A global view on TMT-based quantitative proteomic analysis of rat livers during acute hypoxia

Jin Xu
Qinghai University
Zhan Wang
Tsinghua University
Wen-jie Chen
Qinghai University
Ming-yuan Xin
Qinghai University
Shen-han Gao
Qinghai University
Kai-kun Wang
Qinghai University
Wen-jing Liu
The graduate student of foundation medical of 2020
Jing-wei Ma
Qinghai University
Tawni Tidwell
University of Wisconsin-Madison
Yan-ming Ren (QH202120212021@163.com)
Qinghai University

Research Article

Keywords: Proteomics, TMT, Hypoxia, Metabolism

Posted Date: April 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1528702/v1

Abstract

Background: Acute hypoxia consistently results in altitude sickness and can be fatal. Until present, no studies focusing on global proteomic changes induced by acute hypoxia have been reported. Here, we combined animal experiments and tandem mass tag (TMT)-based proteomic analysis to identify metabolic changes as a result of acute hypoxia.

Methods: We first generated a rat model under acute hypoxia conditions, and combined animal experiments and TMT-based proteomics to identify metabolic changes under acute hypoxia. Then we used qPCR analyses to validate the key regulators, and present a schematic model of acute reactions occurring in the livers of rats subjected to acute hypoxia challenge.

Results: We identified a large number of acute hypoxic responsive proteins in diverse biological pathways, which helped unveil the different mechanisms involved in hypoxia responses in rats. These pathways included those of peroxisome, peroxisome proliferator-activated receptor (PPAR) signaling, lipid metabolism, glycolysis/gluconeogenesis, and amino acid metabolism. According to data obtained from proteomic analysis, rats were able to maintain normal physical activity as a response to acute hypoxia by activating their catabolic capacity in order to get more energy (e.g., lipolysis and amino acid catabolism), and decreasing biosynthesis to reduce energy consumption (e.g., biosynthesis of amino acids and lipids).

Conclusions: We identified a large number of acute hypoxia-responsive proteins associated with diverse biological pathways, and showed rats quickly respond to acute hypoxia by activating lipid biosynthesis to increase lipid storage, and reducing lipolysis to reduce energy consumption. The observed hypoxia-related changes in the liver proteome of rats provide a deeper understanding on the mammalian response to hypoxia.

1. Introduction

Hypoxia is usually triggered by an inadequate supply of oxygen that leads to a global change in the levels of proteins expressed in most human tissues [1]. The liver is involved in most biological processes in the body and plays a pivotal role in maintaining homeostasis. The most renowned hypoxia-responding molecules are the hypoxia inducible factors, also known as HIFs [2]. Although several studies have previously revealed HIF-dependent and HIF-independent effects related to responses to hypoxia stress in multiple tissues across mammal species, the molecular mechanisms associated with these responses remain poorly understood, particularly in liver and adipose tissues.

Hypoxia generally occurs in high altitude areas, where it leads to altitude sickness, and can sometimes result in fatalities following high-altitude cerebral and pulmonary edemas (HACE, HAPE) [3]. For example, in patients with cardiorespiratory diseases, acute hypoxia reduces the production of cytochrome P450 subfamily members, including CYP1A, which further increases the probability of heart failure [4]. Accordingly, from therapeutic and medical perspectives, acute hypoxia most likely blocks the clearance of drugs performed by the P450 subfamilies in the liver, causing potential harm to patients. The liver is well-

recognized as a central organ that metabolizes glucose, glycogen and lipids, supplying energy-producing fuels to the adjacent tissues in order to maintain homeostasis under different conditions, including chronic and acute hypoxia [5]. For example, variation of lipid metabolism caused by acute hypoxia involves the activation of a number of key enzymes and metabolic pathways. However, research studies focusing on global transcriptomic and proteomic changes induced by acute hypoxia remain very limited.

Proteomics is a powerful tool in analytical biochemistry for determining the amount of proteins present in multiple sample types [6]. Tandem mass spectrometry (MS/MS, or MS^2)-based proteomics is a cutting-edge technique that can accurately quantify approximately 10,000 proteins in up to 10 samples within a single run [7]. Proteomics is thus very powerful to detect biomarkers, analyze biological processes, and unveil differentially expressed proteins in different samples and tissues [8]. These characteristics make proteome techniques particularly suitable to explore global changes in protein levels following acute hypoxia treatment.

In this study, we combined animal experiments and TMT-based proteomic analysis to identify metabolic changes resulting from acute hypoxia. Data analyses identified ANGPTL4 and novel proteins regulating lipid metabolism and downstream metabolic pathways. We showed acute hypoxia induces global metabolic changes in the liver and constructed a potential regulatory network of metabolic changes induced by acute hypoxia.

2. Materials And Methods

2.1 Acute Hypoxia Rat models

Sprague-Dawley male rats were randomly allocated to 2 groups (10 animals per group): a group of normal rats (L), and a group of rats exposed to acute hypoxia for 3 days, respectively. Rats in the latter group were exposed to a simulated altitude atmosphere with 5,500 m (380 mmHg), which was implemented using a FLYDWC50-1C low pressure hypoxic experimental cabin (Guizhou Fenglei Air Ordinance LTD, Guizhou, China).

During breeding and experimental procedures, animals in both groups were housed with similar densities per cage under a controlled ambient temperature of 25 ± 2°C, a relative humidity of 50 ± 10%, and a 12 h light/12 h dark cycle. Rats were given standard rodent chow and water ad libitum. Following overnight fasting, rats were sacrificed with anesthesia with 10% chloral hydrate (0.4 ml/100 g body weight, IP). The right lobe of the liver was snap-frozen in liquid nitrogen and then stored at − 80°C for downstream analyses. Control rats (L) were anesthetized and sacrificed on day 1 under similar conditions as described above. The research protocol was approved by the Animal Subject Protection Committee at the Qinghai University School of Medicine (Xining, China)

2.2 RNA preparation and quality control

Total RNA from samples was extracted with Trizol. Quality control tests of extracted RNA were performed using the Thermo Nanodrop 3000 and the Agilent 2100 bioanalyzer using the Agilent RNA 6000 Nano Kit. Quality RNA was subjected to microarray analysis until the following standards were achieved: 1.8 < A260 / A280 < 2.2 with Thermo Nanodrop 3000, and RIN > = 7 and 28S/18S > 1.5 in the Agilent 2100 bioanalyzer.

2.3 Plasma and hepatic lipid profiles

Plasma lipids were measured on Days 0, 15, and 30. Plasma triglycerides (TG), total cholesterol (TC) levels, low-density lipoproteins (LDL), high-density lipoproteins (HDL), and very-low density lipoproteins (VLDL) were measured using the Vitros DT60 II Chemistry System (Johnson & Johnson, Minnesota, MN). The liver samples were homogenized using a Stir-Pak®, (Barrington, Il). TC and TG were extracted in a chloroform-methanol mixture (2:1) and measured with the same Vitros DT60 II Chemistry System.

2.4 Protein Extraction and Digestion

Proteins were extracted with a protein extraction kit (Promega, USA), and quantified with a BCA Protein Assay Kit (Bio-Rad, USA); the protein content of each sample was 1g. We then performed SDS-page gel electrophoresis and Coomassie bright blue staining. Finally, the protein suspension was digested with trypsin (Promega) in NH_4HCO_3 at a temperature of 37 °C overnight. The proteins yields were filtered and approximately 160 mg of protein were collected.

2.5 TMT Protein labelling and High pH Reversed Phase Fractionation (HPRP)

TMT reagents were used for labeling proteins according to the manufacturer's instructions (Thermo Fisher Scientific). The samples were then collected and stored in ice before LC-MS analysis was performed.

2.6 LC-MS Analysis

Each sample was injected once for a total of 16 times for MS/MS analysis. The HPLC liquid phase system was used for phase separation. Proteins were analyzed using the QE-Plus software (Thermo Scientific, Waltham, USA). The resolution of the first-level mass spectrometry was 65,000 at m/z 200. The first-level Maximum IT was set to 49 ms and AGC was set to 1e6. The resolution of the second-level mass spectrometry was set to 35.

2.7 Bioinformatic Analysis

We used the Prisma software and the R statistical computing software to analyze the bioinformatics data. Proteins were screened with a cut off ratio fold-change of > 1.30 and a p-value < 0.05 was considered significant. Hierarchical clustering was used to visualize protein levels. We used R statistical software to compute enrichment analyses with Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Ontology (GO) pathways (Cran Inc., USA).

2.8 Data analysis and statistics

The results were inserted into a database and analyzed using SPSS 22.0 (SPSS, Inc., Chicago, Ill, U.S.A.). The mean, standard deviation, standard error, and confidence intervals were calculated for each parameter. Data are presented as the mean ± SD. Student's t tests were applied, when appropriate, to determine statistically significant differences. We also estimated Pearson's correlations coefficients. The results were considered significant when the p-value was lower than 0.05.

3. Results

3.1 General workflow and plasma lipid metabolic profiles

To explore the influence of acute hypoxia on lipid metabolism, we first built an acute hypoxia animal model in Sprague-Dawley male rats following a previous report (Elmedal et al. 2004). Briefly, rats were randomly allocated to 2 groups (3 animals per group): a group containing normal rats (L) and a group of rats exposed to acute hypoxia for 3 days. The workflow is shown in Fig. 1A. We then profiled biochemical parameters and serum lipids on Day 3. Serum alanine aminotransferase (ALT) and blood glucose (GLU) did not significantly change after exposure to acute hypoxia. However, serum triglycerides (TG), total cholesterol (CHO), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were all significantly affected by acute hypoxia conditions (Fig. 1B). Interestingly, cholesterol and TG levels were highest ($p < 0.05$) after 3 days of exposure, while HDL showed an opposite pattern. The LDL levels were significantly increased ($p < 0.05$), which is consistent with previous reports (Bruder et al. 2005; Siques et al. 2007; Drager et al. 2012; Siques et al. 2014).

These results demonstrated that acute hypoxia may play a pivotal role in lipid and glucose metabolism.

3.2 KEGG enrichment analyses of differentially expressed proteins

We analyzed KEEG pathways enriched to identify canonical pathways associated with responses to acute hypoxia. We surveyed existing data and found protein precursors mainly involved in metabolic pathways, steroid hormone biosynthesis, retinol metabolism, chemical carcinogenesis, PPAR signaling pathways and glutathione metabolism (Fig. 2A). We also found several metabolic pathways, including lipid metabolism, cofactors and vitamins metabolism, amino acid metabolism, carbohydrate metabolism and energy metabolism (Fig. 2B). These results revealed that acute hypoxia significantly affects metabolic pathways in rat livers.

3.3 GO enrichment analysis of differentially expressed proteins

To explore the potential functions associated with differentially expressed proteins after exposure to acute hypoxic conditions, we performed GO analysis. We found the following enriched cellular components: extracellular region, membrane-enclosed lumen, macromolecular complex, membrane, cell part, and cell junction (Fig. 3A). In terms of molecular function, we identified the following enriched categories: catalytic activity, binding, transporter activity, molecular function regulation, structural molecule activity, and transporter activity (Fig. 3A). Finally, we found the following enriched biological processes: cellular process, single-organism process, biological regulation, metabolic process, positive regulation of biological process, response to stimulus, and developmental process (Fig. 6A). Furthermore, we found several enriched metabolic processes, including the carboxylic acid metabolic process, a response to another organism, a response to an external biotic stimulus, a response to a biotic stimulus, the organic acid biosynthesis process, the oxidoreductase activity process, and the oxygen-containing compound process (Fig. 3B). These findings showed that acute hypoxia significantly affects metabolic processes and activates responses to hypoxic stimuli in rat livers.

3.4 Co-expression network analysis of differential expressed proteins

Figure 4 shows the co-expression network containing the most significant differentially expressed proteins in rat livers following after acute hypoxia. The main proteins include SLC24A1, Aldh2, Acly, Acacb, Akr1c3, Cyp1a1, Cyp1a2, Cyp4a8, ACSM2, Rap1a, Lsg15, Phgdh, Hsd17b2 and alox15. These proteins have a close relation with fatty acid metabolism.

3.5 Validation of differentially expressed proteins between control and hypoxia samples using real-time qPCR

After analyzing the differentially expressed proteins with GO, KEGG and co-expression network, we focused on fatty acid metabolism, which was the most enriched pathway following an acute hypoxia stimulus. Figure 5 shows the expression of the genes FADS2, ANGPTL4, SLC24A1, ACSM2, CYP1A1 and CYP1A2 as measured by real-time qPCR. These results were in agreement with the proteomic analysis.

3.6 A schematic model of acute reactions in rat livers subjected to acute hypoxia

The qRT-PCR results analysis revealed that most expressed genes and proteins share similar expression patterns to those observed with proteomic data. Based on bioinformatic analysis and validation data, we constructed a schematic model of the responses to acute hypoxia taking place in rat livers. We provide an overview of the co-expression network, metabolic pathways and regulation effects after acute hypoxia.

4. Discussion

TMT-based profiling has become a powerful and useful biochemical tool for protein quantification in recent years [7]. TMT-based protein quantification explained the relationship between long-term moderate exercise and key neurotrophic signaling pathways in rats [9]. In 3D rat brain cell cultures, a global view on protein pathways were unveiled using TMT-based protein quantification [10]. Accordingly, the screening of deregulated proteins might provide a deeper understanding of the molecular mechanisms regulating responses to specific stimuli.

High altitude exposure often triggers hypoxia [11]. To the best of our knowledge, no study has previously investigated liver metabolism in rats subjected to acute hypoxia using proteomic techniques. The liver is one of the most important organs for an organism in maintaining homeostasis and regulating metabolism, including lipid, glucose, and amino acid metabolism, as well as synthesizing and redistributing metabolic substances [12]. Here, we focused on studying the liver of rats under hypoxia exposure and investigated the global metabolic changes by TMT-based protein quantification techniques. We note that metabolic pathways were amongst the most significantly enriched as estimated by GO and KEGG analyses. Accordingly, we discuss our findings by mainly focusing on metabolic-related proteins.

Metabolic changes are a major cause of illness, including for liver and cardiovascular diseases, and hypoxia-related disorders. Lipid metabolism plays a pivotal role throughout the illness process [12–14], and is closely associated with acute hypoxia disorders, including lipid digestion, absorption, transport, catabolism and biosynthesis [15]. We generated an acute hypoxia rat model and tested several lipid metabolism serum markers, including LDL, HDL, TG, CHO, GLU and ALT. Our results showed that LDL, TG and CHO were significantly elevated in the liver following acute hypoxia. In contrast, HDL levels were significantly decreased in the liver. These results are in accordance with previous studies in mice and rats [15, 16], demonstrating that our acute hypoxia model in rats was successfully generated, and indicating a global inhibition of lipid metabolism.

To uncover the intracellular mechanisms that occur during an acute hypoxia challenge, we performed a TMT-based proteomic experiment on liver samples. KEGG analysis of differentially expressed proteins showed metabolism-related pathways were the mostly significantly enriched, especially those associated with lipid metabolism. These included lipid metabolic pathways, steroid hormone biosynthesis, retinol metabolism, chemical carcinogenesis, PPAR signaling pathway and glutathione metabolism.

These two pathways play important roles in homeostasis and control of lipid metabolism by balancing biosynthesis and lipolysis, and controlling biodegradation after oxidative stress [17–20]. We found the activation of lipid biosynthesis, including the significantly upregulated expression levels of the genes SLC24A1, ACSM2, FADS2, ALOM5, and ANGPTL4, along with the downregulated levels of ACLY and SLC24A1 play an important role in sodium/calcium exchange in retinal rod and cone photoreceptors by mediating the extrusion of one calcium ion and one potassium ion in exchange for four sodium ions. Several diseases have been previously associated with SLC24A1, including night blindness, congenital stationary forms of night blindness, and Type 1 diabetes [21]. Among the related pathways to SLC24A1are metabolism of fat-soluble vitamins and transport of glucose and other sugars, bile salts and

organic acids, metal ions and amine compounds. As for ACSM2, protein is very important for catalyzing fatty acid activation, which is the first step in the metabolism of fatty acids. ACSM2-related pathways include amino acid conjugation and metabolism [22], while gene ontologies-related to this gene include butyrate-CoA ligase activity. FADS2 is a member of the fatty acid desaturase (FADS) gene family that regulates the desaturation of fatty acids through the introduction of double bonds between defined carbons of the fatty acyl chain. Diseases associated with FADS2 include Fanconi anemia complementation group D2 and best vitelliform macular dystrophy (BVMD) [23]. Some of the related pathways to this gene encompass alpha-linolenic acid (ALA) metabolism and fatty acid beta-oxidation (peroxisome). In the case of ANGPTL4, this protein is induced by peroxisome proliferation activators and functions as a serum hormone that regulates glucose homeostasis, lipid metabolism, and insulin sensitivity [24]. ANGPTL4 can also act as an apoptosis survival factor for vascular endothelial cells and can prevent metastasis by inhibiting vascular growth and tumor cell invasion. Diseases associated with ANGPTL4 include quantitative trait locus (QTL) related to plasma triglyceride levels and gastric antral vascular ectasia (GAVE). Among its related pathways are the regulation of lipid metabolism by peroxisome proliferator-activated receptor alpha (PPARalpha) and developmental processes. Taken together, the upregulation of SLC24A1, ACSM2, FADS2, ALOM5, and ANGPTL4 and the downregulation of ACLY indicate acute hypoxia impacts the activation of lipid biosynthesis.

Besides the activation of lipid biosynthesis, we also found a reduction of lipolysis, including significantly upregulated levels of the genes CYP1A1, CYP1A2, both of which play important roles in lipid metabolism, and encode the cytochrome P450 superfamily of enzymes [25]. Cytochrome P450 proteins are monooxygenases that catalyze several reactions in drug metabolism and synthesis of cholesterol, steroids and other lipids. We also found genes associated with oxidative stress-related metabolic activities, including upregulated levels of GSTA3 and downregulation of S100A8 and S100A9. We validated these findings by qPCR analyses (Fig. 5). When compared to the control group, levels of FADS2, SLC24A1, ASCM2, ANGPTL4, CYP1A1 and CYP1A2 in the livers of the rat group submitted to acute hypoxia challenge were in agreement with the proteomic results.

In a global metabolic perspective, we found upregulated levels of PDK4, ND2, and FN3K and downregulated levels of G6PD, UGT21B, ALDH2 and TIMM17A in the livers of rats exposed to acute hypoxia compared to controls. We hypothesize a shift in oxygen delivery given the observed enrichment in the IFG/PI3K/AKT and AMPK pathways based on GO analysis. Finally, the significant upregulation of GIFYF1 and downregulation of ACMSD, ANPEP and SLC38A3 suggest inhibition of amino acid biosynthesis.

5. Conclusion

While it is not possible to completely account for all the mechanisms regulating the observed changes in a rat liver exposed to acute hypoxia, we generated a global view on the proteomic changes in metabolism. Our study identified a large number of acute hypoxic responsive proteins related to several biological pathways. According to our analysis, rats quickly respond to acute hypoxia by activating lipid

biosynthesis and promote lipid storage, and by reducing lipolysis to conserve energy consumption. The observed changes associated with acute hypoxia in the liver proteome provide a deeper understanding of hypoxia-related responses under similar conditions in humans.

Declarations

Ethics approval and consent to participate

Medical Ethics Committee of Qinghai University School of Medicine approved the study (2019-ZJ-876).

Consent for publication

All participants signed informed consent regarding publishing their data.

Availability of data and materials

The data and materials of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by the National Natural Science Foundation of China (grant No.2016-ZJ-924Q).

Authors' contributions

Jin Xu, Zhan Wang: design, statistical analysis, interpretation of results, and manuscript writing. Kai-kun Wang, Wen-jie Chen, Ming-yuan Xin and Shen-han Gao: design, statistical analysis, interpretation of results, and manuscript writing. Wen-jing Liu and Jing-wei Ma: design and reviewing. Tawni Tidwell and Yan-ming Ren: design, interpretation of the result, reviewing. The final manuscript was read and approved by all writers.

Acknowledgements

We thank the institutional resources and related staff for their contributions in facilitating a supportive environment and numerous helpful conditions to complete this study.

References

1. Cai W, Liu Z, Li G, Xiao P, Lv Q, Gong Y, Fan H, Hou S and Ding H. The effects of a graded increase in chronic hypoxia exposure duration on healthy rats at high-altitude. Int J Clin Exp Pathol 2019; 12: 1975–1991.

2. Chen X, Yao JM, Fang X, Zhang C, Yang YS, Hu CP, Chen Q and Zhong GW. Hypoxia promotes pulmonary vascular remodeling via HIF-1alpha to regulate mitochondrial dynamics. J Geriatr Cardiol 2019; 16: 855–871.
3. Huang X, Zhou Y, Zhao T, Han X, Qiao M, Ding X, Li D, Wu L, Wu K, Zhu LL and Fan M. A method for establishing the high-altitude cerebral edema (HACE) model by acute hypobaric hypoxia in adult mice. J. Neurosci. Methods 2015; 245: 178–181.
4. Wang J, Wu Y, Yuan F, Liu Y, Wang X, Cao F, Zhang Y and Wang S. Chronic intermittent hypobaric hypoxia attenuates radiation induced heart damage in rats. Life Sci. 2016; 160: 57–63.
5. Hadjihambi A, De Chiara F, Hosford PS, Habtetion A, Karagiannis A, Davies N, Gourine AV and Jalan R. Ammonia mediates cortical hemichannel dysfunction in rodent models of chronic liver disease. Hepatology 2017; 65: 1306–1318.
6. Riley NM and Coon JJ. Phosphoproteomics in the Age of Rapid and Deep Proteome Profiling. Anal Chem 2016; 88: 74–94.
7. Moulder R, Bhosale SD, Goodlett DR and Lahesmaa R. Analysis of the plasma proteome using iTRAQ and TMT-based Isobaric labeling. Mass Spectrom Rev 2018; 37: 583–606.
8. Morrow BA, Redmond AJ, Roth RH and Elsworth JD. The predator odor, TMT, displays a unique, stress-like pattern of dopaminergic and endocrinological activation in the rat. Brain Res 2000; 864: 146–151.
9. Wei Y, Fang CL, Liu SJ, Yang WQ, Wei LS, Lei XJ, Hu F, Huang HY, Li W, Chen W, Li LM and Long YS. Long-term moderate exercise enhances specific proteins that constitute neurotrophin signaling pathway: A TMT-based quantitative proteomic analysis of rat plasma. J Proteomics 2018; 185: 39–50.
10. Schvartz D, Gonzalez-Ruiz V, Walter N, Antinori P, Jeanneret F, Tonoli D, Boccard J, Zurich MG, Rudaz S, Monnet-Tschudi F, Sandstrom J and Sanchez JC. Protein pathway analysis to study development-dependent effects of acute and repeated trimethyltin (TMT) treatments in 3D rat brain cell cultures. Toxicol. In. Vitro 2019; 60: 281–292.
11. Kelly LP and Basset FA. Acute Normobaric Hypoxia Increases Post-exercise Lipid Oxidation in Healthy Males. Front Physiol 2017; 8: 293.
12. Siques P, Brito J, Naveas N, Pulido R, De la Cruz JJ, Mamani M and Leon-Velarde F. Plasma and liver lipid profiles in rats exposed to chronic hypobaric hypoxia: changes in metabolic pathways. High Alt Med Biol 2014; 15: 388–395.
13. Zhao J, You G, Yin Y, Zhang Y, Wang Y, Chen G, Zhao L and Zhou H. Acute high-altitude exposure shortens survival after uncontrolled hemorrhagic shock in rats. J Surg Res 2018; 226: 150–156.
14. Wende AR. Post-translational modifications of the cardiac proteome in diabetes and heart failure. Proteomics Clin Appl 2016; 10: 25–38.
15. Soler-Argilaga C, Infante R and Polonovski J. Lipid metabolism of rat liver isolated and perfused in hypoxia. Biomedicine 1974; 20: 154–159.

16. Mylonis I, Simos G and Paraskeva E. Hypoxia-Inducible Factors and the Regulation of Lipid Metabolism. Cells 2019; 8:
17. Oh DY, Nam I, Hwang S, Kong H, Lee H, Ha J, Baik M, Oh MH, Kim S, Han K and Lee Y. In vivo evidence on the functional variation within fatty acid synthase gene associated with lipid metabolism in bovine longissimus dorsi muscle tissue. Genes Genomics 2018; 40: 289–294.
18. Xue Z, Hou X, Yu W, Wen H, Zhang Q, Li D and Kou X. Lipid metabolism potential and mechanism of CPe-III from chickpea (Cicer arietinum L.). Food Res Int 2018; 104: 126–133.
19. Maatta J, Sissala N, Dimova EY, Serpi R, Moore LG and Koivunen P. Hypoxia causes reductions in birth weight by altering maternal glucose and lipid metabolism. Sci Rep 2018; 8: 13583.
20. Cao R, Zhao X, Li S, Zhou H, Chen W, Ren L, Zhou X, Zhang H and Shi R. Hypoxia induces dysregulation of lipid metabolism in HepG2 cells via activation of HIF-2alpha. Cell. Physiol. Biochem 2014; 34: 1427–1441.
21. Vinberg F, Wang T, Molday RS, Chen J and Kefalov VJ. A new mouse model for stationary night blindness with mutant Slc24a1 explains the pathophysiology of the associated human disease. Hum Mol Genet 2015; 24: 5915–5929.
22. Ellis JM, Bowman CE and Wolfgang MJ. Metabolic and tissue-specific regulation of acyl-CoA metabolism. PLoS One 2015; 10:
23. Glaser C, Heinrich J and Koletzko B. Role of FADS1 and FADS2 polymorphisms in polyunsaturated fatty acid metabolism. Metabolism 2010; 59: 993–999.
24. Singh AK, Aryal B, Chaube B, Rotllan N, Varela L, Horvath TL, Suárez Y and Fernández-Hernando C. Brown adipose tissue derived ANGPTL4 controls glucose and lipid metabolism and regulates thermogenesis. Molecular metabolism 2018; 11: 59–69.
25. Lu J, Shang X, Zhong W, Xu Y, Shi R and Wang X. New insights of CYP1A in endogenous metabolism: a focus on single nucleotide polymorphisms and diseases. Acta Pharmaceutica Sinica B 2019;

Figures

Figure.1 General workflow of the present study.

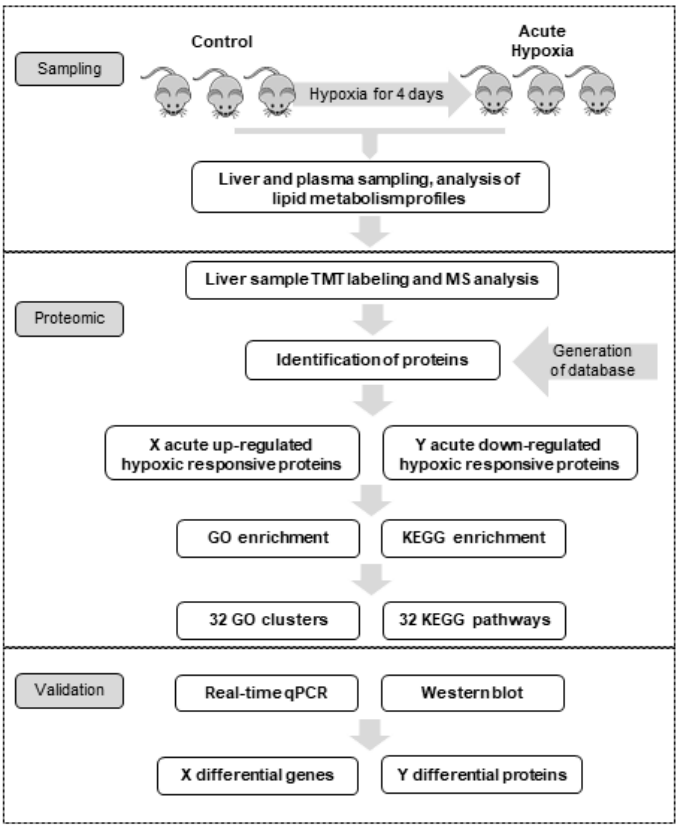

Figure 1

General workflow of the present study.

Figure.2 Plasma lipid metabolism profiles.

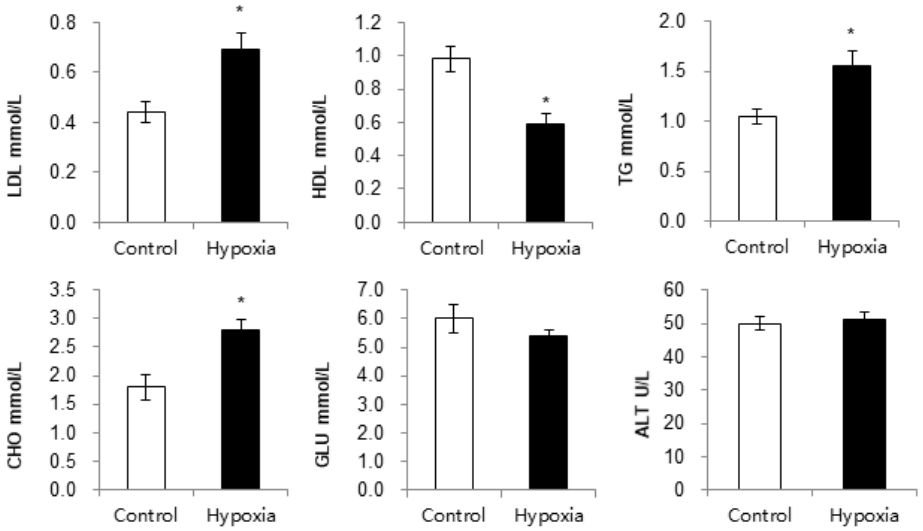

Figure 2

Plasma lipid metabolic profiles. Plasma lipid metabolic profiles, including LDL, HDL, TG, CHO, GLU and ALT levels. Student's T-Test, paired tail, * represents p-value < 0.05.

Figure.3 KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses of all differentially expressed proteins (*p-value* of Fisher's Exact Test < 0.05).

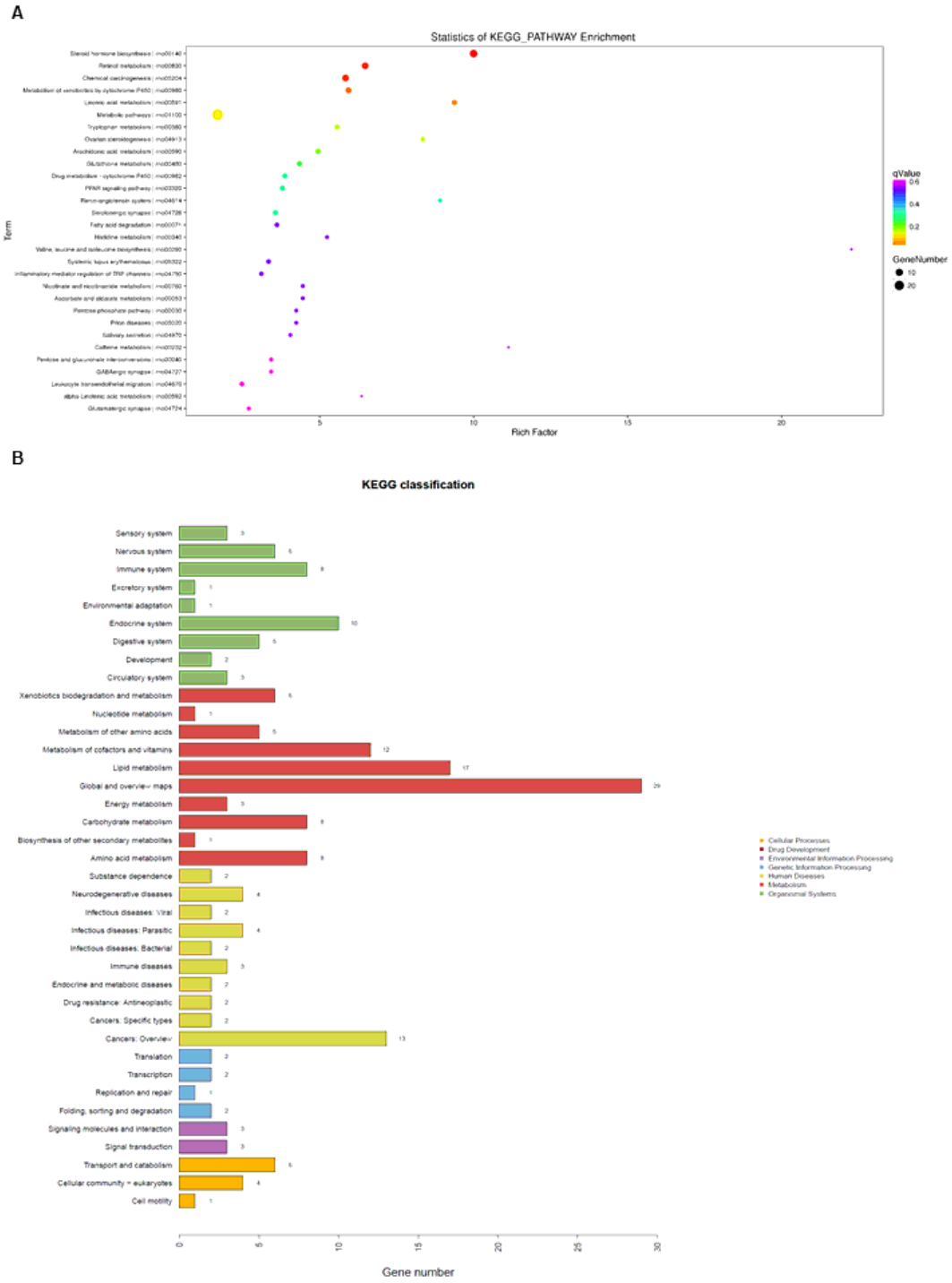

Figure 3

KEGG pathway enrichment analysis. A. KEGG analysis of all significantly regulated proteins (Fisher's Exact Test *p*-value < 0.05). B. KEGG classification of all significantly regulated proteins based on the total number of genes (Fisher's Exact Test *p*-value < 0.05).

Figure.4 GO (Gene Ontology) enrichment analysis of all differentially expressed proteins (P-value of Fisher's Exact Test < 0.05).

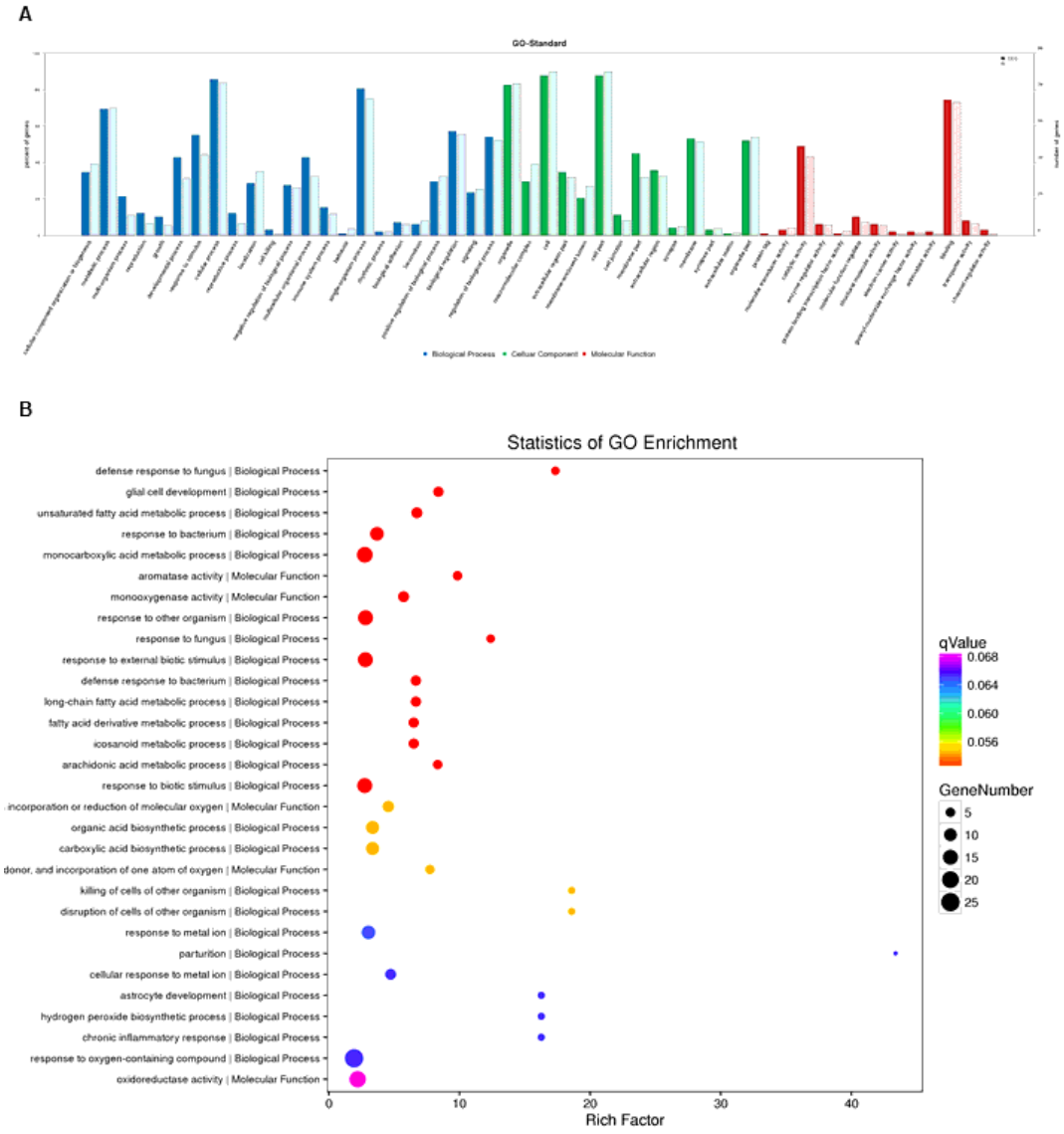

Figure 4

GO enrichment analysis. A. GO analysis of all differentially expressed proteins (Fisher's Exact Test *p*-value < 0.05). B. Statistics of GO enrichment. (Fisher's Exact Test *p*-value < 0.05).

Figure.5 Co-expression network of differential expressed proteins.

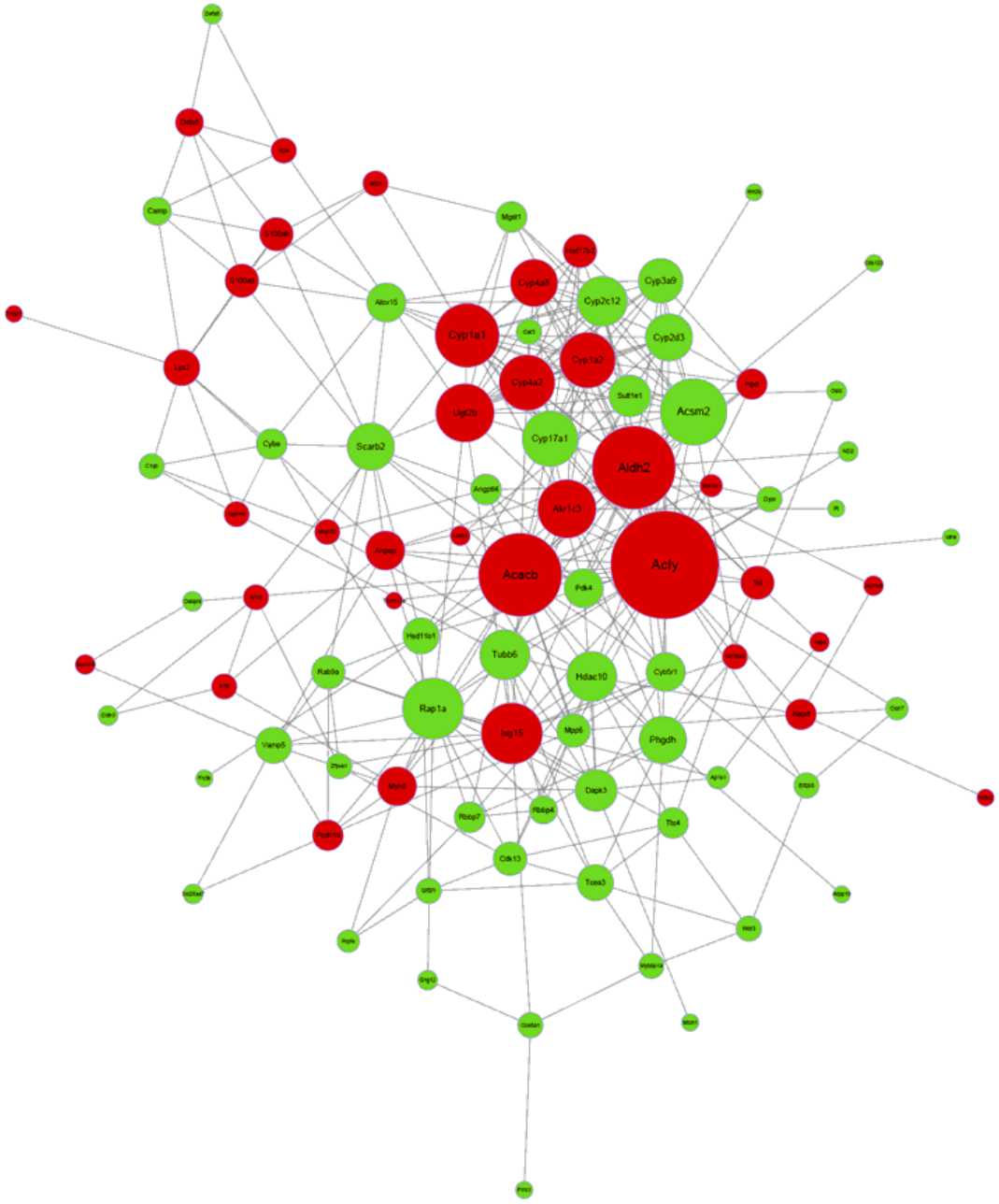

Figure 5

Co-expression network of differentially expressed proteins. Red and green circles indicate upregulated and downregulated proteins after exposure to acute hypoxia, respectively (Fisher's Exact Test *p*-value < 0.05).

Figure.6 Validation of differentially expressed proteins for control and hypoxia samples by real-time qPCR.

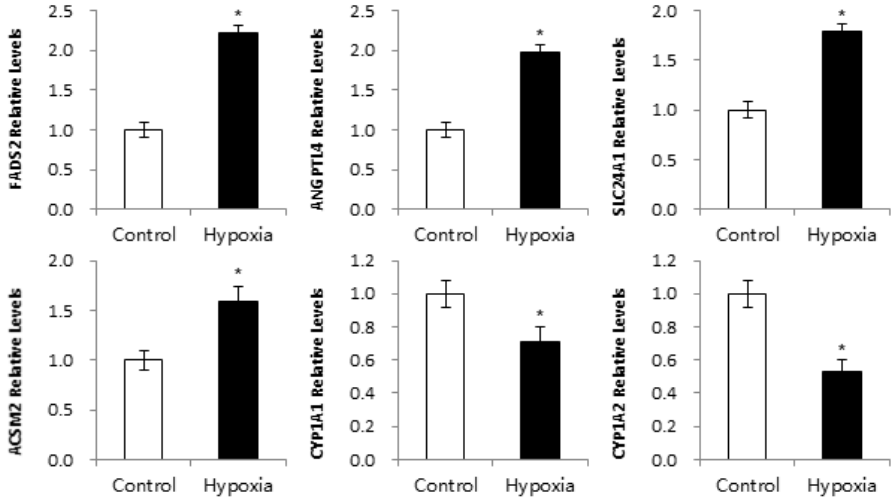

Figure 6

Validation of differentially expressed proteins in control and hypoxia samples using real-time qPCR. A. Real-time qPCR validation of FADS2, ANGPTL4, SLC2A1, ASCM2, CYP1A1 and CYP1A2. GAPDH was used as the internal control. Each experiment was conducted in triplicate to ensure replicability. Student's T-Test, paired tail, * represents *p*-value < 0.05.

Figure.7 A schematic model of acute reactions in the rat liver from acute hypoxia. For abbreviations and explanations, please see the text.

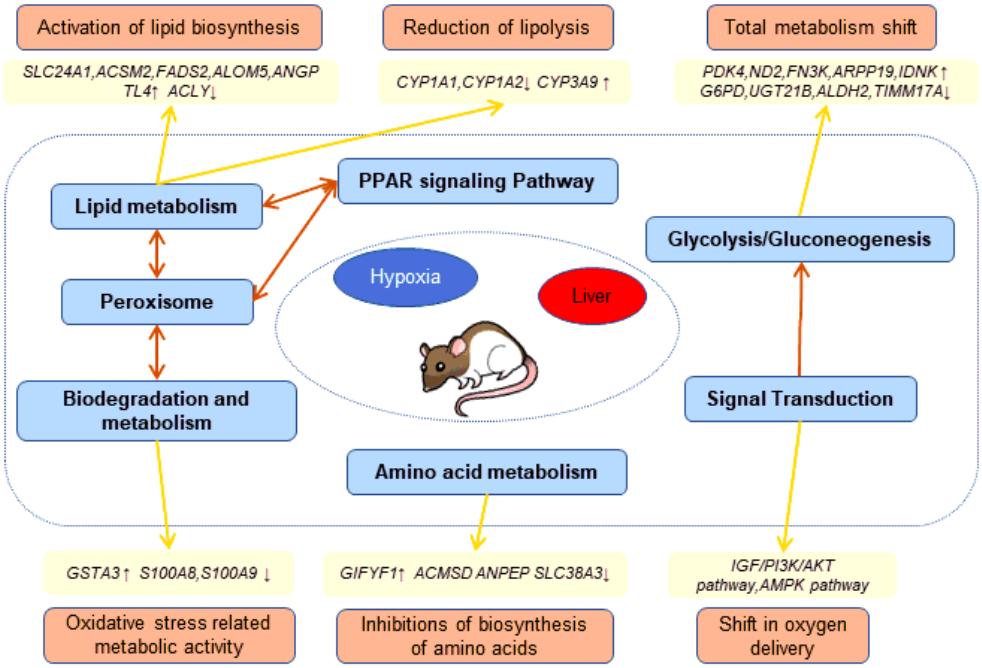

Figure 7

A schematic model of response to acute hypoxia in rat livers.