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Identification of Hypoxia-Regulated Genes in the Liver of Common Sole (*Solea solea*) Fed Different Dietary Lipid Contents

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Abstract:

Coastal systems could be affected by hypoxic events brought about by global change. These areas are essential nursery habitats for several fish species including the common sole (*Solea solea* L.). Tolerance of fish to hypoxia depends on species and also on their physiological condition and nutritional status. Indeed, high dietary lipid content has been recently shown to negatively impact the resistance of sole to a severe hypoxic challenge. In order to study the molecular mechanisms involved in the early response to hypoxic stress, the present work examined the hepatic transcriptome in common sole fed diets with low and high lipid content, exposed to severe hypoxia. The activity of AMP-activated protein kinase (AMPK) was also investigated through the quantification of threonine-172 phosphorylation in the alpha subunit. The results show that hypoxia consistently regulates several actors involved in energy metabolism pathways and particularly AMPKα, as well as some involved in cell growth and maintenance or unfolded protein response. Our findings reveal that (1) the expression of genes involved in biological processes with high energy cost or implicated in aerobic ATP synthesis was down-regulated by hypoxia, contrary to genes involved in neoglucogenesis or in angiogenesis, (2) the consumption of high lipid induced regulation of metabolic pathways going against this energy saving, and (3) this control was fine-tuned by the regulation of several transcriptomic factors. These results provide insight into the biological processes involved in the hepatic response to hypoxic stress and underline the negative impact of high lipid consumption on the tolerance of common sole to hypoxia.

Keywords: Fish ; Hypoxia ; Nutrition ; Liver ; Transcriptome ; AMPK

Introduction

Over the past 50 years, the intensification of anthropogenic activities along riversides and coastlines, combined with global warming, has been connected with the increasing duration and severity of environmental hypoxia in many coastal marine ecosystems. Consequently, hypoxia is now considered as one of the most pressing environmental issues worldwide (Wu 2002). Shallow coastal ecosystems are nursery areas for many benthic fish species and it is believed that hypoxic events can potentially impact them.

A large number of studies have been conducted to examine the physiological consequences and regulatory mechanisms that respond following a reduction in oxygen availability (reviewed by Richards et al. 2009). Although the physiological response to hypoxia varies among fish species (Mandic et al. 2009), it is accepted that they follow a general strategy aimed at inhibiting oxygen- and ATP-demanding metabolic pathways, while favouring the activation of oxygen-independent ATP-production pathways (reviewed by Almeida-Val et al. 2006; Bickler and Buck 2007; López-Barneo et al. 2010; Richards 2011). All these studies showed the common feature that this transition requires adequate stores of glucose, generally in the form glycogen, such as that found in the liver (Richards 2011).

The liver plays a central role in synthesizing or converting molecules that are utilized elsewhere to maintain homeostasis, and in regulating energy balance. Since the regulations of enzyme activity required for metabolic adaptation to hypoxia have been shown to be related to the differential transcription of mRNA (Semenza et al. 1996), a large scale investigation of gene expression would improve understanding of the overall impact of hypoxia on fish physiology. Previous studies performed on hepatic tissue have revealed impacts of hypoxia exposure on the expression of genes involved in energy metabolism (i.e., glucose metabolism), cell growth and proliferation, protein degradation and oxygenase activities (Gracey et al. 2001; Ju et al. 2007; Leveelahti et al. 2011; Everett et al. 2012). As in mammals, Hypoxia Inducible Factors (HIFs) are thought to be involved as transcription factors in the coordination of molecular responses in fish (see review by Nikinmaa and Rees 2005), especially in the liver (Gracey et al. 2001). AMPK, a nutrient and energy sensor that maintains energy homeostasis, is also suggested to play a central role in coordinating the metabolic responses of fish exposed to severe hypoxia (Jibb and Richards 2008). However, numerous key actors and cellular transduction pathways involved in the transcriptional control of metabolism during hypoxia are still unknown.

In the present study, we applied microarray-based gene expression profiling to investigate the short term transcriptional response of metabolic reprogramming during an acute, severe hypoxia challenge in juvenile common sole (*Solea solea*). Moreover, phosphorylation of threonine 172 in the alpha subunit, which is a key determinant of AMPK activity (Hardie 2008), was assessed on the same fish. Common sole is a flatfish of the Soleidae family, which is particularly exposed to hypoxia events during juvenile stages because estuaries have been identified as essential nurseries for this species (Le Pape et al. 2003). Common sole can tolerate quite severe drops in ambient oxygenation and does not show a strong avoidance response to hypoxic estuarine environments (Cannas et al. 2007). Previous studies aiming to better characterise the physiological response of common sole to hypoxia revealed a typical metabolic depression (Dalla Via et al. 1994; 1997). Moreover, recent studies have suggested that the dietary lipid content as a factor influencing hypoxia tolerance of common sole (McKenzie et al. 2008; Zambonino-Infante et al. 2013). In particular, Zambonino-Infante et al. (2013) showed that juvenile sole exhibited a lower tolerance to hypoxia when fed a lipid-rich diet. Even though this effect can probably be related to the known low nutritional tolerance of sole species to high lipid ingestion, which induces perturbation of their energy metabolism, the molecular actors and biological processes

involved in these regulations are poorly documented. To improve our understanding of these processes, we investigated the hepatic transcriptome in juvenile sole fed diets with low and high lipid contents.

2. Material and methods

2.1. Feeding trial, hypoxia challenge stress and sampling

Experiments were conducted in strict compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council 2010). One thousand North Sea sole (*Solea solea*) eggs were purchased from a commercial hatchery (SOLEA BV, Ijmuiden, Netherlands) and brought to the Ifremer larval rearing facility in Brest. Details on larval rearing equipment and conditions can be found in Zambonino-Infante et al. (2013). Eight months post hatching, 160 individuals were randomly drawn from the rearing tanks, tagged subcutaneously (passive integrated transponder: "PIT-tag") and distributed among eight 67-L tanks. For two months, these fish were maintained at 16°C and fed with commercial diets containing either 11% lipids (BP Finition Label Extrudé, Le Gouessant, France) (l-group) or 20% lipids (Neo Grower Extra Marin, Le Gouessant, France) (L-group) with four tanks per dietary group.

Prior to the hypoxia challenge test, 74 individuals selected at random (37 from each dietary group) were moved into a single tank (1 m^3) and left undisturbed and unfed for 48 h. The blood and liver of 36 of these individuals (18 from each dietary group) were sampled in normoxic conditions for biochemical and molecular analysis, respectively.

The hypoxia challenge was applied to the remaining 38 fishes (19 from each dietary group) and consisted in decreasing water oxygenation from 100% air saturation to 10% air saturation within one hour, followed by a slower descent to 1% air sat. over the last 30 minutes. Ambient oxygenation was controlled by bubbling nitrogen at the intake of a submersible pump placed in the tank. After 90 minutes of hypoxia, fish were removed, identified (PIT-tag reading) and their blood and liver sampled. The resulting four final experimental groups were named as follows: Normoxia l-group (Nl), Normoxia L-group (NL), Hypoxia l-group (Hl), Hypoxia L-group (HL). The experimental design was therefore a 2-way full factorial design between two juvenile dietary lipid contents and two oxygenation conditions. Data related to blood (biochemical) parameters measured in these groups were recently published (Zambonino-Infante et al. 2013).

2.2. RNA extraction and purification

Liver tissue from 74 individuals was homogenised in 2 ml Extract All Reagent (Eurobio, Courtaboeuf, France). Total RNA was isolated following the manufacturer's instructions and quantified by measuring absorbance at 260 nm in a spectrophotometer (NanoDrop, Labtech, France). RNA integrity was also evaluated using a Bioanalyzer 2100 (Agilent, Santa Clara, USA) analysis. Following the evaluation of RNA integrity, 71 samples (with RIN> 8) were kept for microarray hybridization: 18 from group Nl, 18 from group NL, 16 from group Hl and 19 from group HL.

2.3. Quantification of Threonine 172 phosphorylation of AMPKα

Total protein extracts were obtained according to Corporeau and Auffret (2003) from the same 74 liver samples. Briefly, to solubilize proteins, powdered tissues were homogenized in

a lysis buffer containing phosphatase and protease inhibitors (150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% Igepal, 5 ml phosphatase inhibitor cocktail II, 2 tablets of cOmplete EDTA-free protease inhibitor cocktail; pH 8.8 at 4°C). Total protein extracts were then quantified using a DC protein assay (Biorad), and protein concentrations were determined quantitatively using 96-well micro-plates (Nunc) and a micro-plate reader connected to KC4 v3 software (Bio-Tek Instruments, Inc).

Prior to quantification of Threonine 172 phosphorylation of AMPKα, each protein lysate was adjusted to a final concentration of 3.5 mg/ml by adding lysis buffer. Thr172 phosphorylation of AMPKα was detected by the PathScan® Phospho-AMPKα (Thr172) Sandwich ELISA Kit following manufacturer's instructions (Cell Signaling Technology, ref #7959). Western blot analysis from protein lysates of sole tissues was previously performed using Rat Anti-AMPKα (23A3) (Rabbit mAb, #2603, Cell Signaling) to ensure that the ELISA assay reported correctly threonine 172 phosphorylation of AMPK in sole. A specific band was detected at approximately 60 kDa confirming high AMPKα amino-acid sequence identity (>90%) between sole and rat species (Additional file 1).

2.4. Microarray design

Gene expression profiling of *S. solea* samples was conducted using an updated version of the Agilent-036353 *S. solea* DNA microarray (GPL16124).

Microarray design was carried out basis of 25,252 contigs (isotigs) and 66,026 singletons (with a minimal length of 200 nt) obtained from the Roche 454 sequencing of a normalized cDNA library of sole larval stages (from 1 to 33 days post hatching) and adult tissues (Ferraresso et al., submitted data). All assembled isotigs are stored in the public database Transcriptome Shotgun Assembly Sequence Database (TSA, http://www.ncbi.nlm.nih.gov/genbank/tsa) under accession number GAAQ00000000 *(submitted)*, while singletons can be directly retrieved from the NCBI Sequence Read Archive (SRA) under accession number SRA058691.

Transcript annotation for both isotigs and singletons was implemented through blastx searches (cut off e-value of < 1.0 E-5) against high quality draft proteomes of *Danio rerio*, *Gasterosteus aculeatus*, *Oryzias latipes*, *Takifugu rubripes*, *Tetraodon nigroviridis* and *Homo sapiens*, available on the Ensembl Genome Browser (release 56), and against the SWISSPROT database. A schematic representation of the approach used for microarray design is given in Additional File 2. Briefly, all annotated isotigs were screened for redundancy using Ensembl Protein IDs. Two or more transcripts were considered "redundant" when they showed the same annotation as at least 3 fish species out of 5, by considering Ensembl Protein ID of five fish species (*D. rerio*, *G. aculeatus*, *O. latipes*, *T. nigroviridis*, *T. rubripes*) and only the longest was considered for microarray design. The same approach was followed for filtering out redundant singletons. A total of 8,750 isotigs and 5,800 singletons were finally represented in the array.

A total of 14,701 probes, targeting 14,531 transcripts, were successfully designed; 8,918 of these had been previously employed on the first version of the array (GEO acc: GPL16124). Probe sequences and further details on the microarray platform can be found in the GEO repository (http://www.ncbi.nlm.nih.gov/geo/) under accession number GPL16714.

2.5. Microarray hybridization and data analysis

Sample labeling and hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol for 8 × 15 K slide format.

Briefly, for each sample 100 ng total RNA were linearly amplified and labeled with Cy3-dCTP. A mixture of 10 different viral poly-adenylated RNAs (Agilent Spike-In Mix) was added to each RNA sample before amplification and labeling, to monitor microarray analysis work-flow. Labeled cRNA was purified with a Qiagen RNeasy Mini Kit, and sample concentration and specific activity (pmol Cy3/µg cRNA) were measured in a NanoDrop® ND-1000 spectrophotometer. A total of 600 ng labeled cRNA were prepared for fragmentation by adding 5 µl 10X blocking agent and 1 µl 25X fragmentation buffer, heated to 60°C for 30 min, and finally diluted by addition of 25 µl 2X GE Hybridization buffer. A volume of 40 µl hybridization solution was then dispensed in the gasket slide and assembled with the microarray slide (each slide containing eight arrays). Slides were incubated for 17 h at 65°C in an Agilent hybridization oven, then removed from the hybridization chamber, quickly submerged in GE Wash Buffer 1 for disassembly of the slides and then washed in GE wash buffer 1 for approximately 1 minute followed by one additional wash in pre-warmed (37°C) GE wash buffer 2.

Hybridized slides were scanned at 5 µm resolution using an Agilent G2565BA DNA microarray scanner. Default settings were modified to scan each slide twice at two different sensitivity levels (XDR Hi 100% and XDR Lo 10%). The two linked images generated were analyzed together and the data were extracted and background subtracted using the standard procedures in the Agilent Feature Extraction (FE) Software version 9.5.1. Cyclic loess normalization was performed using R statistical software. Spike-in control intensities were used to monitor the normalization procedure.

Raw and normalized fluorescence data of all microarray experiments were deposited in the GEO database under accession numbers GSE44579.

2.6. Statistical analysis

Two-way ANOVA (stat soft) was used to compare the phosphorylation levels of AMPKα between groups. The microarray data were also analysed by two-way ANOVA using Tmev (TIGR MultiExperiment Viewer) statistical software, and gene expression was considered significantly different when p-value < 0.005. No multiple test correction (i.e. Bonferroni) was employed as previous analyses indicated that such corrections could be over-conservative (Leaver et al., 2008). However, in order to minimize the type 1 error and related false discovery rates (FDR), p-value was fixed at 0.005. Significant enrichment of GO biological process categories were tested for using EASE software (version 2.0). Benjamini correction was applied for statistical analysis related to GO enrichment.

3. Results and discussion

3.1. Impact of hypoxic challenge

The main objective of the present study was to investigate the short-term response of the liver transcriptome to an acute hypoxic challenge in common sole fed diets of different lipid content. The reliability of datasets was confirmed by the reproducibility of signal obtained from redundant transcript (data not shown). By examining genes regulated by the "hypoxia" factor, we revealed the molecular actors and related processes underlying the physiological response of common sole to hypoxia in liver tissue. ANOVA revealed that 1202 genes (FDR < 7%, with a p-value of 0.005) were significantly regulated following hypoxia, amounting to 8% of the total spotted genes (table 1 and figure 1). Among these regulated genes, 514 were up-expressed under hypoxic conditions and 688 were down-expressed (additional file 3). Gene Ontology enrichment analysis, which enables the identification of GO terms

significantly enriched in the input entity list when compared to the whole array dataset, was performed providing evidence for which biological processes may be particularly altered by hypoxia factor. Four significant GO terms, all interrelated, were identified: carbohydrate metabolism, glucose metabolism, energy pathways and molecular process related to oxidoreductase activity (table 2). Based on the GO categorisation, part of the up-regulated genes were related to gluconeogenesis pathway, regulation of cell growth and maintenance, sulfur amino acid biosynthesis, blood vessel development or transcription regulator activity; among down-regulated genes these were mitochondrial ATP synthesis, TCA (tricarboxylic acid cycle) intermediate metabolism, glycolysis, lipid biosynthesis, and protein folding (tables 3). The present results revealed an important gene-based metabolic reprogramming characterized by the down-regulation of energy-demand and aerobic energy-supply pathways associated with an induction of processes involved in the supply of anaerobic metabolic substrates and cell rescue.

3.1.1. Inhibition of genes related to aerobic ATP supply and energy consumption

We found numerous genes involved in energy metabolism down-regulated by hypoxia (table 3). These include four genes involved in ATP synthesis-coupled electron transport (NADH dehydrogenase ubiquinones: five NDUF genes) and four genes involved in the TCA cycle (ACLY, GAD1, IDH1 and ME1). These down-regulations are consistent with the known reduction of energy production by aerobic metabolic pathways in fish subjected to hypoxic conditions (Krumschnabel et al. 2000). Such down-regulations are also well documented in fish (Wright et al. 1989; Martinez et al. 2006), including common sole (Dalla Via et al. 1994; Van den Thillart et al. 1994), and inhibition of aerobic pathways has been seen to be concomitant with an activation of anaerobic ATP-generating pathways which use glucose as the main substrate. In agreement with these previous results, we found that several genes up-regulated by hypoxia were involved in the key steps of the gluconeogenesis pathway (PCK1 and G6PC) and in the metabolism of glucogenic amino acid, such as the transaminases (AADAT, GOT1, OAT, HAL and TAT) (table 3). Our transcriptomic data, which suggest the stimulation of the expression of genes implicated in glucose synthesis through gluconeogenesis, are consistent with the significant hypoxia-induced increase of glycemia that we measured in these fish (Zambonino-Infante et al., 2013). They are also in agreement with previous transcriptomic results on the liver of longjaw mudsucker (Gracey et al. 2001). However, contrary to data obtained by Gracey and collaborators revealing a stimulation of genes involved in hepatic glycolysis, we observed a significant down-regulation of several genes (ENO1, G6PD, HK2, LDHA, LDHB, PFKL, PGD, PGK1, PKM2 and TALDO1) involved in glucose catabolism pathways, including glycolysis (table 3). The inhibition of glucose catabolism in the liver points to a strategy of re-localization of energy supply in sole. This strategy could consist in maintaining high blood glucose levels for anaerobic ATP production in priority organs, such as the brain and heart, when survival is at stake.

Interestingly, we also found under hypoxic conditions higher hepatic levels of threonine 172 phosphorylation of AMP-activated protein kinase (AMPKα), which is an energy sensor protein kinase that plays a key role in maintaining cellular energy balance (figure 2). Considering that this phosophorylation is a key determinant of AMPK activity (Hardie 2008), our data are consistent with the stimulation of AMPK activity observed by Jibb and Richards (2008) in severely hypoxic goldfish. It is well documented that AMPK inhibits energetically expensive anabolic processes, such as protein, glycogen or fatty acid synthesis and cell growth and proliferation, in response to reduction of intracellular ATP levels (Mihaylova and Shaw 2011). In agreement with the present AMPKα stimulation, we observed 19 genes involved in lipid biosynthesis down-regulated and several genes implicated in the suppression of cell growth and proliferation, such as IGFBP-1, GRB10, DUSP1 and DUSP6, TOB1 and BTG-1 (table 3), up-regulated under hypoxic conditions. While inductions of

IGFBP-1 and GRB10, have been shown to inhibit in vivo IGF action, particularly under hypoxic conditions (Tazuke et al. 1998; Liu and Roth 1995; Kajimura et al. 2005), mitogen-activated proteins DUSP1 and DUSP 6 are known to inactivate the ERK group of MAP kinase involved in cell growth stimulation. The stimulation of IGFBP-1 expression that limits IGF-dependent decrease of growth hormone receptor (GHR) expression (Min et al. 1996) can be related to the increase in hepatic GHR mRNA level that we observed during hypoxia (table 3). TOB1 and BGT-1 are also known to suppress growth through their anti-proliferative function (Ho et al. 2010; Kamaid and Giráldez 2008). The impact of hypoxia on the expression of genes involved in cell growth and proliferation supports the view that fish experiencing reduced oxygen availability must reallocate energy from growth toward life-sustaining processes.

3.1.2. Induction of vascularization

Among the regulated genes , we found some actors involved in the regulation of vascular endothelial cells (table 3). GRB10, known to negatively regulate the insulin pathway, is also involved in angiogenesis by regulating the KDR/VEGFR-2 signalling pathway. The up-regulation of this gene suggests a stimulation of vascular endothelial cell development. In line with this view, we also noted the stimulation of several actors involved in blood vessel development including ERBB4 and EGFR genes, both of which play an essential role as a cell surface receptor of EGF (Russell et al. 1999). ERBB have been shown to play a key role in the regulation of angiogenesis by inducing the vascular endothelial growth factor (VEGF) (Yen et al. 2002). Even though we did not observe any significant differential expression of VEGF in the present work, we found a positive regulation by hypoxia challenge of PRKCA, PDGFRB, FOXF1, JAG1, RAMP2 and STAB1 (table 3), all known to be involved in blood vessel development (Adachi and Tsujimoto 2002; Ichikawa-Shindo et al. 2008; Stankiewicz et al. 2009; Wang et al. 2002; Zimrin et al. 1996). This stimulation of blood vessel formation during hypoxia, which has been widely documented in vertebrates including fish species (reviewed by Nikinmaa and Rees 2005), reflects the need to optimize oxygen supply to tissues. However, contrary to what has been shown in previous studies performed in rainbow trout by Marinsky et al. (1990) or in the euryoxic fish *Gillichthys mirabilis* by Gracey et al. (2001), we could not find any significant regulation of actors involved in iron or oxygen binding. It is likely that these regulations, even if they exist in the liver of common sole, do not represent the first line of adaptive strategy at the transcriptomic level.

As they highlight the impact of a hypoxic stress on several processes involved in the regulation of cellular metabolism and growth, as well as oxygen delivery to tissue cells, these data are consistent with previous reports performed on the liver of different fish species (*Gillichthys mirabilis*: Gracey et al. 2001; *Oryzias latipes*: Ju et al. 2007; *Gasterosteus aculeatus*: Leveelahti et al. 2011; *Fundulus grandis:* Everett et al. 2012).

3.1.3. Regulation of redox potential

It is noteworthy that some of the hypoxia-stimulated genes are also involved in sulfur amino acid biosynthesis (CBS and CTH) and in the glutathione metabolism process (GGT1) (table 3). This regulation probably allows an optimal intracellular glutathione level to be maintained, contributing to cellular antioxidant defense mechanism under hypoxic conditions. In agreement with the above, our transcriptomic data also revealed the differential expression of 56 genes with oxidoreductase activity (table 2), reinforcing the link between reduced oxygen availability and the regulation of redox potential. Altogether, such transcriptomic features can be related to the increase in mitochondrial reactive oxygen species (ROS) production classically observed in hypoxia (Chandel et al. 1998, Mansfield et al. 2005).

3.1.4. Large scale regulation of the hepatic transcriptome by transcriptomic factors

As mentioned in table 1, our results indicate that a large number of genes (n=1202) are significantly regulated 90 minutes after the onset of hypoxia. The immediate large-scale effect of hypoxia on the hepatic transcriptome may be partly explained by early regulation of the expression of several transcription factors (i.e., JUN, FOS, EGR1, HIF3, CREB, ATF7IP and CEBPD) listed in table 3. These transcription factors are indeed involved in a cascade of regulation involving a battery of genes that act in concert to facilitate the supply of oxygen and nutrients, regulate energy metabolism and promote cell survival and growth control (Hochachka et al. 1996; Cummins and Taylor 2005). This result demonstrates that, in addition to the regulations occurring at the post-transcriptomic level (van den Beucken et al. 2011), a severe hypoxic stress has a rapid and profound effect on the hepatic transcriptome.

3.1.5. Activation of the unfolded protein response (UPR)

We found several genes involved in protein folding (9 genes) or related to endoplasmic reticulum (RE) (21 genes) down-regulated by hypoxia challenge (table 3). In vertebrates it is well documented that regulation of the protein folding process is associated with RE stress triggers UPR (Kim et al. 2008). Interestingly, HSPA5 was shown to be up-regulated following hypoxia (table 3). HSPA5 is a key calcium-dependant chaperone involved in the setting off of the downstream signalling of the UPR through its association with IRE1, ATF6 and PERK (Kim et al. 2008). In a similar way as in the results obtained by Tagliavacca et al. (2012) and Kim et al. (2008) in mammals, the present data suggest that these pathways were also triggered by hypoxia in fish. In mammals, it is well documented that the PERK pathway is initiated by the phosphorylation of eIF2α, thereby blocking protein translation and activating the transcription factor ATF4. ATF4 controls the expression of genes involved in redox balance, amino acid metabolism, protein folding and apoptosis (Ameri and Harris 2008). In the present work, we observed up-regulation of challenge target genes of ATF4 such as the previously mentioned folding protein HSPA5, the amino acid transporter SLC7A3 as well as IGFBP-1 and GRB10, which are implicated in cell growth mechanisms (Luo et al. 2003; Harding et al. 2003) (table 3). This suggests that the PERK pathway is also activated during hypoxia in sole. With respect to the other UPR pathways, it is known that ATF6 and XBP1 in mammals are able to transactivate genes encoding chaperones and proteins involved in endoplasmic reticulum-associated degradation (ERAD) (Jäger et al. 2012; Kim et al. 2008). The stimulation of the ATF6-target gene Herpud1 (table 3), involved in ERAD (Ma and Hendershot 2004), also suggests the activation of the ATF6 pathway within the 90 minutes of hypoxia tested in the present study.

While UPR consists of mechanisms decreasing the synthesis of proteins and their influx into the ER, we found only a few genes involved in this process that were regulated during environmental hypoxia. This observation suggests that the main mechanisms responsible for the decrease in protein synthesis shortly after the initiation of hypoxia require post-transcriptional regulation.

Our data revealed that most of the molecular actors involved in ER stress, as they were described for mammals, can also be found in common sole. Moreover, these data are totally consistent with a recent study of Ishikawa et al. (2011) indicating that the process of UPR is conserved across vertebrates including fish.

3.2. Impact of dietary lipid content

The second objective of this work was to determine the biological processes underlying the lower tolerance of sole to hypoxia when fish were fed a lipid-rich diet (Zambonino-Infante et al., 2013). Statistical analysis revealed 801 genes (FDR < 10%, with a p-value of 0.005) regulated by the dietary lipid content, whatever the oxygenation conditions (table 1; figure 1). These included 424 genes down-expressed and 377 genes up-expressed in fish fed the high lipid diet (additional file 3). Interestingly, we found only 200 genes with expression differentially affected by hypoxia depending on the dietary lipid content. It is noteworthy that gene ontologies related to macromolecule biosynthesis and protein folding, mitochondrial ATP synthesis and defense activity were significantly enriched within genes regulated by diet condition whatever the oxygenation conditions (table 4).

3.2.1. Regulation of glucose metabolism

We found that hepatic nuclear factor 6 (ONECUT1) was stimulated in fish fed the high fat diet (table 5). It is known that ONECUT1 is involved in the regulation of the transcription of gluconeogenic enzymes such as glucose-6-phosphatase (G6PC) (Streeper et al. 2001). In line with this, we also observed a stimulation of TCA enzymes involved in gluconeogeneis (MDH1 and MDH2) in the same dietary group (table 5). Together with all the mechanisms involved in high-fat diet-induced diabetes, this result could explain the higher glycemia rate observed in fish fed the high lipid diet (Zambonino-Infante et al., 2013).

3.2.2. Stimulation of an ATP-expensive anabolic process and aerobic ATP synthesis

Analysis of GO revealed that up-regulated genes were also related to protein biosynthesis and folding (61 genes, see table 5). The stimulation of genes involved in protein synthesis and maturation could be due to an increased need for lipoproteins, required to transport the overload of dietary lipids (Lim et al. 2009; Yildirim-Aksoy et al. 2009). This result contrasts with data obtained in mice fed a high fat diet, where a down-regulation of protein synthesis was observed (Deldicque et al. 2010; Oyadomari et al. 2008). This discrepancy may be likened to the low lipid tolerance of flatfish species (Borges et al. 2009). As mentioned by Zambonino-Infante et al. (2013), the relative difficulty that sole have at handling high dietary lipid contents may have a metabolic cost. In agreement with this idea, the present study revealed some up-regulated genes involved in oxidative phosphorylation and ATP biosynthesis (NDUFs and ATP synthases, table 5). Globally, around 10% of the genes up and down-regulated by hypoxia factor were found to be down and up-regulated by lipid factor, respectively (figure 1); GO over-represented within those genes being related to mitochondria and hydrogen ion transporter activity (table 6). These data suggest an increased activity of aerobic ATP production in response to high-fat feeding in common sole, probably through the positive regulation of fatty acid beta-oxidation and the TCA pathway.

The stimulation of ATP-expensive anabolic process, supported by the lower rates although not significant ($p = 0.07$) of AMPKα phosphorylation (figure 2), goes against the principle that hypoxic fish should save oxygen. These regulations were unrelated to the oxygen conditions and could therefore explain the impaired tolerance to hypoxia in fish fed the high lipid diet.

3.2.3. Low significant hypoxia x lipid interaction on the hepatic transcriptome

Our statistical analysis revealed only a slight interaction between hypoxia and lipid factors on the hepatic transcriptome (i.e., 200 genes, see table 1 and additional file 3). However, biological data mining resulting from this analysis is ambiguous since FDR is expected to be

around 30% using a p-value of 0.005. Moreover, no gene ontology was enriched among the 200 genes.

3.2.4. Impact of dietary lipid content on defence activities

Finally, our transcriptomic data revealed that feeding fish a high lipid content diet down-regulated genes associated with blood coagulation, immune response and homeostasis (table 5). These data confirm earlier studies that demonstrated an effect of the dietary lipid content on the fish immune system, particularly on complement activity (Geay et al. 2011). Together with the impact of high lipid ingestion on the energy metabolism of common sole, this last result suggests deleterious effects on the processes of defence, and further undermines the tolerance of this fish species to lipids.

4. Conclusion

The present data reveal new relevant information about the short term effect of acute hypoxia on the fish liver transcriptome. In particular, the data highlight a finely tuned regulation of different biological processes, including metabolic pathways and UPR response, resulting in metabolic depression. Our results revealed that the regulation of these processes could be related to the stimulation of several transcription factors, explaining the large-scale regulation of the hepatic transcriptome. Moreover, our data provide valuable insight on the influence of high lipid consumption on liver metabolic pathways. While several other tissues (i.e., muscle, heart and brain), as well as post-transcriptomic levels of regulations, are certainly affected by lipid content in diet, we can hypothesize that the regulations induced by high lipid diets in liver are detrimental to the energy-saving required for cellular homeostasis under hypoxia. This effect could partly contribute to explaining why fish fed high level of fat exhibited lower tolerance to hypoxia compared to those fed a low lipid diet. It is indeed accepted that hypoxia-tolerance is largely based on an organism's ability to down-regulate ATP production and consumption in a coordinated way during energy limiting conditions (Krumschnabel et al. 2000). It is suggested that climate warming will induce changes on zooplankton communities and especially lipid-storing species, which would affect the lipid flux of the entire system (Lee et al. 2006). Assessing the molecular mechanisms underlying the effects of dietary lipid content on hypoxia tolerance in common sole is crucial for a better understanding the effects that global change will have on the physiology of this specific taxon, on its life-traits and the possible consequences in term of population.

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Tables

Table 1: Number of genes regulated by the factors “hypoxia” and “lipid”, and responding to the “hypoxia × lipid” interaction, as revealed by two-way ANOVA on transcriptomic data.

4.1. Factors	4.2. Hypoxia	4.3. Lipid	4.4. Hypoxia × Lipid
4.5. Number of regulated genes	4.6. 1202 up: 514 down: 688	4.7. 801 up: 424 down: 377	4.8. 200

Table 2: Gene Ontologies over-represented among genes regulated by the factor "hypoxia"

System	Gene Ontology	Number of genes	Corrected (Benjamini) p-value
Biological process	carbohydrate metabolism	40	0.04
	glucose metabolism	16	0.03
	energy pathways	31	0.03
Molecular function	oxidoreductase activity	56	0.01

Table 3: Summary of selected down- and up-regulated transcripts in the liver of *Solea solea* 90 min after the beginning of the hypoxic challenge

Transcripts	Official gene name	p-value
Down-regulated		
ATP synthesis-coupled electron transport		
NADH dehydrogenase 1 beta subcomplex subunit 2	*NDUFB2*	6.92E-4
NADH dehydrogenase 1 beta subcomplex subunit 8	*NDUFB8*	4.63E-4
NADH-ubiquinone oxidoreductase 75 kDa subunit	*NDUFS1*	0.0028
NADH dehydrogenase flavoprotein 2	*NDUFV2*	0.0026
TCA intermediate metabolism		
ATP-citrate synthase	*ACLY*	2.63E-4
Glutamate decarboxylase 1	*GAD1*	6.03E-4
Isocitrate dehydrogenase	*IDH1*	0.0014
NADP-dependent malic enzyme	*ME1*	0.0047
Glycolysis		
Eno1 protein	*ENO1*	4.64E-9
Glucose-6-phosphate 1-dehydrogenase	*G6PD*	2.46E-9
Hexokinase-2	*HK2*	1.37E-4
L-lactate dehydrogenase A	*LDHA*	4.20E-4
L-lactate dehydrogenase B	*LDHB*	7.22E-5
6-phosphofructokinase	*PFKL*	0.0031
6-phosphogluconate dehydrogenase	*PGD*	1.50E-7
Phosphoglycerate kinase 1	*PGK1*	3.93E-5
Pyruvate kinase isozyme M1/M2	*PKM2*	0.0027
Transaldolase	*TALDO1*	6.53E-7
Lipid biosynthesis		
Arachidonate 12-lipoxygenase	*ALOX12*	8.42E-4
Delta(14)-sterol reductase	*TM7SF2*	0.0041
Squalene monooxygenase	*SQLE*	1.24E-4
Acyl-CoA desaturase	*SCD*	1.93E-6
Lathosterol oxidase	*SC5DL*	0.0049
Phosphatidylinositol N-acetylglucosaminyl transferase H	*PIGH*	2.77E-4
Phosphatidylinositol N-acetylglucosaminyl transferase B	*PIGB*	1.75E-4
Ethanolamine-phosphate cytidylyltransferase	*PCYT2*	0.0015
Methionine adenosyltransferase 2 subunit beta	*MAT2B*	5.28E-4
Lanosterol synthase	*LSS*	0.0048
3-keto-steroid reductase	*HSD17B7*	0.0037
3-hydroxy-3-methylglutaryl-coenzyme A reductase	*HMGCR*	4.20E-6
Fatty acid synthase	*FASN*	2.45E-6
7-dehydrocholesterol reductase	*DHCR7*	2.28E-5
Arachidonate 5-lipoxygenase	*ALOX5*	3.49E-4

ATP-citrate synthase	*ACLY*	2.26E-4
Acetyl-CoA carboxylase 1s	*ACACA*	0.0029
Protein folding		
T-complex protein 1 subunit gamma	CCT3	0.0027
T-complex protein 1 subunit epsilon	*CCT5*	1.33E-4
DnaJ homolog subfamily B member 2	*DNAJB2*	7.88E-4
ERO1-like protein alpha	*ERO1L*	0.0019
Peptidyl-prolyl cis-trans isomerase FKBP3	*FKBP3*	0.0020
Heat shock cognate 71 kDa protein	*HSPA8*	0.0012
10 kDa heat shock protein, mitochondrial	*HSPE1*	4.58E-4
Tubulin-specific chaperone D	*TBCD*	0.0014
Tuberin	*TSC2*	1.72E-5
Endoplasmic reticulum		
Cytochrome P450 2J2	*CYP2J2*	2.25E-4
Cytochrome P450 2S1	*CYP2S1*	9.65E-5
7-alpha-hydroxycholest-4-en-3-one 12-alpha-hydroxylase	*CYP8B1*	0,0037
7-dehydrocholesterol reductase	*DHCR7*	2.28E-5
ERO1-like protein alpha	*ERO1L*	0.0019
3-hydroxy-3-methylglutaryl-coenzyme A reductase	*HMGCR*	4.20E-5
Gamma-soluble NSF attachment protein	*NAPG*	0,0035
Protein-L-isoaspartate(D-aspartate) O-methyltransferase	*PCMT1*	4.66E-4
Phosphatidylinositol N-acetylglucosaminyl transferase B	*PIGB*	1.75E-4
Phosphatidylinositol N-acetylglucosaminyl transferase H	*PIGH*	2.77E-4
Proteolipid protein 2	*PLP2*	3.62E-5
Proteasome subunit alpha 2	*PSMA2*	6.69E-5
Proteasome subunit beta 4	*PSMB4*	3.26E-6
Proteasome subunit beta 5	*PSMB5*	0,0030
Proteasome subunit beta 7	*PSMB7*	3.29E-5
Proteasome subunit delta 5	*PSMD5*	1.15E-6
Proteasome activator complex subunit 1	*PSME1*	4.40E-4
Reticulon-4	*RTN4*	8.08E-10
Lathosterol oxidase	*SC5DL*	0.0049
Acyl-CoA desaturase	*SCD*	1.93E-6
Delta(14)-sterol reductase	*TM7SF2*	0,0042
Up-regulated		
Gluconeogenesis pathway		
Phosphoenolpyruvate carboxykinase	*PCK1*	5.86E-4
Glucose-6-phosphatase	*G6PC*	1.32E-4
Metabolism of glucogenic amino acid		
Kynurenine/alpha-aminoadipate aminotransferase	*AADAT*	0.0018
Aspartate aminotransferase	*GOT1*	5.63E-5
Ornithine aminotransferase	*OAT*	1.09E-4
Histidine ammonia lyase	*HAL*	8.58E-7
Tyrosine aminotransferase	*TAT*	2.10E-4
Regulation of cell growth and proliferation		

Insulin-like growth factor-binding protein 1	*IGFBP-1*	6.94E-6
Growth factor receptor-bound protein 10	*GRB10*	2.88E-4
Dual specificity protein phosphatase 1	*DUSP1*	0.0012
Dual specificity protein phosphatase 6	*DUSP6*	0.0014
Transducer of erbB-2 1	*TOB1*	0.0025
B-cell translocation gene 1 protein	*BTG-1*	5.69E-6
Vessel development		
Stabilin-1	*STAB1*	0.0017
Growth factor receptor-bound protein 10	*GRB10*	2.88E-4
Receptor tyrosine-protein kinase erbB-4	*ERBB4*	0.0049
Epidermal growth factor receptor	*EGFR*	7.02E-4
Protein kinase C alpha type	*PRKCA*	1.55E-4
Platelet-derived growth factor receptor beta	*PDGFRB*	3.33E-4
Forkhead box protein F1	*FOXF1*	0.0029
Protein jagged-1	*JAG1*	2.36E-4
Receptor activity-modifying protein 2	*RAMP2*	1.80E-4
Sulfur amino acid biosynthesis		
Cystathionine beta-synthase	*CBS*	2.38E-8
Cystathionine gamma-lyase	*CTH*	8.29E-5
Gamma-glutamyltranspeptidase 1	*GGT1*	7.59E-4
Transcription factors		
Transcription factor jun-B	*JUNB*	1.69E-8
Proto-oncogene c-Fos	*FOS*	3.36E-4
Early growth response protein 1	*EGR1*	1.67E-4
CCAAT/enhancer-binding protein delta	*CEBPD*	0.0033
Hypoxia-inducible factor 3-alpha	*HIF3*	1.56E-4
Cyclic AMP-responsive element-binding protein 1	*CREB1*	0.0037
Activating transcription factor 7-interacting protein 1	*ATF7IP*	8.47E-4
Other		
Cationic amino acid transporter 3	*SLC7A3*	0.0031
78 kDa glucose-regulated protein	*HSPA5*	1.80E-4
Growth hormone receptor	*GHR*	3.78E-4
Homocysteine-responsive endoplasmic reticulum-resident ubiquitin-like domain member 1 protein	*HERPUD1*	9.46E-4

Table 4: Gene Ontologies over-represented among genes regulated by high dietary lipid

System	Gene Ontology	Number of genes	Corrected (Benjamini) p-value
Biological process	protein biosynthesis	52	2,06E-06
	complement activation	8	2,50E-03
	hydrogen transport	11	0.03
	protein folding	15	0.04
	blood coagulation	12	0.04
Cellular component	ribosome	39	1,02E-06
	cytosol	46	2,06E-06
	proton-transporting ATP synthase complex	6	2,50E-03
	mitochondrial inner membrane	14	0.04
Molecular function	hydrogen ion transporter activity	24	2,06E-06
	RNA binding	37	0.01
	defense/immunity protein activity	11	0.03

Table 5: Summary of selected transcripts down and up-regulated in the liver of *Solea solea* by high dietary lipid

Transcripts	Official gene name	p-value
Down-regulated		
Immune response		
Beta-2-glycoprotein 1	*APOH*	4.68E-4
Attractin	*ATRN*	0.0026
Complement C1r subcomponent	*C1R*	5.04E-4
Complement C3	*C3*	3.95E-5
Complement C5	*C5*	0.0036
Complement component C8 alpha chain	*C8A*	0.0049
Complement component C8 gamma chain	*C8G*	8.36E-4
Immunoglobulin-binding protein 1	*IGBP1*	9.03E-4
Lysozyme C	*LYZ*	0.0028
Mannan-binding lectin serine protease 1	*MASP1*	2.14E-7
Phosphatidylinositol 3-kinase regulatory subunit alpha	*PIK3R1*	0.0046
Parathymosin	*PTMS*	8.06E-4
E-selectin	*SELE*	3.04E-4
Alpha-1-antitrypsin	*SERPINA1*	0.0034
Plasma protease C1 inhibitor	*SERPING1*	7.32E-4

Up-regulated

Protein biosynthesis

Alanine--tRNA ligase, cytoplasmic	*AARS*	7.18E-6
Cysteine--tRNA ligase, cytoplasmic	*CARS*	0,0034
T-complex protein 1 subunit beta	*CCT2*	5.17E-4
Elongation factor 1-gamma	*EEF1G*	5.05E-5
Elongation factor 2	*EEF2*	0,0012
Translation initiation factor eIF-2B subunit epsilon	*EIF2B5*	0.0028
Eukaryotic translation initiation factor 2 subunit 2	*EIF2S2*	0.0037
Eukaryotic translation initiation factor 2 subunit 3	*EIF2S3*	1.13E-5
Eukaryotic translation initiation factor 5A-1	*EIF5A*	0.0028
Bifunctional glutamate/proline--tRNA ligase	*EPRS*	1.98E-4
Clustered mitochondria protein homolog	*KIAA0664*	0.0042
39S ribosomal protein L1, mitochondrial	*MRPL1*	0,0040
39S ribosomal protein L15, mitochondrial	*MRPL15*	1.05E-5
39S ribosomal protein L17, mitochondrial	*MRPL17*	0,0011
39S ribosomal protein L4, mitochondrial	*MRPL4*	0.0019
39S ribosomal protein L42, mitochondrial	*MRPL42*	3.49E-5
28S ribosomal protein S14, mitochondrial	*MRPS14*	4.54E-4
28S ribosomal protein S2, mitochondrial	*MRPS2*	0,0049
28S ribosomal protein S35, mitochondrial	*MRPS35*	0,0048
Glycylpeptide N-tetradecanoyltransferase 1	*NMT1*	7.55E-4
Serine/threonine-protein phosphatase 2A 65 kDa regulatory	*PPP2R1B*	0.0017
Glutamine--tRNA ligase	QARS	2.92E-4
Glutamyl-tRNA(Gln) amidotransferase subunit A	QRSL1	1.70E-5
Arginine tRNA ligase, cytoplasmic	RARS	2.09E-4
60S ribosomal protein L11	RPL11	5.63E-5
60S ribosomal protein L13	RPL13	7.26E-5
60S ribosomal protein L18a	RPL18A	1.77E-5
60S ribosomal protein L24	RPL24	5.58E-4
60S ribosomal protein L27	RPL27	8.19E-4
60S ribosomal protein L3	RPL3	7.41E-5
60S ribosomal protein L30	RPL30	0,0036
60S ribosomal protein L35a	RPL35A	0.0029
60S ribosomal protein L36	RPL36	0,0013
60S ribosomal protein L38	RPL38	0,0017
60S ribosomal protein L4	RPL4	1.37E-5
60S ribosomal protein L7	RPL7	6.62E-4
60S ribosomal protein L7a	RPL7A	5.86E-5
60S ribosomal protein L8	RPL8	1.44E-4
40S ribosomal protein S13	RPS13	0,0030
40S ribosomal protein S15a	RPS15A	4.43E-4
40S ribosomal protein S19	RPS19	0,0012
40S ribosomal protein S2	RPS2	8.28E-4
40S ribosomal protein S21	RPS21	0,0041
40S ribosomal protein S26	RPS26	0,0041
40S ribosomal protein S27a	RPS27A	1.52E-4
40S ribosomal protein S28	RPS28	5.82E-4
40S ribosomal protein S3	RPS3A	0,0014
40S ribosomal protein S5	RPS5	0,0033
40S ribosomal protein S9	RPS9	3.39E-4
Toll-like receptor 3	TLR3	2.95E-4

Protein folding		
T-complex protein 1 subunit beta	*CCT2*	5.17E-4
T-complex protein 1 subunit gamma	*CCT3*	5.15E-6
T-complex protein 1 subunit delta	*CCT4*	0.0012
T-complex protein 1 subunit epsilon	*CCT5*	9.58E-8
T-complex protein 1 subunit eta	*CCT7*	6.34E-6
T-complex protein 1 subunit theta	*CCT8*	0.0037
DnaJ homolog subfamily B member 2	*DNAJB2*	6.40E-4
DnaJ homolog subfamily C member 7	*DNAJC7*	0.0016
10 kDa heat shock protein, mitochondrial	*HSPE1*	0.0021
T-complex protein 1 subunit alpha	*TCP1*	1.45E-8
Prefoldin subunit 3	*VBP1*	0.0010
Glucose metabolism		
Hepatocyte nuclear factor 6	*ONECUT1*	0.0031
Gluconeogenesis and TCA		
Malate dehydrogenase cytoplasmic	*MDH1*	1.81E-4
Malate dehydrogenase mitochondrial	*MDH2*	0.0011
ATP biosynthesis		
ATP synthase subunit alpha	*ATP5A1*	0.0011
ATP synthase subunit gamma	*ATP5C1*	0.0011
ATP synthase subunit delta	*ATP5D*	3.59E-5
ATP synthase subunit f	*ATP5J2*	0.0016
ATP synthase subunit O	*ATP5O*	3.76E-5
NADH dehydrogenase 1 beta subcomplex subunit 10	*NDUFB10*	1.59E-5
NADH dehydrogenase 1 beta subcomplex subunit 4	*NDUFB4*	1.32E-4
NADH dehydrogenase 1 beta subcomplex subunit 8	*NDUFB8*	0.0024
NADH dehydrogenase [ubiquinone] iron-sulfur protein 3	*NDUFS3*	3.59E-5
NADH dehydrogenase [ubiquinone] iron-sulfur protein 5	*NDUFS5*	0.0023
Cytochrome b-c1 complex subunit 7	*UQCRB*	4.05E-5

Table 6: Gene Ontologies over-represented among genes inversely regulated by "hypoxia" and “high dietary lipid” factors

System	Gene Ontology	Number of genes	Corrected (Benjamini) p-value
Cellular component	mitochondrion	16	0.01
Molecular function	hydrogen ion transporter activity	8	0.01

Figures

Figure 1: Venn diagram showing the number of genes significantly up and down-regulated by hypoxia and lipid factors.

Figure 1:

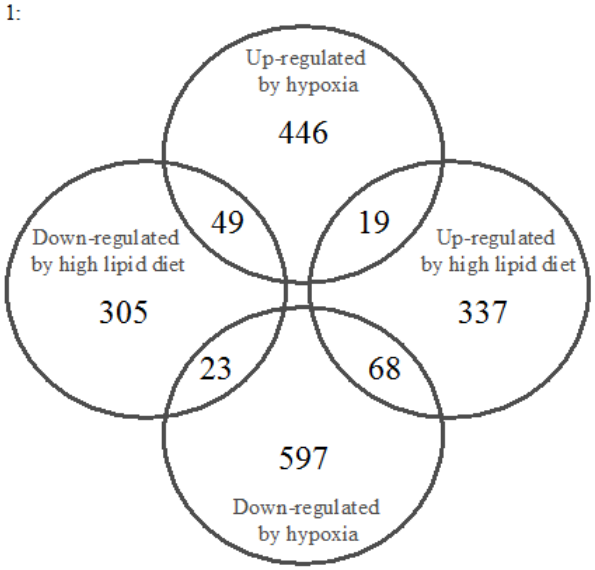
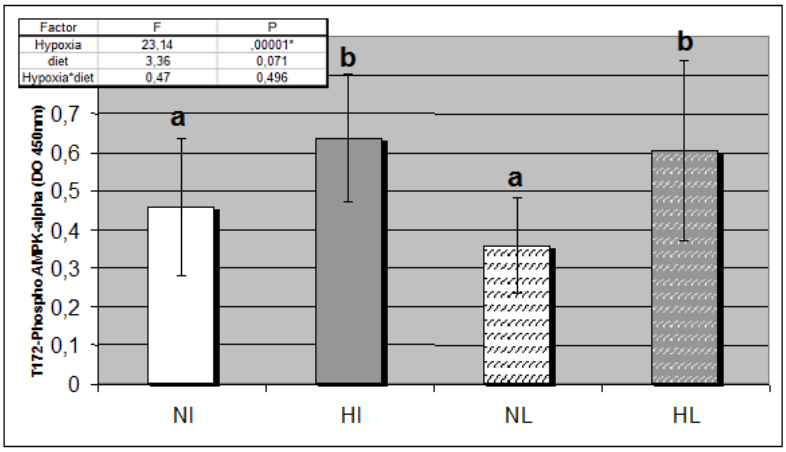
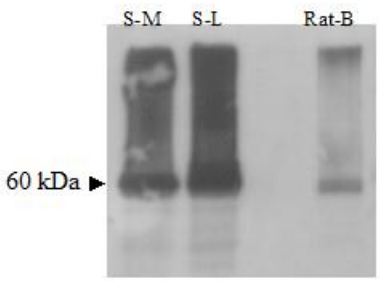


Figure 2: Quantification of threonine 172 phosphorylation in the alpha subunit of AMPK in the Normoxia l-group (Nl), Normoxia L-group (NL), Hypoxia l-group (Hl) and Hypoxia L-group (HL). Different letters (a, b) indicate a significant difference between the groups ($p<0.05$).

Figure 2:

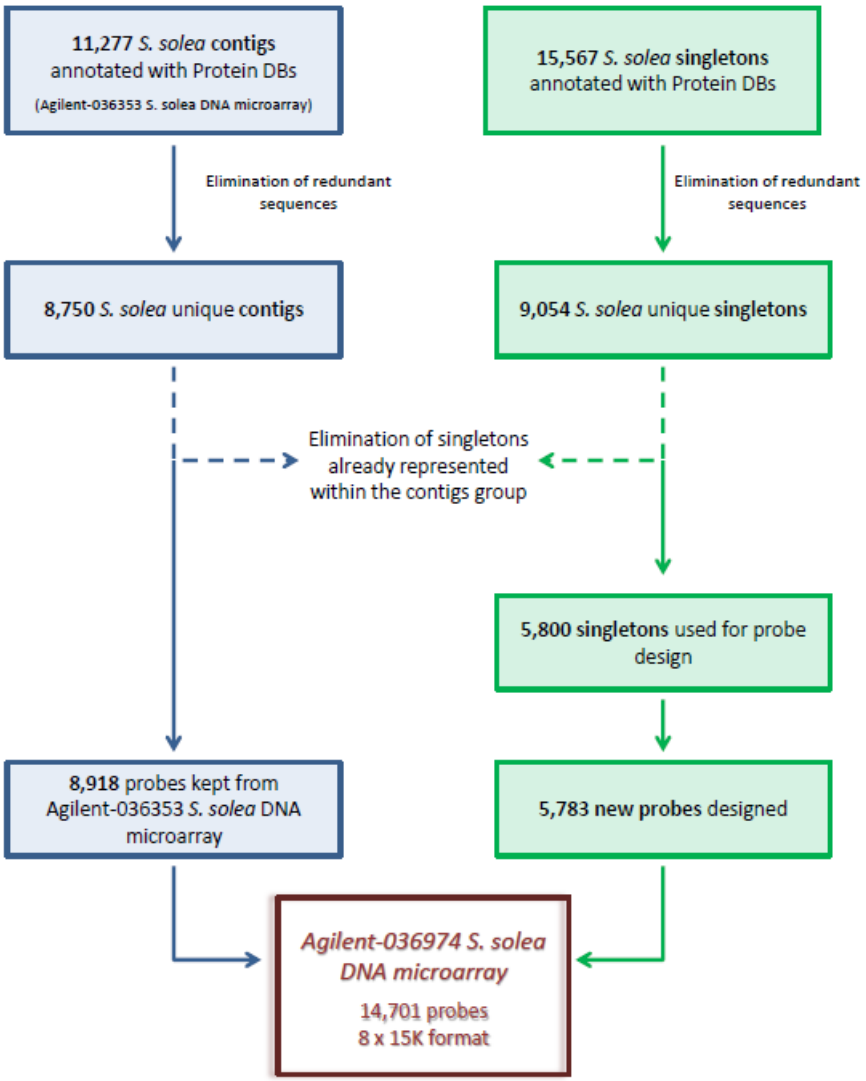


Additional file 1: Western blot analysis from sole tissues performed using Rat Anti-AMPKα



Supplementary data 1: Western blot analysis from protein lysates of sole tissues (sole muscle S-M, sole liver S-L) and rat brain (Rat-B) using Rat Anti-AMPKα (23A3) (Rabbit mAb, #2603, Cell Signaling). A specific band was detected at approximately 60 kDa.

Additional file 2: schematic representation of the approach used for microarray design



Additional file 3: Full list of genes regulated by "hypoxia", "lipid" and "hypoxia" x "lipid" factors interaction.

List of genes up-regulated by hypoxia (p<0,005)
RPS6KA5
SLC44A5
NAA25
TMEM240
CABZ01089777.1
C5H9orf171
K1211
G6W9YEI02J3XJ7
PAPSS1
FGD4
TIA1
SERP1

HPDL
CDK14
TNRC6C
PCDHAC2
G6W9YEI01APVEA
isotig20588
HRAS
USPL1
BX927253.1
G6W9YEI02GLJEM
SUMO4
git2b
atrx
C22orf39
MOSPD2
isotig20018
MAT2A
SFXN1
isotig07752
PDSS1
EPB41L2
PHACTR4
ETNK1
CDK17
ide
NCKAP5
G6W9YEI02J4MC0
TIMM10
TMEM184A
IRF3
nlgn4a
ADAMTS16
G6W9YEI02JCQ41
G6W9YEI01A09VR
NDRG1
SGCE
isotig13652
NDUFB1
NDUFA3
chd7
HNRNPA1
STRN
isotig20947
G6W9YEI02HW1BT
TTC14
ENAH
isotig14551
ZFHX3
ZFHX3
ZFHX3
ZFHX3
CHD4
isotig03400
C3 (6 of 8)
AADAT

PLRG1
SND1
BX537277.1
DDA1
G6W9YEI01AZVAH
ANKRD11
isotig09984
h2afv
HDAC4
PNP
FGA
PENK
ACVR2B
PDE4DIP
LPL
SLC40A1
slc40a1
G6W9YEI02GCJEH
ASNSD1
DUSP1
POC1B-GALNT4
isotig13247
ahr2
HIPK3
SERINC5
GADD45G
stat5.1
C1orf116
zmiz1
CSNK1G2
ELMOD2
G6W9YEI02GH9NO
OAT
CTH
F11R
NFIX
plxna4
isotig09491
CU660013.2
SMURF1
ERC1
BX511270.1
BX511270.1
HES5 (7 of 9)
ZNF710
FAM73B
MMP24
CABZ01038928.1
FKBP15
isotig16206
arhgap10
dynll1
ubtfl
SEC16A
G6W9YEI02FM73N

G6W9YEI01D5RMC
hs3st1l1
FRMD4B
CABZ01118775.1
PLEKHG5
IER5L
LZTR1
S2535
G6W9YEI01BML1Z
G6W9YEI02HXZFW
ALCAM
MAP1LC3C
BX005256.1
PAK2
IRF2BP2
egr1
junbl
FOS
JUNB
isotig05643
isotig03993
ITPKC
fam46c
FAM46A
isotig21478
isotig05160
SLC12A2
MYO9B
FRMPD1
HOXB2
PRKCA
NFE2L2
NFE2L2
SLC43A1
G6W9YEI01CSBV1
hmgb1a
MYCBP2
SLC22A14
PIAS1
RBFOX2
arid1ab
isotig14780
GLI2
DCN
MYOF
RGS2
isotig20719
syt16
H3F3B
LRP1
RBPMS
FOXF1
ITPK1
SMOC2
llgl2

CRHBP
TMEM27
cdc42ep1
G6W9YEI02I8DW5
RAB11FIP1
CABZ01024770.1
BT1A1
CHCHD3
rhoab
DNAJC8
CTDSPL2
ZNF598
ZGPAT
DLST
AASDHPPT
map3k12
USP24
TNRC6A
TNRC6A
CPSF6
chd7
sept-07
PRPF39
isotig02784
BCKDHB
DHX40
MYCL1
RPAC2
MFN2
CPT1A
RAB35
foxp1b
SPEN
SLC37A2
CDC42SE2
SBNO2
G6W9YEI01DTAP0
ADCK5
isotig16486
MOB2
SLMO2
PSME4
psme4b
INSIG1
CCNL1
G6W9YEI01EKBF8
C20H1orf9
COG3
HM13
RBM25
UFSP2
VAPA
HNRNPAB
TOP1
DYNC1LI2

ddx3
isotig18388
ddx3
ddx3
FBXW11
isotig02477
FARS2
G6W9YEI02GJ4DG
WDR18
ITPR3
BSDC1
RBP5
TULP4
G6W9YEI01A9J45
ABLIM1
pcdh10b
isotig07100
RBM12
MYO7A
AGRN
TRIM29 (7 of 21)
MINK1
PRPSAP1
CTDSPL2
isotig06783
fbxw7
MAN1C1
SLC25A22
isotig06262
epha7
POLB
GYG1
G6W9YEI02H48U0
G6W9YEI01D7VLA
RPS6KA1
TAT
HAL
ucp1
DIABLO
SLC43A1
CBS
CBS
isotig08370
CDO1
AFMID
HACL1
KCNV2
G6W9YEI01A4FFR
atf7ip
nfil3-6
FAM20A
isotig03014
TNRC6C
CSDE1
SDHB

gsk3b
GSK3B
c1galt1b
nfil3-6
DOCK1
GGTL2
ggt1
GHR
PPP1R37
C17orf103
TOB1
isotig17702
PLEKHA5
ZNF648
irx7
B4GALT1
isotig08127
ABCB11
HNRNPA3
SPTBN2
PTPRD
MTMR4
wu:fk48d07
GOT1
KIAA1191
G6W9YEI02G2V09
VTN
HEXIM1
I17RC
DPAGT1
TOM1
zufsp
VAV1
AP1S3
NIT2
FAM46B
VATE1
isotig04514
KL
cirbp
GK
IVD
C1TC
TRAPPC4
PPIF
CERS1
SMYD4
KAT5
ADO
KIAA1467
IPPK
isotig02107
STK19
C20orf30
HES1

isotig02749
TOPORS
G6W9YEI01EOZR3
IUNH
JAG1
isotig22014
RRM2
ACTL6A
SET
POLE3
isotig12437
HMGB4
APOB (1 of 5)
ACAA2
ACAA2
SRP68
LIN54
CHST15
C1orf50
KIAA1715
G6W9YEI01CV8F2
isotig07264
SLC35A1
TBX1
RFK
C1orf27
BET1
HNRNPA0
DPP3
C5orf43
SGSM3
DERL2
ATOX1
SSR3
ACAD11
isotig08678
isotig12919
HNRNPA1
C16orf58
ARL1
isotig09834
TMEM214
UFSP2
sec22bb
herpud1
herpud1
G6W9YEI01BYVMH
HSPA5
C20orf24
FICD
SLC35B1
MOGS
LRRC59
SEC61A1
erp44

PDIA6
G6W9YEI01C0L5P
NEIL3
isotig11784
ERBB4
isotig22007
AL929150.1
GRB10
tdh
slc7a3
SLC7A3
CABZ01044048.1
CABZ01044048.1
PNP
isotig02146
ZFP36
CEBPD
WNK2
G6W9YEI02ITYVR
CREB1
SGK1
keap1a
CR388163.2
G6PC
CYP24A1
G6W9YEI02JCJ2C
ZNF654
HIF3A
FKBP5
DDIT4
dusp6
PCK1
SGK1
FAM160A1
CDH5
RAB14
UBR4
RBMS3
STAB1
ARRB2
gyg2
SYNE1
PEPE
PDGFRB
STARD13
RPL22L1
EEF1B2
RPS10
RS17
RPL28
SLC25A33
IGFBP1
IGFBP1
FAM13B
G6W9YEI01A8WLM

FAM13B
EWSR1
ASCC1
ubr3
hnrnpa0
PHOX2B
ADCYAP1R1
isotig21026
SIK1
MTA1
VAV2
SPTBN1
ABCB4
ATP1A1
G6W9YEI01EWQVH
asah2
ca4b
VDAC3
ATP8A1
SERPINA10
CD68
isotig09333
RGPD2
isotig18665
isotig18665
rgs3
clk4a
RAMP2
RBM5
SF3B1
IQSEC1
G6W9YEI01B62IP
RNF144B
RASSF4
MCOLN2
cx43
MYCT1
EGFR
YAP1
SSFA2
NOS1AP
IER2
gas1b
gas1a
ppap2b
DLL4
RXRA
UBTF
UBTF
efnb2a
isotig03796
isotig07135
G3BP2
ZSWIM5
isotig07045

isotig02709
GRB2
ZBED4
btg1
BTG1
PITPNC1
rc3h1

List of genes down-regulated by hypoxia (p<0,005)
EPB41
CREBZF
CYP27A1
ubr7
MTHFD2
G6W9YEI02FKBQJ
ELP2
SIRT6
rpp21
agfg1b
ZNF697
AADACL2
isotig03847
DDX42
SENP7
chp2
ANXA3
ANXA3
HBM
TMOD4
isotig21816
rbm38
GAD1
thbs1
F13A1
slc25a37
alox12
ALOX5
jph1a
RHAG
FAM78A
WBP4
GFI1B
isotig18989
SLC4A1
ALAS2
isotig02473
cahz
ba1
ba1
KEL
NDUFV2
BX088712.3
TRA2B
ppp1r10
FHOD3

LIPS
DOM3Z
PPAP2A
G6W9YEI02J2FXB
SRSF5
SRSF5
LDHB
isotig12370
BX088712.3
LACE1
STRADA
NEK4
METTL5
TM109
CNOT10
CNO
WDR11
CLCN6
isotig07006
isotig06870
CASP2
ZDHHC16
isotig05781
ZNF784
isotig13777
isotig03903
ZNF292
isotig09815
mll4a
GBAS
MEPCE
TASP1
TNPO2
isotig10755
isotig03451
MBD5
BCORL1
ciz1
PRPF38B
isotig11003
isotig07794
RFX7
GIT2
SRSF9
SLC17A7
ARIH2
DCTN2
PIP5K1A
TARDBP
RS27
ENOPH1
MAPKAP1
IAH1
GGNBP2
isotig02830

isotig22031
DOCK1
sox4a
GPR137B
RXRB
ORMDL2
LEPROT
GTF2B
cldn15lb
C21orf2
C21orf2
G6W9YEI02GSPOV
isotig19097
PFKFB4
PKM2
PLOD1
BRD2
TAX1BP1
SLC39A8
isotig00842
TMEM39B
SLC35C1
FBXL20
OTUB2
UPRT
chd2
mxi1
APOA1
apoa4
isotig13249
GSK3A
ncor1
ECE1
RDH14
KIAA1161
SOD3
DCAF17
TRIM2
FKBP3
NDUFB2
PSMB4
HSPE1
C22orf28
PSMA2
POMP
PSMB5
ap2m1a
ABCC2
TSTD1
GSR
TKTL2
npsn
GSTA
TXNDC2
GSTO2

ZNF711
BX470254.2
SNAPC1
HTD2
CYP8B1
TMC7
BCL6
CXXC5
ncor1
im:7151068
METTL1
TAF10
COX8B
FBXO38
HABP2
G6W9YEI01BR086
TADA3
trpm7
ZMYND8
isotig06993
SRSF11
CCDC115
NDUFS1
HMG20B
CUL4A
UNKL
G6W9YEI01B58UW
NEU1
CK046
ING1
MUTED
CLN5
VPS18
FAM173A
TFDP3
TEX10
H1F0
CPOX
SNX14
CENPM
ero1l
PLCD1
cratb
KDM4C
isotig08263
WDR37
G6W9YEI02FKX89
LDHA
JAKMIP1
KDM5B
eno1
isotig03538
hk2
RSG1
BBS12

PIGH
G6W9YEI01CP2B9
CMBL
MTSS1
TMEM214
TRIM13
URI1
PDE4A
ALAD
G6W9YEI01EII6W
TRMT5
GTPBP6
ZMYM4
AASDH
DDB2
RABL5
ddt
CNOT7
C6H6orf125
RDM1
isotig09595
PCYT2
ATF7IP2
DPCD
PFDN6
isotig13262
G6W9YEI02G8CXG
CR932000.1
MLF2
PLP2
fkbp1ab
CHCHD6
ttc25
PFKL
ITPA
HBXIP
POFUT2
C15orf61
KNG1
idh1
MSMO1
HMGCR
SHMT1
HSD17B7
TM7SF2
C14orf1
DHCR7
LSS
SQLE
SC5DL
UCHL3
isotig12715
PSMD5
TMEM147
MMGT1

TMEM241
TM111
isotig02419
ZNHIT3
PTPMT1
SRSF7
sf3b14
FDX1L
AATF
TCIRG1
LRG1
isotig06988
selt1a
DCTN1
NAT8
GNPTG
TPC2L
EXOSC7
C18orf21
FHOD1
TPMT
isotig00666
MTX2
rfx2
CHTOP
MRP63
MRPS7
PCGF1
CCBL1
PINX1
isotig00155
UTP6
CCT5
CCT3
MDH2
VDAC2
ID3
LSM12
CCDC88C
LLPH
RNMTL1
MRPL21
ATP5H
68MP
ATP5J
NDUFB8
ATP5F1
COX5A
ATP5C1
ATP5A1
MDH1
NDUFA10
SLC25A11
METTL20
SLC25A32

TBRG4
CD2BP2
TMEM177
MRPS2
TMEM70
MRPL17
LYRM7
MRPS14
XRCC6BP1
SNRNP25
SNAP47
TMEM69
SMN2
CCDC97
COIL
C1orf109
G6W9YEI02INZL9
CETN2
VTI1B
GPR149
TSC2
isotig20201
RBM5
RBM5
G6W9YEI02JUAL4
MYL1
ANXA1
G6W9YEI02IP0J1
SYPL1
tpm1
PARVA
MYH9
WDR1
ACTG1
CNN2
VCL
isotig07370
PPARA
DGKH
TMEM79
bactin2
DPYSL5
CAPZB
bactin2
capzb
NCSTN
KLHDC5
TMEM111
ZMIZ1
flrt3
GOT1
PPFIA3
isotig11927
PSMD6
COL10A1

CCDC127
ZHX1-C8ORF76
SRCRB4D
isotig15112
YBEY
FAM102A
TXLNG
RABGAP1L
isotig16608
UCN3
SART1
NAPG
sox6
selt2
sumo3a
PIGV
C19H16orf80
UBE2I
EEF1D
FGF6
ITGA5
LAMB3
FDX1
G6W9YEI02HFJGV
RRN3
WDR19
HDHD3
isotig02411
fabp10a
EIF5
ilf3b
G6W9YEI01CRVFL
MFF
PSME1
AASS
NR2C2AP
VWA1
G6W9YEI02HD500
CECR2
EIF2AK1
PCMT1
METAP2
synj1
TSPAN31
ACTR8
RPAP3
NR2C1
G6W9YEI02I82H8
RSAD1
GRHL1
INTS4
BRD9
MED27
TMEM134
IRAK1BP1

COPS2
PPDPF
DNAJB2
DNAJB2
NAPRT1
NFKB2
FAM92A1
GPR137
GPR137
FABP7
TM6S2
ACACA
PAPSS2
TKT
isotig14665
CBY1
isotig11803
SCAMP3
isotig07677
mettl7a
ZCRB1
PORCN
isotig05979
SLC20A2
cited3
SOD2
ACSL3
TBX2
PM20D1
isotig00614
FBXO45
NPC1L1
G6W9YEI02IBUL8
GLO1
ACSL3
CHSY1
ELOVL5
GSTA4
fads2
FASN
SCD
FABP3
EFNA2
alg14
alg14
TMEM126B
PIGY
C5H9orf142
FP067396.1
CXorf38
COQ6
HSPA8
FAM96A
RPP30
gnai1

BTBD1
PGRMC1
HNRNPK
C8orf33
MBNL1
CSNK1A1L
tmem106a
GLT1D1
POLR3K
IFRD1
KANK4
MOGAT3
slc35d1a
G6W9YEI02I1LJH
NFKBIL1
UBIAD1
POP7
ENPP5
C9orf46
G6W9YEI01BB8B9
PNPLA3
AGMO
ABHD12B
FRIM
PAIP2B
OAZ1
eno1
FAH
KLF10
MYL7
ICK
TMEM184C
ELOVL6
ACLY
CNP
G6PD
PSME2
CYP2J2 (3 of 6)
CYP2J2 (3 of 6)
CAMKV
cyp3a65
ME1
PGD
G6PD
PRDX3
PQLC3
PSMB7
DKEY-122A22.2
tuba1
tuba1l
RNASEH1
ADCK3
isotig04451
KIAA0913
G6W9YEI01BX5M5

G6W9YEI01DJJX7
FAM82B
JMJD7
PLS3
capns1b
pgm1
oip5
WDSUB1
APEH
G6W9YEI02HKIW0
PPP1R3B
MAPK15
isotig21844
KLHL24
MYSM1
SLC1A3
G6W9YEI01CHHTX
NARS2
RPUSD1
PIGB
VPS41
FBXO46
isotig02224
ALKBH3
ALKBH3
CLDN12
GORASP1
SMCR7
EGLN1
TF
UBL7
NUBP1
GPN2
TBCD
METAP1
CDC37
MAT2B
RNMT
NME7
CD81
RPP14
WDR41
STOML1
CD40
SGSH
FSD2
isotig06561
CYP2S1
CYP2S1
PGK1
peli1b
pcgf5b
C9orf78
KDM1B
TCEB2

SPRYD7
SERINC1
ZNF672
DCAF11
TMEM50A
isotig09430
Y2408
MAX
ACTR6
COMMD4
FIG4
BLOC1S1
FYTTD1
fxr1
LOH12CR1
DFFB
UBXN7
HMG20A
DCP2
PUS7L
ATP6V1H
VPS41
HSDL1
C16H7orf30
ZFAND1
OGFOD2
G6W9YEI02HVW18
ANGEL1
AHI1
ahi1
ALLC
fabp10a
HPX
RABEPK
AHCY
GSTZ1
CR388231.2
DIO1
SEPHS2
TALDO1
CBR3
AS3MT
AS3MT
AS3MT
CIZ1
RTN4
FABP1
ALDH1L1
RDH12
COMTD1
BPHL
HECA
ASB15
NT5DC2
GPN1

RNF123
gabarapl2
isotig08456
SUMF1
PINK1
TMUB1
BTBD2
C12orf49
FAM168B
BX927314.1
RNF165
RNF165
SSU72
FBXL5
gtf2a1
FBXO18
TDO2
TARDBP
UGDH
WAC
G6W9YEI02FLGRR
isotig17856
isotig10755
NGDN
G6W9YEI01DKO9B
UBN2
MITD1
BX957329.1
G6W9YEI02IAMBA
CAP2
isotig03223
TMEM9B
ATP6V1H
AMT
CHMP4C
PDCD4
isotig03943

List of genes up-regulated by the high lipid diet (p<0,005)
cldni
ATP5G3
G6W9YEI02INLD9
UBTD1
dysfip1
apba2
PVRL1
C3orf17
RLBP1
isotig14534
SNRPE
G6W9YEI02IPW04
RPS27A
SMARCE1
QPRT
ANTXR2

SQSTM1
DBR1
SCUBE2
luc7l
ITPR1
PLK2
PTPRG
isotig20752
PLEC
isotig20203
RBP5
CHD1
hoxb5a
TTC14
MACD1
RTP3
G6W9YEI02F55KE
HERC5
G6W9YEI01A16BO
ISG15
MIA
SLC25A48
PCDH1
G6W9YEI01DOW7D
atp5ib
C7orf44
QCR10
ugt5c1
PBX4
fgfr2
KIF1B
DNAJC7
isotig08508
POLR1A
PARVA
bactin2
CAP1
G6W9YEI02FLGRR
isotig17856
CNN2
ACTR3B
isotig10755
UGDH
isotig07006
RBM5
BCORL1
CECR2
WAC
UBR2
GCFC1
ANGPTL4
isotig12954
EPDR1
G6W9YEI01BZAJH
isotig09430

synj1
PROM1
TLR3
RBM25
ATHL1
IGFBP2
G6W9YEI02HD500
isotig21599
SC6A6
ATP5J2
FZD2
isotig10603
INTS4
isotig09387
VWA1
SELENBP1
RAB24
WDR13
G6W9YEI02FJ9UC
MRPL35
atp5ia
DNAJB2
DNAJB2
TSPAN31
HMGCR
TMEM79
APOA1
tuba1l
G6W9YEI02ISREK
ID3
MDH2
CCT7
CCT2
AK2
CCT4
ATP5G1
ATP5G3
CCT5
ATP5O
NDUFA12
CHCHD10
PFDN1
ATP5F1
COX5A
ATP5C1
ATP5A1
ATP5D
ASPDH
MDH1
COQ9
NDUFA10
SLC25A11
INHBE
INHBE
PLA2G12B

SEC23B
ITSN2
pmt
G6W9YEI02GEBJ2
BLVRA
G6W9YEI02IVFAL
G6W9YEI02JILHM
AGAP9
JUN
TMED5
isotig03795
CCND2
TPD52L2
UQCR10
ZNF346
SLC1A5
EPN3
SC6A2
BNIP2
isotig12762
TIMM17A
isotig19865
VPS13B
SERPINE1
HSPA14
TRIM63
isotig07629
SLC6A19
EIF2B5
IPO4
G6W9YEI01C7W27
BAZ1A
FASTK
G6W9YEI02I9745
MTMR11
CNOT4
RSF1
C10orf76
NMT1
isotig02038
CABZ01055715.1
WDR60
CC2D2A
HSPE1
TSTD1
C5orf35
isotig16889
MYL7
C7orf25
TXNDC2
AS3MT
AS3MT
TKTL2
npsn
TXNDC2

HPD
CCT8
UQCRC1
MRPL15
INPP5K
COX5B
UQCRB
ATP5EP2
ATP5H
68MP
NDUFB10
ATP5J
NDUFB4
USMG5
NDUFA13
MRPL18
ATP5L2
NDUFS5
MGST3
tmem150c
QARS
RARS
AARS
EPRS
HARS2
CHAC1
PSPH
aimp1
DPH5
RPP40
RPP40
PTCD3
G6W9YEI02IOKTL
NUP107
RTCD1
MRPL1
MRPS2
MRPS14
MRPL4
RNMTL1
MRPL17
QRSL1
MRPL42
BOP1
FDX1L
NLE1
IMP4
PINX1
BX005022.2
EXOSC7
isotig10755
NGDN
RIOK1
GUF1
CPSF3

isotig18174
AATF
POLR3F
UTP23
PRKRIP1
ppargc1b
ppargc1b
ppargc1b
SRSF7
MRPS35
WDR74
RG9MTD1
DDX49
NOM1
ATP5J2-PTCD1
BRIX1
MRPS30
RUVBL1
ABT1
CWC15
AHSA1
NIPA1
PDCD11
RBMX2
FBL
WDR36
pprc1
RNF40
ctnnb1
lef1
LEF1
NIPBL
BPTF
MTF2
FITM2
CD68
GET4
SPEN
KIAA2022
DNAJC13
SGPP1
G6W9YEI01ANZJW
ZNF740
PABPN1
FERMT2
FAM161A
LPCAT4
ANGPTL6
MAPT
NT5E
CYP2S1
CYP2S1
DACT2
SLC6A18
CROCC

ONECUT1
isotig05204
CLDN3
EIF3M
TGFB3
PDCL3
IFT122
HHATL
SENP7
RPL38
SLC25A28
NDUFS3
SAG
CASS4
RF12B
isotig16333
BX547998.1
TOP2B
FBF1
MRPL37
MRPL38
SRA1
cplx2
cplx2
ZC3H7B
RGPD2
G6W9YEI01A9J45
C9orf102
G6W9YEI02F0MCT
CERS2
PPTC7
slc6a13
FBLN1
isotig12833
PIK3CB
TP53
TCERG1
NCOA6
CYC1
PPDPF
DLG1
rho
anp32b
ABHD1
ABHD1
COX1
PEPE
isotig15568
VBP1
NAP1L1
EIF3F
TCP1
VDAC2
CCT3
CCT3

HSP90AB1
eef1g
EIF3H
EIF3K
PNO1
GTPBP4
EIF3A
RPS13
hnrnpa0
mibp2
EIF3B
RPL8
RPL27
eif5a
EEF2
RPL3
pabpc1a
GNB2L1
RPL24
RPL7A
RPL11
RPS15A
RPS3A
RPL35A
rps21
RPS9
RPS2
RPS19
RPL30
rps28
RPL36
RPL32
RPS5
RPL13
RPL18A
RPL7
RPS26
RPL4
IUNH
slc6a13
isotig18625
MAST2
MRPL41
NDUFA6
NDUFB8
SMS
SRSF6
CXorf26
LUZP1
myca
isotig08716
SLIT2
isotig14230
nsd1a
KIAA0664

PPP2R1B
SLC7A1
EIF2S2
PSAT1
CARS
YRDC
C20H1orf131
EIF2S3
NDUFAB1
CR396586.2
NPM1
FAM136A
BMS1
PRPF40A
EXOSC2
GLTSCR2
IGF2BP1
mycn
SLMO2
G6W9YEI01A4FFR
ZAR1
atf7ip
nfil3-6

List of genes down-regulated by the high lipid diet (p<0,005)
DAO
C5orf32
C4orf33
CU855789.1
RBM47
ATP1B4
PCDH18
SOST
GNG13
SMOC2
ENPP7
acsbg2
RALB
AL929434.1
isotig01231
ENPEP
KCNMA1
GNAI2
TUBB
XYLA
TGFBR3
SELE
bcl11aa
hs3st1l1
SPTBN2
PTPRD
NFE2L2
GHR
slco2a1
C20orf30

btg1
isotig08370
CDO1
GRB2
PRKCA
gas1b
gas1a
ppap2b
efnb2a
zufsp
USP12
isotig13873
DSC1
SGK2
SGK2
isotig03982
khdrbs1b
RNASEH2B
isotig11803
PORCN
DGAT2
RUNX3
nfia
isotig01458
ugt5e1
pik3r1
gpx1b
ahsg
OVGP1 (3 of 5)
OVGP1 (3 of 5)
G6W9YEI02HXFIM
RNF170
GOSR1
FAM96B
IGBP1
VMA21
ETHE1
PTMS
F13A1
MED25
PEX16
BX957234.1
TAF3
ncoa2
CWC25
isotig08966
LOX
ubl3
isotig09571
PRR12
isotig06900
plekhn1
rybpb
IGBP1
NCAPD2

CR932000.1
NXPH2
isotig14656
PEX5
isotig05892
sox6
SHD
RAB7A
SMNDC1
KANSL3
UBE2I
HSPB8
ZC4H2
fkbp1ab
COMTD1
HSBP1
DIDO1
PER2
HNRNPD
AKIP1
USF1
ATP6
CYTB
isotig08422
serpina1
ITIH3
TF
isotig21886
sb:cb37
FGB
FGG
FGA
C8A
CCDC39
selt2
GP1BB
ARHGAP6
GPR89B
G6W9YEI02GWCQG
isotig13920
PPP4R2
EFNA1
FGFR4
isotig21290
PSMD6
PPFIA3
isotig11927
arrdc1b
SYTL2
CLTC
TRPM1
GGT7
PTPN23
HSF1
isotig17631

OSBPL5
TECR
xpo1a
CECR1
PPP2R5E
isotig00711
DNAJC5
G6W9YEI02HTO63
EVL
B3GNTL1
IGF2
PHLPP1
MYO5C
GPM6A
ZNF185
WNT5B
SLC9A6
PCNX
rho
PPP2R2A
PDE4DIP
prdm16
TFCP2L1
TFCP2L1
FMO4
VSTM2A
NFIX
isotig17702
NFIX
isotig14854
TTYH2
TMPRSS4
GALNTL1
cyp1c1
C20orf30
isotig18697
C17orf103
AGXT
SLC2A9
GOT1
foxo3b
GPD1
nucks1a
HSD17B4
HS2ST1
DECR2
HP1BP3
NRARP
nrarpa
C16orf87
SLC22A18
SLC22A18
ALDH7A1
CAMK2D
pglyrp2

KHK
isotig19287
ZBTB20
F9
ETFA
UCK1
DCTN3
RAD9A
TMCO6
isotig06463
AQP12A
IVD
ANKRD54
POLR2G
LRRC8D
BAP1
isotig06002
isotig04853
G6W9YEI01A7IYK
PDGFRL
AHSG
CYSP1
GMNN
FBX6
BAG2
C11orf54
TMEM59
SDHAF1
G6W9YEI02GW7KA
PEX2
PCBD1
PPP1R37
CUX1
CNPY1
plxna4
GATM
DNAJC9
HDGFL1
APOH
NUDCD2
LYZ
APOH
G6W9YEI02I4IL5
isotig22014
col2a1a
pgm1
RFK
MASP1
FAM53B
nfil3-6
G6W9YEI02GAED7
SEPP1
isotig05773
gna11b
ppp4r2b

gnb1b
HNF4A
HEXIM1
GPT
CLIC5
SERINC2
ptgds
C5
FTHL17
PPAP2B
DOCK1
GGTL2
ggt1
cfhl2
SERPING1
C1R
IFNAR1
PAM
DAB2
PPP1R3D
PRELID1
ZZEF1
ALD2
TUBB4B
TUBB4A
G6W9YEI01C22WX
AP2S1
G6W9YEI01DGYL6
F9
GGCX
SCAMP2
FAM46A
DNAJC14
isotig16640
YWHAB
BZW1
C5orf43
RNF185
UBA3
CPN2
CPN2
CPN2
SERF2
SNAP29
MCFD2
SRPR
SRPRB
isotig06872
sept-15
isotig11426
OSTC
FKBP14
PARP16
FKBP7
SPCS1

AP3M1
AP3M1
TMEM53
SGSM3
DERL2
TMED1
SEC61B
LRRC59
erp44
ALG6
cfhl3
EXOC3L4
MOB3A
YJEFN3
QPCTL
MSN
CHID1
ADD1
isotig07582
isotig19237
KATNAL1
CR626907.1
PRR15L
kcnq1
C3 (5 of 8)
C3 (4 of 8)
CO4
G6W9YEI01ALGJS
FP236513.2
FP236513.2
HAAO
HAAO
thpo
SLC40A1
slc40a1
SYMPK
ASNSD1
SLC35F5
VAMP3
HIPK3
POC1B-GALNT4
isotig13247
arhgap10
NFIX
BX005380.1
isotig13241
FFAR3
CRJ1A
C12orf65
ATRN
ARSE
ATXN10
CYP1A2
MARCH6
trpm7

CLEC4M
MGAT2
CYP20A1
ST3GAL3
ST7
IGFALS
pdcd4a
isotig12739
MTHFD1
HABP2
G6W9YEI01BR086
ALKBH2
isotig02047
ppp1cb
LANCL1
IER3IP1
C8G

List of genes whose expression respond to hypoxia x lipid interaction (p<0,005)
isotig18740
ICK
SLC6A1
G6W9YEI01BZVW9
G6W9YEI01ART0C
FYTTD1
EIF3B
ALYREF
isotig08966
NCOA5
PLD3
sema3fb
ZFYVE20
MACD1
TMEM220
GOLIM4
G6W9YEI02IOKTL
isotig13846
USMG5
NEDD4L
TRIM63
eef1g
PRR18
rdh1
isotig16889
B4GALT5
ppial
SLC25A36
isotig03898
HMGXB4
GTF2A1L
RHOT1
DRG2
ALLC
LOX
BX927362.1

NOL7
BCL7B
GCA
APTX
TCP11L2
isotig06992
SYF2
DCAF8
TNRC6B
HIBADH
VAMP3
ZRANB1
npsn
DNM2
TPPP
FRMD8
RANBP3
TCP11
ARHGDIA
C21orf2
XPC
IL16
AIDA
G6W9YEI02F1UZ9
CR392001.1
PDLIM3
FAM195A
G6W9YEI02J3XJ7
TBPL1
G6W9YEI02F219I
C16orf93
VAV1
ARCN1
TMEM214
ELOVL6
CR388231.2
STAT2
OAZ1
C23H20orf24
LCK
GNL3L
POLDIP3
PABPC4
CDC16
MKLN1
LRIG2
GNB2L1
ASB8
ARF4
eno1
WDR77
HNF4A
ddx3
EPB41
ANGEL1

LMNB2
C20orf24
G6W9YEI02IXA30
FAM175A
CCNB1
PLP2
hdlbp
C16H7orf30
hsp70.3
polr3glb
IK
MTIF2
PCNA
CGREF1
G6W9YEI01EHV0K
isotig06617
APLF
ALKBH3
DDX1
setd8a
BX936371.3
isotig12092
TMC7
COL11A1
GTPBP4
cd63
TARDBP
SCAMP2
SPSB1
CDKAL1
NUDT14
ACP6
isotig11759
cldn17
G6W9YEI02G9SYT
CCNG2
C5orf35
isotig06065
ACTR1B
mapk14b
CHL1
RIMS2
G6W9YEI02G0YDA
HPGD
ZCCHC13
isotig12919
METAP1
DHPS
LRRC39
APOB (1 of 5)
SNRPD3
G6W9YEI02F7Y1I
ACO2
PTPRN2
dok1a

isotig05450
WIPI2
PI4K2B
ASMTL
APLF
G6W9YEI01B58UW
SSRP1
AP1M1
ARL1
isotig18697
CCNDBP1
HSP90AB1
PFN2
NEU1
HEXIM1
ATP2A2
ALKBH3
MYL7
AL929434.1
isotig03341
HDLBP
TXNDC2
TMEM220
psmd11a
KDELR2
isotig20717
CCND2
UROC1
fancg
TMEM53
hspb11
NPEPL1
C20orf20
RP71-7L19.6
SLC25A39
MAPK15
PDLIM7
fam120c
MKS1
EEF2
KHDRBS1
PEF1
MAPK11
EFTUD2
TMED1
AP2A1
ARR3
pcdh17
PLEC
FAM53B
ISG15
EPB41
GLTSCR2
EIF6