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WIDE-TARGETED GENE EXPRESSION INFERS TISSUE-SPECIFIC MOLECULAR SIGNATURES OF LIPID METABOLISM IN FED AND FASTED FISH.

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Abstract

European sea bass (*Dicentrarchus labrax*) is one of the most important species for Mediterranean aquaculture. It is therefore crucial to establish appropriate feeding management regimens and to gain better knowledge of nutritional requirements for this species, exploring not only new feed ingredients and feeding strategies, but also understanding the molecular mechanisms that regulate the metabolism of nutrients. Accordingly, transcriptomic analysis represents a useful nutrigenomic discovery tool for identifying the molecular basis of biological responses to nutrition as well as nutritional biomarkers in fish. This study evaluated how the transcriptional activity of genes controlling lipid metabolism in European sea bass were modulated in a tissue-specific manner in response to fasting and refeeding. Such approach focused on a panel of 29 genes in which desaturases, elongases, triacylglycerol lipases, fatty acid-binding proteins, β-oxidation and oxidative phosphorylation enzymes, phospholipid-related enzymes, and transcription factors that regulate lipid homeostasis were represented. Fasting activated the lipolytic machinery in adipose tissue, liver and muscle of European sea bass, whereas markers of lipogenesis were downregulated in liver and adipose tissue. Genes involved in phospholipid and oxidative metabolism were differentially regulated in liver and skeletal muscle of fasted European sea bass. However, ten days of refeeding were sufficient, for the most part, to reverse the expression of key genes. Overall, our data clearly showed a tissue-specific regulation of lipid-related genes according to the different metabolic capabilities of each tissue, being the brain the most refractory organ to changes in nutrient and energy availability and liver the most responsive tissue.

Keywords: Aquaculture**,** transcriptome, lipid metabolism, fasting and refeeding, biomarkers.

Introduction

Marine fish are considered a healthy component of the human diet due to relatively high ratios of polyunsaturated to saturated fatty acids (PUFA:SAFA) in comparison to other animal food sources (Givens and Gibbs, 2006; Pérez-Sánchez et al., 2013). In particular, marine fish contain high concentrations of essential n-3 long-chain PUFA (LC-PUFA), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), whose beneficial effects upon cardiovascular health, inflammatory diseases and neurological disorders have been well established (Yates et al., 2014). However, intensive fish farming with the advent of new fish feed formulations have the potential to alter the lipid content and fatty acid (FA) profile of fish meat in both salmonid (Kiessling et al., 2005, Hixson et al., 2014) and non-salmonid fish (Kaushik et al., 2004; Benedito-Palos et al., 2008). Certainly, high levels of n-3 LC-PUFA are important nutrient factors in human foods, and even the culture of salmonids and freshwater fish, which do not have specific requirements in n-3 LC-PUFA, is facing increasing pressures to include EPA and DHA in their finishing diets. This reinforces the interest in predictive modeling of fish fillet FA composition. A recent multivariate-dummy regression model fits well for flat fish and typically Mediterranean farmed fish (Ballester-Lozano et al., 2014a; 2014b), helping to comply with human nutritional recommendations and policies for sustainable utilization of finite fishery resources (fish meal, FM; fish oil, FO) as ingredients for fish feeds. However, this is a complex trade-off and the use of vegetable oils can compromise the nutritive value of fish meat but also fish health, due to changes in tissue FA composition and re-allocation of tissue lipid depots as evident in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) (Montero et al., 2005; Izquierdo et al., 2005).

Of course, ration size affects in a large extent lipid metabolism, too, but it is noteworthy that its effect on FA composition is more evident on phospholipids (PL) rather than triacylglycerols (TAG) (Kiessling et al., 1991; Velázquez et al., 2006; Bonaldo et al., 2010; Suárez et., 2010; Benedito-Palos et al., 2013). PL metabolism is also highly influenced by environmental temperature and salinity (Los & Murata, 2004; Ibarz et al., 2005), whereas TAG are mostly influenced by dietary FA composition, which is indicative of the different role and regulation of PL and TAG (Tocher, 2003). The localization and composition of body lipid depots strongly affects fish nutritional value, organoleptic properties, transformation yields and storage time of fish carcass (Peres & Oliva-Teles, 1999). Therefore, disturbances of lipid metabolism represent a major problem in cultured fish, and correct management of lipid metabolism is a priority for aquaculture nutritionists and physiologists. In this regard, recent studies in gilthead sea bream have addressed at the molecular level the allostatic regulation of lipid metabolism in response to fasting or feed restriction (Benedito-Palos et al., 2013, 2014). Fasting imposes a vast array of adaptive adjustments that are, at least in part, fish species-specific (Wang et al., 2006). Hence, typically carnivorous fish, such as European sea bass, should be better adapted to caloric restriction than omnivorous and herbivorous fish since the former experiences short and long fasting periods under natural conditions (Bond 1996). Fasting-associated growth retardation is completely overcome, or at least reduced, if an abundant food supply becomes available after a prolonged period of food shortage. Then, fish display a rapid growth spurt known as compensatory growth (Terova et al., 2007; 2008). Compensatory growth is mainly supported by the rapid restoration of fish metabolic profile (Metón et al. 2003; Morales et al. 2004, Pérez-Jiménez et al., 2007, 2012), which depends on age, environmental conditions, duration and intensity of food-deprivation period, and nutritional background.

Accordingly, the aim of the present study was to evaluate how the transcriptional profile of a panel of 29 selected markers of lipid metabolism is modulated in a tissue-specific manner by fasting and refeeding in juveniles of European sea bass. Target markers included FA desaturases, FA elongases, phospholipid-related enzymes, acylglycerol lipases, enzymes of β-oxidation and oxidative phosphorylation, peroxisome proliferator-activated receptors, and transcription

factors that regulate lipid homeostasis. Sea bass is a high valuable fish for the European aquaculture, and improving the nutrient utilization throughout the production cycle of this species represents a major challenge in the agenda of the European Aquaculture Technological Platform (EATP). To achieve this, intense and concerted research needs to be undertaken on fish nutrition and physiology, to better understand the molecular and cellular mechanisms that regulate energy and nutrient partitioning under energy-deficit or -overflow conditions. Accordingly, our attention was focused on sea bass adipose tissue, liver, skeletal muscle, and brain -- since these tissues have different lipid deposition rates -- as well as oxidative and lipid biosynthetic capabilities.

Methods

Animals care, feeding and tissue sampling

Juveniles of European sea bass were obtained from the Nuova Azzurro® hatchery in Civitavecchia (Rome), and reared in 2.5 m^3 tanks in an indoor experimental facility of Department of Biotechnology and Life Science, University of Insubria (Varese). The tanks were connected to a water recirculation system where salinity was 20 g/l, temperature 23±1°C, pH 8.4, total ammonia <0.2 mg/l, and dissolved oxygen over 99% of saturation. At the beginning of the trial, 160 sea bass of 28.0 ± 1.5 g mean body mass were transferred to four tanks of 800 l. Fish were acclimatized for 10 days under natural photoperiod and fed to visual satiety with a commercial diet produced by Naturalleva (VRM S.r.l.), Italy. After the acclimation, fish of two tanks continued to be fed to visual satiety during all the experiment (CTRL group), whereas fish of the two other tanks were fasted for 15 days and then refed for 10 days (fasted group). Feed consumption was estimated from the difference between feed delivered and uneaten feed, collected from the bottom of the tank. Five fish from each replicate (10 per group) were sampled at the following time points: at the beginning of the trial (T0), at the end of fasting (T1), and at 10 days following refeeding (T2). Fish were sampled 15 min before the scheduled feeding time. They were rapidly anaesthetized with 3-aminobenzoic acid ethyl (MS222, 100 ppm) and body weight and standard body length were measured. For gene expression analysis liver, skeletal muscle, mesenteric adipose tissue and brain were dissected out, frozen immediately in liquid nitrogen and then stored at −80°C until the molecular analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from individual samples of liver, muscle, brain and mesenteric adipose tissue. The tissue lysis and homogenization was carried out in a closed system using gentle MACS Dissociator and single use gentle MACS M tubes (MiltenyiBiotec). The procedure of total RNA isolation was proceed by an automated purification process using the Maxwell® 16 Instrument and Maxwell® 16 Tissue LEV total RNA purification Kit (Promega, Italy). The quantity and purity of RNA was assessed spectrophotometrically by NanoDrop (Thermo Scientific) and the RNA integrity was checked by electrophoresis on 1% agarose gel stained with ethidium bromide. Reverse transcription of 1 µg total RNA was performed with random decamers in a volume of 100 µl using High-Capacity cDNA Archive Kit (Life Technologies, Italy) according to manufacturer's instructions.

Gene expression analysis

Quantitative PCR was performed using a CFX96 Connect™ Real-Time PCR Detection System (Bio-Rad). The real-time PCR protocol consisted of an initial denaturation step of 95 °C for 3 min, followed by 40 cycles of denaturation for 15 s at 95 °C and annealing/extension for 60 s at 60°C. The efficiency of PCR reactions was higher than 90% and negative controls without sample templates were routinely performed for each primer set. The 96-well PCR array layout was designed for the simultaneous profiling of a panel of 29 genes in a triplicate format. This set of genes (Table 1) included four FA elongases (*elovl1*, *elovl4*, *elovl5*, *elov6*), two FA desaturases (*fads2*, *scd1b*), four phospholipid synthesis and remodeling enzymes (*lpcat1*, *lpcat2*, *pemt*, *pla2g12b*), eight acylglycerol lipases (*lpl*, *lpl-like*, *hl*, *el*, *atgl*, *cel*, *hsl*, *lipa*), seven genes of FA β-oxidation and oxidative phosphorylation (*cpt1a*, *hadh*, *cs*, *nd5*, *sdhc*, *cyb*, *cox1*), one enzyme of cholesterol metabolism (*cyp7a1*) and three related-lipid transcription factors (*pparα*, *pparβ*, *pparγ*). The housekeeping gene (β-actin) and controls of general PCR performance were included on each array and all the pipetting operations were performed using the EpMotion 5070 Liquid Handling Robot (Eppendord). Briefly, RT reactions were diluted to convenient concentrations and the equivalent of 660 pg of total input RNA was used in a 25 µl volume for each PCR reaction. PCR-wells contained a 2 x SYBR Green Master Mix (Bi-Rad), and specific primers at a final concentration of 0.9 µM were used to obtain amplicons of 50-150 bp in length (Supplementary Table 1).

The specificity of PCR reactions was verified by analysis of melting curves (ramping rates of 0.5°C/10 s over a temperature range of 55-95°C, yielding a single peak for each sample and gene), linearity of serial dilutions of RT reactions, electrophoresis and sequencing of PCR amplified products. Fluorescence data acquired during the PCR extension phase were normalized by the $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001) using β-actin as housekeeping gene for the data normalization procedure. Fold-change calculation for each gene in a given tissue was expressed as a ratio between fasted and CTRL group or refed and CTRL group (values>1 indicate up-regulated genes, conversely values <1 indicate down-regulated genes). For multi-gene analysis comparisons, in each tissue all data were in reference to the expression level of PPARγ in CTRL group to which an arbitrary value of 1 was assigned.

Statistical analysis

Data were subjected to Levene's test for homogeneity of variances. Changes in gene expression for a given tissue and nutritional condition were analyzed by one-way analysis of variance followed by the Student–Newman–Keuls tests. The fasting and refeeding mediated effects on growth performance and gene expression were analyzed by Student t-test. The differences were considered statistically significant at a significance level of $P < 0.05$. All analyses were performed using the SPSS package version 20.0 (SPSS Inc., USA).

Results

Growth performance

Data on body weight are reported in Figure 1. CTRL fish grew normally during the trial with a feed conversion efficiency (FCE) (wet weight gain/dry feed intake) of 0.63 and 53% weight gain (final average body weight, 43.21 ± 1.41 g). After 10 fasting days (T1), weight loss of fasted fish was 13%. After 10 days of refeeding (T2), their body weight remained significantly lower than that of CTRL fish. However, weight gain at T2 with respect to T1 was 36% and their FCE was 1.07. In the same period, weight gain was 18% for CTRL fish.

Gene sequence analysis

Most nucleotide sequences (21 out of 29) used in this study were obtained from the IATS transcriptomic sea bass database (www.nutrigroup-iats.org/seabassdb). This allowed the unequivocal identification (E-value < 4e-37) of 21 new European sea bass sequences, which were uploaded to GenBank database with accession numbers KF857288-KF857308 (Table 2). The sequences include three FA elongases (*elovl1*, *elovl4*, *elovl6*), four phospholipid-related genes (*lpcat1*, *lpcat2*, *pemt*, *pla2g12b*), seven acylglycerol lipases (*lpl-like*, *hl*, *el*, *atgl*, *cel*, *hsl*, *lipa*), six FA catabolic genes (*cpt1a*, *hadh*, *cs, nd5, sdhc*, *cox1*) and one cholesterol-related gene (*cyp7a1*). Among them, nine nucleotide sequences (*elovl1*, *pemt*, *pla2g12b*, *cel*, *hadh*, *cs*, *nd5*, *sdhc*, *cyp7a1*) are complete coding sequences.

Functional gene expression analysis

The relative gene expression of all genes included in the array is reported for adipose tissue, liver, muscle, and brain in Supplementary Tables 2, 3, 4, and 5, respectively. Notably, *pla2g12b* and *cyp7a1* were mostly undetectable in skeletal muscle. *elovl4* and *elovl5* were mostly detected in brain, while *hl* was exclusively expressed in liver. The remaining genes were found at quantifiable levels in all analyzed tissues. Among them, markers of oxidative phosphorylation (OXPHOS) (*cox1* and *nd)* were constitutively expressed at the highest level.

Tissue-specific molecular signatures in well-nourished fish

Data on gene expression in CTRL fish sampled at T0, T1, and T2 were considered together in defining tissue-specific molecular signatures of lipid metabolism in well-nourished fish. Hence, regardless of OXPHOS markers, the highest gene expression level was registered in the mesenteric adipose tissue for vascular/intracellular lipases (*cel, lpl* > *hsl* > *lpl-like*) and Δ9 desaturase (*scd1b*) enzymes, followed by *cs*, *pparγ* and a lysosomal lipase (*lipa)* (Fig 2A). Likewise, the hepatic tissue displayed high expression levels of acylglycerol lipases with *cel* showing the highest relative expression followed in a decreasing pattern by *lpl, hl,* and *el*. In addition to this, the liver also displayed a high expression of *scd1b*, the phospholipase *pla2g12b*, and the cholesterol-related *cyp7a1* gene (Fig. 2B). The skeletal muscle showed an oxidative molecular signature with high expression levels of citrate synthase (*cs*) and of FA β-oxidation enzymes (*hadh*, *sdhc* > *cpt1a*) (Fig. 2C). Like muscle, the brain transcriptome showed high levels of oxidative markers (*cs* > *sdhc* > *cpt1a*), but FA elongases (*elovl1* > *elovl5*), desaturases (*scd1b*) and acyltransferase (*lpcat2*) were co-expressed at a relatively high level (Fig. 2D).

Transcriptionally mediated effects by fasting and refeeding

In adipose tissue, fasting triggered a statistically significant downregulation of Δ9 (*scd1b*) and Δ6 (*fads2*) desaturases, lipoprotein lipases (*lpl*, *lpl-like*, *el*), *pparγ*, and OXPHOS enzymes (*cox1* and *nd5*). In contrast, *elovl5*, *cpt1a* and *pparα* expression increased during fasting (Fig. 3A), with a recovery or slight rebound for *scd1b*, *lpl*, *lpl-like*, *hadh*, *nd5*, *cs,* and *sdhc* genes during the refeeding period (Fig. 3B). Overall, the magnitude of gene expression changes was highest in liver tissue, in which a consistent upregulation of lipoprotein lipases was found (Fig. 3C). Among these, *lpl-like* showed the highest-fold change increase at 18.88. The expression of *elovl6*, *pemt*, *plag12b*, *atgl*, *lipa*, *cpt1a*, *hadh*, *cs*, *pparα,* and *pparβ* increased in response to fasting, too. In contrast, the desaturase *scd1b* and acylglycerol lipase *cel* were strongly

downregulated (33.3- and 12.5-fold decrease, respectively). Expression of desaturase *fads2,* OXPHOS genes (*nd5*, *cyb*, *cox1*), *pparγ*, and *cyp7a1* decreased in fasted liver, too (Fig. 3C). Ten days of refeeding were sufficient to reverse most of these fasting-mediated effects with a slight, but statistically significant rebound effect of *hsl*, *cpt1a,* and *pparγ*, while *hadh* remained slightly downregulated (Fig. 3D).

The response of skeletal muscle to fasting was characterized by the up-regulation of lipoprotein lipases, with the exception of *el*, which was strongly down-regulated (6.3 fold decrease). *Fads2* was also markedly downregulated by fasting. In contrast, FA catabolic markers (*cpt1a*, *cs*, and *sdhc*) as well as *scd1b*, *lpcat2* and *pemt* were upregulated (Fig. 4A). A rebound effect of lipoprotein lipases (*lpl-like* and *el*) and oxidative enzymes (*cs* and *cyb)* was seen during refeeding (Fig. 4B). In brain, the analyzed genes remained almost unaltered by fasting and only few of them were slightly upregulated by fasting, with fold-changes varying between 1.15 and 1.49 (Fig. 4C).

Discussion

The present study clearly showed a tissue-specific regulation of a selected panel of lipid-related genes in European sea bass, reflecting the different role and metabolic capabilities of each tissue. Brain was highly refractory to changes in nutrient and energy availability, whereas liver was the most reactive tissue with changes in gene expression affecting not only the biosynthetic, but also the oxidative and lipolytic machinery as previously reported in gilthead sea bream (Benedito-Palos et al., 2014). Also microarray gene expression profiling of gilthead sea bream cardiac and skeletal muscle tissues highlighted a characteristic transcriptomic profile for each muscle tissue following changes in oxidative capacity (heart>red skeletal muscle>white skeletal muscle). The transcriptome of heart and secondly that of red skeletal muscle were highly responsive to nutritional changes, whereas that of glycolytic white skeletal muscle showed less ability to respond (Calduch-Giner et al., 2014). The different expression profile of red and white muscle fibers has also been evidenced by deep RNA-seq in rainbow trout (Palstra et al., 2013). Likewise, the transcriptome of human and rodents, which is usually clustered according to tissue function and development (Son et al., 2005; Zheng-Bradley et al., 2010), revealed that up to 90% of genes in the "Human Gene Expression Atlas" show tissue-specific molecular signatures (Lukk et al., 2010). However, how robust and tissue- and species-specific is a given gene expression pattern remains to be established from a functional and evolutionary point of view and the present study provide new insights on this field.

In marine fish, the inability to synthetize n-3 LC-PUFA from α-linolenic acid C18 FA is primarily due to a defect in Δ-5 desaturases activity (Tocher, 2010). In contrast, FADS2 enzymes with Δ-6 desaturase activity have been characterized in several marine fish species, such as gilthead sea bream (Seiliez et al., 2003), cobia (*Rachycenton canadum*) (Zheng et al., 2009), Atlantic cod (*Gadus morhua*) (Tocher et al., 2006), turbot (*Scophthalmus maximus*) (Zheng et al. 2004) black sea bream (*Acanthopagrus schlgelii*) (Kim et al., 2014) and European sea bass (Santigosa et al, 2011). The results of our study showed that in a normal feeding regimen European sea bass had relatively high levels of expression of *fads2* gene in almost all the analyzed tissues, with the highest levels in brain and liver. High brain *fads2* expression was also previously reported by other authors in this species (Santigosa et al., 2011) and in other species, such as Atlantic cod (Tocher et al., 2006) and cobia (Zheng et al., 2009). Benedito-Palos and colleagues (2014) found, instead, a high expression of *fads2* in liver of continuously fed gilthead sea bream and related this to diet composition. All the evidence indicates that FADS2 is a nonlimiting enzyme in the LC-PUFA biosynthetic pathway in European sea bass and in other marine species; however, measurements of enzyme activity are needed to confirm this assumption. Indeed, when FM was replaced completely with a plant meal-based diet, the upregulation of *fads2* expression was not associated with the induction of enzymatic activity in European sea bass (Geay et al., 2011). In the present study, the expression of *fads2*

was significantly downregulated by fasting in muscle and liver and slightly in adipose tissue. This might indicate a low FA bioconversion of 18:3n-3 to 18:4n-3 and 18:2n-6 to 18:3n-6 when the feed intake is reduced. Similarly, in gilthead sea bream, the expression of *fads2* decreased in liver of fasted fish, whereas in muscle and adipose tissue its expression seemed to be unaffected by starvation (Benito-Palos et al., 2014). However, after 10 days of refeeding, *fads2* expression levels in our study were comparable to those of the CTRL fish, confirming the great capacity of European sea bass to recover from fasting, which is typical of carnivorous fish.

Another key desaturase involved in *de novo* synthesis of monounsaturated FA (MUFA) is the stearoyl-CoA deasaturase 1b (SCD1B), also termed delta-9 desaturase. In this study, a relative high expression of *scd1b* isoform was observed in brain, liver and adipose tissue of CTRL fish. The expression in liver and adipose tissue was in accordance with the expression of *scd1* genes observed in the vertebrates analyzed so far (Castro et al., 2011), whereas the high *scd1b* expression in the brain was an unexpected result. However, in zebrafish (*Danio rerio) scd1b* was found to be expressed almost uniquely in brain, suggesting that a selective pressure is present to maintain a SCD "brain function" in fish species (Evans et al., 2008). Experimental evidence also indicates that the expression of *scd1b* was strongly downregulated by fasting in adipose tissue and liver of European sea bass, followed by a recovery of CTRL values during refeeding phase with even a rebound effect in the case of the adipose tissue. A strong fasting-mediated effect was also reported in gilthead sea bream not only in liver and adipose tissue, but also at a lower extent, in skeletal muscle (Benedito-Palos et al., 2014). Similarly, a decreased expression pattern was observed during starvation in liver of rodents, chickens and humans (Liang et al, 2002; Désert et al., 2008; Castro et al., 2011). All this evidence supports our hypothesis that *scd1* genes are strong markers of lipogenesis in a wide range of tissues and species, including mammals, birds and fish.

Elongase enzymes (ELOVLs) determine the rate of overall FA elongation and display differential substrate specificity, tissue distribution, and regulation, making them important regulators of cellular lipid composition and of specific cellular functions (Jakobsson et al., 2006). Fish ELOVL5 has been cloned and functionally characterized in several fish species, including freshwater (Agaba et al., 2004), salmonids (Morais et al., 2009) and marine fish (Gregory et al., 2010; Mohd-Yusof et al., 2010; Monroig et al., 2013; Morais et al., 2012), whereas a gene coding for ELOVL2 has, to date, only been found in zebrafish and Atlantic salmon (Monroig et al., 2009; Morais et al., 2009). The lack of ELOVL2 in marine fish is consistent with the inability of these species to perform the last elongation steps of the LC-PUFA biosynthetic pathway. Thus far, only the nucleotide sequence of *elovl5* was available in public databases for European sea bass. After exhaustive search in our transcriptomic database, we have now completed the ELOVLs dataset for this species by uploading *elovl1*, *4* and *6* (acc. number KF857295, KF857296, KF857297). From a functional point of view, it is noteworthy that the expression pattern of *elovl5* resembled that of genes involved in the LC-PUFA biosynthetic pathway of carnivorous marine fish, in which both gene expression and enzyme activity are low in liver but high in brain (Tocher et al., 2006). Furthermore, in our experimental model, both *elovl4* and *elovl5* were mostly detected in the brain, whereas *elovl1* and *elovl6* were ubiquitous. In any case, the hepatic ELOVL6 was the only FA elongase influenced at the transcriptional level by nutritional conditions, showing a pronounced upregulation in fasted fish and a restoration of CTRL values during refeeding. This contrasted with the general idea that starvation should cause a significant decrease in lipogenic enzymes, including elongases, in both liver and adipose tissue, whereas refeeding should promote an increase in their expression (Turyn et al., 2010).

The FA composition of first synthesized PLs is altered at the sn-2 position in the remodeling Land's cycle through the concerted action of phospholipases (PLA) and acyltransferases (LPCAT) (Lands, 1958). Other important regulatory steps of PL metabolism are the sequential methylations catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT). Here, we focused on transcriptional regulation of these enzymes, and, after search in our transcriptomic database,

pemt, *pla2g12b* and *lpcat1-2* isoforms were uploaded to GenBank as new European sea bass sequences (acc. number KF857298, KF857299, KF857301, KF857300). These genes were expressed at detectable levels in all the analyzed tissues, with the exception of *pla2g12b* that was barely detected in the skeletal muscle. Similarly to gilthead sea bream, none of these genes was transcriptionally regulated in adipose tissue in response to fasting (Benedito-Palos et al., 2013; 2014). As for the other tissues, we found an overall fasting-mediated upregulation that contrasted with the data obtained in gilthead sea bream, in which *pemt*, and *lpcat1-3* isoforms were downregulated in liver or remained unchanged in brain and muscle. *Pla2g12b* showed the same pattern of expression, being upregulated in liver and brain of fasted European sea bass. The upregulation of *pla2g12b* in liver and brain of fasted sea bass is in agreement with recent studies using *Pla2g12b* knockout mice and mutagenesis (Guan et al., 2011; Alijakna et al., 2012). These studies demonstrated a potential role of this gene in lipid mobilization since lack of PLA2G12B was associated with decreased serum lipids and increased liver fatty droplets in mouse.

Most of the important metabolic arrangements that occur during fasting also involve several vascular enzymes of the lipase superfamily. The current research is one of the few studies in fish in which an almost entire set of lipoprotein lipases (*lpl*, *lpl-like*, *hl*, *el*) was analyzed after being unequivocally annotated and uploaded to public databases as new European sea bass *lpl-like*, *hl* and *el* nucleotide sequences (acc. number KF857288, KF857289, KF857290). As expected, the expression of *hl* was restricted to liver, whereas two *lpl* isoforms were ubiquitously expressed with an enhanced relative gene expression in adipogenic tissues, particularly evident for *lpl*. Regardless of this, *lpl-like* was particularly sensitive to changes in nutrient supply in all the analyzed tissues, whereas in gilthead sea bream this pattern was mostly restricted to skeletal muscle in which the ratio *lpl/lpl-like* was lower than in the liver, adipose tissue, and brain (Benedito-Palos et al., 2014). In any case, very often the regulation trend of *lpl* in adipose tissue is the opposite of that in liver in order to drive the lipid flux from adipose tissue to liver and vice versa (Benedito-Palos et al., 2014). Here, this trend was observed for both *lpl* and *lpl-like*, and importantly, the fasting up-regulated expression of hepatic *lpl* and *lpl-like* occurred in parallel to that of *hl*. This can be viewed as an adaptive response to cover all the spectrum of circulating lipoproteins, improving then the liver FA uptake. However, it is noteworthy that this is not always the case in gilthead sea bream and other Sparid fish (Benedito-Palos et al., 2014; Oku et al., 2006). The ultimate physiological significance of this finding remains to be established, but probably this is a consequence of the differences in fish adiposity and allocation of body fat depots. Importantly, few data exist on the regulation of endothelial lipase (*el*) in fish (Morais et al., 2011; Betancor et al., 2014) and this is one of the first reports addressing this issue in this group of lower vertebrates. In this regard, it must be noted that the highest expression ratio of *el* and *lpl/lpl-like/hl* was found in skeletal muscle, in which *el* was more regulated by fasting. In humans, the role of sterol regulatory element binding proteins 1 and 2 (*srebp-1*, *-2*) on *el* activation (Kivelä et al., 2012) has been demonstrated. Both *srebp-1* and *srebp-2* are able to bind to *el* promoter, but they might act not only as inducers but also as repressors of the target gene transcription, since it cannot be determined whether they are regulating *el* expression cooperatively or at some level leading to a net inhibitory effect.

The list of enzymes that belongs to the lipolytic superfamily is increasing in the last years, but to date, few lipolytic genes are characterized at the molecular level in European sea bass. We uploaded to GenBank database four new European sea bass sequences unequivocally annotated as *atgl*, *hsl*, *lipa* and *cel* (acc. number KF857294, KF857293, KF857292, and KF857291). *Atgl* acts sequentially with *hsl* for the proper hydrolysis of tri- and diglycerides, respectively. Both in mammals (Kershaw et al., 2006) and gilthead sea bream (Benedito-Palos et al., 2014), the expression of *atgl,* termed also desnutrin, is significantly upregulated by fasting, following the increased expression of oxidative enzymes involved in the mitochondrial FA β-oxidation. This was further evidenced herein: the expression of *atgl*, *hadh* and *cpt1a* was significantly upregulated by fasting not only in liver, but also in muscle and brain. Conversely, in gilthead sea bream,

atgl and *hsl* were not co-regulated in liver, which might suggests that the time course and magnitude of response of these lipolytic enzymes is dependent on tissue, physiological condition and fish-species. This notion could be extended also to other lipolytic enzymes, such as *lipa*, which is upregulated in the liver of European sea bass in the present study, but it was downregulated by fasting in the adipose tissue of fasted gilthead sea bream. Likewise, *cel* was downregulated in the liver and adipose tissue of fasted gilthead sea bream, whereas herein this response was found only in the liver, which could be a sign of complete depletion of mesenteric fat. This hypothesis is supported by the study of Kittilson and colleagues (2011) in trout. Fasting trout for 6 weeks led to an increase in *hsl* expression in liver and red muscle, whereas its expression in mesenteric fat increased until the 4th week of fasting but then (at the 5th and 6th week) declined, coinciding with mesenteric fat tissue depletion.

Concurrent to changes in lipogenic and lipolytic enzymes, the regulation of genes linked to oxidative phosphorylation (OXPHOS) is highly informative of the metabolic condition and capabilities of a given tissue. Such approach is limited by the availability of nuclear and mitochondrial sequences encoding for OXPHOS genes with catalytic, regulatory or assembly roles. However, this situation is now changing and almost a complete set of enzyme subunits that belong to five enzyme complexes (Complex I-V) of the mitochondrial respiratory chain have recently been characterized at the DNA level in gilthead sea bream (Bermejo-Nogales et al., 2015). The authors of this study also showed that 72 out of 88 enzyme subunits were downregulated by fasting in the liver tissue, whereas an opposite trend of regulation of a lower magnitude and intensity, affecting 10-29 genes, was found in cardiac and skeletal muscle tissues, respectively. Similar results are reported in other mammalian experimental models (Da Costa et al., 2004; Suzuki et al., 2002). This might reflect the reduced energy demand of liver with the fasting-related inhibition of lipogenesis as well as the increased oxidative capacity of muscle tissues with the reduced nutrient supply for energy purposes. In our case, this notion was confirmed by the observation that *cs*, a good marker of the activity of tricarboxylic acid cycle, was mostly upregulated in skeletal muscle, whereas some markers of OXPHOS such as *nd5*, *sdhc*, *cyb*, and *cox1* were consistently downregulated in liver but not in the other analyzed tissues.

Cholesterol 7-alpha-monooxygenase (CYP7A1), also known as cholesterol 7 alpha-hydroxylase, catalyzes the first and rate-limiting step in the bile acid biosynthetic pathway in liver (Murashita et al., 2013). Bile acids are synthesized from the precursor cholesterol and are released into the gut upon ingestion of a meal. This means that bile acid synthesis has a key role in intestinal absorption of nutrients, which also represents the major pathway for cholesterol elimination. *Cyp7a1* is downregulated when plasma cholesterol levels are low and upregulated when cholesterol levels are high (Kalaany et al., 2006). Furthermore, bile acids are versatile molecules that activate nuclear receptors and cell-signaling pathways and play critical roles in regulating lipid, glucose, and energy metabolism (Lefebvre et al., 2009; Thomas et al., 2008). European sea bass showed a downregulation of *cyp7a1* in liver in response to fasting, in line with what was reported previously in trout (Murashita et al., 2013). This result pointed out a decrease in cholesterol synthesis in response to starvation, as already reported in higher vertebrates, too, after 24-48 h of fasting (Desert et al, 2008; Bauer et al., 2004).

Peroxisome proliferator-activated receptors (PPARs) are ligand-depended transcription factors, activated by binding FA or their oxidized derivatives and regulate expression of genes of lipid degradation and biosynthesis. PPARα activates lipid catabolism by regulating the expression of genes encoding enzymes involved in the peroxisomal and mitochondrial β-oxidation of FAs in response to changing energy requirements and nutritional status (Ji et al., 2011). In gilthead sea bream, like in other fish species and mammals, hepatic expression of *pparα* increased in response to fasting (Leaver et al., 2005). Recently, Betancor and coworkers (2014) showed a significant daily expression rhythmicity of *pparα* in Atlantic salmon, in which *pparα* reached a peak of expression at Zeitgeiber time (ZT) 14:00, i.e. under low feeding

condition just before scotophase (dark phase in a cycle of light and darkness, especially artificially induced). Our results in European sea bass fit well with previous reported data; indeed, *pparα* expression was strongly increased in liver in response to fasting and its pronounced upregulation was associated with an increasing expression of *hadh* and *ctp1a*. PPARβ, in mammals, seems to act as a regulator of fat burning (Wang et al., 2003), and like PPARα is involved in FA catabolism. This might explain why herein *pparβ* expression followed a pattern similar to that of *pparα* upon fasting and refeeding, increasing in the fasted state and decreasing to control values following refeeding. Lastly, PPARγ has instead, a central role in fat storage by promoting and maintaining the adipocyte phenotype (Desvergne et al., 2006). In accordance with this, *pparγ* was expressed at relatively high levels in adipogenic tissues of European sea bass, showing a downregulation during fasting. Conversely *pparγ* was upregulated in liver of European sea bass once refed, indicating an induction of fat deposition phase.

Conclusions

In summary, this study investigated how 29 genes selected as markers of lipid metabolism are co-regulated in fish during fasting and refeeding. We uploaded to GenBank database 21 new European sea bass nucleotide sequences related to lipid metabolism. Among them, nine nucleotide sequences are complete codifying sequences. Our findings clearly indicated tissue-specific molecular signatures that are regulated in a large extent by nutrient supply in liver and secondly in adipose tissue and skeletal muscle, whereas brain was the most refractory tissue to changes in gene expression. Depending on the feeding status, lipogenesis, lipolysis, lipoprotein metabolism, and mitochondrial β-oxidation were activated or inhibited in a tissue-specific manner. From our data, *scd1b* proved to be one of the most informative markers of lipogenesis in liver and adipose tissue, which is also inferred from changes in energy demand and the expression of OXPHOS enzymes. In parallel, *lpl* and *lpl-like* gene expression provided a clear and accurate indication of the lipid flux between adipose tissue and liver, which is confirmed by changes in *hl*. Conversely, up-regulation of *pla2g12b*, *atgl* and *pparα* could be a good indicator of the activation of the hepatic lipolytic machinery. In muscle, however, *lpl*, *lpl-like* and *el* together with *cpt1a* and *sdhc* are the best markers for monitoring nutritional status of European sea bass. Accordingly, the results presented here provide valuable, novel, and interesting information on fish lipid metabolism, which could be successfully applied by the aquaculture industry to monitor the metabolic status of farmed fish in order to optimize feeding protocols and new diet formulations.

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Table 1 List of European sea bass genes analyzed by real-time PCR

Physiological process	Gene	Symbol	Acc. N°
LC-PUFA metabolism	Elongation of very long chain fatty acids 1	*elovl1*	KF857295
	Elongation of very long chain fatty acids 4	*elovl4*	KF857296
	Elongation of very long chain fatty acids 5	*elovl5*	FR717358
	Elongation of very long chain fatty acids 6	*elovl6*	KF857297
	Fatty acid desaturase 2	*fads2*	EU647692
	Stearoyl-CoA desaturase 1b	*scd1b*	FN868643
Phospholipid metabolism	Lysophosphatidylcholine acyltransferase 1	*lpcat1*	KF857298
	Lysophosphatidylcholine acyltransferase 2	*lpcat2*	KF857299
	Phosphatidylethanolamine N-methyltransferase	*pemt*	KF857300
	Group XIIB secretory phospholipase A2	*pla2g12b*	KF857301
Lipoprotein and Triacylglycerol metabolism	Lipoprotein lipase	*lpl*	AM411614
	Lipoprotein lipase-like	*lpl-like*	KF857288
	Hepatic lipase	*hl*	KF857289
	Endothelial lipase	*el*	KF857290
	Adipose triglyceride lipase	*atgl*	KF857294
	Carboxyl ester lipase	*cel*	KF857291
	Hormone sensitive lipase	*hsl*	KF857293
	Lysosomal acid lipase	*lipa*	KF857292
Fatty acid β-oxidation and Oxidative phosphorylation	Carnitine palmitoyltransferase 1A	*cpt1a*	KF857302
	Hydroxyacyl-CoA dehydrogenase	*hadh*	KF857303
	Citrate synthase	*cs*	KF857304
	NADH dehydrogenase subunit 5	*nd5*	KF857307
	Succinate dehydrogenase cytochrome b560 subunit	*sdhc*	KF857305
	Cytochrome b	*cyb*	EF427553
	Cytochrome c oxidase subunit I	*cox1*	KF857308
Cholesterol metabolism	Cholesterol 7-alpha-monooxygenase	*cyp7a1*	KF857306
Transcriptional regulation	Peroxisome proliferator-activated receptor α	*pparα*	AY590300
	Peroxisome proliferator-activated receptor β	*pparβ*	AY590302
	Peroxisome proliferator-activated receptor γ	*pparγ*	AY590303
Housekeeping	β-Actin	*actb*	AY148350

Table 2 Characteristics of new assembled sequences according to BLAST searches

GenBank	Contig(s)	F*	size (nt)	Annotation†	Best match‡	E§	CDS
KF857295	L12_83314	149	1674	ELOVL1	XP_003974086	0.0	160-1143
KF857296	L2_46617	1	733	ELOVL4	ADG59898	1e-42	<1->237
KF857297	L2_31165	1	541	ELOVL6	XP_005794848	2e-96	<1->541
KF857298	L2_65576	1	900	LPCAT1	XP_005745523	8e-160	<1->900
KF857299	L12_81695	35	1543	LPCAT2	XP_003969581	0.0	<1-1283
KF857300	L12_89146	70	2823	PEMT	AFP97555	1e-139	168-881
KF857301	L12_73756	32	1169	PLA2G12B	XP_003448024	4e-111	90-728
KF857288	L2_31709	1	222	LPL-like	BAB20997	4e-37	<1->222
KF857289	L3_72288	3	968	HL	ACI32419	0.0	<1->968
KF857290	L12_80726	31	1476	EL	XP_005798464	0.0	<1-1109
KF857294	L3_77722	1	593	ATGL	XP_003967745	7e-90	87->593
KF857291	L12_84525	174	1790	CEL	XP_003978424	0.0	25-1692
KF857293	L12_73755	18	1167	HSL	AGU42438	0.0	32->1167
KF857292	L12_84249	50	1601	LIPA	AFV39805	0.0	<1-1119
KF857302	L12_89028	47	2751	CPT1A	ADH04490	0.0	<1-1304
KF857303	L3_73675	10	1165	HADH	XP_003972444	0.0	49-978
KF857304	L12_89830	145	2272	CS	AEH27542	0.0	104-1513
KF857307	L12_90240	587	2354	ND5	YP_003795709	0.0	79-1920
KF857305	L12_44135	21	705	SDHC	XP_004555215	2e-91	33-542
KF857308	L3_83395	89	1527	COX1	YP_003795701	0.0	<1->1527
KF857306	L1_88647	120	2587	CYP7A1	XP_004575762	0.0	205-1746

*Number of sequences.

†Gene identity determined through BLAST searches. ELOVL1, elongation of very long chain fatty acids protein 1; ELOVL4, elongation of very long chain fatty acids protein 4; ELOVL6, elongation of very long chain fatty acids protein 6; LPCAT1, lysophosphatidylcholine acyltransferase 1; LPCAT2, lysophosphatidylcholine acyltransferase 2; PEMT, phosphatidylethanolamine N-methyltransferase; PLA2G12B, group XIIB secretory phospholipase A2; LPL-like, lipoprotein lipase-like; HL, hepatic lipase; EL, endothelial lipase, ATGL, adipose triglyceride lipase; CEL, carboxyl ester lipase; HSL, hormone sensitive lipase; LIPA, lysosomal acid lipase; CPTIA, carnitine palmitoyltransferase 1A; HADH, hydroxyacyl-CoA dehydrogenase; CS, Citrate synthase; ND5, NADH dehydrogenase subunit 5; SDHC, succinate dehydrogenase cytochrome b560 subunit; COX1, cytochrome c oxidase subunit 1; CYP7A1,Cholesterol 7-alpha-monooxygenase.

‡ Best BLAST-X protein sequence match. § Expectation value.

Figure captions

Fig. 1 Mean body weight of European sea bass (fasted and CTRL group) prior to fasting (T0), at the end of fasting (T1), and at the end of refeeding (T2). All value are expressed as mean ± SEM (Standard Error of the Mean). (*) indicates significant differences (*T*-test, $P < 0.05$) between two groups at each sampling point

Fig. 2 Expression levels for the top genes with highest expression in different tissues (A, mesenteric adipose tissue; B, liver; C, white muscle; D, brain) of European sea bass control group. We have used different bar pattern to discriminate for genes biological function. Data represents the mean of 30 fish + SEM (Standard Error of the Mean). β-actin was used as housekeeping gene. The relative expression of mitochondrial genes *cox1* and *nd5* was omitted since the magnitude of their expression was too high in comparison to the genes presented. Different superscript letters indicate significant differences of expression between genes (Student-Newman-Keuls test, $P < 0.05$)

Fig. 3 Graphical representation of fold-changes of differentially expressed genes (fasted vs control and refeeding vs control) for mesenteric adipose tissue (A, B) and liver (C, D) of European sea bass

Fig. 4 Graphical representation of fold-changes of differentially expressed genes (fasted vs control and refeeding vs control) for muscle (A, B) and brain (C) of European sea bass

Figure 1

Click here to download Figure Fig1.tif

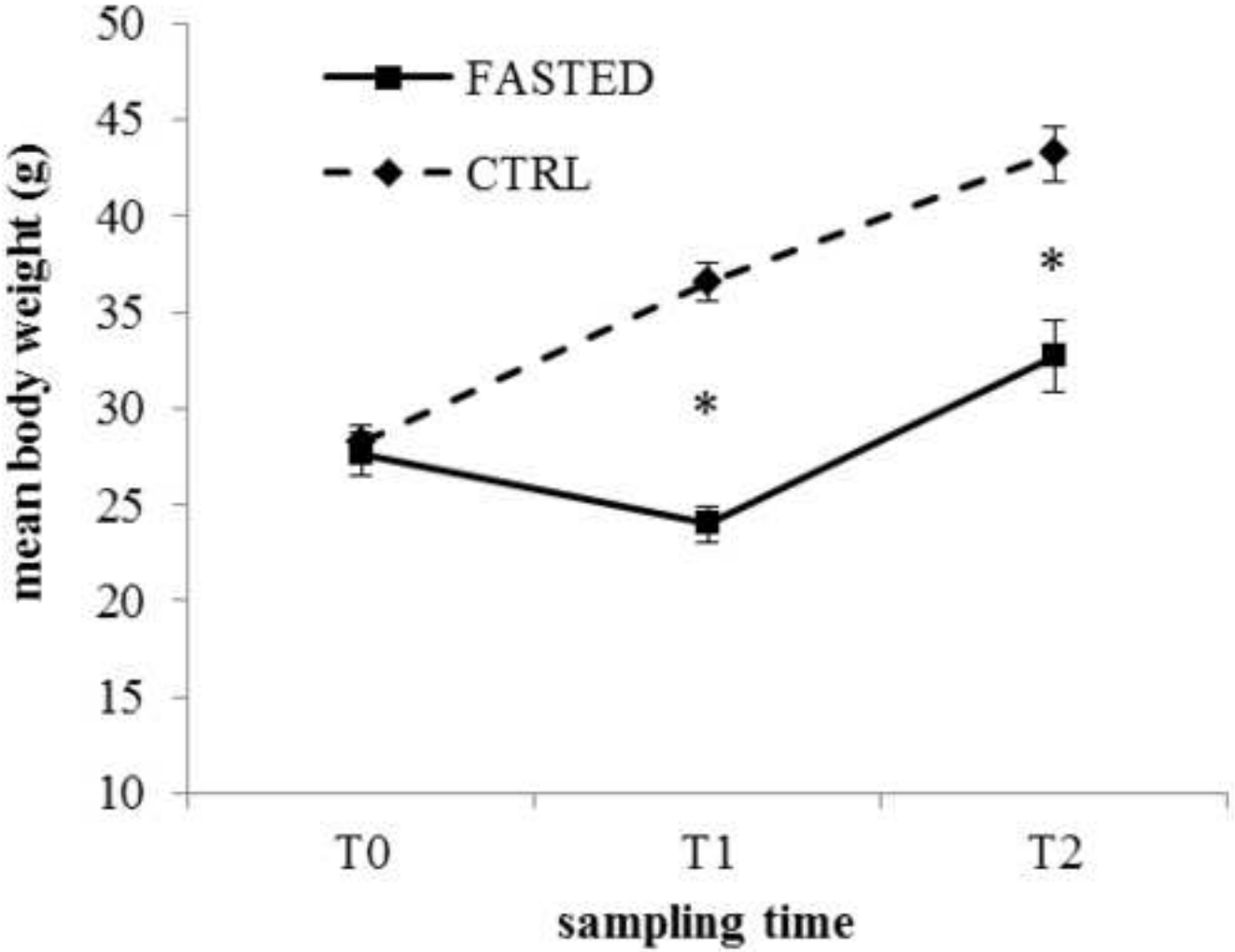

Figure 2A

Click here to download Figure Fig2A.tif

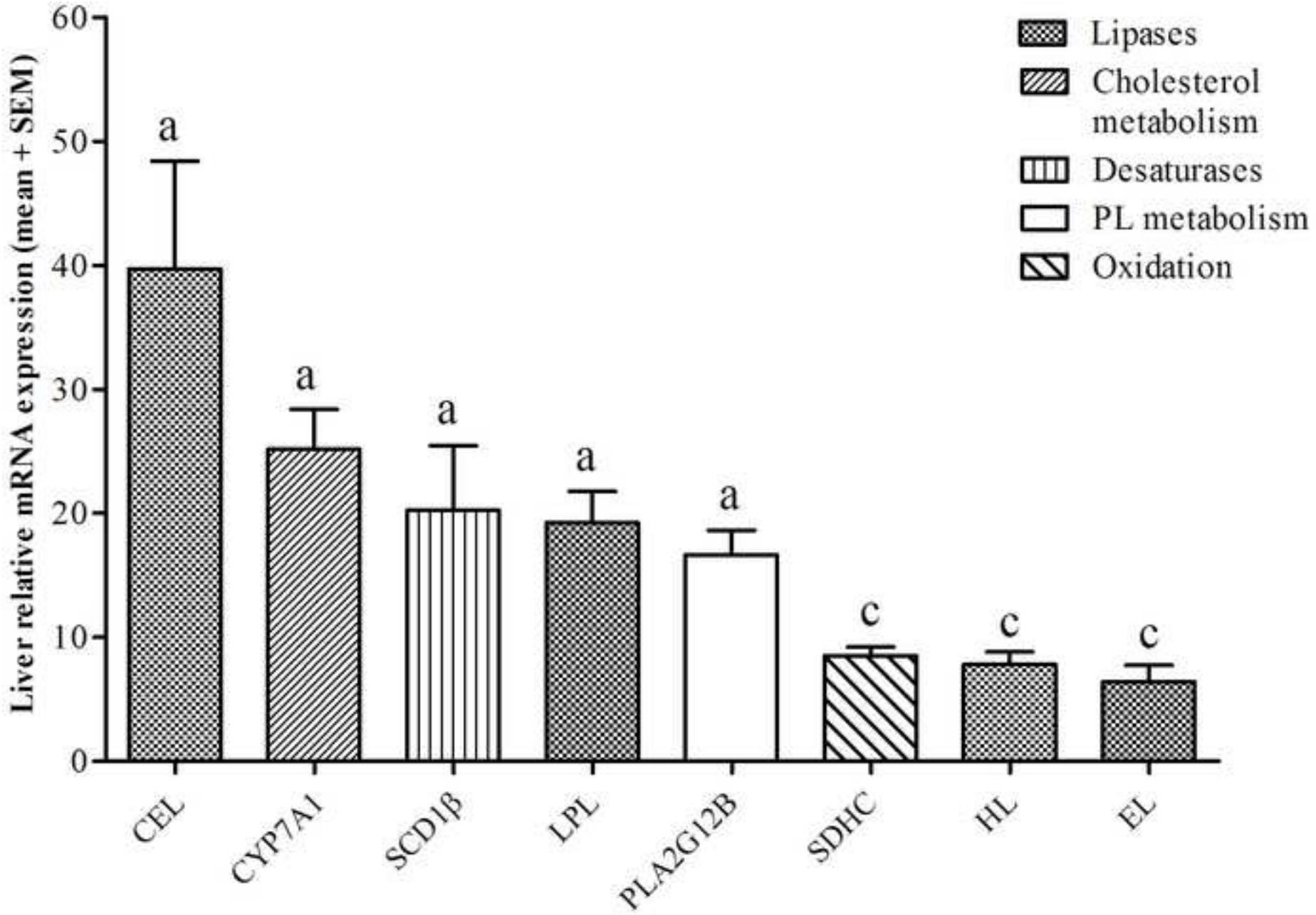

Figure 2B

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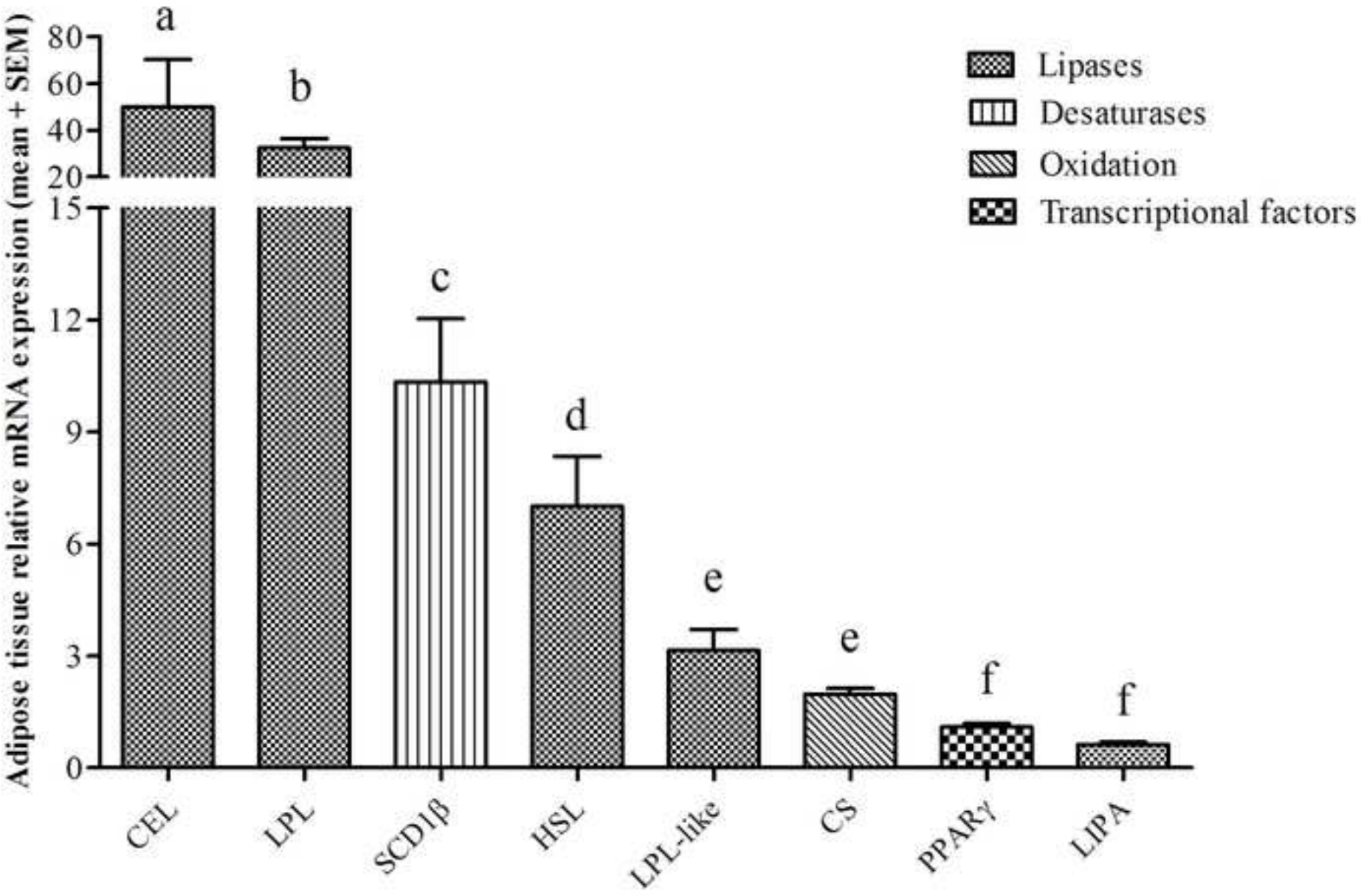

Figure 2C

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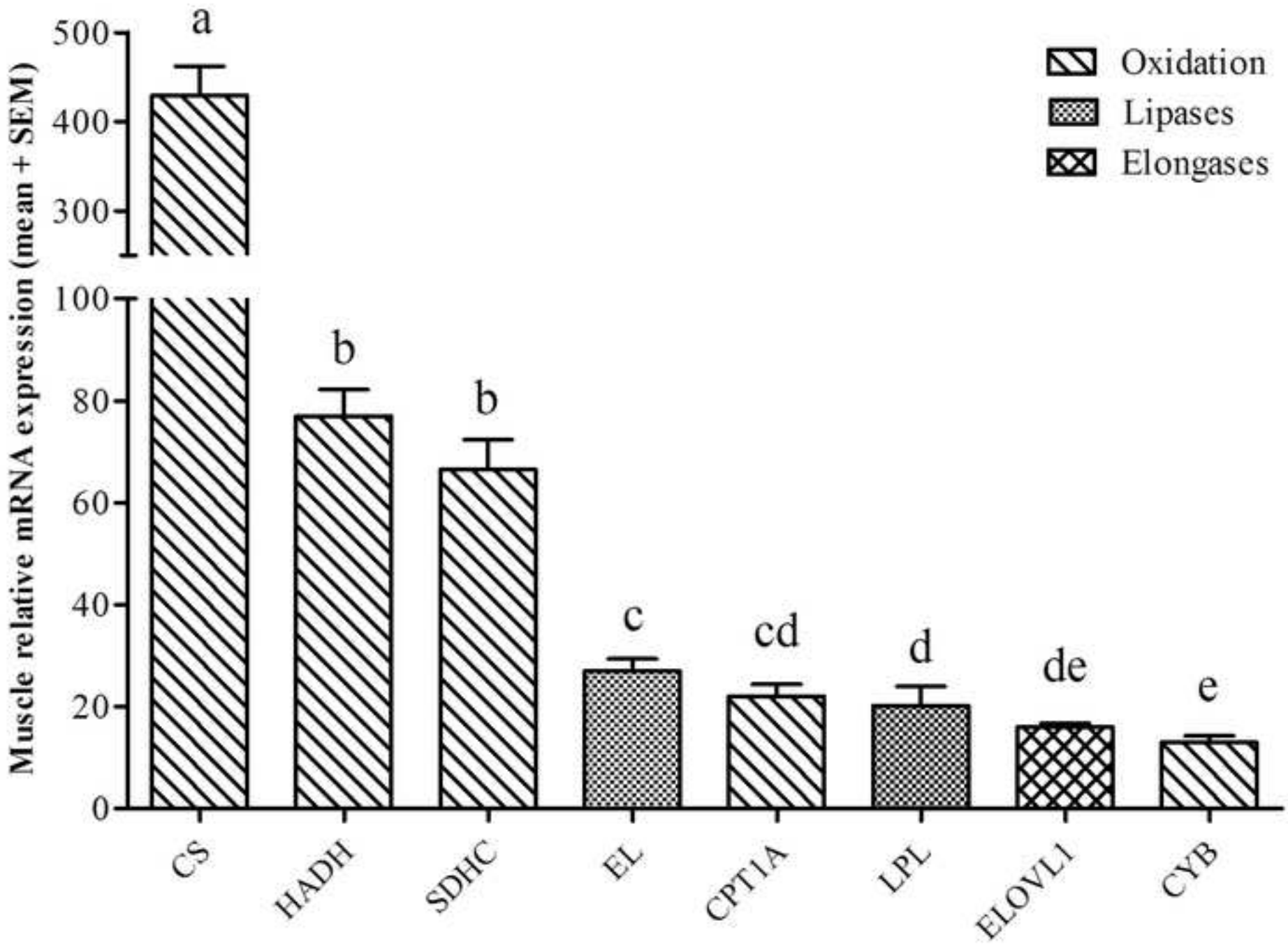

Figure 2D

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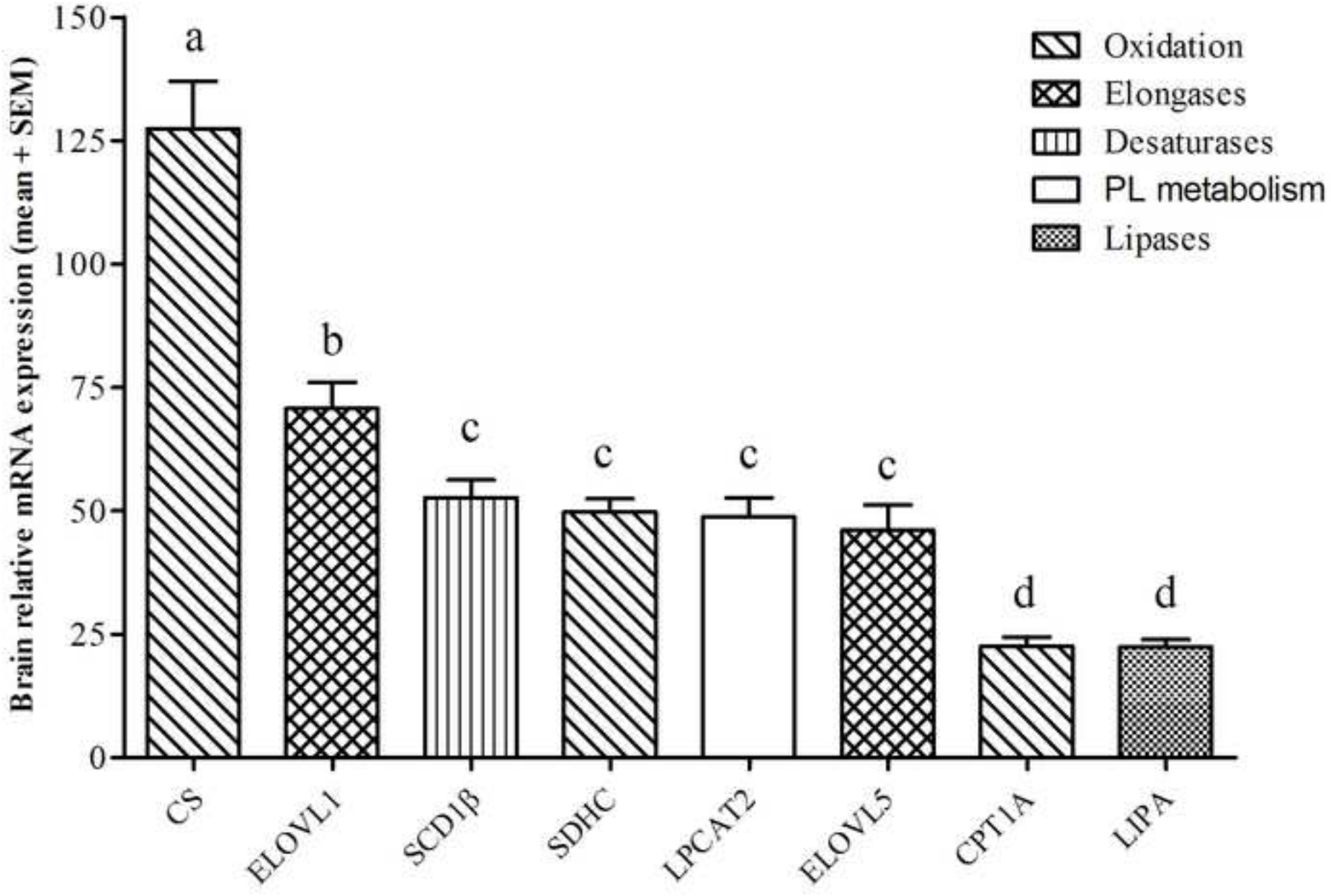


Figure 3

Click here to download Figure Figure 3 mean SEM.tif

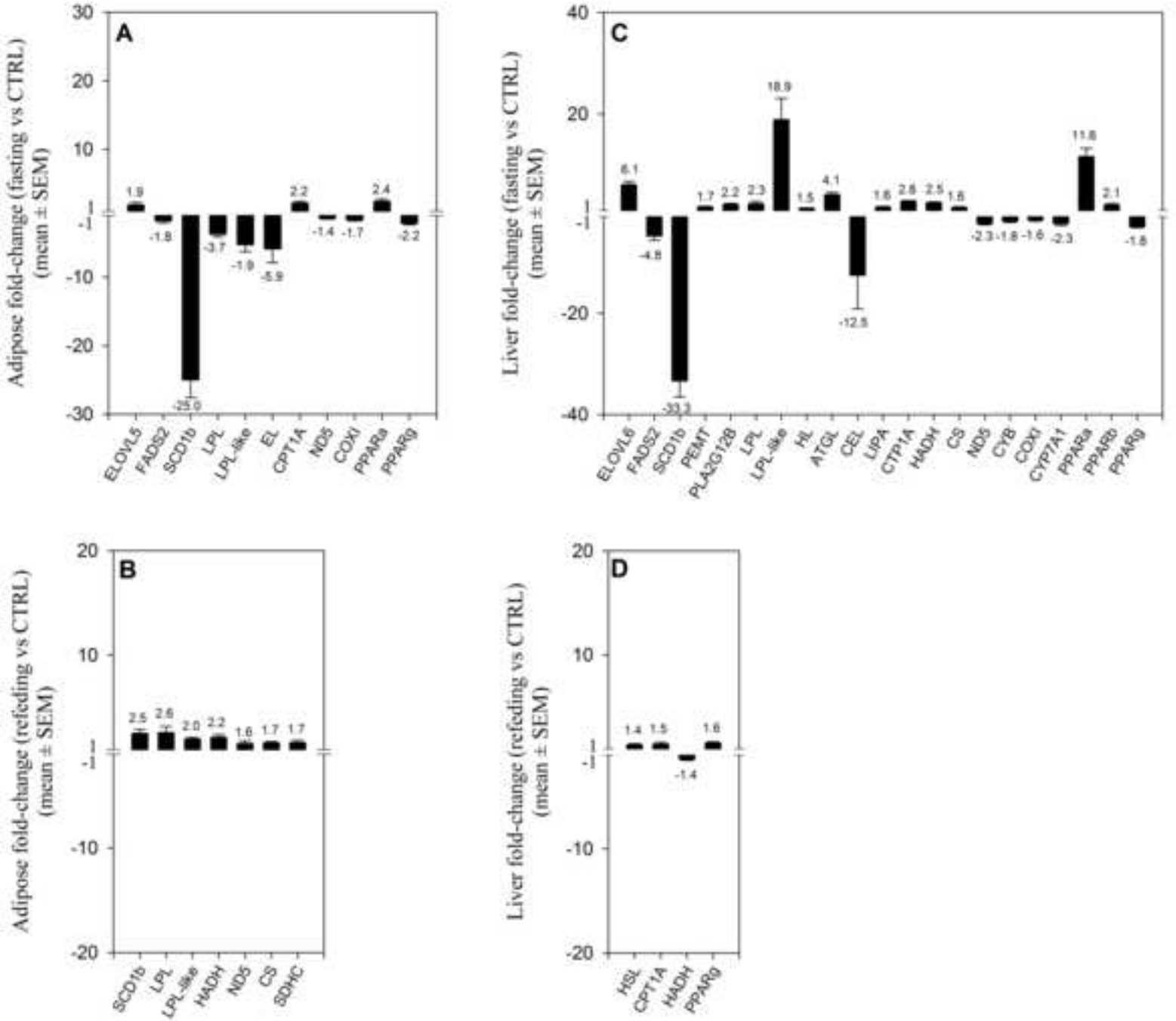
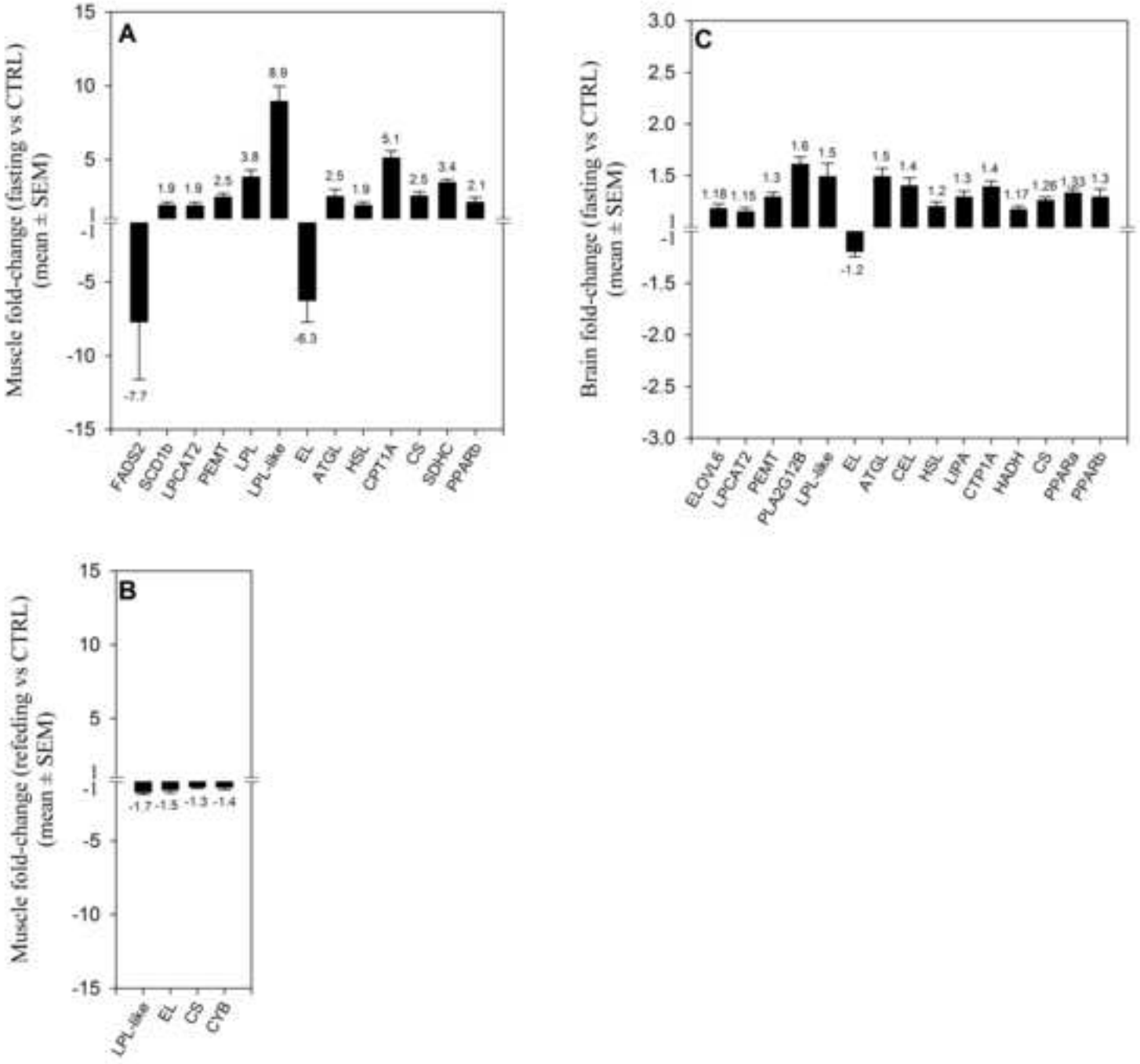


Figure 4

Click here to download Figure Figure 4 mean SEM.tif



Supplementary material

Supplementary Table 1. Forward and reverse primers for quantitative real-time PCR

Gene	Symbol	Acc. N°	Primer sequence
Elongation of very long chain fatty acids 1	*elovl1*	KF857295	F TAC ACA TCT TCC ACC ACT CCT TCA T R CCA TTC CAC CAG GAG CAT AGG
Elongation of very long chain fatty acids 4	*elovl4*	KF857296	F ACC ATG CTT ACC GAC GCA AAC CTT R CGA CGT GCT TGC CTC CCT TCT G
Elongation of very long chain fatty acids 5	*elovl5*	FR717358	F CAG TCA TGT ACC TTC TGA TCG TGT GGA TGG R GGA GTA CGG CTG CCT GTG TTT CAT
Elongation of very long chain fatty acids 6	*elovl6*	KF857297	F ACA TCA CCG TGC TGC TCT ACT CCT G R CCG CCA CCT GGT CCT TGT AGC A
Fatty acid desaturase 2	*fads2*	EU647692	F CCG CCG TGA CTG GGT GGA T R GCA CAG GTA GCG AAG GTA GTA AGA CAT AGA
Stearoyl-CoA desaturase 1b	*scd1b*	FN868643	F GCT TGT GGC ATA CTT CAT CCC TGG ACT C R GGT GGC GTT GAG CAT CAC GGT GTA
Lysophosphatidylcholine acyltransferase 1	*lpcat1*	KF857298	F CAG GGA GAC AGG CTG GGT TTG G R GTC TGT AAC TGG CAG GTT GAG GAA TTG G
Lysophosphatidylcholine acyltransferase 2	*lpcat2*	KF857299	F ACA GTG TCT TGG ACT TGG CAG GGT TTC R CAC AGA GTC AGA AGC AGC AGC GTC TT
Phosphatidylethanolamine N-methyltransferase	*pemt*	KF857300	F CGC AGC CAC AGT ATG ACG ATA GC R TCC TGA CCA TCA CCT CCC ACT T
Group XIIB secretory phospholipase A2	*pla2g12b*	KF857301	F TTG AGT CAG TTG GCG GCT ACT T R CGG TAT TGG CAC ACT CCA TCA C
Lipoprotein lipase	*lpl*	AM411614	F CAA TGT GAT CGT GGT GGA CTG R CGT CGG GTA GTG CTG GTT
Lipoprotein lipase-like	*lpl-like*	KF857288	F TAA ATG GGA GGA GAC AAA CGG TTG GT R GAA CTT CCA TAT TAG CGT TGT CGG TGT CA
Hepatic lipase	*hl*	KF857289	F CGC AGT GGC ACC AGC AAG A R CGG CAT CCG AGA CCG TGT T
Endothelial lipase	*el*	KF857290	F GGA CCA TCG GCA GAA TCA CT R CGA CGC CCT CAA ACA TTG G
Adipose triglyceride lipase	*atgl*	KF857294	F GGA GCC CTC ACT GCC ACT R ATT CGC ACC AGT CTC TCC AAG A
Carboxyl ester lipase	*cel*	KF857291	F CCG CAC CTA CTC CTA CCT CTT CTC T R TGC CAA TGC CGC CCA TAC G
Hormone sensitive lipase	*hsl*	KF857293	F GCC CTG TCT CCA GAC TAT TGC TAT C R GCT GCT ACA CCT ATT CCT GAC TGA T
Lysosomal acid lipase	*lipa*	KF857292	F CGT AGC GAC CGT AGC GTT CAC R GCA GGA CGG ACA GCT TGG T

Supplementary Table 1 (continued)

Gene	Symbol	Acc. N°	Primer sequence
Carnitine palmitoyltransferase 1A	*cpt1a*	KF857302	F TGC CAA GAG GTC ATC CAG AGT TCT R AGT CCA CAT CAT CCG CCA GAG A
Hydroxyacyl-CoA dehydrogenase	*hadh*	KF857303	F TGA TGG GTG GTC TGC AAT GGA T R CTT CTT GTT CAA CAG TTC GCT CGG
Citrate synthase	*cs*	KF857304	F GTG TAT GAG ACC TCC GTG TTG G R AGC AAC TTC TGA CAC TCT GGA ATG
NADH dehydrogenase subunit 5	*nd5*	KF857307	F CCC GAT TTC TGT GCC CTA CTA R AGG AAA GGA GTG CCT GTG A
Succinate dehydrogenase cytochrome b560 subunit	*sdhc*	KF857305	F ACA TGG GCA AGG GCT TCA AA R CGA TGA TGG ACA GAC CGA TAA CG
Cytochrome b	*cyb*	EF427553	F TGC CTA CGC TAT CCT TCG CTC GAT CC R TAA CGC CAA CAC CCC GCC CAA T
Cytochrome c oxidase subunit 1	*cox1*	KF857308	F ATA CTT CAC ATC CGC AAC CAT AA R AAG CCT CCG ACT GTA AAT AAG AAA
Cholesterol 7-alpha-monooxygenase	*cyp7a1*	KF857306	F TGC CAT CAA AGT CCC ACC TCT T R CAC ATC ATA GGT AGG CTG GAG GAT TC
Peroxisome proliferator-activated receptor α	*pparα*	AY590300	F CGT GCC TCT AGT GGA ACA GC R AGC AGG TGG AGC CGT AGT
Peroxisome proliferator-activated receptor β	*pparβ*	AY590302	F GCC CTG TTT GTT GCT GCC ATT ATT CTC T R TCC TGA CTC TGC TCC ACC TGC TTA
Peroxisome proliferator-activated receptor γ	*pparγ*	AY590303	F CAG GAC ACG CAC AAC TCA ATC A R GGA GAA CAC GGG ACA GTC AGA A
β-Actin	*actb*	AY148350	F TCC TGC GGA ATC CAC GAG A R AAC GTC GCA CTT CAT GAT GCT

Long chain fatty acid (LC-PUFA) metabolism: *elovl1*, *elovl4*, *elovl5*, *elovl6*, *fads2*, *scd1b*; Phospholipid metabolism: *lpcat1*, *lpcat2*, *pemt*; Lipoprotein and triacylglycerol metabolism: *lpl*, *lpl-like*, *hl*, *el*, *atgl*, *cel*, *hsl*, *lipa*; Fatty acid β-oxidation and OXPHOS: *cpt1a*, *hadh*, *cs*, *nd5*, *sdhc*, *cyb*, *cox1*; Cholesterol metabolism: *cyp7a1*; Transcription factors: *pparα*, *pparβ*, *pparγ*.

Supplementary Table 2. Molecular profiling of a panel of lipid-metabolic genes in adipose tissue

	T0		Fasting period				Refeeding period			
	CTRL		CTRL		Fasted		CTRL		Fasted	
Gene	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
elovl1	1.19	0.30	0.45	0.08	0.62	0.06	0.59	0.10	0.67	0.11
elovl4	-	-	-	-	-	-	-	-	-	-
elovl5	0.04	0.01	0.02	0.00	0.04*	0.01	0.02	0.00	0.04	0.01
elovl6	0.25	0.04	0.15	0.02	0.26	0.07	0.22	0.09	0.23	0.05
fads2	0.04	0.01	0.04	0.01	0.02*	0.00	0.05	0.02	0.07	0.01
scd1b	9.92	3.01	9.80	2.12	0.43*	0.05	10.89	2.74	27.63*	4.18
lpcat1	0.22	0.02	0.08	0.02	0.09	0.02	0.08	0.01	0.12	0.02
lpcat2	1.30	0.11	0.66	0.05	0.66	0.06	0.77	0.08	0.99	0.13
pemt	0.08	0.01	0.05	0.01	0.07	0.01	0.05	0.01	0.09	0.02
pla2g12b	0.05	0.01	0.06	0.03	0.07	0.03	0.44	0.41	0.15	0.08
lpl	25.85	5.87	33.89	6.36	9.11*	1.01	30.99	5.43	81.32*	17.84
lpl-like	1.59	0.30	3.24	0.95	0.62*	0.11	3.04	0.68	6.09*	0.54
hl	0.03	0.01	0.02	0.02	0.04	0.01	0.02	0.01	0.05	0.03
el	0.15	0.03	0.31	0.08	0.05*	0.01	0.30	0.12	0.76	0.26
atgl	2.06	0.38	0.96	0.25	1.00	0.13	1.11	0.30	1.41	0.39
cel	21.32	7.65	84.91	37.74	20.81	9.35	14.98	5.45	40.74	15.38
hsl	15.69	3.26	6.67	2.00	4.67	0.43	7.34	1.91	7.73	1.60
lipa	0.98	0.17	0.63	0.14	0.88	0.20	0.62	0.03	1.19	0.36
cpt1a	0.44	0.10	0.16	0.03	0.36*	0.04	0.29	0.11	0.27	0.06
hadh	0.85	0.09	0.65	0.11	0.45	0.04	0.58	0.05	1.26*	0.15
cs	2.35	0.20	1.76	0.13	1.54	0.11	2.18	0.28	3.45*	0.27
nd5	132.08	18.33	51.51	6.42	35.76*	1.90	86.27	12.99	142.03*	20.84
sdhc	1.09	0.10	0.71	0.08	0.55	0.04	0.93	0.07	1.55*	0.20
cyb	0.31	0.06	0.16	0.04	0.08	0.01	0.23	0.05	0.32	0.07
cox1	1484.67	675.22	264.71	35.68	158.08*	11.01	609.35	200.40	597.73	133.38
cyp7a1	0.06	0.04	0.11	0.09	0.05	0.02	0.05	0.02	0.37	0.31
pparα	0.05	0.01	0.03	0.01	0.08*	0.01	0.04	0.01	0.04	0.01
pparβ	0.31	0.08	0.20	0.03	0.24	0.03	0.20	0.05	0.25	0.03
pparγ	1.28	0.29	1.03	0.09	0.47*	0.04	1.16	0.17	1.49	0.14

ELOVL1, elongation of very long chain fatty acids protein 1; ELOVL4, elongation of very long chain fatty acids protein 4; ELOVL6, elongation of very long chain fatty acids protein 6; LPCAT1, lysophosphatidylcholine acyltransferase 1; LPCAT2, lysophosphatidylcholine acyltransferase 2; PEMT, phosphatidylethanolamine N-methyltransferase; PLA2G12B, group XIIB secretory phospholipase A2; LPL-like, lipoprotein lipase-like; HL, hepatic lipase; EL, endothelial lipase, ATGL, adipose triglyceride lipase; CEL, carboxyl ester lipase; HSL, hormone sensitive lipase; LIPA, lysosomal acid lipase; CPTIA, carnitine palmitoyltransferase 1A; HADH, hydroxyacyl-CoA dehydrogenase; CS, Citrate synthase; ND5, NADH dehydrogenase subunit 5; SDHC, succinate dehydrogenase cytochrome b560 subunit; COX1, cytochrome c oxidase subunit 1; CYP7A1, cholesterol 7-alpha-monooxygenase.

Mean values and standard error mean (SEM) of each fish group are presented (n = 10). β-actin was used as housekeeping gene and expression value of *pparγ* in control group was used as reference gene. Within fasting and refeeding period, means between groups with an asterisk are different ($P < 0.05$).

Supplementary Table 3. Molecular profiling of a panel of lipid-metabolic genes in liver

	T0		Fasting period				Refeeding period			
	CTRL		CTRL		Fasted		CTRL		Fasted	
Gene	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
elovl1	0.86	0.03	0.96	0.15	1.21	0.19	1.10	0.17	1.40	0.13
elovl4	-	-	-	-	-	-	-	-	-	-
elovl5	-	-	-	-	-	-	-	-	-	-
elovl6	4.80	0.54	5.20	0.96	31.54*	3.39	5.89	1.01	7.41	1.54
fads2	1.75	0.75	5.56	0.78	1.19*	0.19	5.71	1.24	6.98	2.45
scd1b	2.84	0.72	20.49	7.71	0.62*	0.06	19.99	7.74	27.90	7.43
lpcat1	0.08	0.01	0.11	0.02	0.12	0.01	0.13	0.02	0.16	0.02
lpcat2	0.82	0.08	0.88	0.09	0.97	0.10	1.19	0.18	1.33	0.15
pemt	1.58	0.39	1.67	0.13	2.75*	0.27	2.29	0.26	2.07	0.26
pla2g12b	7.16	0.75	14.38	2.34	31.53*	2.80	18.90	3.11	21.21	4.67
lpl	19.01	5.10	17.08	3.78	39.54*	8.08	21.39	3.48	19.81	2.54
lpl-like	0.35	0.21	0.15	0.04	2.87*	0.64	0.45	0.16	0.25	0.04
hl	7.19	0.53	5.89	0.91	8.54*	0.43	9.67	1.57	9.39	1.00
el	1.81	0.59	4.56	1.01	3.91	1.00	8.23	2.32	5.10	1.30
atgl	2.38	0.32	1.15	0.35	4.69*	0.51	2.41	0.54	2.69	0.64
cel	5.60	2.22	53.91	14.31	4.11*	0.94	25.45	7.02	24.40	4.64
hsl	2.35	0.41	1.87	0.48	3.00	0.25	2.33	0.22	3.16*	0.26
lipa	3.30	0.34	5.22	0.43	8.58*	0.61	5.28	0.46	5.40	0.73
cpt1a	0.74	0.10	0.63	0.14	1.79*	0.17	0.71	0.09	1.04*	0.11
hadh	3.66	0.26	3.38	0.24	8.49*	0.52	5.25	0.30	3.79*	0.28
cs	3.73	0.33	3.76	0.30	5.88*	0.52	5.94	0.86	6.92	0.84
nd5	261.47	28.02	274.67	12.50	118.73*	9.51	459.73	75.23	568.71	63.50
sdhc	6.80	0.47	7.62	0.32	8.43	0.84	9.40	1.29	7.08	0.79
cyb	1.35	0.16	1.43	0.10	0.79*	0.10	1.94	0.23	2.17	0.18
cox1	1360.09	121.78	1238.92	105.96	779.71*	87.13	1928.81	364.91	2346.52	97.26
cyp7a1	18.12	2.97	25.94	3.64	11.11*	1.49	24.35	5.69	23.22	2.33
pparα	0.10	0.01	0.19	0.05	2.24*	0.31	0.14	0.04	0.19	0.03
pparβ	3.09	0.46	2.18	0.44	4.54*	0.62	5.37	0.74	4.57	0.71
pparγ	1.01	0.07	1.02	0.09	0.35*	0.02	1.05	0.14	1.62*	0.13

ELOVL1, elongation of very long chain fatty acids protein 1; ELOVL4, elongation of very long chain fatty acids protein 4; ELOVL6, elongation of very long chain fatty acids protein 6; LPCAT1, lysophosphatidylcholine acyltransferase 1; LPCAT2, lysophosphatidylcholine acyltransferase 2; PEMT, phosphatidylethanolamine N-methyltransferase; PLA2G12B, group XIIB secretory phospholipase A2; LPL-like, lipoprotein lipase-like; HL, hepatic lipase; EL, endothelial lipase, ATGL, adipose triglyceride lipase; CEL, carboxyl ester lipase; HSL, hormone sensitive lipase; LIPA, lysosomal acid lipase; CPTIA, carnitine palmitoyltransferase 1A; HADH, hydroxyacyl-CoA dehydrogenase; CS, Citrate synthase; ND5, NADH dehydrogenase subunit 5; SDHC, succinate dehydrogenase cytochrome b560 subunit; COX1, cytochrome c oxidase subunit 1; CYP7A1, cholesterol 7-alpha-monooxygenase.

Mean values and standard error mean (SEM) of each fish group are presented (n = 10). β-actin was used as housekeeping gene and expression value of *pparγ* in control group was used as reference gene. Within fasting and refeeding period, means between groups with an asterisk are different ($P < 0.05$).

Supplementary Table 4. Molecular profiling of a panel of lipid-metabolic genes in muscle

	T0		Fasting period				Refeeding period			
	CTRL		CTRL		Fasted		CTRL		Fasted	
Gene	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
elovl1	14.25	1.29	16.03	1.34	15.90	2.32	15.80	1.14	14.57	0.75
elovl4	-	-	-	-	-	-	-	-	-	-
elovl5	-	-	-	-	-	-	-	-	-	-
elovl6	1.10	0.37	0.94	0.11	1.20	0.17	0.92	0.09	1.02	0.22
fads2	0.19	0.03	0.74	0.08	0.09*	0.02	0.62	0.15	1.04	0.17
scd1b	4.52	0.21	6.07	0.54	11.39*	1.43	7.29	0.97	6.85	0.50
lpcat1	0.75	0.10	2.93	2.33	1.04	0.35	0.77	0.08	0.61	0.07
lpcat2	8.57	0.49	9.31	0.71	17.33*	2.27	9.30	0.57	10.41	0.94
pemt	0.94	0.08	0.86	0.12	2.10*	0.22	0.99	0.12	0.90	0.11
pla2g12b	-	-	-	-	-	-	-	-	-	-
lpl	19.31	2.19	14.21	3.68	54.09*	6.73	26.07	6.32	17.94	3.65
lpl-like	3.60	0.40	4.57	0.56	40.82*	4.69	5.70	0.99	3.38*	0.33
hl	-	-	-	-	-	-	-	-	-	-
el	11.67	1.10	24.98	4.10	3.91*	0.74	28.95	2.71	18.76*	2.24
atgl	1.90	0.27	1.08	0.26	2.70*	0.51	2.13	0.36	1.34	0.25
cel	1.43	0.91	3.76	3.47	0.84	0.54	0.11	0.06	0.36	0.16
hsl	4.16	0.56	4.25	0.38	8.05*	1.03	5.02	0.24	4.21	0.33
lipa	8.92	1.24	10.19	1.04	10.30	1.09	11.39	1.18	11.61	0.87
cpt1a	33.13	3.74	16.20	2.54	82.60*	7.80	27.83	3.06	20.23	4.02
hadh	73.96	8.48	67.89	7.73	63.39	8.30	86.13	5.69	87.91	9.26
cs	388.32	23.91	338.70	22.94	853.99*	99.98	520.16	44.17	389.27*	35.46
nd5	2530.19	198.14	2995.62	318.35	2667.63	344.41	4197.55	403.18	3359.81	285.77
sdhc	71.96	4.45	53.45	5.71	183.18*	12.02	79.51	8.34	92.43	9.64
cyb	11.06	1.13	10.53	2.03	12.05	3.56	15.34	1.14	11.27*	1.19
cox1	12816.11	1309.45	16617.71	1841.99	14317.72	2467.22	18767.29	745.72	15754.94	1615.58
cyp7a1	-	-	-	-	-	-	-	-	-	-
pparα	2.85	0.37	2.26	0.38	3.63	0.55	3.50	0.41	2.88	0.43
pparβ	2.63	0.24	2.21	0.26	4.65*	0.71	3.56	0.38	3.11	0.27
pparγ	1.04	0.13	1.22	0.30	0.72	0.06	1.06	0.14	0.81	0.08

ELOVL1, elongation of very long chain fatty acids protein 1; ELOVL4, elongation of very long chain fatty acids protein 4; ELOVL6, elongation of very long chain fatty acids protein 6; LPCAT1, lysophosphatidylcholine acyltransferase 1; LPCAT2, lysophosphatidylcholine acyltransferase 2; PEMT, phosphatidylethanolamine N-methyltransferase; PLA2G12B, group XIIB secretory phospholipase A2; LPL-like, lipoprotein lipase-like; HL, hepatic lipase; EL, endothelial lipase, ATGL, adipose triglyceride lipase; CEL, carboxyl ester lipase; HSL, hormone sensitive lipase; LIPA, lysosomal acid lipase; CPTIA, carnitine palmitoyltransferase 1A; HADH, hydroxyacyl-CoA dehydrogenase; CS, Citrate synthase; ND5, NADH dehydrogenase subunit 5; SDHC, succinate dehydrogenase cytochrome b560 subunit; COX1, cytochrome c oxidase subunit 1; CYP7A1, cholesterol 7-alpha-monooxygenase.

Mean values and standard error mean (SEM) of each fish group are presented (n = 10). β-actin was used as housekeeping gene and expression value of *pparγ* in control group was used as reference gene. Within fasting and refeeding period, means between groups with an asterisk are different ($P < 0.05$).

Supplementary Table 5. Molecular profiling of a panel of lipid-metabolic genes in brain

	T0		Fasting period				Refeeding period			
	CTRL		CTRL		Fasted		CTRL		Fasted	
Gene	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
elovl1	63.97	3.99	68.66	9.25	74.63	5.80	72.83	5.94	78.82	3.46
elovl4	3.61	0.16	3.69	0.09	4.08	0.27	4.27	0.48	4.07	0.40
elovl5	51.16	3.85	33.44	2.35	40.67	3.43	58.70	6.95	59.11	4.30
elovl6	9.37	0.37	6.86	0.23	8.12*	0.32	10.58	1.56	10.67	0.91
fads2	17.44	0.44	11.94	1.02	13.41	0.50	19.93	2.95	19.03	1.77
scd1b	44.74	2.84	44.27	2.66	43.28	1.07	61.03	4.49	62.39	4.01
lpcat1	2.31	0.22	1.54	0.16	1.86	0.10	2.27	0.26	2.46	0.39
lpcat2	43.59	1.79	42.21	2.03	48.42*	1.65	55.21	6.83	56.05	7.61
pemt	4.57	0.31	3.80	0.23	4.88*	0.18	4.18	0.39	4.50	0.29
pla2g12b	0.26	0.06	0.23	0.04	0.37*	0.02	0.26	0.04	0.29	0.01
lpl	5.19	0.80	6.84	1.41	8.03	0.79	5.90	1.09	3.70	0.29
lpl-like	0.90	0.10	1.03	0.15	1.54*	0.13	1.70	0.35	0.97	0.20
hl	-	-	-	-	-	-	-	-	-	-
el	7.90	0.47	6.61	0.30	5.54*	0.27	7.76	0.70	8.15	0.43
atgl	1.98	0.12	2.08	0.16	3.10*	0.17	2.53	0.26	2.28	0.16
cel	0.81	0.08	0.32	0.03	0.45*	0.03	0.56	0.12	0.46	0.10
hsl	20.91	1.62	20.36	0.93	24.39*	0.90	24.05	5.56	23.23	2.14
lipa	24.60	2.25	19.13	1.02	24.71*	1.10	25.67	2.18	31.09	2.69
cpt1a	25.06	1.75	19.22	1.49	26.81*	1.10	25.74	3.22	27.30	0.90
hadh	18.81	2.04	22.18	1.04	25.93*	0.81	20.21	0.73	20.75	0.71
cs	166.70	13.37	102.55	3.89	128.98*	3.49	152.21	12.22	161.71	6.63
nd5	2768.52	163.57	2677.27	183.72	3181.18	229.18	3306.83	279.21	3636.73	177.37
sdhc	56.50	4.73	44.27	2.49	48.73	1.09	55.19	3.72	57.57	2.05
cyb	5.90	0.49	15.39	1.15	17.15	1.62	7.01	0.69	7.06	0.48
cox1	29141.04	4664.21	17160.18	1617.50	17892.35	962.41	27065.76	7706.25	24986.83	8245.65
cyp7a1	0.25	0.06	0.64	0.21	0.43	0.07	0.43	0.19	0.26	0.09
pparα	3.67	1.01	5.65	0.26	7.54*	0.24	5.55	0.59	4.36	0.58
pparβ	5.55	1.06	5.33	0.27	6.88*	0.43	6.43	0.56	5.90	0.70
pparγ	1.20	0.24	1.01	0.06	1.21	0.08	1.06	0.14	1.21	0.10

ELOVL1, elongation of very long chain fatty acids protein 1; ELOVL4, elongation of very long chain fatty acids protein 4; ELOVL6, elongation of very long chain fatty acids protein 6; LPCAT1, lysophosphatidylcholine acyltransferase 1; LPCAT2, lysophosphatidylcholine acyltransferase 2; PEMT, phosphatidylethanolamine N-methyltransferase; PLA2G12B, group XIIB secretory phospholipase A2; LPL-like, lipoprotein lipase-like; HL, hepatic lipase; EL, endothelial lipase, ATGL, adipose triglyceride lipase; CEL, carboxyl ester lipase; HSL, hormone sensitive lipase; LIPA, lysosomal acid lipase; CPTIA, carnitine palmitoyltransferase 1A; HADH, hydroxyacyl-CoA dehydrogenase; CS, Citrate synthase; ND5, NADH dehydrogenase subunit 5; SDHC, succinate dehydrogenase cytochrome b560 subunit; COX1, cytochrome c oxidase subunit 1; CYP7A1, cholesterol 7-alpha-monooxygenase.

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