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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) (I-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<sup>3</sup>) 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 I-group (NI), Normoxia L-group (NL), Hypoxia I-group (HI), 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 NI, 18 from group NL, 16 from group HI 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 AMPK $\alpha$ , each protein lysate was adjusted to a final concentration of 3.5 mg/ml by adding lysis buffer. Thr172 phosphorylation of AMPK $\alpha$  was detected by the PathScan® Phospho-AMPK $\alpha$  (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-AMPK $\alpha$  (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 AMPK $\alpha$  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 Cy3dCTP. 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 workflow. 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  $\mu$ m 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 AMPK $\alpha$  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 cvcle (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 (AMPK $\alpha$ ), 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 AMPK $\alpha$  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), mitogenactivated 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 upregulation 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 eIF2a, 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 AMPK $\alpha$  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 downregulated 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 dehvdrogenase 1 beta subcomplex subunit 2	NDUFR2	6.92E-4
NADH dehydrogenase 1 beta subcomplex subunit 8 NADH-ubiquinone oxidoreductase 75 kDa subunit NADH dehydrogenase flavoprotein 2	NDUFB8 NDUFS1 NDUFV2	4.63E-4 0.0028 0.0026
TCA intermediate metabolism		
ATP-citrate synthase	ACLY	2.63E-4
Glutamate decarboxylase 1 Isocitrate dehydrogenase NADP-dependent malic enzyme	GAD1 IDH1 ME1	6.03E-4 0.0014 0.0047
Glycolysis		
Eno1 protein	ENO1	4.64E-9
Glucose-6-phosphate 1-dehydrogenase Hexokinase-2 L-lactate dehydrogenase A L-lactate dehydrogenase B 6-phosphofructokinase 6-phosphogluconate dehydrogenase Phosphoglycerate kinase 1 Pyruvate kinase isozyme M1/M2 Transaldolase	G6PD HK2 LDHA LDHB PFKL PGD PGK1 PKM2 TALDO1	2.46E-9 1.37E-4 4.20E-4 7.22E-5 0.0031 1.50E-7 3.93E-5 0.0027 6.53E-7
	TALDOT	0.00
Lipid biosynthesis Arachidonate 12-lipoxygenase Delta(14)-sterol reductase Squalene monooxygenase Acyl-CoA desaturase Lathosterol oxidase Phosphatidylinositol N-acetylglucosaminyl transferase H	ALOX12 TM7SF2 SQLE SCD SC5DL PIGH	8.42E-4 0.0041 1.24E-4 1.93E-6 0.0049 2.77E-4
Phosphatidylinositol N-acetylglucosaminyl transferase B Ethanolamine-phosphate cytidylyltransferase	PIGB PCYT2	1.75E-4 0.0015
Methionine adenosyltransferase 2 subunit beta Lanosterol synthase 3-keto-steroid reductase 3-hydroxy-3-methylglutaryl-coenzyme A reductase Fatty acid synthase 7-dehydrocholesterol reductase	MAT2B LSS HSD17B7 HMGCR FASN DHCR7	5.28E-4 0.0048 0.0037 4.20E-6 2.45E-6 2.28E-5
Aracnidonate 5-lipoxygenase	ALUX5	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 88F-4
ERO1-like protein alpha	ER01L	0.0010
Pentidyl-prolyl cis-trans isomerase FKRP3	EKRP3	0.0019
Heat shock cognate 71 kDa protein	HSPA8	0.0020
10 kDa heat shock protein mitochondrial	HSPE1	0.0012 4 58E-4
Tubulin-specific chaperone D	TBCD	0.0014
Tuberin	TSC2	1 705 5
	1002	1.72E-3
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	ER01L	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	3.20E-0
Proteasome subunit beta 5	PSMBS	
Protoasomo subunit dolta 5	PSIVIDI DSMD5	3.29E-3 1 15E 6
Proteasome activator complex subunit 1	PSME1	4 40E-4
Reticulon-4	RTN4	8.08E-10
Lathosterol oxidase	SC5DI	0.002 10
AcvI-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
I yrosine aminotransterase	IAI	2.10E-4

### Regulation of cell growth and proliferation

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

System	Gene Ontology	Number genes	of (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 4: Gene Ontologies over-represented among genes regulated by high dietary lipid

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 Attractin Complement C1r subcomponent Complement C3 Complement C5 Complement component C8 alpha chain Complement component C8 gamma chain Immunoglobulin-binding protein 1 Lysozyme C Mannan-binding lectin serine protease 1 Phosphatidylinositol 3-kinase regulatory subunit alpha Parathymosin E-selectin Alpha-1-antitrypsin	APOH ATRN C1R C3 C5 C8A C8G IGBP1 LYZ MASP1 PIK3R1 PIK3R1 PTMS SELE SFRPINA1	4.68E-4 0.0026 5.04E-4 3.95E-5 0.0036 0.0049 8.36E-4 9.03E-4 0.0028 2.14E-7 0.0046 8.06E-4 3.04E-4 0.0034
Plasma protease C1 inhibitor	SERPING1	7.32E-4

## Up-regulated

Protein biosynthesis		
AlaninetRNA ligase, cytoplasmic	AARS	7.18E-6
CysteinetRNA 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/prolinetRNA 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
GlutaminetRNA ligase	QARS	2.92E-4
Glutamyl-tRNA(GIn) amidotransferase subunit A	QRSL1	1.70E-5
Arginine tRNA ligase, cytoplasmic	RARS	2.09E-4
60S ribosomal protoin 1 11		
003 11008011181 01018111 E 1 1	RPLII	5.63E-5
60S ribosomal protein L13	RPL11 RPL13	5.63E-5 7.26E-5
60S ribosomal protein L13 60S ribosomal protein L13	RPL11 RPL13 RPL18A	5.63E-5 7.26E-5 1.77E-5
60S ribosomal protein L13 60S ribosomal protein L13 60S ribosomal protein L18a 60S ribosomal protein L24	RPL11 RPL13 RPL18A RPL24	5.63E-5 7.26E-5 1.77E-5 5.58E-4
60S ribosomal protein L13 60S ribosomal protein L18a 60S ribosomal protein L24 60S ribosomal protein L27	RPL11 RPL13 RPL18A RPL24 RPL27	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4
60S ribosomal protein L13 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3	RPL11 RPL13 RPL18A RPL24 RPL27 RPL23	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5
60S ribosomal protein L13 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L3	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0.0036
60S ribosomal protein L13 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0.0013
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL38	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0.0017
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL38 RPL4	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L4	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL38 RPL38 RPL4 RPL7	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL36 RPL38 RPL4 RPL7 RPL7 RPL7A	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L18a 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7a 60S ribosomal protein L8	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL38 RPL38 RPL4 RPL7 RPL7A RPL7 RPL7A RPL8	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein L8 40S ribosomal protein S13	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL35A RPL36 RPL38 RPL4 RPL7 RPL7 RPL7A RPL7 RPL7A RPL7 RPL7A RPL8 RPS13	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0.0030
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein S13 40S ribosomal protein S13	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL36 RPL38 RPL4 RPL7 RPL7A RPL7A RPL7A RPL7A RPL7A RPL8 RPS13 RPS15A	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein L8 40S ribosomal protein S13 40S ribosomal protein S15 40S ribosomal protein S19	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL36 RPL38 RPL4 RPL7 RPL7 RPL7A RPL7 RPL7A RPL7 RPL7A RPL13 RPS13 RPS15A RPS19	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4 0.0012
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L38 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7a 60S ribosomal protein L7a 60S ribosomal protein S13 40S ribosomal protein S15a 40S ribosomal protein S19 40S ribosomal protein S19 40S ribosomal protein S2	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL38 RPL4 RPL7 RPL7 RPL7A RPL7 RPL7A RPL7 RPL7A RPL8 RPS13 RPS15A RPS19 RPS2	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4 0,0012 8.28E-4
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein S13 40S ribosomal protein S15a 40S ribosomal protein S19 40S ribosomal protein S2 40S ribosomal protein S2	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL36 RPL38 RPL4 RPL7 RPL7A RPL7 RPL7A RPL7 RPL7A RPL7 RPL7A RPL33 RPS13A RPS15A RPS15A RPS19 RPS2 RPS21	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4 0,0012 8.28E-4 0.0041
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein S13 40S ribosomal protein S15a 40S ribosomal protein S19 40S ribosomal protein S2 40S ribosomal protein S21 40S ribosomal protein S21	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL36 RPL38 RPL4 RPL7 RPL7A RPL7A RPL7A RPL7A RPL7A RPL7A RPL7A RPL7A RPS13 RPS15A RPS19 RPS2 RPS21 RPS26	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4 0,0012 8.28E-4 0,0041 0.0041
60S ribosomal protein L11 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein S13 40S ribosomal protein S15a 40S ribosomal protein S19 40S ribosomal protein S2 40S ribosomal protein S21 40S ribosomal protein S21	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL36 RPL38 RPL4 RPL7 RPL7A RPL7 RPL7A RPL7 RPL7A RPS13 RPS15A RPS19 RPS21 RPS21 RPS26 RPS27A	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4 0,0012 8.28E-4 0,0041 0,0041 1.52E-4
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein S13 40S ribosomal protein S15a 40S ribosomal protein S15a 40S ribosomal protein S15a 40S ribosomal protein S2 40S ribosomal protein S21 40S ribosomal protein S27a 40S ribosomal protein S27a 40S ribosomal protein S27a	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL36 RPL38 RPL4 RPL7 RPL7 RPL7A RPL7 RPL7A RPL7 RPL7A RPS13 RPS15A RPS15A RPS19 RPS21 RPS21 RPS22 RPS21 RPS22 RPS27A RPS28	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4 0,0012 8.28E-4 0,0041 0,0041 1.52E-4 5.82E-4
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L30 60S ribosomal protein L30 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein S13 40S ribosomal protein S15a 40S ribosomal protein S15a 40S ribosomal protein S21 40S ribosomal protein S21 40S ribosomal protein S27a 40S ribosomal protein S27a 40S ribosomal protein S27a 40S ribosomal protein S27a 40S ribosomal protein S28 40S ribosomal protein S28 40S ribosomal protein S28 40S ribosomal protein S28	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL38 RPL4 RPL7 RPL7A RPL7A RPL7A RPL7A RPL7A RPS13 RPS15A RPS15A RPS15A RPS21 RPS26 RPS27A RPS28 RPS23A	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4 0,0012 8.28E-4 0,0041 1.52E-4 5.82E-4 0,0014
60S ribosomal protein L17 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein S13 40S ribosomal protein S15a 40S ribosomal protein S19 40S ribosomal protein S21 40S ribosomal protein S26 40S ribosomal protein S27a 40S ribosomal protein S27a 40S ribosomal protein S28 40S ribosomal protein S33 40S ribosomal protein S33 40S ribosomal protein S27a 40S ribosomal protein S3 40S ribosomal protein S3 40S ribosomal protein S3 40S ribosomal protein S3	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL36 RPL38 RPL4 RPL7 RPL7A RPL7A RPL7A RPL7A RPS13 RPS15A RPS19 RPS21 RPS21 RPS26 RPS27A RPS28 RPS27A RPS28 RPS3A RPS3A RPS5	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4 0,0012 8.28E-4 0,0041 0,0041 1.52E-4 5.82E-4 0,0014 0,0033
60S ribosomal protein L11 60S ribosomal protein L13 60S ribosomal protein L24 60S ribosomal protein L27 60S ribosomal protein L3 60S ribosomal protein L30 60S ribosomal protein L35a 60S ribosomal protein L36 60S ribosomal protein L38 60S ribosomal protein L4 60S ribosomal protein L7 60S ribosomal protein L7 60S ribosomal protein S13 40S ribosomal protein S15a 40S ribosomal protein S15a 40S ribosomal protein S19 40S ribosomal protein S2 40S ribosomal protein S3 40S ribosomal protein S3 40S ribosomal protein S3 40S ribosomal protein S5 40S ribosomal protein S5 40S ribosomal protein S9	RPL11 RPL13 RPL18A RPL24 RPL27 RPL3 RPL30 RPL35A RPL36 RPL36 RPL38 RPL4 RPL7 RPL7A RPL7 RPL7A RPL7 RPL7A RPS19 RPS21 RPS21 RPS21 RPS22 RPS21 RPS22 RPS221 RPS28 RPS2	5.63E-5 7.26E-5 1.77E-5 5.58E-4 8.19E-4 7.41E-5 0,0036 0.0029 0,0013 0,0017 1.37E-5 6.62E-4 5.86E-5 1.44E-4 0,0030 4.43E-4 0,0012 8.28E-4 0,0041 0,0041 1.52E-4 5.82E-4 0,0014 0,0033 3.39E-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
Glucoso motabolism		
Hopatocyte nuclear factor 6		0.0031
	ONLOOTT	0.0001
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	ATP50	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 On	tology	Number of genes	Corrected (Benjamini) p-value
Cellular component Molecular function	mitochondrion hydrogen ion activity	transporter	16 8	0.01 0.01

### Figures

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



Figure 2: Quantification of threonine 172 phosphorylation in the alpha subunit of AMPK in the Normoxia I-group (NI), Normoxia L-group (NL), Hypoxia I-group (HI) 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-AMPKa



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-AMPK $\alpha$  (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 isotiq03223 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 ATP50 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 ΤF 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 isotiq13846 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