicate for each oil concentration) in a linear regression design for 42 wk (**Table 1**). Diets, produced by Nutreco ARC, differed only in their oil composition. The chemical compositions of diets were (g/kg): lipid, 300; protein, 450; moisture, 60; ash, 70; and nitrogen-free extract, 120.

Due to low expected differences in gene expression between groups at low RO or OO inclusion, lower mean body weight (bulk weighing) in the 100% RO group after 22 wk and the high analytical cost of microarray construction and analysis, the 100% FO and 75% RO diets were selected as candidates for technical validation of microarrays and microarray analysis of a number of hepatic lipid metabolism genes.

Sampling procedure

Samples were collected during wk 22 and 42 for Q-PCR analysis, and during 42 wk for microarray analysis. The fish were deprived of food for 24 h before sampling. Randomly sampled fish from each tank, (i.e., dietary treatment) were anesthetized with methomidate (7 g/L; Norsk Medisinaldepot) before being killed with a blow to the head. Liver samples taken from 30 individual fish from each dietary group were pooled and preserved in RNAlater® (Ambion) according to the manufacturer's recommendations. For RT-PCR analysis, liver samples were immediately removed from 6 individual fish from each dietary group, snap-frozen in liquid nitrogen, and stored at −80°C. The protocol was approved by the local representatives for the Norwegian State Board of Biological Experiments with Living Animals.

TABLE 1

Dietary fatty acid composition of the experimental diets[1]

	Diet											
Fatty acid	100% FO	25% RO	50% RO	75% RO	100% RO	50% OO	100% FO	25% RO	50% RO	75% RO	100% RO	50% OO
	g/100 g fatty acid						*mg/g diet*					
14:0	6.7	5.0	3.6	2.1	0.4	3.4	14.4	10.8	8.5	5.1	1.0	7.2
16:0	11.6	10.0	8.9	7.5	5.7	11.7	25.0	21.6	21.0	17.9	13.7	24.9
18:0	1.0	1.2	1.4	1.6	1.7	1.9	2.1	2.6	3.3	3.8	4.1	4.1
Σ SFA	20.4	17.4	14.9	12.5	9.1	18.0	43.9	37.5	35.1	29.9	21.7	38.2
16:1(n-9)	0.2	0.2	0.2	—	—	0.2	0.5	0.5	0.4	—	—	0.4
18:1(n-9)	11.2	22.5	32.4	42.4	53.6	37.3	24.1	48.5	76.2	101.5	128.6	79.1
20:1(n-11)	0.4	0.3	0.3	0.2	—	0.3	0.9	0.6	0.7	0.6	—	0.6
20:1(n-9)	17.1	13.3	9.8	6.1	2.1	9.0	36.9	28.5	23.0	14.7	5.1	19.1
22:1(n-11)	13.3	10.1	7.3	4.3	1.0	5.4	28.6	21.8	17.1	10.3	2.3	11.6
22:1(n-9)	2.0	1.6	1.3	0.9	0.5	1.1	4.4	3.5	3.0	2.2	1.2	2.3
Σ MUFA	57.1	58.4	59.6	60.4	61.1	61.2	123.0	125.6	140.3	144.5	146.9	130.0
18:2(n-6)	3.5	7.6	11.5	15.4	19.5	7.7	7.6	16.3	27.0	36.9	46.9	16.4
20:4(n-6)	0.3	0.3	0.1	—	—	0.2	0.6	0.5	0.2	—	—	0.4
20:2(n-6)	0.2	0.2	—	—	—	—	0.5	0.5	—	—	—	—
Σ (n-6)	4.1	8.1	11.5	15.4	19.5	7.9	8.7	17.3	27.2	36.9	46.9	16.8
18:3(n-3)	1.1	3.0	4.7	6.6	8.6	2.6	2.3	6.4	11.0	15.9	20.7	5.5
20:4(n-3)	0.4	0.3	0.2	—	—	0.2	0.9	0.6	0.5	—	—	0.5
20:5(n-3)	5.9	4.5	3.4	2.1	0.7	3.2	12.6	9.7	8.0	5.1	1.7	6.9
22:5(n-3)	0.4	0.3	0.2	0.2	—	0.3	0.9	0.7	0.6	0.4	—	0.5
22:6(n-3)	4.6	3.7	3.0	2.1	1.0	2.9	10.0	8.0	7.1	5.0	2.5	6.2
Σ (n-3)	15.8	14.2	13.3	11.9	10.6	10.9	34.1	30.63	1.4	28.6	25.4	23.1
(n-3):(n-6)	3.9	1.8	1.2	0.8	0.5	1.4	3.9	1.8	1.2	0.8	0.5	1.4
Σ FA	100.0	100.0	100.0	100.0	100.0	100.0	215.5	215.1	235.4	239.9	240.9	212.2
Σ Identified[2]	97.5	98.6	99.4	100.0	100.0	98.0	210.1	212.0	234.0	239.9	240.9	208.1

[1] Values are means, $n = 2$. Values < 0.1 are denoted by —. The remaining constituent in vegetable oil diets was FO. All diets, produced by Nutreco ARC were composed (g/kg diet) of fish meal (338.3), maize gluten (200.0), soybean meal (HP; 100.0), wheat (79.0), oil (257.7) and premixes (25.0). Diets were supplemented with vitamins and minerals according to the nutritional requirements of fish (25).

[2] Includes 14:1(n-9), 15:0, 16:1(n-7), 17:0, 16:2(n-4), 16:3(n-3), 18:1(n-11), 18:1(n-7), 16:4(n-3), 18:4(n-3), 20:0, 20:1(n-7), 20:3(n-3) and 24:1(n-9).

Fatty acid composition

Lipids from livers and diets were extracted by homogenization in chloroform:methanol (2:1, v:v). After extraction of total lipid, an aliquot was saponified and methylated using 12% BF_3 in methanol. The fatty acid composition of total lipids was analyzed using methods described previously by Lie and Lambertsen (26). Briefly, methyl esters were separated using a trace gas chromatograph 2000 ("cold on column" injection, 60°C for 20 s, 25°C/min; 160°C for 28 min, 25°C/min; 190°C for 17 min, 25°C/min; 220°C for 9 min), equipped with a 50-m CP-sil 88 (Chromopack) fused silica capillary column (i.d., 0.32 mm). The fatty acids were identified by retention time using standard mixtures of methyl esters (Nu-Chek-Prep), and quantified using Totalchrom software (version 6.2, Perkin Elmer). The amount of fatty acid per gram tissue was calculated using 19:0 methyl ester as an internal standard.

Microarray analysis

Minimum information about a microarray experiment (MIAME). MIAME standards (27) were used to define the content and the structure of the information for this microarray experiment. In accordance with these standards features used (Online Supplemental Table 1), the experimental conditions (Microarray hybridization protocol), the experiment (Experimental design), and array design (Microarray construction) as well as a description of samples, hybridizations, and normalizations controls (RNA isolation and preparation, Microarray hybridization protocol, and Normalization and statistical analysis) were reported.

Experimental design. Two arrays (i.e., technical replicates) were scanned using cohybridized labeled total RNA from control and experimental samples labeled with cyanine (Cy)3 and Cy5, respectively. Each of these experiments was replicated with Cy dyes switched to control for labeling bias. The same experimental design was used for cohybridized mRNA samples from fish fed the 2 diets.

Microarray construction. PCR amplicons from selected Atlantic salmon expressed sequence tags (ESTs) (28) were printed on barcoded CMT-GAPS coated slides (Corning Microarray Technology) using a manual pin caster microarray printing system (Micro Caster 8 pin system, Schleicher & Schuell). cDNA clones were selected on the basis of their similarity [BLAST search of EST sequence (29)] to features involved in lipid metabolism. The Microarray Assistant Clone Organizer and Array Simulator (30) was used to organize clones and amplicons. A total of 73 features, mainly from Atlantic salmon, were spotted on the array in quadruplicate (4 subarrays with 96 probes on each subarray). Some features were spotted in duplicate on different areas of each subarray to control for changes in signal/hybridization in different parts of the slide. In total, there were 89 different amplicons, 2 empty spots and 5 controls consisting of a concentration gradient of an *Arabidopsis thaliana* chlorophyll synthetase G4 (GenBank Accession U19382). Several ribosomal proteins and β-actin were included to monitor the expression of housekeeping genes. Controls were used only to ensure quality. Empty spots were included on the array for controlling the background intensity. All features were spotted using 1 spot length distance between spots to avoid interference of signals.

RNA isolation and preparation. Total RNA was purified from 30 pooled livers using the RNAwiz protocol™ (Ambion). RNA was subjected to DNase treatment (DNA-*free*™, Ambion) and quality and quantity determined by denaturing gel electrophoresis and spectrophotometry ($A_{260/280}$). mRNA was isolated from total RNA using the Oligotex® mRNA kit (Qiagen).

Microarray hybridization protocol. A total of 8 arrays were analyzed, 4 using total RNA and 4 using mRNA as templates for direct cDNA labeling. Both sources had an $A_{260/280}$ ratio of 1.9:2.0 and were of sufficient quality for hybridization. Total RNA (50 μg) and mRNA (3 μg) were subjected to separate cDNA syntheses using a direct labeling protocol with Cy3- or Cy5-labeled dCTP (Perkin Elmer Life Sciences). *Arabidsopsis thaliana* chlorophyll synthetase G4 mRNA (80 pg) was added to the labeling reaction as an internal control; 5 μL concentrated and purified labeled cDNAs were combined, and 1 μL Poly (A)-DNA (20 g/L) (Amersham Pharmacia, Cat #27-7836-01) was added. The mixture was incubated at 95°C for 3 min, followed by quick cooling on ice for 2 min to block renaturation. Subsequently, 12 μL hybridization buffer (Amersham Pharmacia Cat#PRK0325) and 24 μL deionized formamide (Sigma, Cat #F9037) were added and the mixture was hybridized to preincubated arrays (80 μL hybridization buffer, 42°C) in a humid chamber at 42°C overnight. Scans were performed at a resolution of 10 mm using a microarray scanner (ScanArray® 5000XL, Packard BioChip Technologies).

Normalization and statistical analysis. Background subtraction and normalization to total spots were performed using the QuantArray® microarray analysis software (Packard BioChip Technologies), and low intensity spots (intensity < 1000 pixels) were eliminated from further analysis. All 8 scanned arrays were subjected to a significance analysis of microarrays (SAM) (31) using separate mRNA and total RNA data sets as well as the combined dataset. A simple gene specific *t* test analysis of the mean log ratio (32) was also used to analyze the separate mRNA and total RNA datasets (data not shown). Pooling of 30 individual samples was done in accordance with the central limit theorem (33,34) to remove sources of individual variation due to genetic differences and to ensure a low SD and a normal distribution within the population. Significance analysis was performed with a high number of technical replicates of normally distributed treatment groups.

RT-Q-PCR analysis

RNA isolation and preparation. Total RNA was purified using TRIZOL® (Invitrogen) and subjected to DNase treatment (DNA-*free*, Ambion). RNA quality and quantity were determined as described above.

RT-PCR. cDNA (125 ng) was synthesized following a modified protocol from the Taq Man Reverse Transcription Reagents kit (Applied Biosystems). Modifications included increasing the total reaction volume to 30 μL and accordingly increasing the volume of all reagents used. Oligo $d(T)_{16}$ primers were used for Δ5 FAD and liver basic fatty acid binding protein (Lb-FABP), and random hexamers were used for 18S rRNA. The reactions were incubated at 25°C for 10 min and 48°C for 60 min, and the reverse transcriptase enzyme was inactivated at 95°C for 5 min followed by a decrease to 4°C. RT-PCR efficiency was monitored using a 4-step, 2-fold dilution curve of RNA.

Q-PCR. BLAST searches (29) for Atlantic salmon 18S rRNA, Δ5 FAD, and Lb-FABP were performed to identify highly conserved regions for primer design. Selected sequences were translated using the Expasy translate tool (35) and intron-exon borders were identified from alignments of genomic and cDNA sequence using ClustalW software (36). Primer and probe sequences were constructed using the Assay by Design service (Applied Biosystems). The primer and probe sequences (5′ to 3′) for 18S rRNA (AJ427629) were: CCCCGTAATTGGAATGAGTACACTTT (F), ACGCTATTGGAGCTGGAATTACC (R), **FAM**-CACCAGACTTGCCCTCC-**MGBNFQ** (reverse probe), for Δ5 FAD (AF478472.1) GGAACCACAAACTGCACAAGT (F), GTGCTGGAAGTGACGATGGT (R) and **FAM**-CAGAGGCACCCTTTAGGTG-**MGBNFQ** (reverse probe), and for Lb-FABP (BG935057) CCGACATCACCACCATGGA (F), GCTTCCCTCCC TCCAGTTTG(R) and FAM-CAGTGCA CTTGAGCTT- **MGBNFQ** (reverse probe).

Q-PCR was performed using FAM fluorescent chemistry on an ABI prism 7000 (Applied Biosystems). The reaction mixture (25 μL) contained primers (900 nmol/L each), FAM probe (200 nmol/L), 1X TaqMan universal PCR master mix (Applied Biosystems art. no. 430 4437) and 5 μL cDNA. All samples were assayed in triplicate with nontemplate controls on the same plate. The reaction was incubated at 50°C for 2 min followed by 95°C for 10 min and 50 cycles of 95°C for 10 s and 60°C for 15 s. Liver RNA samples from 6 fish from each diet were tested.

Normalization and statistical analysis. 18S rRNA levels were used to normalize the results and to calculate relative expression levels using the Q gene method (37). The normalized relative expression data were analyzed using the nonparametric Kruskal-Wallis method (Statistica 6.1, Statsoft; $n = 6$ and $n = 5$) due to heterogeneity in variance within the 75% RO group. Differences were considered significant at $P < 0.05$.

Statistical analysis of dietary and liver fatty acid composition

For fatty acid composition in the experimental diets (Table 1, $n = 2$ analytical parallels) and in liver samples (**Table 2**, $n = 3$ and 6 pooled samples), means and medians (ranges) were calculated, respectively, using descriptive statistics (Statistica 6.1, Statsoft). Data were not analyzed for significant differences.

RESULTS

The body weight of Atlantic salmon fed the experimental diets increased from 0.142 kg initially to 1.463 kg after 42 wk. The body weight data were based on bulk weighing and counting of all fish. The body weight in the 100% RO-fed group after 22 wk (0.926 kg) was notably lower than that for groups fed FO (1.026 ± 0.020 kg) (mean ± SD, $n = 2$). Mortalities were negligible throughout the experimental period. More details on data for growth and feed efficiency are reported elsewhere (24).

The concentration of 20:5(n-3) was 60% lower in the 75% RO diet compared with the control (FO) diet (Table 1). The concentrations of 22:5(n-3) and 22:6(n-3) were 56 and 50% lower, respectively. The total highly unsaturated fatty acid (HUFA) content, the sum of SFA, and the sum of (n-3) fatty acids were 58, 32, and 16% lower, respectively, whereas the sum of (n-6) fatty acids was 3-fold greater in the 75% RO diet compared with control. In addition, the amount of total (n-9) fatty acids was almost 2-fold greater in the 75% RO diet compared with the control diet; 18:1(n-9) was 3-fold greater. The concentrations of 18:1(n-9) (40%) and 18:2(n-6) (15%) fatty acids were high in the 75% RO diet. Those of 18:2(n-6) and 18(3n-3) were ~4- and 6-fold greater, respectively, in the 75% RO diet. The (n-3):(n-6) ratio in the 75% RO diet was 79% less than the control.

Irrespective of diet, 22:6(n-3) dominated in the salmon liver total lipid (Table 2). The concentrations of 18:1(n-9) and 18:2(n-6) were ~2-fold greater, and that of 18:3(n-3) > 6-fold greater (median values) compared with livers from salmon fed the control diet. In contrast, concentrations of 22:5(n-3), 20:5(n-3), and 22:6(n-3) in liver of salmon fed the 75% RO diet were 46, 34, and 31% lower, respectively, than those of controls. Further details on fatty acid composition in livers of salmon fed different RO diets were published (11).

Microarray analysis demonstrated that expression of hepatic fatty acid Δ5 desaturase was significantly greater in livers of 75% RO-fed salmon than FO-fed salmon, whether total RNA or mRNA was analyzed (**Table 3**). Expression of apo A1 precursor was upregulated in analyses using labeled total RNA, and the expression of apo C-II was upregulated in analyses using combined and mRNA datasets. Long-chain ACS3 was among several genes that were downregulated in livers of 75% RO-fed salmon in all analyses.

SAM analysis of combined and mRNA datasets identified SCD and acyl carrier protein (mitochondrial precursor) as genes that were downregulated in livers of 75% RO-fed salmon, whereas SAM analysis of the combined and total RNA datasets identified translocase of outer mitochondrial membrane (tom70), outer mitochondrial membrane translocase, and CPT-II as genes that were downregulated in livers of 75% RO-fed salmon. A single analysis from arrays hybridized with total RNA from fish fed control and experimental diets identified enoyl CoA hydratase, PPARγ, and catalase as downregulated genes in livers of 75% RO-fed salmon. Data from Q-PCR analysis confirmed results from the microarray screening showing an ~2-fold increased gene expression of Δ5-FAD in livers of 75% RO-fed salmon after 42 wk of feeding (**Fig. 1**). Gene expression of Δ5-FAD in livers of 75% RO-fed salmon after 22 wk of feeding, was higher ($P < 0.05$, nonparametric statistics, $n = 5$) than in the control ($n = 6$).

TABLE 2

Fatty acid composition of livers from salmon fed a 100% FO or 75% RO diet for 42 wk[1]

Fatty acid[2]	100% FO	75% RO
	g/100 g fatty acids	
14:0	2.2 (2.2–2.3)	1.1 (1.0–1.2)
16:0	16.7 (15.6–17.6)	12.4 (11.7–13.5)
18:0	3.6 (3.5–3.7)	4.4 (4.2–4.5)
Σ SFA	24.0 (22.9–24.9)	19.1 (18.7–20.9)
18:1(n-9)	9.0 (8.4–10.1)	26.2 (21.0–26.6)
18:1(n-11)	1.8 (1.7–1.8)	0.7 (0.6–0.8)
20:1(n-9)	5.4 (5.0–5.5)	3.6 (3.6–4.3)
20:1(n-11)	0.6 (0.3–0.7)	0.3 (0.3–0.3)
22:1(n-11)	1.5 (1.3–1.7)	0.8 (0.8–0.8)
Σ MUFA	25.3 (24.0–26.5)	36.5 (31.3–37.5)
18:2(n-6)	2.3 (2.2–2.7)	8.6 (7.3–8.6)
20:2(n-6)	0.4 (0.4–0.5)	1.3 (1.1–1.5)
20:4(n-6)	1.7 (1.7–1.8)	2.0 (2.0–2.1)
Σ (n-6)	4.4 (4.2–5.1)	11.9 (10.4–12.1)
18:3(n-3)	0.3 (0.0–0.5)	2.3 (2.0–2.4)
20:4(n-3)	1.6 (1.5–1.6)	1.4 (1.4–1.4)
20:5(n-3)	10.0 (9.9–10.8)	6.6 (6.5–8.0)
22:5(n-3)	4.1 (3.7–4.4)	2.2 (2.1–2.6)
22:6(n-3)	28.3 (27.6–29.1)	19.6 (18.6–22.6)
Σ (n-3)	44.5 (43.6–45.8)	32.3 (31.4–36.8)
(n-3):(n-6)	10.2 (8.7–10.8)	2.7 (2.6–3.5)

[1] Values are medians (range), $n = 3$ (75% RO) or $n = 6$ (FO) pooled samples from each net pen (FO, 2 net pens). The 75% RO diet was also composed of 25% FO.

[2] Selected from all fatty acids including 14:1(n-9), 15:0, 16:1(n-7), 17:0, 16:2(n-4), 16:3(n-3), 18:1(n-7), 16:4(n-3), 20:0, 20:1(n-7), 20:3(n-3), 22:1(n-9), and 24:1(n-9).

DISCUSSION

Microarray analysis can be used as a tool for studying homeostatic metabolic systems (38) and can potentially reveal small but significant changes in gene expression if reliable controls are used. The internal control and reference genes, used only to ensure quality, yielded expected values of expression, validating this approach for studying the expression of genes involved in liver lipid metabolism in Atlantic salmon. Therefore, microarray analysis gave a preliminary indication of the metabolic processes that may be responding to changes in dietary oil and thus changes in tissue fatty acid composition. Because a primary objective was to validate the suitability of the array for further studies, technical replicates of several types were selected (mRNA, total RNA, and fluor flips plus replicates within these groups). Additional quality assurance was also prioritized through duplicate feature spotting and quadruplicate subarrays. Pooling of livers before RNA isolation and microarray analysis was performed to control for individual genetic variation, similar to the strategy described by Douglas and co-workers (39). The degree of technical replication allows a thorough technical validation of the array as well as examination of variation between treatment groups (40).

Q-PCR analysis, performed to validate expression data from microarray analysis, using essays for Lb-FABP (results not shown) (Online Supplemental Table 1) confirmed the lack of gene specific variation between dietary treatments. However,

TABLE 3

Differentially expressed genes in liver of salmon fed 75% RO compared with control (fed 100% FO) as identified by microarray analysis[1]

	Gene name[2]	E value	Combined		mRNA		Total RNA	
			Ratio[3]	δ[4]	Ratio	δ	Ratio	δ
Transport								
JH0472	Apo A-I precursor (A-I-I)	2E-99					1.1	+
AAG11410	Apo C-II	2E-42	1.1	+	1.1	+		
JC4858	VLDL receptor precursor	3E-11	−0.9	+	−0.9	+	−0.9	+
Mitochondrial activity								
NP004448	Long-chain ACS 3 (*M. glutinosa*)[5]	8E-13	−0.9	+	−0.95	+	−0.9	+
NP055635	Translocase of outer mitochondrial membrane	E-74	−0.9	+			−0.8	+
O14561	Acyl carrier protein	6E-40	−0.9	+	−0.95	+		
CAB89422	Outer mitochondrial membrane translocase	2E-53	−0.9	+			−0.8	+
NP000089	CPT-II	9E-78	−0.9	+			−0.8	+
P14604	Enoyl CoA hydratase (*M. mizolepsis*)	E-69					−0.9	+
Desaturation and elongation								
AF478472	Δ5 FAD[6]		1.3	+	1.3	+	1.4	+
CAA42997	SCD (*M. mizolepsis*)	6E-55	−0.9	+	−0.9	+		
Peroximal activity								
CAB64949	Catalase	E-17					−0.9	+
Nuclear receptors and transcription regulators								
P19838	Nuclear factor $\kappa\beta$ P105 subunit	3E-45	−0.9	+	−0.9	+	−0.9	+
BB054438	Nucleic acid binding factor	8E-20	−0.9	+	−0.9	+	−0.9	+
AAC50890	p300/CBP-associated factor (*P. americanus*)	3E-75	−0.9	+	−0.9	+		+
NP035980	Nuclear receptor subfamily 0, Group B	8E-19	−0.9	+			−0.8	+
P54864	Transcription factor AP 1 (jun)	5E-06	−0.9	+			−0.8	+
AJ416952	PPARγ						−0.8	+
Others								
S35704	Protein kinase C Δ	E-62	−0.9	+	−0.9	+	−0.9	+
NP002722	Protein kinase (cAMPdept)	E-110	−0.9	+	−0.9	+	−0.9	+
CAB40545	Lipoprotein lipase	E-60			−0.9	+		+
AF512561	Bile salt-dependent lipase (*P. americanus*)[5,6]				−0.9	+		+
AW013173	Phospholipase C (*P. americanus*)						−0.9	+
P51913	Enolase	E-88	−0.9	+	−0.95	+	−0.9	+
NP004155	Retinol binding protein 2	4E-20	−0.9	+	−0.9	+	−0.9	+
NP001896	CTP synthase	8E-52	−0.9	+	−0.9	+	−0.9	+
AF231707	Eggshell protein	E-156	−0.9	+			−0.8	+
NP004558	6 phosphofructo 2 kinase/fructose 2,6 biphosphatase	2E-66					−0.8	+

[1] Genes presented are those for which the hepatic expression pattern differed between Atlantic salmon fed 100% FO (control) and 75% RO, $P < 0.05$.
[2] Gene name is designated on the basis of the BLAST search; for each designated gene name, the E value and GenBank accession numbers from the BLAST search are shown.
[3] A plus sign indicates greater gene transcript levels, and a minus sign indicates lower gene transcript levels in livers of salmon fed 75% RO diet compared with control.
[4] δ is the cut-off value for significance in SAM determined by the user based on the false-positive rate.
[5] Probes are from Atlantic salmon unless otherwise denoted in brackets.
[6] Only GenBank accession numbers are presented for fully sequenced gene products.

both the microarray and Q-PCR results demonstrated that Δ5 FAD gene expression was higher in livers of 75% RO-fed salmon (Table 3 and Fig. 1). This is in agreement with Zheng and co-workers (15) who observed an increase in the expression of hepatic Δ5 desaturase and fatty acid elongase genes in salmon fed increased levels of dietary linseed oil for 20 wk. The values for expression of fatty acid elongase obtained in the present microarray study were too low to be deemed reliable. Bell and co-workers (13,14) suggested that the stimulation of hepatic desaturation and elongation by vegetable oils presumably reflects increased substrate availability [18:2(n-6) and/or 18:3(n-3)] coupled with reduced end product concentration [20:5(n-3) and 22:6(n-3)], with high concentrations of the latter inhibiting the pathway.

In contrast, SCD gene expression was reduced in livers of 75% RO-fed salmon compared with control salmon. Supporting these data, Berge et al. (41) demonstrated that SCD enzyme activity was reduced in salmon fed conjugated linoleic acid (CLA), and gene regulation of SCD was shown to differ from regulation of Δ5- and Δ6-FAD (42). However, SCD has not been cloned in salmon, and further studies are required.

Dietary 20:4(n-6), 20:5(n-3), and 22:6(n-3) were reduced in the 75% RO diet (Table 1) compared with control diet, and 20:4(n-6) and 22:6(n-3) were reported to affect hepatic gene expression (3). Therefore, this long-term dietary fatty acid change may reduce mRNA transcript synthesis in livers of 75% RO-fed salmon.

The HUFA, 20:5(n-3) and 22:6(n-3), were reported to be 2- to 4-fold more potent inducers of fatty acid β-oxidation than 18:2(n-6) and 18:3(n-3) in mammals (43). Although there was an increase in the amount of 18:2(n-6) and 18:3(n-3), the reduced dietary 20:5(n-3) and 22:6(n-3) in the 75% RO diet compared with control correlated with a significant downregulation of several mitochondrial genes. These include

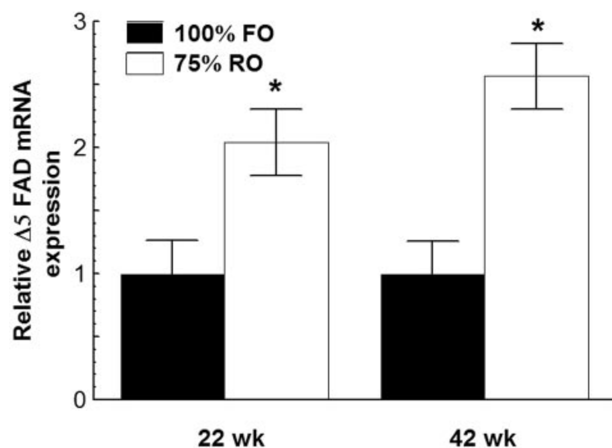

FIGURE 1 Q-PCR analysis of Δ5 FAD mRNA expression in livers of salmon fed 75% RO and 100% FO diets after 22 and 42 wk of feeding. Values are presented as relative to control, 100% FO (set to 1), for each sampling. Data were normalized to 18S rRNA levels. Values are means ± SD, $n = 5$ or 6 individual samples. *Different from control (FO), $P < 0.05$.

long-chain ACS3, enoyl CoA hydratase, 2 mitochondrial outer membrane proteins, CPT-II, and mitochondrial acyl carrier protein (precursor). Furthermore, several additional mitochondrial genes (malate dehydrogenase, translocase of inner mitochondrial membrane, and mitochondrial solute carrier protein) were identified as significantly downregulated in fish fed 75% RO when gene-specific *t* tests, allowing genes with smaller fold changes to be detected, were used for statistical analysis (results not shown).

CPT-I and CPT-II activate and transport long-chain fatty acids into the mitochondrial matrix (44) and therefore occupy a key regulatory step in their catabolism (23,44). CPT-II was significantly downregulated in livers of 75% RO-fed salmon, possibly indicating lower β-oxidation capacity in this group (23,45). Turchini and co-workers (21) showed that hepatic CPT-II in brown trout (*Salmo trutta* L.) had significantly higher activities in fish fed a FO compared with fish fed a canola (rapeseed) oil diet.

Two long-chain ACS features were present on the microarray, one from hagfish, *Myxine glutinosa,* which had the highest sequence similarity to the rat long-chain ACS3 (NM_057107) and one from *Salmo salar*, which had the highest similarity to the rat long-chain ACS1 gene (NM_012820). Only the former showed a significant change in mRNA levels due to diet, with a downregulation in livers from 75% RO-fed fish. Rat long-chain ACS3 and ASC1 are from different subfamilies with distinct functions, and share only 30% amino acid sequence identity (46). Rat long-chain ACS3 activates 20:5(n-3) and 20:4(n-6) most efficiently and has a 4-fold higher capacity for utilization of C20 HUFA than rat ASC1 (47). Long-chain ACS3 is thought to direct fatty acids to mitochondrial oxidation, whereas long-chain ACS1 may play a role in channeling fatty acids to triacylglycerol synthesis (46). Higher expression of the ACS3-like isoform could be expected in FO-fed salmon, but a fully characterized clone of salmon long-chain ACS3 is required to completely elucidate the effects of dietary fatty acids on ACS expression.

High dietary 20:5(n-3) (48) and (n-3):(n-6) ratio (49) both induce mitochondrial proliferation in rodents; thus, the reduced 20:5(n-3) and (n-3):(n-6) ratio in the 75% RO diet may also reduce mitochondrial proliferation in livers of 75% RO-fed salmon compared with the control. Mootha and co-workers (50) used proteomic and mRNA expression profiling to reveal several genes and transcriptional regulators exhibiting expression patterns that correlated with mitochondrial biogenesis. These included cellular nucleic acid binding protein and PPARγ, which is consistent with the expression data in the present study. Furthermore, PPARγ was downregulated by CLA and sunflower oil compared with FO in gilthead sea bream (*Sparus aurata*) (51), suggesting that vegetable oil inclusion may negatively affect PPARγ expression. Whether PPARγ is involved in mitochondrial biogenesis in salmon fed dietary RO remains to be elucidated.

The apo A-I precursor [sequence similarity to apo A-I-I (BE 518583)] and apo C-II were affected by the dietary oil source, showing upregulation in livers of 75% RO-fed salmon compared with control. Apo A-I–like protein was associated with HDL, and apo-C–like protein with LDL in rainbow trout (52). In contrast to the apolipoprotein expression results in the present study, the amount of HDL was significantly decreased in plasma of 75% RO-fed salmon, compared with fish fed FO (11). Torstensen and co-workers (11), however, measured the total lipoprotein levels in plasma, which is a relatively crude measurement compared with measuring the expression levels of a single apolipoprotein. Furthermore, the recirculation time of HDL particles in salmon plasma is not known, and increases in the expression of apo A-I-I in the 75% RO group may be a response to decreased plasma HDL levels as a result of increased HDL uptake in liver. Unraveling the complex gene and protein expression patterns of apolipoproteins in Atlantic salmon awaits further characterization of these individual physiological components.

The microarray data presented here as well as other recent data utilizing microarrays (53), confirm the effects of (n-3) HUFA on the regulation of gene expression, suggesting that cDNA microarray technology is a powerful tool for studying nutrient-gene interactions. Therefore, further work may be performed using the present array, or other high-density arrays developed recently using features of salmonid origin (39,54,55), as a complement to the specific microarray utilized in study.

In conclusion, the current thoroughly validated and quality controlled microarray study (technical replicates, internal controls, and PCR) showed differential, although relatively minor, regulation of several genes related to hepatic lipid metabolism in Atlantic salmon in response to changes in dietary fatty acids. These included Δ5 FAD, PPARγ, and several genes whose gene products are involved in mitochondrial functions. Salmon have a partially tetraploedic genome, and genes may be present in more than one isotype, which may differ in expression patterns (56,57). Thus, there is an argued need for a more thorough examination of genes and gene expression patterns. However, the effects of nutrients are under strict homeostatic control (58), and microarray analysis is a valuable tool with which to screen for nutritional effects on a gene-expression level in Atlantic salmon, and should be further pursued.

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LITERATURE CITED

1. Barlow S. Fish meal and fish oil: sustainable feed ingredients for aquafeeds. Global Aquacult Advocate. 2000;4:85–8.
2. Jump DB. Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr Opin Lipidol. 2002 Apr;13(2):155–64.
3. Jump DB. Fatty acid regulation of gene transcription. Crit Rev Clin Lab Sci. 2004;41:41–78.

4. Tobin KAR, Steineger HH, Alberti S, Spydevold O, Auwerx J, Gustafsson JA, Nebb HI. Cross-talk between fatty acid and cholesterol metabolism mediated by liver X receptor-alpha. Mol Endocrinol. 2002;14:741–52.

5. Mak PA, Laffitte BA, Desrumaux C, Joseph SB, Curtiss LK, Mangelsdorf DJ, Tontonoz P, Edwards PA. Regulated expression of the apolipoprotein E/C-I/C-IV/C-II gene cluster in murine and human macrophages—a critical role for nuclear liver X receptors alpha and beta. J Biol Chem. 2002 Aug 30;277(35): 31900–8.

6. Joseph SB, Laffitte BA, Patel PH, Watson MA, Matsukuma KE, Walczak R, Collins JL, Osborne TF, Tontonoz P. Direct and indirect mechanisms for regulation of fatty acid synthase gene expression by liver X receptors. J Biol Chem. 2002 Mar 29;277(13):11019–25.

7. Miyazaki M, Ntambi JM. Role of stearoyl-coenzyme A desaturase in lipid metabolism. Prostaglandins Leukot Essent Fatty Acids. 2003;68:113–21.

8. Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Yoshikawa T, Hasty AH, Tamura Y, Osuga J, Okazaki H, et al. Dual regulation of mouse Delta(5)- and Delta(6)-desaturase gene expression by SREBP-1 and PPAR alpha. J Lipid Res. 2002;43:107–14.

9. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev. 1999 Oct;20(5):649–88.

10. Torstensen BE, Lie O, Froyland L. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)—effects of capelin oil, palm oil, and oleic acid-enriched sunflower oil as dietary lipid sources. Lipids. 2002;35:653–64.

11. Torstensen BE, Froyland L, Lie O. Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil—effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities. Aquacult Nutr. 2004 Jun;10(3):175–92.

12. Torstensen BE, Lie O, Hamre K. A factorial experimental design for investigation of effects of dietary lipid content and pro- and antioxidants on lipid composition in Atlantic salmon (*Salmo salar*) tissues and lipoproteins. Aquacult Nutr. 2001 Dec;7(4):265–76.

13. Bell JG, McEvoy J, Tocher DR, McGhee F, Campbell PJ, Sargent JR. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J Nutr. 2001 May;131(5):1535–43.

14. Bell JG, Henderson RJ, Tocher DR, McGhee F, Dick JR, Porter A, Smullen RP, Sargent JR. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. J Nutr. 2002;132:222–30.

15. Zheng XZ, Tocher DR, Dickson CA, Bell JG, Teale AJ. Effects of diets containing vegetable oil on expression of genes involved in highly unsaturated fatty acid biosynthesis in liver of Atlantic salmon (*Salmo salar*). Aquaculture. 2004 Jun;236(1–4):467–83.

16. Leaver MJ, Boukouvala E, Autonopoulou E, Diez A, Favre-Krey L, Ezaz MT, Bautista JM, Tocher DR, Krey G. Three peroxisome proliferator-activated receptor isotypes from each of two species of marine fish. Endocrinology. 2005 July;146(7):3150–62.

17. Henderson RJ, Sargent JR. Chain-length specificities of mitochondrial and peroxisomal beta-oxidation of fatty-acids in livers of rainbow-trout (*Salmo-gairdneri*). Comp Biochem Phys B. 1985;82:79–85.

18. Crockett EL, Sidell BD. Substrate selectivities differ for hepatic mitochondrial and peroxisomal beta-oxidation in an Antarctic fish, *Notothenia-gibberifrons*. Biochem J. 1993;289:427–33.

19. Torstensen BE, Stubhaug I. β-Oxidation of 18:3n-3 in Atlantic salmon (*Salmo salar* L.) hepatocytes treated with different fatty acids. Lipids. 2004;39:153–60.

20. Totland GK, Madsen L, Klementsen B, Vaagenes H, Kryvi H, Froyland L, Hexeberg S, Berge RK. Proliferation of mitochondria and gene expression of carnitine palmitoyltransferase and fatty acyl-CoA oxidase in rat skeletal muscle, heart and liver by hypolipidemic fatty acids. Biol Cell. 2000 Aug;92(5):317–29.

21. Turchini GM, Mentasti T, Froyland L, Orban E, Caprino F, Moretti VM, Valfre F. Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout (*Salmo trutta* L.). Aquaculture. 2003 Jul 14;225(1–4):251–67.

22. Egginton S. Effect of temperature on the optimal substrate for β-oxidation. J Fish Biol. 1996 Oct;49(4):753–8.

23. Froyland L, Madsen L, Eckhoff KM, Lie O, Berge RK. Carnitine palmitoyltransferase I, carnitine palmitoyltransferase II, and acyl-CoA oxidase activities in Atlantic salmon (*Salmo salar*). Lipids. 1998;33:923–30.

24. Torstensen BE, Froyland L, Ornsrud R, Lie O. Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. Food Chem. 2004 Oct;87(4):567–80.

25. National Research Council. Nutrient requirements of fish. Nutrient requirements of domestic animals. Washington: National Academy Press; 1993.

26. Lie Ø, Lambertsen G. Fatty acid composition of glycerophospholipids in seven tissues of cod (*Gadus morhua*), determined by combined high-performance liquid chromatography and gas chromatography. J Chromatogr. 1991; 565:119–129.

27. Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W, Ball CA, et al. Minimum information about a microarray experiment (MIAME)—toward standards for microarray data. Nat Genet. 2001 Dec;29(4):365–71.

28. Tsoi SCM, Ewart KV, Penny S, Melville K, Liebscher RS, Brown LL, Douglas SE. Identification of immune-relevant genes from Atlantic salmon using suppression subtractive hybridization. Mar Biotechnol. 2004 May-Jun;6(3):199–214.

29. Basic local alignment search tool. [Online] [cited Jan 2005]; Available from: http://www.ncbi.nlm.nih.gov/BLAST/.

30. Anbazhagan R. Microarray Assistant clone organizer and array simulator. Biotechniques. 2002;32:1398–402.

31. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A. 2001 Apr 24;98(9):5116–21.

32. Cui XQ, Churchill GA. Statistical tests for differential expression in cDNA microarray experiments. http://genomebiology.com/2003/4/4210. Available from: <Go to ISI>://000182696200003.

33. Bhattacharyya GK, Johnson RA. Statistical concepts and methods. New York, NY: John Wiley & Sons, Inc; 1977.

34. Zar JH. Biostatistical analysis. 3rd ed. Upper Saddle River, NJ: Prentice-Hall Inc; 1999.

35. The Expert Protein Analysis System. [Online] [cited Jan 2005]; Available from: http://au.expasy.org/tools/dna.html.

36. Clustal W. [Online] [cited Jan 2005]; Available from: http://www.ebi.ac.uk/clustalw/.

37. Simon P. Q-Gene: processing quantitative real-time RT-PCR data. Bioinformatics. 2003 Jul 22;19(11):1439–40.

38. Muller M, Kersten S. Nutrigenomics: goals and strategies. Nat Rev Genet. 2003;4:315–22.

39. Ewart KV, Belanger JC, Williams J, Karakach T, Penny S, Tsoi SC, Richards RC, Douglas SE. Identification of genes differentially expressed in Atlantic salmon (*Salmo salar*) in response to infection by *Aeromonas salmonicida* using cDNA microarray technology. Dev Comp Immunol. 2005;29:333–47.

40. Churchill GA. Fundamentals of experimental design for cDNA microarrays. Nat Genet. 2002;32(Suppl):490–5.

41. Berge GM, Ruyter B, Asgard T. Conjugated linoleic acid in diets for juvenile Atlantic salmon (*Salmo salar*); effects on fish performance, proximate composition, fatty acid and mineral content. Aquaculture. 2004 Aug 2;237(1–4): 365–80.

42. Nakamura MT, Nara TY. Structure, junction, and dietary regulation of Δ 6, Δ 5, and Δ 9 desaturases. Annu Rev Nutr. 2004;24:345–76.

43. Jump DB, Clarke SD, Thelen A, Liimatta M, Ren B, Badin M. Dietary polyunsaturated fatty acid regulation of gene transcription. Prog Lipid Res. 1996 Sep;35(3):227–41.

44. Power GW, Newsholme EA. Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. J Nutr. 1997;127:2142–50.

45. Power GW, Calder PC, Newsholme EA. The influence of dietary fatty acids on the activity and metabolic control of peroxisomal carnitine palmitoyltransferase in the liver. Nutr Res. 1997;17:847–60.

46. Coleman RA, Lewin TM, Van Horn CG, Gonzalez-Baro MR. Do long-chain Acyl-CoA synthetases regulate fatty acid entry into synthetic versus degradative pathways? J Nutr. 2002;132:2123–6.

47. Fujino T, Kang MJ, Suzuki H, Iijima H, Yamamoto T. Molecular characterization and expression of rat acyl-CoA synthetase 3. J Biol Chem. 1996 Jul 12;271(28):16748–52.

48. Willumsen N, Vaagenes H, Lie O, Rustan AC, Berge RK. Eicosapentaenoic acid, but not docosahexaenoic acid, increases mitochondrial fatty acid oxidation and upregulates 2,4-dienoyl-CoA reductase gene expression in rats. Lipids. 1996;31:579–92.

49. Vamecq J, Vallee L, Delaporte PL, Fontaine M, Decraemer D, Vandenbranden C, Lafont H, Grataroli R, Nalbone G. Effect of various n-3/n-6 fatty-acid ratio contents of high-fat diets on rat-liver and heart peroxisomal and mitochondrial beta-oxidation. Biochim Biophys Acta. 1993 Oct 13;1170(2):151–6.

50. Mootha VK, Bunkenborg J, Olsen JV, Hjerrild M, Wisniewski JR, Stahl E, Bolouri MS, Ray HN, Sihag S, et al. Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. Cell. 2003 Nov 26;115(5):629–40.

51. Diez ADM, Bautista JM, Vega-Rubin de Celis S, Gómez-Requeni P, Perez-Sánchez J, Bores G, Obach A, Leaver MJ, Tocher DR, et al. Regulation the expression of peroxisome proliferator-activated receptor (PPARs) subtypes in Sparus aurata by dietary fat supplementation. 11th International Symposium on Nutrition and Feeding in Fish; 2004; Phuket, Thailand; 2004.

52. Babin PJ. Plasma-lipoprotein and apolipoprotein distribution as a function of density in the rainbow-trout (*Salmo gairdneri*). Biochem J. 1987 Sep 1;246(2):425–9.

53. Lapillonne A, Clarke SD, Heird WC. Polyunsaturated fatty acids and gene expression. Curr Opin Clin Nutr Matab Care. 2004 Mar;7(2):151–6.

54. Rise ML, Jones SR, Brown GD, von Schalburg KR, Davidson WS, Koop BF. Microarray analyses identify molecular biomarkers of Atlantic salmon macrophage and hematopoietic kidney response to Piscirickettsia salmonis infection. Physiol Genomics. 2004 Dec 15;20(1):21–35.

55. Rise ML, von Schalburg KR, Brown GD, Mawer MA, Devlin RH, Kuipers N, Busby M, Beetz-Sargent M, Alberto R, et al. Development and application of a salmonid EST database and cDNA microarray: data mining and interspecific hybridization characteristics. Genome Res. 2004;14:478–90.

56. Hordvik I, Berven FS, Solem ST, Hatten F, Endresen C. Analysis of two IgM isotypes in Atlantic salmon and brown trout. Mol Immunol. 2002 Oct;39(5–6):313–21.

57. McKay SJ, Trautner J, Smith MJ, Koop BF, Devlin RH. Evolution of duplicated growth hormone genes in autotetraploid salmonid fishes. Genome. 2004 Aug;47(4):714–23.

58. van Ommen BSR. Nutrigenomics: exploiting systems biology in the nutrition and health arena. Curr Opin Biotechnol. 2002 Oct;13(5):517–21.

ONLINE SUPPORTING MATERIAL

SUPPLEMENTAL TABLE 1.

Designated gene names for all amplicons printed on the microarray.

GenBank accession numbers	Gene name[1]	E value
Transport		
AAF67743	Liver - fatty acid binding protein	9E-34
AAC60350	H6-fatty acid binding protein	1E-61
Q91488	Apolipoprotein A-I (Apo-A-I-ii)	7E-31
X81856	Apolipoprotein B	3E-16
JH0472	Apolipoprotein A-I (A-I-i)	2E-99
AAG11410	Apolipoprotein C-II	2E-42
JC4858	VLDL receptor precursor	3E-11
Mitochondrial activity		
P08249	Malate dehydrogenase	4E-82
NP005908	Malate dehydrogenase, NAD	3E-90
AAD34966	Acetyl Co-A acetyltransferase	1E-69
NP006326	Translocase of inner mitochondrial membrane	5E-57
NP004448	Long chain acyl-CoA synthetase 3 (*M. glutinosa*) #[2 3]	8E-13
BE518505	Long-chain acyl Co-A synthetase #	
BM082412 and BM082589	Enoyl-Co-A hydratase (mitochondrial precursor)(*D. rerio*) #	
P14604	Enoyl Co-A hydratase (*M.mizolepsis*) #	1E-69
A40141	Mitochondrial solute carrier protein	6E-51
Q02368	NADPH-Ubiquionine Oxidoreductase	9E-46
AAD41391	Cytochrome oxidase III	3E-48
CAB89422	Outer mitochondrial membrane translocase	2E-53
NP055635	Translocase of outer mito membrane (tom70)	1E-74
O14561	Acyl carrier protein	6E-40
AAH06668	Carnitine acyl transferase (*H.hippoglossus*) #	1E-125
NP000089	Carnitine palmitoyltransferase (II)	9E-78
P56384	ATP synthase lipid binding protein P3	3E-32
Desaturation and elongation		
CAA42997	Δ9 fatty acid desaturase (*M.mizolepsis) #*	6E-55
AF478472	Δ5 Fatty acid desaturase *[4]	
AY170327	Elongase *	
Peroximal activity		
BE518489	Electron transfer flavoprotein #	
CAB64949	Catalase	1E-17
Nuclear receptors and transcription regulators		
AJ416952	PPAR- γ *	
AJ41695	PPAR- β1 *	
	Nuclear receptor subfamily 1 (no useful data)	
AAC50890	p300/CBP-associated factor (*P. americanus*) #	3E-75
NP035980	Nuclear receptor subfamily 0.Group B	8E-19
BG729054	SREBP (*D. rerio*) #	
Q64152	Transcription factor BTF3	4E-77
P19838	Nuclear factor NF-kappa β-P105 subunit	3E-45
BB054438	Nucleic acid binding factor	8E-20
P54864	Transcription factor AP-1 (jun)	5E-06
P53996	Cellular nucleic acid binding protein	1E-65
Others		
AJ299018	Cyclooxygenase 1 (*O. mykiss*) *[5]	
PMID: 10229670	Cyclooxygenase 2 (*O. mykiss*) *[6]	
AF512562	Triacylglycerol lipase (*P. americanus*)*	
Q08758	Phosphatidylcholine-sterol-acyltransferase	7E-25
BAA13672	Phosphatidylserine specific phospholipase A1 alpha	7E-310

SUPPLEMENTAL TABLE 1 (continued).

Designated gene names for amplicons printed on the microarray.

GenBank accession numbers	Gene name	E value
Others		
NP001896	CTP synthase	8E-52
P48999	Arachidonate-5-lipoxygenase	6E-73
D83712	Prostaglandin D synthase (*P. americanus*) #	2E-18
P53447	Fructose bisphosphate aldolase B (*P. americanus*) #	5E-71
AAB94003	Succinyl-Co-A synthetase	1E-104
BAA76974	Glutathione S-transferase	4E-98
S35704	Protein kinase C- Δ	E-62
NP002722	Protein kinase (cAMPdept)	E-110
S14113	Phospholipase C (*P. americanus*) #	4E-23
BM082549	1-phosphatidylinositol phosphodiesterase precursor (*D. rerio*) #	
AY386248	Bilesalt dependent lipase (*S. viviparus*) *	
AF512561	Bilesalt dependent lipase (*P. americanus*) *	
CAB40545	Lipoprotein lipase	1E-60
AW013173	Phospholipase (PI specific) (*P. americanus*) #	
NP004558	6-phosphofructo-2-kinase/fructose-2,6 biphosphatase	2E-66
BE518590	3-hydroxy-3methylglutaryl coenzyme A reductase #	
S39781	Phosphoprotein phosphatase	1E-82
NP004155	Retinol-binding protein 2	4E-20
AAC25677	Phosphatidylinositol 3-kinase	5E-85
AF231707	Eggshell protein	1E-156
P51913	Enolase	1E-88
Controls		
P29316	Ribosomal protein L23A (rpl23A)	2E-66
NP000989	Ribosomal proteinL37A(rpl37A)	4E-43
NP001014	Ribosomal protein S20 (rps20)	3E-61
JN0273	Ribosomal protein L10 (rpl10)	2E-65
P53484	Beta actin (act1)	1E-114
U19382	Arabidopsis chlorophyll synthetase	

[1] Gene name is designated based on BLAST search of EST sequenced cDNA, and for each designated gene name E value and GenBank accession numbers from the BLAST search are shown.

[2] Genes denoted by # are EST sequences and received from other sources. These include published salmon EST sequences BE518489, BE518505 and BE518590 (late Dr. Richard Powell, Galway), and IMAGE clones (*D. rerio*) (BM082549, BM082412 and BM082589, and BG729054), and winter flounder and mud load EST sequences (Dr. Dong Soo Kim and Yoon Nam, Pusan, South Korea. in collaboration with Dr. Sue Douglas)

[3] Probes were from Atlantic salmon unless otherwise denoted in brackets.

4 Genes denoted by * are fully sequenced, and only GenBank accessions numbers are presented.

[5] Cyclooxygenase 1 and -2 (Dr. Jun Zuo, Aberdeen) are fully sequenced, but only small PCR products were printed on the array.

[6] Cyclooxygenase 2 is identified through Pubmed ID: PMID: 10229670.