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Liver and muscle metabolic changes induced by dietary energy content and genetic selection in rainbow trout (*Oncorhynchus mykiss*)

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Kolditz C, Borthaire M, Richard N, Corraze G, Panserat S, Vachot C, Lefèvre F, Médale F. Liver and muscle metabolic changes induced by dietary energy content and genetic selection in rainbow trout (*Oncorhynchus mykiss*). *Am J Physiol Regul Integr Comp Physiol* 294: R1154–R1164, 2008. First published January 30, 2008; doi:10.1152/ajpregu.00766.2007.—We combined genetic selection and dietary treatment to produce a model to study metabolic pathways involved in genetic and nutritional control of fat deposition in fish muscle. Two experimental lines of rainbow trout, selected for a lean (L) or fat (F) muscle, were fed with diets containing either 10 or 23% lipids from the first feeding, up to 6 mo. At the end of the feeding trial, trout were distinguished by very different muscle fat content (from 4.2 to 10% wet weight), and line × diet interactions were observed for parameters related to fat storage. We analyzed the activity and gene expression of key enzymes involved in lipid metabolism (fatty acid synthase, hydroxyacyl-CoA dehydrogenase, carnitine palmitoyltransferase 1 isoforms, and peroxisome proliferator-activated receptor α) and glycolysis (hexokinase 1 and pyruvate kinase) as well as energy production (isocitrate dehydrogenase, citrate synthase, and cytochrome oxidase) in the liver and the white muscle of rainbow trout. The lipid-rich diet repressed the activity of the lipogenic enzymes and stimulated enzymes involved in fatty acid oxidation and glycolysis in liver but had little effect on muscle enzymes assessed in this study. Regarding the selection effect, enzyme activity and expression suggest that compared with the L line, the F line presented reduced hepatic fatty acid oxidation as well as reduced mitochondrial oxidative capacities and enhanced glucose utilization in both liver and muscle. Very few line × diet interactions were found, suggesting that the two factors (i.e., dietary energy content and selection) used in this study to modify muscle lipid content exerted some additive but mostly independent effects on these metabolic actors.

fish nutrition; metabolism; enzyme activity; gene expression

MUSCLE LIPID STORES CONSTITUTE an important energy source for muscle functions [e.g., during a prolonged muscular exercise (18, 35) or during fasting (17)]. In addition, muscle lipid level has become of considerable interest for the farm animal industry, since it strongly affects meat quality in terms of its nutritional value and sensory properties (1, 36, 44). Dietary manipulation and genetic selection are currently the main tools used to manage muscle fat content in farm animals. Energy intake and genetic factors also have a major influence on obesity in humans (30).

Increasing dietary energy supply promotes fat deposition in the whole body, including muscle in most fish (1, 36), as well as in other farm animals (11, 45). Salmonids have a high ability to store fat in muscle and show a wide range of variation for muscle lipid content under dietary manipulation, from 3% to as much as 18% (12, 36, 8), thus making salmonids particularly suitable species in which to study the mechanisms involved in muscle fat deposition and mobilization.

As mentioned above, selective breeding is another commonly used way to manage body fat content (9, 28). Recently, Quillet et al. (32) developed two experimental lines of rainbow trout through divergent selection for low or high muscle fat content, using a nondestructive measurement on live fish. Analysis of body composition after two generations of selective breeding showed that the selection procedure was efficient. Feeding the lines with diets differing in energy content enlarged the range of muscle fat level (33), suggesting a cumulative effect of the genetic background and the dietary treatment on muscle fattening. Combining genetic selection and dietary treatment thus resulted in a relevant model to gain further knowledge about the metabolic changes that occur when manipulating muscle fat content by these ways. Muscle lipid content results from the metabolic balance among dietary fat supply, de novo fatty acid synthesis in the liver, uptake of plasma triglycerides by muscle, and subsequent partitioning of fatty acids toward storage or oxidation for energy requirement. An increase in dietary fat level has been shown to depress the activity of different lipogenic enzymes in salmonids (12), but the effects on the other metabolic actors involved in energy utilization have been scarcely explored. With regard to the genetic selection effect, nothing is presently known about the contribution of these metabolic pathways in the differences in muscle fat content observed between lines. The question we addressed was whether these two factors, i.e., genetic selection and dietary manipulation, used to modify muscle fat content, affect in the same way key actors involved in the main metabolic pathways of energy utilization.

In the present study, we used trout from the two divergent lines described above but resulting from the third generation of selective breeding, and we fed them two diets, either with added fish oil (23% lipids) or without (10% lipids) from the first feeding onward, over a period of 6 mo. We analyzed the effects of genetic selection and dietary energy supply on body composition and growth parameters as well as feed utilization

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and examined the response of several key actors involved in fatty acids uptake by tissue (lipoprotein lipase), lipogenesis (fatty acid synthase, glucose-6-phosphate dehydrogenase, malic enzyme, and acetyl-CoA carboxylase), fatty acid oxidation (hydroxyacyl-CoA dehydrogenase, carnitine palmitoyltransferase 1, acyl-CoA oxidase, and peroxisome proliferator-activated receptor α), glycolysis (hexokinase 1 and pyruvate kinase), and energy production (isocitrate dehydrogenase, citrate synthase, and cytochrome oxidase). The objectives were *1*) to examine the response of these key metabolic actors to the dietary treatment, *2*) to identify those that were modified by the genetic selection, and *3*) to evaluate the possible cumulative effects of the genetic background and long-term feeding of a high-energy diet on the selected metabolic actors. These latter analyses were conducted in the liver, which is the center of intermediary metabolism and the main site of lipogenesis in fish, and in the muscle, the target tissue of the selection applied.

MATERIAL AND METHODS

Experimental Fish and Diets

The two lines of rainbow trout, designed as L (lean muscle line) and F (fatty muscle line), were obtained after three generations of divergent selection for high or low muscle fat content using a nondestructive method (Distell Fish Fatmeter). The selection process has been detailed by Quillet et al. (32). Fish were reared in the Institut National de la Recherche Agronomique (INRA) experimental facilities (PEIMA, Drennec, Sizun, Finistère, France) at a constant water temperature of 11.5 ± 0.5°C. The experiment was conducted following the *Guidelines of the National Legislation on Animal Care of the French Ministry of Research*.

Two experimental diets, designed as LE (low-energy diet) and HE (high-energy diet), were formulated by INRA and manufactured by a feed producer as extruded pellets. They were made from the same fishmeal-based mixture. About 15% fish oil was added to the HE diet to induce a large difference in lipid content between the two diets. The LE diet contained 9.8% lipids, provided by fishmeal, whereas the HE diet contained 23% lipids (Table 1), which is close to the highest values of fat content of commercial diets for young rainbow trout. The increase in dietary crude fat content was accompanied by a decrease in the proportions of protein and starch in the HE diet and an increased ratio of *n*-3/*n*-6 polyunsaturated fatty acids (Table 1).

Feeding Trial

Just before the first feeding time, fish of each line were distributed into six tanks of 500 animals (mean body weight: 0.16 g). Triplicate groups of each genotype were fed either the LE or the HE diet for 6 mo. During the first 4 mo, food was distributed in excess to ensure that food supply was not limiting. From the fifth mo, food pellet size was high enough to enable collection of uneaten food. The fish were group-weighted every 2 wk and counted to calculate the average body weight. Feed intake was recorded daily during the last 2 mo of the trial and feed efficiency calculated for the 2-mo period.

Table 1. *Appoximate composition of experimental diets*

Diets	LE	HE
DM, %	93.0	93.3
Protein, %DM	57.6	51.1
Lipid, %DM	9.8	23.1
n-3/*n*-6 FA	1.5	2.5
Starch, %DM	12.1	9.2
Energy, kJ/g DM	21.0	23.8

LE, low-energy diet; HE, high-energy diet; DM, dry matter; FA, fatty acids.

Sampling Procedure

To study the long-term effect of dietary treatment and not the effect of a test meal, fish were sampled 24 h after the meal to ensure that digestion and transit were completed. This postfeeding time does not correspond to a fasting state, since in trout, as in other ectothermic animals, basal metabolic levels are reached later than in mammals. This is due to a slower transit and a longer gastric emptying time at low temperature compared with that in endothermic animals. Ten fish per tank were sampled at the beginning of the "feed intake measurement period" (5th month) and at the end (6th month). They were pooled per tank, ground, and freeze-dried before being analyzed for protein and fat content of whole body.

At the end of the 6-mo feeding trial, all fish were anesthetized with 2-phenoxyethanol at the recommended dose for surgical procedures (0.2 ml/l) 24 h after a meal. They were individually measured for weight and length. Nineteen fish per tank were killed by a sharp blow on the head. Liver and viscera were weighed to calculate the hepatosomatic index [HSI (%) = 100 × (liver weight/body weight)], and the viscerosomatic index [VSI (%) = 100 × (total viscera weight/body weight)]. Pieces of white muscle were excised from the dorsal right fillet for enzyme activity measurements. Fillets from the left side of the fish were kept after trimming and skin withdrawal as samples for analysis of lipid content. Three additional fish per tank were sampled for liver and white muscle under RNase-free conditions to perform gene expression analysis. All the tissues samples were frozen in liquid nitrogen and stored at −80°C until analysis.

Chemical Analysis

The chemical composition of the diets and the freeze-dried samples of whole body collected at the fifth and sixth months was analyzed using the following procedures: dry matter after drying at 105°C for 24 h, lipids after petroleum ether extraction at 160°C, protein by the Kjeldahl method, gross energy in an adiabatic bomb calorimeter, and starch in the diets by the glucose-amylase-glucose-oxidase method (40). Total lipid content in the muscle was measured according to the Folch method (10) with dichloromethane instead of chloroform as a solvent.

Metabolic Pathways

The effects of genetic selection and dietary treatment on lipogenesis, fatty acid oxidation, glycolysis, and energy production were studied through the analysis of gene expression and activity of the key enzymes involved in these pathways. The lipogenic enzyme acetyl-CoA carboxylase (ACC; EC 6.4.1.2) as well as glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) and malic enzyme (ME; EC 1.1.1.40), which provide NADPH for fatty acid synthesis, were analyzed only for activity because of the absence of sequence information for rainbow trout in the databases. Gene expression of the transcription factor peroxisome proliferator-activated receptor α (PPARα) was measured as a key regulator of fatty acid oxidation.

Gene expression analysis: quantitative RT-PCR. Total RNAs were extracted from liver and white muscle using the TRIzol reagent method (Invitrogen, Carlsbad, CA). Nine individual samples per experimental condition were used as biological replicates. Total RNA was quantified using spectrophotometry based on absorbance at 260 nm, and integrity was controlled using the Agilent 2100 bioanalyzer (Agilent Technologies, Kista, Sweden). Total RNA (1 μg) was reverse-transcribed to cDNA with the SuperScript III RNase H reverse transcriptase kit (Invitrogen) using oligo$(dT)_{15}$ primers (Promega). Reverse transcription of each RNA extract was done in duplicate. Real-time PCR was performed with the iCycler iQ (Bio-Rad, Hercules, CA) on triplicates of 10 μl of the RT reaction mixtures using

the iQ SYBR green Supermix (Bio-Rad). The total volume of the PCR reaction mixture was 25 μl, containing 200 nM of primers. Specific primer pairs were designed using Primer3 software and chosen to contain an overlapping intron when it was possible, using known sequences in nucleotide databases (GenBank, http://www.genome.ad.jp/htbin/www_bfind?dna-today; National Institute of Agronomic Research INRA-Sigenae, http://ensembl-sigenae.jouy.inra.fr/; Tigr Gene Index, http://www.tigr.org/tdb/tgi/). Database accession numbers and the sequences of the forward and reverse primers used for each gene tested for its expression are shown in Table 2. All the different PCR products were controlled by sequencing the amplicon. Thermal cycling was initiated with the incubation at 95°C for 90 s for hot-start iTaq DNA polymerase activation. Thirty-five steps of PCR were performed, each one consisting of heating at 95°C for 20 s for denaturing and 30 s at the corresponding primer pair's annealing temperature (see Table 3) for hybridization and extension. After the final cycle of the PCR, melting curves were systematically monitored (increasing set point temperature from 59 to 95°C by 0.5°C/10 s). Negative controls (sample without reverse transcriptase, samples without RNA) were done for each reaction. mRNA levels of all target genes were normalized with the housekeeping gene α-elongation factor 1 (EF1α), previously used as a gene reference in salmonids (29). Expression levels were calculated according to threshold cycle ($\Delta\Delta C_T$) method (31).

Enzyme activities. Enzyme activities were measured on thawed samples of liver and white muscle. Activities of fatty acid synthase (FAS; EC 2.3.1.85), ACC, G6PDH, and ME were measured in liver as described by Richard et al. (34), using homogenates obtained from individual whole liver. Lipoprotein lipase (LPL; EC 3.1.1.34) activity was measured in muscle samples as described by Richard et al. (34).

Activities of the following enzymes were measured in liver and muscle samples. Hydroxyacyl-CoA dehydrogenase (HAD; EC. 1.1.1.35) enzyme activity was performed according to Kobayashi et al. (22). The glycolysis pathway was studied by measuring activity of hexokinase 1 (HK1; EC 2.7.1.1) and pyruvate kinase (PK; EC 2.7.1.40) as previously described (21). Citrate synthase (CS; EC 4.1.3.7) was measured according to Singer et al. (38) by following the reduction of DTNB at 412 nm. To measure NADP-dependent isocitrate dehydrogenase (ICDH-NADP; EC 1.1.1.42) activity, samples were homogenized in an ice-cold buffer (20 mM Tris·HCl, 250 mM mannitol, 2 mM EDTA, 100 mM NaF, 10 mM mercaptoethanol, and 0.5 mM PMSF), sonicated, and centrifuged at 12,000 *g*. Activity was assessed following NADP reduction at 340 nm after addition of 89.7 mM tetraethylammonium chloride at pH 7.4, 41 mM NaCl, 0.34 mM NADP, 0.43 mM $MnSO_4$, and 4.17 mM D,L-isocitrate to supernatants. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the hydrolysis of 1 μmol of substrate per minute at assay temperature. Enzyme activities were expressed per milligram of soluble protein. Protein concentration was determined according to the Bradford method, using a Bio-Rad protein assay kit (Munich, Germany) with bovine serum albumin as a standard.

Statistics

Data are means ± SE. Angular transformation was applied to proportions (lipid, protein, and moisture content, VSI, and HSI) before performing analyses to meet assumptions of variance analysis (homogeneity of variance and normally distributed residuals). The effects of dietary treatments, lines, and line × diet interactions on the different parameters were tested using the statistical software SAS by means of a two-way (line and diet) analysis of variance (ANOVA). Differences were considered significant when the probability level was <0.05. When interactions were significant, means were compared using the Student-Newman-Keuls test.

RESULTS

Growth Performance, Feed Intake, and Nutrient Efficiency

Data on growth and feed utilization are reported in Table 4. Feed intake was the same irrespective of the line or the diet. As expected, the HE diet improved feed and protein efficiencies ($P = 0.003$ and 0.0001, respectively), with a protein sparing effect provided by the higher amount of lipids of this diet. The higher energy content of the HE diet enhanced weight gain ($P = 0.0001$) and growth ($P = 0.043$) of fish irrespective of the line. Protein retention was not affected by dietary treatment, contrary to lipid retention that was increased in fish fed the LE diet ($P = 0.0006$), with values above 100%, indicating an active de novo lipid synthesis.

Higher feed efficiency and protein efficiency were observed in L fish ($P = 0.03$ and 0.026, respectively), particularly when fed the HE diet, but no significant line × diet interaction was recorded for these traits ($P = 0.09$ and 0.07, respectively). At the end of the feeding trial, fish of the L line showed higher body weight ($P = 0.0001$) and total length ($P = 0.0001$) than fish of the F line. Daily protein and lipid gains were not significantly different among the two lines.

Whole Body and Tissue Composition

Dietary treatment modified whole body composition of fish of both lines (Table 5). Fish fed the HE diet had lower moisture and protein content ($P = 0.0001$ and 0.0007, respectively) and

Table 2. *Accession number and corresponding database of target gene sequences*

Target Gene	Abbreviation	Database	Accession No.
Lipoprotein lipase	LPL	GenBank	AJ224693
Fatty acid synthase	FAS	Sigenae	tcaa0001c.m.06_5.1.om.4
Hydroxyacyl-CoA dehydrogenase	HAD	Sigenae	tcad0001a.i.15_3.1.om.4
Carnitine palmitoyl transferase 1a	CPT1a	GenBank	AF327058
Carnitine palmitoyl transferase Ib	CPT1b	GenBank	AJ606076
Carnitine palmitoyl transferase Ic	CPT1c	GenBank	AJ619768
Carnitine palmitoyl transferase Id	CPT1d	GenBank	AJ620356
Acetyl-CoA oxidase	ACO	GenBank	BX085367
Peroxisome proliferator-activated receptor α	PPARα	GenBank	AY494835
Hexokinase 1	HK1	GenBank	AY864082
Pyruvate kinase	PK	Sigenae	tcav0004c.c.07_3.1.om.4
Isocitrate dehydrogenase-NADP	ICDH	Sigenae	15072560.1.om.4
Cytochrome oxidase subunit 4	COX4	Sigenae	tcav0004c.i.22_3.1.s.om.8
Citrate synthase	CS	Tigr	TC89195
Elongation factor 1α	EF1α	GenBank	AF498320

Table 3. *Sequences of rainbow trout primers used for amplification of target genes by qRT-PCR*

Gene	5′-3′ Forward Primer	5′-3′ Reverse Primer	Annealing Temperature, °C	Amplicon Size, bp
LPL	taattggctgcagaaaacac	cgtcagcaaactcaaaggt	59	164
FAS	gagacctagtggaggctgtc	tcttgttgatggtgagctgt	54	186
HAD	ggacaaagtggcaccagcac	gggacggggttgaagaagtg	59	126
CPT1a	tcgattttcaagggtcttcg	cacaacgatcagcaaactgg	55	166
CPT1b	ccctaagcaaaaagggtcttca	catgatgtcactcccgacag	55	149
CPT1c	cgcttcaagaatggggtgat	caaccacctgctgtttctca	59	187
CPT1d	ccgttcctaacagaggtgct	acactccgtagccatcgtct	59	154
ACO	gcgccaagtacttcctcaac	tcacaaactcctgtgtgctg	55	210
PPARα	ctggagctggatgacagtga	ggcaagtttttgcagcagat	55	195
HK1	ctgggacgctgaagaccaga	cggtgctgcatacctccttg	59	159
PK	cctgtatcgcgggatcttcc	ccacacgcatggtgttggta	59	192
ICDH	gacagcaccaacagggcaa	aagccagcctcgatggtctc	59	163
COX4	tacgtgggggacatggtgtt	cccaggagcccttctccttc	59	150
CS	ggccaagtactgggagttca	ctcatggtcactgtggatgg	55	229
EF1	tcctcttggtcgtttcgctg	acccgagggacatcctgtg	59	159

higher lipid content ($P = 0.0001$) than fish fed the LE diet. Muscle lipid content and VSI were higher in fish fed the HE diet compared with fish fed the LE diet ($P < 0.0001$ and $P = 0.0001$, respectively).

Whole body composition was similar for the two lines fed the same diet. A line × diet interaction was observed for this trait ($P = 0.003$), with a marked increase in F fish fed the HE diet. Muscle lipid contents were different among each experimental group: the lowest values were seen in L fish fed the LE diet (4.2% of wet weight) and the highest in F fish fed the HE diet (10.1% of wet weight). Furthermore, similar values (6.3% of wet weight) were recorded for the L fish fed the HE diet and F fish fed the LE diet. HSI was not modified by the genetic selection or the diet. VSI was high in L fish ($P = 0.0001$), indicating an increased fat deposition as visceral adipose tissue compared with F fish. A line × diet interaction was recorded for VSI ($P = 0.003$), with L fish fed the HE diet showing the highest values.

Hepatic Metabolic Actors

Metabolic actors in liver were studied at the enzymatic level (Table 6) and at molecular level (Fig. 1) when trout sequences of the corresponding genes were available in the databases. Lipogenesis was assayed in liver only, since this organ is the major site for de novo lipid synthesis in fish (26, 27). Activities of FAS, G6PDH, ME, and ACC were decreased when fish were fed the HE diet ($P = 0.0002$, 0.0003, 0.03, and 0.0002, respectively). There was no significant difference in FAS mRNA level among dietary treatments ($P = 0.56$). Regarding fatty acid oxidation pathway, the HE diet enhanced specific activity of HAD ($P = 0.04$) as well as gene expression of acetyl-CoA oxidase (ACO; $P = 0.0004$), carnitine palmitoyltransferase 1 (CPT1) a and b isoforms ($P < 0.0001$ for both). We did not find any change in expression of PPARα among diets. The HE diet led to higher HK1 and PK activities ($P = 0.003$ and $P < 0.00010$) as well as HK1 and PK mRNA level

Table 4. *Growth performance and nutrient utilization of trout from L and F lines fed LE and HE diets for 6 mo*

	L Line		F Line		*P* Values		
	LE	HE	LE	HE	Diet	Line	Line × Diet
Final body weight, g	73.0±2.9	92.4±2.9	57.8±2.0	77.4±2.5	0.0001	0.0001	0.66
Length, mm	174.9±2.1	186.3±1.7	165.5±1.8	176.9±1.7	0.0001	0.0001	0.38
Daily growth index, %/day	2.57±0.04	2.67±0.05	2.37±0.08	2.52±0.03	0.053	0.011	0.64
Feed intake, g DM/kg	16.5±0.1	16.3±0.5	16.6±0.1	15.8±0.2	0.10	0.53	0.34
Feed efficiency	1.22±0.01	1.27±0.02	1.21±0.01	1.23±0.01	0.003	0.03	0.10
Protein efficiency ratio	2.11±0.01	2.49±0.02	2.10±0.02	2.41±0.02	0.0001	0.026	0.07
Nitrogen gain, $mg \cdot kg^{-1} \cdot day^{-1}$	572±8	471±20	550±22	467±10	0.0005	0.44	0.61
Fat gain, $g \cdot kg^{-1} \cdot day^{-1}$	1.93±0.04	3.50±0.28	2.23±0.07	3.48±0.17	0.0001	0.34	0.29
Retention, %							
Protein	37.6±0.5	35.6±2.7	35.9±1.4	36.1±0.8	0.58	0.72	0.59
Fat	119.2±2.6	93.5±10.2	140.6±6.0	95.3±5.6	0.0006	0.11	0.16

Weight and length are expressed as means ± SE of 57 individuals in all groups. The other traits were calculated for each tank, which represented 1 experimental unit. Daily growth index was calculated as 100 × (mean final body weight$^{[1/3]}$ − mean initial body weight$^{1/3}$)/day. Feed intake was recorded only during the last 2 mo of trial, so these traits were calculated for this period. Daily feed intake was calculated as the total amount of ingested food (kg DM) divided by the mean biomass over the trial [(initial biomass + final biomass)/2, expressed in kg wet weight (WW)] and the number of days. Feed efficiency was estimated as the gain in total biomass [(final biomass − initial biomass) (kg WW)] divided by the amount of ingested dry matter (kg DM). Protein efficiency ratio was the gain in total biomass divided by the amount of ingested crude protein (kg). Nitrogen and fat gain were calculated as (final carcass nutrient content − initial carcass nutrient content) divided by the mean biomass over the trial [(initial biomass + final biomass)/2 (kg WW)] and the number of days, where nutrient refers to nitrogen and fat. Protein and fat retention were calculated as [100 × (final body weight × final carcass nutrient content) − (initial body weight × initial carcass nutrient content)]/nutrient intake, where nutrient refers to protein and lipid. Values are expressed as means ± SE ($n = 3$ in all groups). P values <0.05 indicate significant differences [multivariate analysis of variance (MANOVA), 2 factors].

Table 5. *Whole body composition, morphological parameters, and muscle lipid content of trout from L and F lines fed LE and HE diets for 6 mo*

	L Line		F Line		*P* Values		
	LE	HE	LE	HE	Diet	Line	Line × Diet
Whole body composition, %WW							
Moisture	70.7±0.3	65.9±0.6	69.9±0.2	66.9±1.3	0.0001	0.75	0.08
Protein	17.3±0.4	15.6±0.3	16.8±0.7	15.4±0.4	0.0003	0.21	0.67
Lipid	9.5±0.2	15.3±1.1	10.8±0.5	15.2±1.0	0.0001	0.27	0.16
HSI, %	1.3±0.2	1.2±0.2	1.3±0.2	1.3±0.3	0.14	0.99	0.33
VSI, %	8.3±0.8[c]	12.4±1.3[a]	7.7±0.9[d]	11.0±1.9[b]	0.0001	0.0001	0.0034
Muscle lipid content, %WW	4.3±0.8[c]	6.4±1.2[b]	6.3±1.2[b]	10.1±2.3[a]	<0.0001	<0.0001	0.0026

Values are means ± SE (n = 57 individus in all groups, except for muscle lipid content, for which n = 30). HSI, P values <0.05 indicate significant differences (MANOVA, 2 factors). [a,b,c,d]$P < 0.05$, means with different superscript letters are significantly different from each other (ANOVA).

(P = 0.003 and 0.02), despite the lower starch content of this diet [9.2% dry matter (DM)] compared with LE diet (12.1% DM). Activity of glucokinase was also assayed in the liver, but the levels were too low 24 h after meal to be informative. The HE diet depressed the activity and gene expression of ICDH (P = 0.0001 and $P < 0.0001$) but did not modify those of CS. An interaction was observed for cytochrome oxidase 4 (COX4) at the molecular level (P = 0.013), with the HE diet decreasing COX4 mRNA level in the F line only. A slight interaction was also observed for CS gene expression, with higher values observed in L fish fed the LE diet (P = 0.053).

Activities of FAS, G6PDH, and ME were not significantly different between lines, but that of ACC was higher in F fish compared with L fish ($P < 0.0001$). Concerning fatty acid catabolism, no difference was recorded between lines for HAD activity; however, an interaction was observed at the molecular level (P = 0.006), since the HE diet increased HAD mRNA level in the L line only. Gene expression of ACO, CPT1a, and CPT1b was increased in fish of the L line (P = 0.007, 0.01, and <0.0001, respectively). CPT1c gene expression was similar in L-HE and F-LE groups and higher than in L-LE and F-HE groups (P = 0.0003). PPARα was upregulated in the liver of L fish compared with F fish. Regarding glycolysis, lines differed only for HK1 activity ($P < 0.0001$) and mRNA level (P = 0.03), which were increased in F fish. Regarding enzymes involved in energy production (CS, ICDH, and COX4), only ICDH was affected, with a lower activity and gene expression in the F line (P = 0.012 and 0.001, respectively).

Muscle Metabolic Actors

Data on enzyme activities and gene expression are reported in Table 7 and Fig. 2, respectively. Dietary treatment induced no change in LPL activity or gene expression. No change was recorded for HAD activity, but gene expression of HAD was increased in fish fed the HE diet (P = 0.008). This diet decreased gene expression of CPT1b (P = 0.04), whereas mRNA levels of CPT1a and CPT1c, as well as ACO and PPARα, were not significantly affected by the diets. No diet effect was recorded for either of the two glycolytic enzymes tested. Specific activity and gene expression of ICDH were increased with the LE diet (P = 0.008 and $P < 0.0001$) as well as the COX4 mRNA level (P = 0.05). Gene expression and activity of CS were unchanged with dietary treatments, although CS activity tended to be higher with the LE diet (P = 0.06). No line × diet interaction was found for enzymes involved in glycolysis and energy production in muscle.

LPL activity and gene expression were not modified by selection; however, the activity of LPL tended to be lower in muscle of L fish fed the LE diet (P = 0.08). No change in gene expression was recorded between L and F fish for CPT1a, CPT1c, ACO, HAD, and PPARα, whereas CPT1b gene expression was enhanced in F fish (P = 0.02). Activities and gene

Table 6. *Specific enzyme activities in liver of trout from the L and F lines fed the LE and HE diets for 6 mo*

	L Line		F Line		*P* Values		
	LE	HE	LE	HE	Diet	Line	Line × Diet
Lipogenesis							
FAS	36.5±2.0	24.8±1.3	34.8±3.5	26.7±0.7	0.0002	0.95	0.42
G6PDH	444.4±39.1	308.8±16.3	492.4±35.5	348.3±33.5	0.0003	0.19	0.90
ME	76.2±6.2	60.7±3.7	80.1±5.9	66.1±8.4	0.03	0.46	0.93
ACC	3.8±0.2	2.9±0.2	6.1±0.3	4.6±0.3	<0.0001	<0.0001	0.20
β-Oxidation							
HAD	109.9±5.9	129.3±13.7	97.6±4.6	115.3±9.2	0.04	0.13	0.92
Glycolysis							
HK1	2.9±0.1	3.6±0.2	4.8±0.1	5.8±0.2	<0.0001	<0.0001	0.31
PK	117.5±7.9	230.3±8.8	174.3±4.0	229.9±2.0	<0.0001	0.80	0.84
Energy metabolism							
CS	115.7±5.4	118.4±5.0	103.3±7.6	113.3±6.7	0.33	0.19	0.61
ICDH	415.4±9.9	357.6±9.1	395.6±6.9	330.4±8.0	0.0001	0.012	0.67

Values are specific activities expressed in mUI/mg protein, except for FAS, for which values are expressed in μUI/mg protein, and are means ± SE (n = 6 in all groups except for HAD and CS, for which n = 5). G6PDH, glucose-6-phosphate dehydrogenase; ME, malic enzyme; ACC, acetyl-CoA carboxylase.

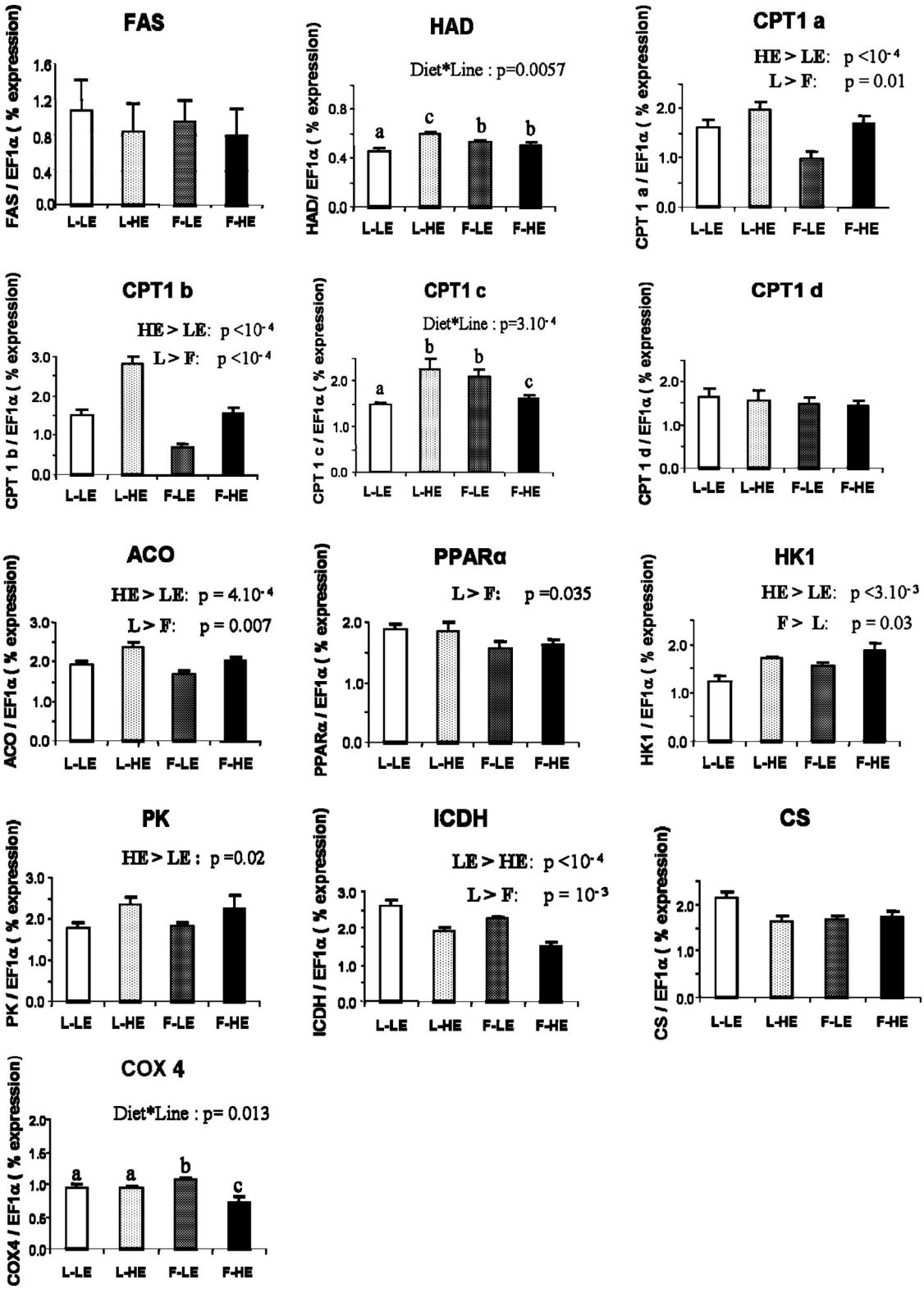

Fig. 1. Gene expression of selected enzymes in the liver of rainbow trout of a lean muscle line (L) and a fatty muscle line (F) fed a high- (HE) or low-energy (LE) diet, measured using real-time quantitative RT-PCR. Lipid metabolism: fatty acid synthase (FAS), hydroxyacyl-CoA dehydrogenase (HAD), carnitine palmitoyltransferase 1 isoforms (CPT1a, CPT1b, CPT1c, and CPT1d), acetyl-CoA oxidase (ACO), and peroxisome proliferator-activated receptor α (PPAR α). Glycolysis: hexokinase 1 (HK1) and pyruvate kinase (PK). Energetic metabolism: isocitrate dehydrogenase (ICDH), citrate synthase (CS), and cytochrome oxidase 4 (COX4). mRNA was prepared from individual livers (n = 9 per group). Data are means ± SE of 9 samples performed in triplicate. Expression values are normalized with α-elongation factor 1 (EF1α)-expressed transcripts. [a,b,c,d]$P < 0.05$, means not sharing a common letter are significantly different from each other.

Table 7. *Specific enzyme activities in muscle of trout from the L and F lines fed the LE and HE diets for 6 mo*

	L Line		F Line		*P* Values		
	LE	HE	LE	HE	Diet	Line	Line × Diet
Fatty acid uptake							
LPL	0.40±0.05	0.60±0.09	0.60±0.07	0.55±0.06	0.28	0.26	0.083
Fatty acid β-oxidation							
HAD	40.8±1.7	45.3±3.3	40.9±4.5	47.6±2.9	0.10	0.71	0.74
Glycolysis							
HK	0.42±0.01	0.42±0.02	0.51±0.01	0.51±0.01	0.79	<0.0001	0.54
PK	7.8±0.3	7.7±0.2	9.2±0.2	9.3±0.2	0.87	<0.0001	0.60
Energy metabolism							
CS	253.4±9.4	212.9±8.2	185.3±11.7	170.3±8.1	0.06	0.0001	0.21
ICDH	422.2±9.3	395.7±12.8	432.2±12.9	392.9±8.8	0.008	0.74	0.57

Values are specific activities expressed in mUI/mg protein, except for PK, for which values are expressed in UI/mg protein, and are means ± SE ($n = 6$ in all groups except for LPL and CS, for which $n = 7$ and 4, respectively).

expression of HK1 ($P < 0.0001$ and $P = 0.007$) and PK ($P < 0.0001$ and $P = 0.03$) were higher in F fish muscle than in L fish. Specific activity of CS was significantly lowered in fish of this line ($P = 0.001$). There were no significant differences in gene expression of enzymes involved in energy metabolism (CS, ICDH, and COX4), although the CS mRNA level was slightly lower in F fish ($P = 0.055$) than in L fish.

DISCUSSION

The combination of genetic selection and dietary energy level led to the obtention of animals having very different values for muscle lipid content: from 4.2 to 10.1% of wet weight, with similar values (6.4%) recorded for the L line fed the HE diet and the F line fed the LE diet. The objective of our study was to identify the metabolic actors that were modified by genetic selection for muscle fat content compared with those affected by long-term feeding with a HE diet.

HE Diet Enhances Fish Growth and Whole Body Fat Content

The HE diet improved growth of fish of the two lines and resulted in increased body fat content with a concomitant decrease in moisture, in agreement with previous observations (19, 41). In salmonids, like rainbow trout, fat deposition mainly occurs in visceral adipose tissue, and to a lesser extent in muscle, within adipocytes scattered between myofibers (6, 42). Accordingly, the HE diet resulted in increased visceral fat deposition (as reflected by a higher VSI) and increased muscle lipid content in both lines.

Divergent Selection for Muscle Fat Content Affects Growth Performance and Fat Allocation Without Modifying Whole Body Fat Content of Fish

According to the selection target, muscle lipid content was significantly higher in the F line than in the L line, whereas L fish had a higher VSI, reflecting a higher fat deposition in visceral adipose tissue in this line. There was no significant difference in either fat gain or whole body fat content between the L and F lines. The data indicate that L fish deposited fat preferentially in visceral adipose tissue compared with F fish, which stored comparatively more fat in the muscle. Body weight and length of F fish were significantly lower than those of L fish, irrespective of the diet. This seems not to be attributable to a difference in feed intake (expressed as g DM/kg body wt), since similar values were found for both lines, but rather to a better feed efficiency and protein utilization for growth in L fish. It is noteworthy that the effect of the HE diet on these two storage sites differed between L and F fish. Whereas visceral and muscle fat deposition were similarly enhanced by the HE diet compared with the LE diet in the L genotype (+49.4 and 48.8%, respectively), in the F genotype, a change of greater magnitude was observed in muscle (+58.7%) compared with visceral adipose tissue (+42.8%).

Actors of Liver Metabolism

Activity of key enzymes involved in hepatic lipogenesis are affected by the dietary treatment but not by the genetic selection. Liver is the major site of de novo fatty acid synthesis in fish (15, 26, 27), as in human and most avian species (16). The lower activity of lipogenic enzymes, i.e., FAS, G6PDH, ME, and ACC, when fish were fed the HE diet is in accordance with an inhibition of lipogenesis by dietary lipid content higher than 10% DM, already described in fish (12). Furthermore, fish oil has a high content of *n*-3 polyunsaturated fatty acids (PUFAs), known to depress lipogenesis in mammalian liver (16), as well as in rainbow trout hepatocytes (2). In the present study, the lower amounts of dietary protein and carbohydrate that were provided by feeding the HE diet were also expected to account for the decrease of the activity of the lipogenic enzymes.

A study by Corraze et al. (6) evidenced that in rainbow trout, neosynthesized lipids were preferentially incorporated in the muscle, rather than in the storage sites such as visceral adipose tissue. This led us to hypothesize that enhanced lipogenic capacities might have contributed to the preferential partition of fat toward muscle in the F line compared with the L line. This hypothesis was not confirmed, since we found that activities of lipogenic enzymes were not modified between lines, except for ACC, the first step enzyme of fatty acid biosynthesis, which was higher in the F line. This could not be related to an increase in lipogenesis in F fish, since neither FAS activity nor activities of G6PDH and ME, which provide NADPH for fatty acid synthesis, were changed. It thus seems that these lipogenic actors do not account for differences in muscle fat deposition between lines.

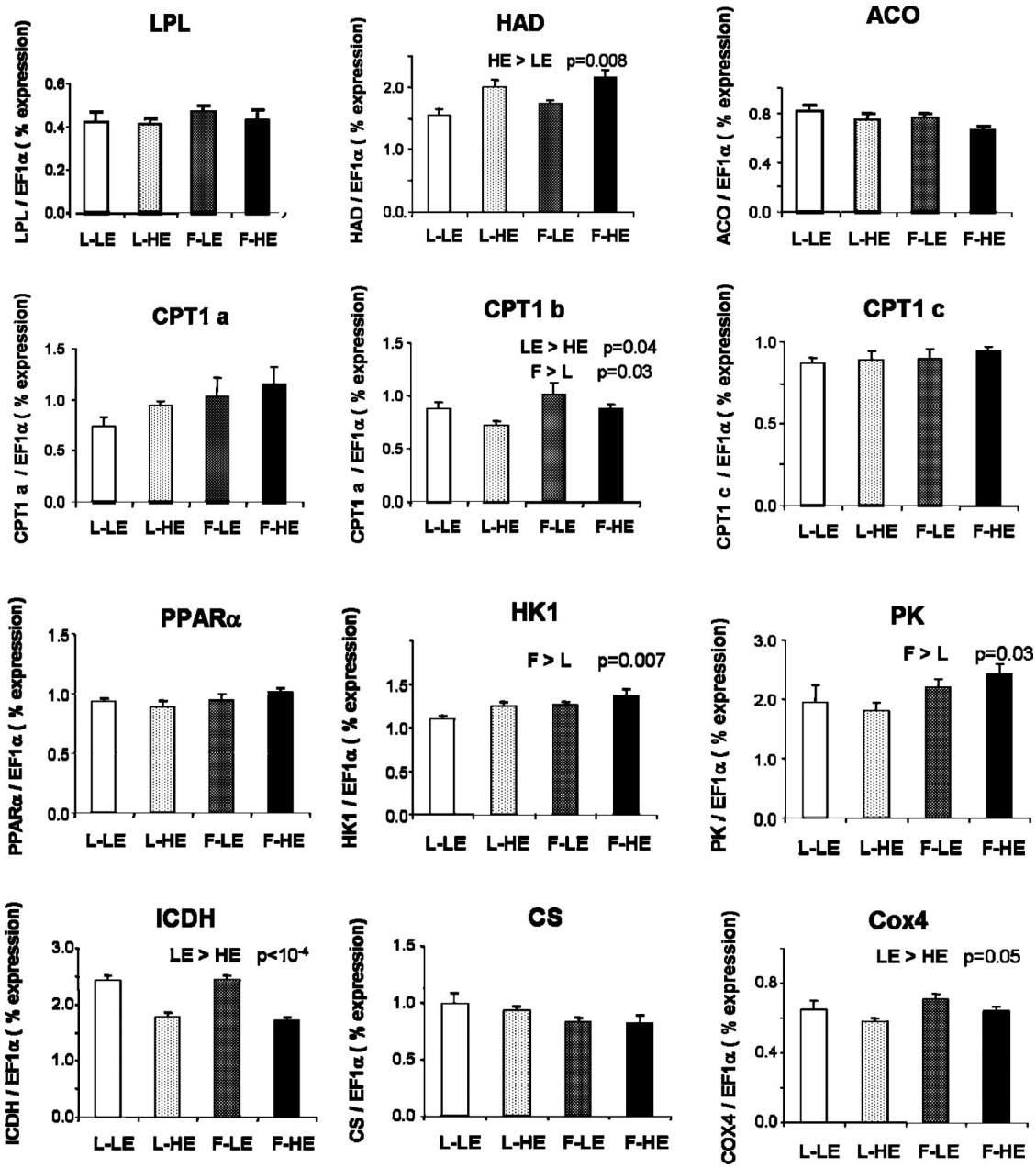

Fig. 2. Gene expression of selected enzymes in the white muscle of rainbow trout of the L and F lines fed a HE or LE diet, measured by real time quantitative RT-PCR. Lipid metabolism: lipoprotein lipase (LPL), HAD, CPT1a, CPT1b, CPT1c, ACO, and PPARα. Glycolysis: HK1 and PK. Energetic metabolism: ICDH, CS, and COX4. mRNA was prepared from individual samples of white muscle ($n = 9$ per group except for HAD, CPT1a, CPT1b, and PPARα, for which $n = 8$). Data are means ± SE of 9 samples performed in triplicate. Expression values are normalized with EF1α-expressed transcripts.

Actors of fatty acid oxidation in the liver are stimulated with the HE diet but repressed in the F line. Gene expressions of enzymes catalyzing fatty acid β-oxidation, i.e., ACO, CPT1a, and CPT1b, as well as HAD activity, were upregulated in the liver of fish fed the HE diet. ACO and CPT1 are rate-limiting enzymes of peroxisomal and mitochondrial fatty acid oxidation, respectively, which suggests that both mitochondrial and peroxisomal fatty acid oxidation were induced in liver by the higher dietary lipid supply. In mammals, fatty acids, especially *n*-3 PUFAs, enhance peroxisomal and mitochondrial fatty acid oxidation through the activation of the transcription factor PPARα, leading to an upregulation of the corresponding genes (37). The major role played by PPARα in upregulating the expression of several key enzymes involved in fatty acid oxidation such as CPT1 and ACO has been demonstrated in rodent liver (23), but little is known yet about nutritional regulation involving PPARs in fish. Recently, Kennedy et al. (20) showed that PPARα was upregulated in Atlantic salmon fed a diet rich in fish oil. In the present study, gene expression of PPARα was not affected by the dietary treatments. One possible reason for this discrepancy might be that the diet used by Kennedy and coworkers contained much more lipids (~33% DM) than the HE diet (23% DM) in the present experiment. Potential interspecies differences also have to be

considered. Nevertheless, because PPARs are activated by binding to fatty acids, notably PUFAs, to act on gene expression, a posttranscriptional activation of PPARα might be responsible for the stimulation of fatty acid β-oxidation related genes in fish fed the HE diet.

Gene expression of ACO, CPT1a, and CPT1b was significantly depressed in F fish liver, suggesting that both peroxisomal and mitochondrial pathways of fatty acid β-oxidation were repressed in the liver of the F line, although no differences were found between lines for liver HAD activity and mRNA level. PPARα gene expression was significantly lower in the liver of the F line compared with the L line. This weaker expression of PPARα gene might be at least in part responsible for the lower expression observed for ACO, CPT1a, and CPT1b in F fish liver.

Liver HK1 activity and gene expression are stimulated with the HE diet and in the F line. Regarding to the dietary effect, the elevated gene expression and activity of HK1 and PK in the liver suggest that hepatic glycolysis was enhanced in trout fed the HE diet. This result was quite unexpected because of the slightly lower starch intake by trout fed this diet and the usual depressive effect of dietary PUFA on hepatic glycolysis through the inhibition of PK gene expression (25). Further investigations are needed to explain such an effect. This could reflect a disturbance of carbohydrate metabolism in response to an alteration of lipid homeostasis after long-term feeding a high-fat diet in fish.

Regarding to the selection effect, the F line showed higher hepatic HK1 activity and mRNA level, suggesting enhanced glucose utilization in the liver. Further studies monitoring glucose utilization by the two genotypes, i.e., using radiolabeled glucose, are required to confirm this observation. These data show that feeding the HE diet and upward selection for muscle fat content affected HK1 in the same way at both the molecular and activity level.

Hepatic ICDH activity and gene expression are depressed in fish fed the HE diet and in the F line. Hepatic activity and gene expression of ICDH were lowered with the HE diet. ICDH is one of the key enzymes of the tricarboxylic acid cycle (TCA). Our results suggest a stimulation of both hepatic fatty acid oxidation and glycolysis by the HE diet. Hence, the reduction of TCA cycle with the HE diet, as suggested by the lowered ICDH activity and mRNA level, might be due to a lowered catabolism of dietary amino acid in the liver, since the overall protein intake was lower with the HE diet. Indeed, proteins appear as important lipogenic precursors in rainbow trout hepatocytes (5). The reduced activity of ICDH, which produces NADPH, is in line with the lower activity of lipogenic enzymes.

Hepatic activity and gene expression of ICDH were lower in the F line than in the L line. These data suggest a depressed mitochondrial oxidative metabolism in liver of the F line compared with the L line, probably related to the lower liver fatty acid oxidation in this line.

Actors of Muscle Metabolism

Increased muscle fat content induced by dietary treatment and selection are not attributable to a difference in LPL activity or gene expression. LPL is described in mammals as the rate-limiting enzyme for the import of triglyceride-derived fatty acids into muscle (24). We thus hypothesized that a higher LPL activity could be responsible for the higher muscle fat content resulting from feeding the HE diet and from upward selection for muscle fat content. The data did not confirm this hypothesis. There was no significant difference for LPL gene expression or activity level whatever the diet or the genotype, with great interindividual variations. Nevertheless, it is noteworthy that in muscle, LPL activity was slightly lower in L fish fed the LE diet, which had the lowest muscle fat content among the four experimental groups. Very little information is available in fish with regard to dietary regulation of LPL in white muscle, and to our knowledge, the effects of a high dietary energy supplied mainly as fat on LPL activity or mRNA level in muscle has never been investigated until now. It would be interesting to study the postprandial kinetics of muscle LPL activity and gene expression in response to a high-fat diet to definitively deal with the role of the LPL in muscle fattening.

Dietary treatment and genetic selection have little effect on the enzymes involved in fatty acid oxidation in muscle. No change in gene expression or activity was recorded between the two dietary groups for the fatty acid oxidative enzymes assayed in muscle, except for the HAD mRNA level, which was increased in fish fed the HE diet, and CPT1b gene expression, which was concomitantly lowered. This opposite nutritional effect on the gene expression of these two fatty acid oxidative enzymes remains to be clarified. CPT1 is described as the rate-limiting enzyme of β-oxidation flux in liver, heart, and skeletal muscle of mammals (7). In rainbow trout, four isotypes of CTP1 with all of the structural features characteristics of mammalian CPT1 have been identified (13). All of the four predicted amino acid sequences share a better similarity with human CPT1A protein sequence (65–68%) than with human CPT1B (58–62%) and CPT1C (50–52%) protein sequences. The present data show that rainbow trout CPT1a, -b, and -c are expressed in both the liver and the skeletal muscle, whereas CPT1d expression is not detectable in the skeletal muscle. Regulation of each isoform needs to be further investigated, since we observed differential expression between these isoforms for the first time in fish.

Regarding the lines, no significant differences were recorded for any of the enzymes involved in fatty acid oxidation that we tested, except CPT1b gene expression, which was increased in F fish. Hence, these data suggest that the increased muscle fat level observed in fish from the F line did not result from a decrease in fatty acid oxidation capacity, as we first hypothesized.

Muscle activity and gene expression of HK and PK are stimulated in the F line and remain unchanged with dietary treatment. There was no change in gene expression or activity for HK and PK between the two dietary treatments. Activity levels of these two glycolytic enzymes were also low compared with the liver. This is in conformity with the general observation that in rainbow trout, white muscle poorly utilizes glucose as an energy source (43).

In contrast, activity and gene expression of HK and PK were higher in muscle of the F line, whatever the diet, suggesting that glycolysis was enhanced in the F line compared with the L line. This is particularly interesting, since, as mentioned previously, dietary glucose usually contributes weakly as an oxidative fuel for muscle energy need. One possibility is that the F line might have a genetic predisposition to higher glucose utilization in muscle than the L line. Increased capacity to

oxidize glucose in this tissue for energy purpose might have led to a concomitant relative decrease in the use of lipid, thus increasing availability of lipids for subsequent storage in muscle of the F line. Another possibility would be that this increased glucose oxidation capacity might have been installed consequently to an impaired capacity of the F line to increase fatty acid oxidation in a context of elevated muscle triglyceride level. The underlying mechanism remains to be defined, but it appears to be an interesting lead to explore in the future.

Enzymes involved in energy production are depressed in muscle of fish fed the HE diet and in the F line. Enzymes involved in energy production, i.e., ICDH and COX4, were depressed in muscle of fish fed the HE diet, likely reflecting a decrease in their mitochondrial oxidative capacities. Such a phenomenon has been already described in skeletal muscle of mammals after long-term feeding of a high-energy diet (4, 39). A negative relationship between mitochondrial oxidative capacities and triglyceride content in muscle has also been reported (3, 14). Whether reduced muscle oxidative capacities are a cause or a consequence of the increased muscle lipid content in fish fed the HE diet is unclear and needs further investigation.

In muscle, CS activity was significantly lower in the F line than in the L line. The activity of this enzyme is generally correlated with the number of mitochondria and the capacity of a tissue in aerobic oxidative metabolism. A lower mitochondrial oxidative capacity in the muscle of F fish could have contributed to reduce triglyceride depletion in the muscle of this line.

Conclusion

Dietary treatment induced significant changes in metabolic actors involved in lipid metabolism, glycolysis, and energy production in the liver but had little effect on those assayed in the muscle. It is likely that the higher muscle lipid content resulting from the 6-mo feeding of the high-energy diet in both genotypes is directly related to an oversupply of dietary fat in this tissue. Our data show that the genetic selection for high muscle fat content does not affect feed intake, with similar whole body fat content between the two lines fed the same diet. The higher muscle fat level observed in the F line was associated with neither a stimulation of lipogenic enzymes or muscle LPL-mediated lipid uptake nor alteration of fatty acid oxidation enzymes in muscle. Instead, our data suggest that the F line has enhanced glycolytic capacity in both liver and muscle and decreased hepatic fatty acid oxidation capacity compared with the L line. With regard to their muscle-fattening effect, the dietary treatment and the genetic selection seem to act through different metabolic actors, since additive effects were observed only for enzymes involved in glycolysis (HK) and the TCA cycle (ICDH) in the liver.

Perspectives and Significance

The use of more global approaches such as microarrays and proteomics might allow us to gain further insight into metabolic differences existing between the two strains and to improve our understanding of the effect of nutrient-gene interactions on the regulation of metabolism. By combining dietary treatment and genetic selection for muscle fat content, we obtained a relevant model to investigate mechanisms conditioning not only muscle fattening but also fat partitioning in different body compartments in fish. The identification of markers discriminating the two genotypes would be of critical interest to our understanding of the mechanisms that are responsible for differences of fat allocation in not only fish but also other animals, including humans.

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