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Differential effects of medium- and long-term high-fat diets on the expression of genes or proteins related to nonalcoholic fatty liver disease in mice

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is now considered to be the most common liver disease worldwide, caused by fat deposition in hepatocytes. High-fat diet is considered to be a major lifestyle factor predisposing to NAFLD. However, the effect of different cycles of high-fat diets on changes in NAFLD-related gene and protein expression is unclear. In this study, NAFLD mouse models were established by feeding C57BL/6 male mice a high-fat diet for 16 and 38 weeks. The transcriptome and proteome of mouse liver were profiled by RNA sequencing and high-resolution mass spectrometry, respectively. The results show that accumulation of liver lipids was observed at 38 weeks of treatment on a high-fat diet. At the same time, the expression profiles of 1329 genes and 802 proteins involved in NAFLD were changed, with a total of 234 genes and 37 proteins significantly changing by more than twice. These differentially expressed genes and proteins were significantly enriched in fatty acid metabolism and organic acid biosynthesis. 18 genes and their corresponding protein overlaps were identified using Venn diagrams, and most of them were regulated by high-fat diet in an aging-dependent manner. All in all, our study is valuable for understanding the high-fat diet on the developmental process of NAFLD.

Keywords: high-fat diet; NAFLD; data mining; biomarkers; transcriptome.

Practical Application: The investigation provides the important information for consumers and researchers to understand the effect of high fat diet on NAFLD.

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease worldwide, with a global prevalence of 25% (Younossi et al., 2016). Recent studies have shown that NAFLD is a multisystemic disease associated with metabolic diseases such as obesity, diabetes, and dyslipidemia (Kasper et al., 2021; Lu et al., 2018; Younossi et al., 2019). However, there are no approved drug treatments for NAFLD, which requires an in-depth understanding of the pathogenesis of NAFLD in order to find effective therapeutic targets (Nassir., 2022).

The pathogenesis of NAFLD is complex, and in recent years it is often explained by the "multiple-hit" hypothesis. This hypothesis suggests that multiple factors, including hepatic lipid accumulation, oxidative stress, insulin resistance, inflammation and mitochondrial dysfunction, can contribute to the development of NAFLD (Buzzetti et al., 2016). Insulin resistance is one of the key factors in the development of steatosis or hepatitis, leading to increased hepatic de novo lipogenesis (DNL) and lipolysis of adipose tissue, which in turn leads to increased fatty acid influx into the liver (Smith et al., 2020). Fat accumulates in the liver in the form of triglycerides, along with increased lipotoxicity due to high levels of free fatty acids, free cholesterol and other lipid metabolites, ultimately leads to mitochondrial dysfunction accompanied by oxidative stress (Bessone et al., 2019). All of the above factors lead to hepatic fat accumulation and inflammation, thus inducing NAFLD through heterogeneous hepatocyte damage pathways (Buzzetti et al., 2016).

These biological courses are orchestrated by a wide spectrum of genes, and uncovering important signature genes among them is particularly critical in the diagnosis and treatment of NAFLD. Recent innovations in transcriptomic and proteomic technologies may help to solve this problem (Wang et al., 2022). However, few studies have explored NAFLD biomarkers using complementary transcriptomic and proteomics simultaneously. Therefore, we conducted a literature and database search to collect 1329 NAFLD genes, and used RNA-Seq technology and high-resolution mass spectrometry technology to find the NAFLD genes and their coding proteins that were significantly affected by high fat diet, and analyze how they are affected by both age and high-fat diet. It is hoped that this study will contribute to the diagnosis of NAFLD progression and the development of effective therapeutic approaches.

2 Materials and methods

2.1 Editorial policies and ethical considerations

This study was in accordance with the requirements of Animal Experiment Ethics Committee of Zunyi Medical University.

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2.2 Materials

Active blood glucose test strips were purchased from Roche Accu-Chek (Shanghai, China). Normal diet (23.2% of calories from protein, 12.1% from fat, and 64.7% from carbohydrate) was purchased from Jiangsu Xietong Biomedical Engineering Co., Ltd. (Jiangsu, China). High fat diet (20.0% of calories fromprotein, 60.0% from fat, and 20.0% from carbohydrate) was purchased from Research Diets Inc. (New Brunswick, USA).

2.3 Animal experiments

Male C57BL/6 mice (23-25 g) were purchased from Huafu Kang Biological Technology Co., Ltd. (Beijing, China; approval number: SCXK 2014-0004). Male C57BL/6 mice were kept in the well-controlled animal room on the SPF (Specific Pathogen Free) level. Room temperature was set to 21-23 °C, while the humidity adjustment ranged from 50 to 60% and a 12 h light/dark cycle was used. Animals were free to ingest food and water, monitored body weight trends every day. In this study, we involved 55 mice in total and set. three time points including day 0, week 16, and week 38. At the start of the experiment, 11 mice in group day-0 were sacrificed without any treatment. The other 44 mice were divided into 4 groups (n = 11 per group), treated by normal diet (ND) or high fat diet (HFD) for 16 or 38 weeks. At the end point of each group, mice were executed and a slice of liver samples was fixed in 10% formaldehyde solution, while rest of the livers and other tissues were placed in liquid nitrogen for quick-freezing, and then stored in -80 °C freezer.

2.4 Measure liver weight

Liver weight was measured after execution of mice at week 38 of the experiment.

2.5 Histology analysis

The liver tissues were fixed in 10% formalin solution for 24 h, dehydrated with alcohol and placed in xylene. The fixed liver tissues were embedded in the paraffin (Leica EG1150, Wetzlar, Germany) and then cut into slices of 4-6 μm by a Leica RM2245 Biosystems (Wetzlar, Germany). Tissue slices were washed, dehydrated, and stained by hematoxylin and eosin for Olympus BX43 microscopic examination (Tokyo, Japan).

2.6 RNA preparation and sequencing

For each group, 5 out of 11 mice livers were randomly picked for RNA sequencing. Total RNA was extracted from mouse liver tissues according to following procedures. Approximately 20 mg of liver tissues was homogenized in 1 mL Trizol on ice, incubated at room temperature for 5 min and added 200 μL chloroform. After low temperature centrifugation, 500 μL isopropanol was used to precipitate RNA. The precipitate was washed with 75% ethanol (DEPC water configuration), and then dissolved by 30 μL DEPC water after evaporated all ethanol. RNA integrity was evaluated by Agilent 2100 Bioanalyzer (Agilent Technologies). RNA sequencing was performed on an Illumina Hiseq 2000 platform.

2.7 Protein extraction

The sample was grinded by liquid nitrogen into cell powder and then transferred to a 5-mL centrifuge tube. After that, four volumes of lysis buffer (8 M urea, 1% Protease Inhibitor Cocktail) was added to the cell powder. After sonicate spallation, the protein extracts were centrifuged at 12,000×g for 10 min. Finally, the supernatant was collected and the protein concentration was determined with BCA kit according to the manufacturer's instructions.

The protein was purified by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Trapped proteins were digested overnight by trypsin. Peptides were eluted, desalted and centrifuged at 14,000×g for 15 min, and the supernatant was collected for LC-MS/MS analysis.

2.8 LC-MS/MS analysis

The tryptic peptides were dissolved in 0.1% formic acid (solvent A), directly loaded onto a home-made reversed-phase analytical column (15-cm length, 75 μm i.d.). The gradient was comprised of an increase from 6% to 23% solvent B (0.1% formic acid in 98% acetonitrile) over 26 min, 23% to 35% in 8 min and climbing to 80% in 3 min then holding at 80% for the last 3 min, all at a constant flow rate of 400 nL/min on an EASY-nLC 1000 UPLC system. The peptides were subjected to NSI source followed by tandem mass spectrometry (MS/MS) in Q ExactiveTM Plus (Thermo) coupled online to the UPLC. The electrospray voltage applied was 2.0 kV. The m/z scan range was 350 to 1800 for full scan, and intact peptides were detected in the Orbitrap at a resolution of 70,000. Peptides were then selected for MS/MS using NCE setting as 28 and the fragments were detected in the Orbitrap at a resolution of 17,500. A data-dependent procedure that alternated between one MS scan followed by 20 MS/MS scans with 15.0s dynamic exclusion. Automatic gain control (AGC) was set at 5E4. Fixed first mass was set as 100 m/z.

2.9 Data process and mining

In the current study, we generated a list of 1329 genes involved in NAFLD systems, and matched to 802 corresponding proteins using proteomic data. This gene set was obtained from the nash-profiler database (http://www.nash-profiler.com.). R program was used to visualize the expression levels of all investigated NAFLD gene expressions and profile the global changes. Principal component analysis was used for gene expression statistics, and individual mice were used as samples, and each gene expression value of a single sample was used as a variable to construct a data matrix, which was processed by the PCA function of mixOmics in the R language open source toolkit. GO analysis was performed through enrichGO function in the ClusterProfiler package. Differences between two groups were calculated by t-test in R, and differences between multiple groups were calculated by one-way ANOVA in R. $P< 0.05$ represented statistical significance, and all data were expressed as "mean ± standard error (SEM)" (Figure 1).

3 Result

3.1 Histological analyses

38 weeks of high-fat diet significantly increased the liver weight and its ratio to body weight of the mice (Figure 2A).

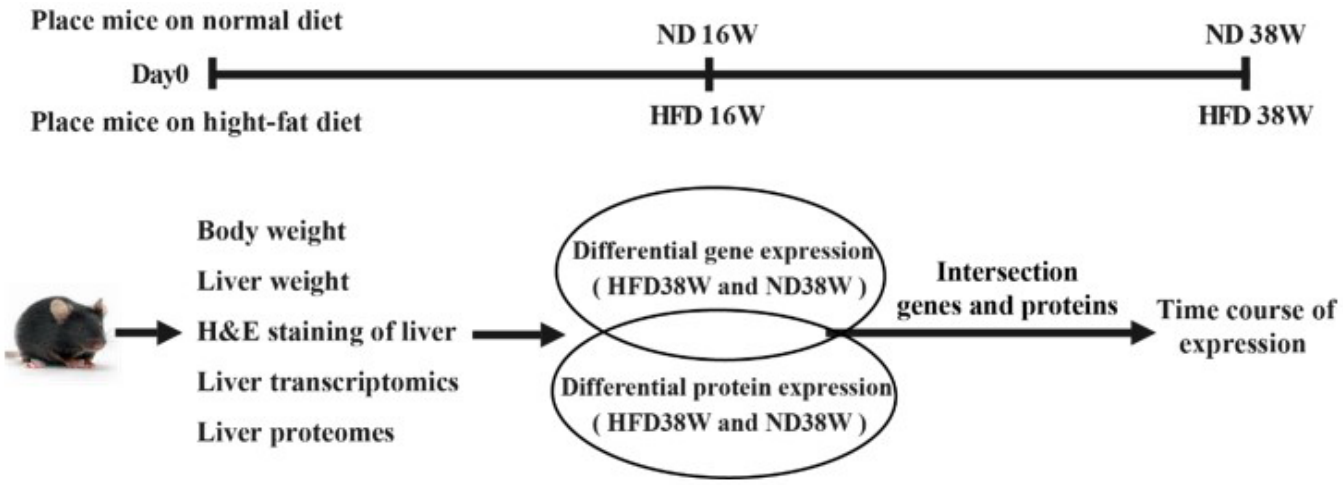

Figure 1. Flowchart of the experimental design. Abbreviation: Day0, Mice euthanized at the beginning of the experiment; ND38w, normal diet for 38 weeks; HFD38w, high fat diet for 38 weeks.

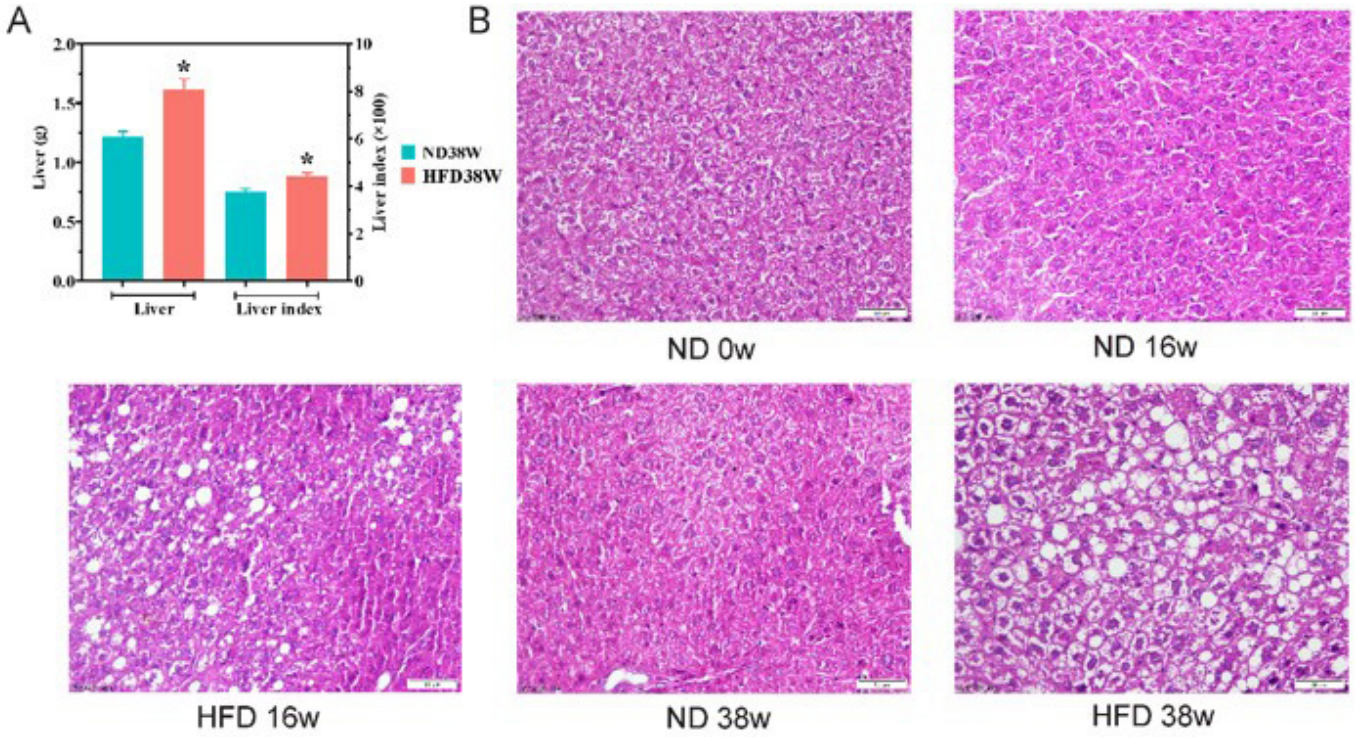

Figure 2. Establishment of animal models of nonalcoholic fatty liver disease (NAFLD). (A) Liver weight and Liver index (liver weight/body weight × 100); (B) Images for H&E staining of liver sections. Abbreviation: ND, normal diet group; HFD, high fat diet group; 0w, 0week; 38w, 38weeks; 16w, 16weeks. Data were presented as mean ± SEM. "*" indicates $p < 0.05$.

HE staining showed obvious fat vacuoles in liver sections from 16-week high-fat diet mice, while 38-week high-fat diet mice showed severe fat accumulation in the liver (Figure 2B).

3.2 Transcriptomic analyses

A total of 1329 NAFLD-related genes were included in this study. Based on this gene set, the hepatic expression profile of high-fat diet-treated mice at 38 weeks were significantly different from that of normal diet treatment. Principal component analysis (PCA) showed that normal diet and high-fat diet mice were completely separated in the PC1 direction, indicating that expression profiles of gene transcriptions involved in NAFLD systems were significantly affected by high fat diet (Figure 3A).

During the identification of differentially expressed genes, a fold change ≥ 2 and p value < 0.05 were used as screening criteria, and we found that there were 234 differential genes, including 150 up-regulated genes and 84 down-regulated genes (Figure 3B).

The biological process (BP) of these 234 genes were analyzed by GO pathway enrichment, and the top 10 most significantly enriched BP terms were listed. GO BP analysis showed that these 234 DEGs were mainly enriched in fatty acid metabolism progress, organic acid biosynthetic process and carboxylic acid biosynthetic process categories (Figure 3C).

3.3 Proteomics analyses

Subsequently, we analyzed the effect of a long-term high-fat diet on NAFLD-associated protein profiles in the same way. we generated a list with proteins involved in NAFLD systems. In total 802 proteins were collected. The PCA showed completely different location between mice treated with normal diet and high fat diet in score plots (Figure 4A). Therefore, a high-fat diet can also affect the NAFLD protein system profile in mice.

Volcano maps show that hepatic expression levels of 37 proteins were significantly altered more than twice, including 13 up-regulated proteins and 24 down-regulated proteins (Figure 4B). GO BP analysis showed that these 37 proteins were significantly enriched in fatty acid metabolic process, sulfur compound metabolic process and organic acid biosynthetic process categories (Figure 4C).

3.4 Time course of on NAFLD gene and protein expression levels

In the current study, a 38-week high-fat diet altered the expression profile of NAFLD-related genes and proteins, and the expression of 234 NAFLD genes and 37 NAFLD proteins changed more than 2-fold. Subsequently, cross-linking analysis of differentially expressed genes and differentially expressed proteins using Venn diagrams revealed that 18 genes were not only significantly regulated at the transcriptional level by the high-fat diet, but also the proteins encoded by these 18 genes were significantly regulated by the high-fat diet at the same time, with 6 genes being up-regulated and 12 genes being down-regulated (Figure 5A). These genes are likely to play an important role in the development of NAFLD. At the beginning of the experiment, the average age of the mice was 8 weeks, and after 38 weeks of the rearing, the mice were already 46 weeks old and could be used as an aging model. To observe how young mice are affected by short-term high-fat diet, we set another time point of 16 weeks to further analyze how these 18 significantly regulated genes and their corresponding proteins are affected by both age and diet.

Transcriptomically, most of these 18 genes were affected by the high-fat diet in an aging-dependent manner (Figure 5B). Among the genes significantly up-regulated by the 38-week high-

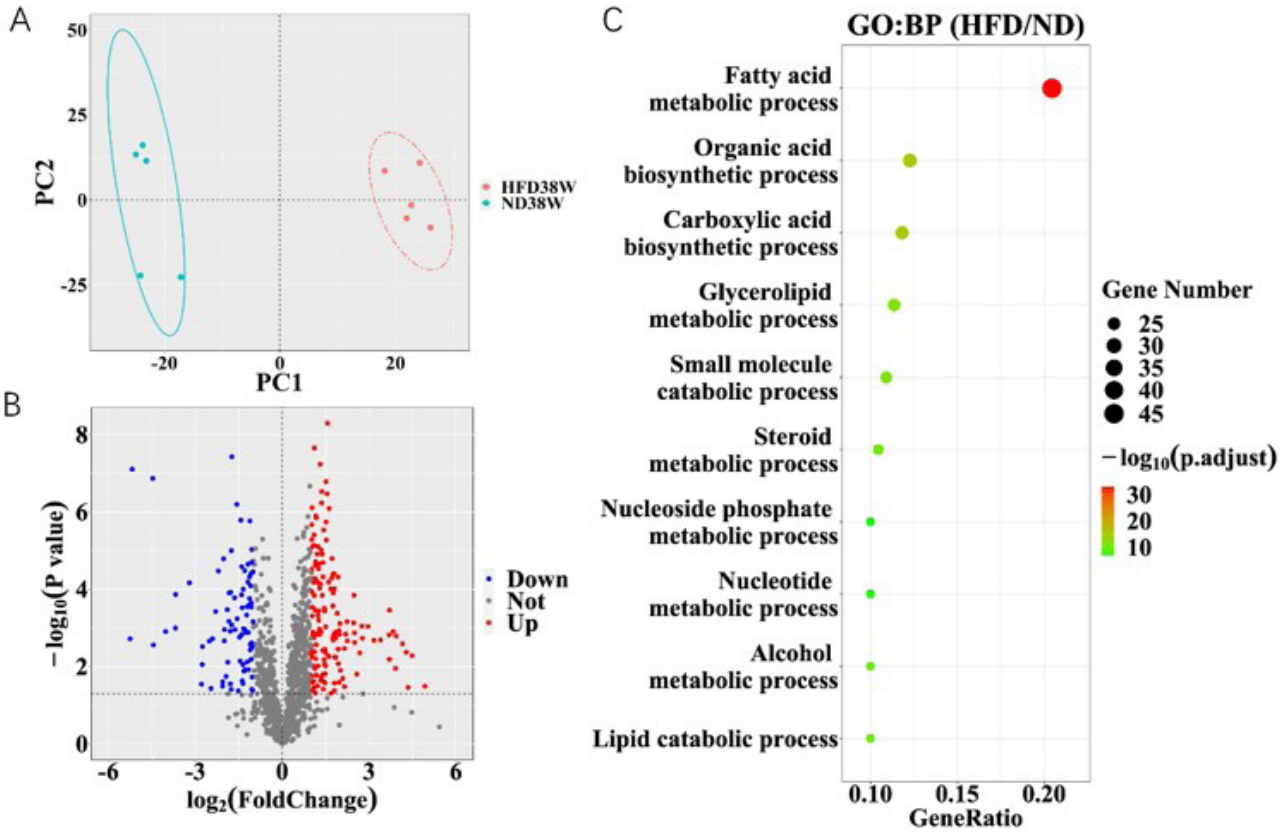

Figure 3. Liver gene expression analysis. (A) Score plots of principal component; (B) Volcano plots showing the significantly regulated genes; (C) GO annotation enrichment analysis of differentially expressed genes. The top 10 enriched GO BP. Abbreviation: ND38w, normal diet for 38 weeks; HFD38w, high fat diet for 38 weeks; Up, significantly up-regulated; Down, significantly down-regulated; Up, significant up-regulated. Down, significant down-regulated; GO, gene ontology; BP, biological process.

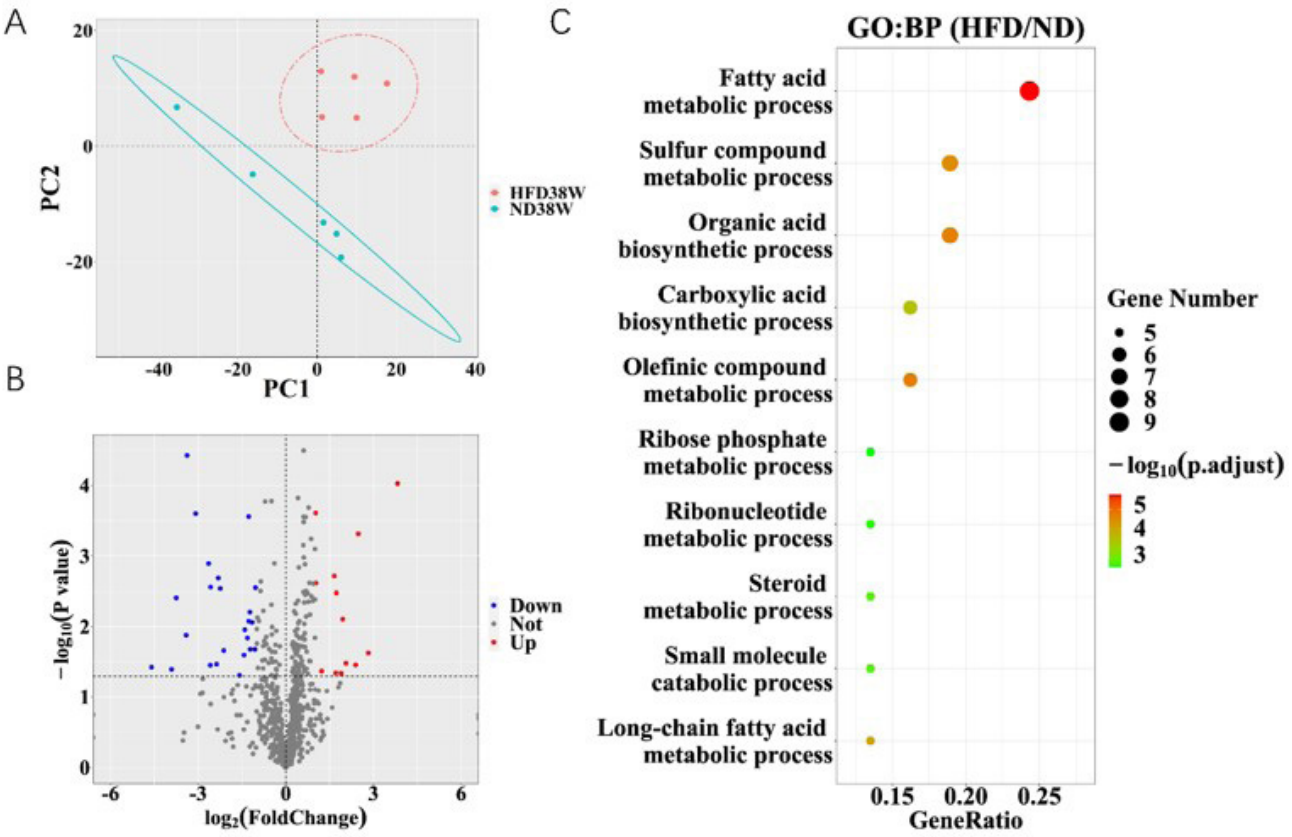

Figure 4. Liver proteins expression analysis. (A) Score plots of principal component analysis; (B) Volcano plots showing the significantly regulated proteins; (C) GO annotation enrichment analysis of differentially expressed proteins. Abbreviation: ND38w, normal diet for 38 weeks; HFD38w, high fat diet for 38 weeks; Up, significantly up-regulated; Down, significantly down-regulated; Up, significant up-regulated. Down, significant down-regulated; GO, gene ontology; BP, biological process.

fat diet, Dio1, Syvn1, Dhx58 were not significantly regulated by the 16-week high-fat diet, while Msmo1, Mvd, Tmem176a were significantly down-regulated by the 16-week high-fat diet. Among the genes significantly downregulated by 38-week high-fat diet, Ehhadh, Cyp4a14, Cyp4a10, Retsat, Etnppl, Acot3, St3gal5, Pdk4 were not significantly affected by 16-week high-fat diet, while Lpin2 was significantly upregulated by 16-week high-fat diet, and only Csad, Cyp3a11, and Cyp4a12a were downregulated by both 16-week and 38-week high-fat diet.

Proteomically, most of these proteins were also affected by the high-fat diet in an aging-dependent manner (Figure 5C). Among the proteins significantly upregulated by the 38-week high-fat diet, MSMO1, MVD, DIO1, DHX58, and TMEM176A did not change significantly at 16 weeks of high-fat diet, whereas SYVN1 was significantly downregulated at 16 weeks of high-fat diet. Among the proteins significantly down-regulated by 38-week high-fat diet, CSAD, CYP4A14, RETSAT, ETNPPL, ACOT3, ST3GAL5, PDK4, and LPIN2 were not significantly changed by 16-week high-fat diet, while EHHADH and CYP4A10 were significantly up-regulated by 16-week high-fat diet, and only CYP3A11 and CYP4A12A were down-regulated by both 16-week and 38-week high-fat diet.

4 Discussion

Currently, liver biopsy is considered the gold standard for the diagnosis of NAFLD, which has inherent limitations, such as being invasive, prone to bleeding, and sampling bias. Indeed, the ideal diagnostic marker for hepatic steatosis should be a noninvasive marker suitable for early detection of the disease and reflecting its severity. In this regard, proteomics and transcriptomics hold great promise, as they can provide information on the mechanisms of disease progression (Li et al., 2022).

Animal models are an important part of the study of high-throughput histology. prior studies have demonstrated the feasibility of establishing a mouse model of NAFLD with a high-fat diet. A long-term high-fat diet can lead to overnutrition. A long-term high-fat diet can lead to excess nutrition. When fat tissue is unable to store all of its excess nutrients as triglycerides, lipids begin to accumulate in various tissues such as muscle, liver, pancreas and heart. This accumulation is called ectopic lipids. Various mechanisms by which ectopic lipids are harmful in different tissues have been proposed, and these disorders will trigger metabolic risk factors (Grundy, 2016; Mi et al., 2022). Our previous data demonstrated that a high-fat diet leads to liver fat accumulation and significant increased levels of serum alanine

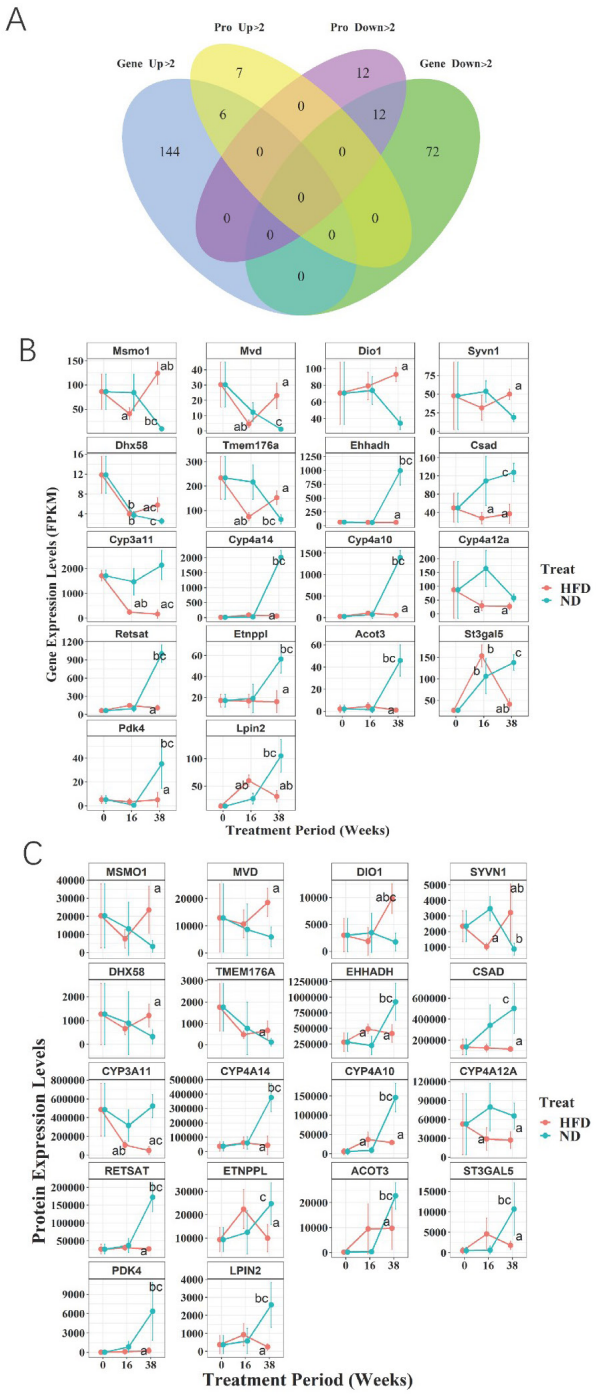

Figure 5. Time course of on NAFLD gene and protein expression levels. (A) Venn diagram showing the intersections of differentially expressed genes and protein; (B) Time course of hepatic expression levels of 18 genes in mice treated by normal diet or high fat diet for 0 week, 16 weeks and 38 weeks; (C) Time course of hepatic expression levels of 18 proteins in mice treated by normal diet or high fat diet for 0 week, 16 weeks and 38 weeks. Abbreviation: Gene Up > 2, gene significantly up-regulated by more than twice; Gene Down > 2, gene significantly down-regulated by more than twice. Pro Up > 2, protein significantly up-regulated by more than twice; Pro Down > 2, protein significantly down-regulated by more than twice; ND, normal diet group; HFD, high fat diet group. Data were presented as mean ± SEM. $^{a}p< 0.05$ between ND and HFD groups at a specified time point; $^{b}p< 0.05$ vs the previous time point with the same treatment; $^{c}p< 0.05$ vs the week 0.

aminotransferase (ALT), serum aspartate aminotransferase (AST), serum total cholesterol (TC), serum low-density lipoprotein cholesterol (LDL-C), blood glucose and body weight in mice (He et al., 2020). This study further investigated the effect of high-fat diet on NAFLD in mice using transcriptomic and proteomic data with the aim of finding biomarkers of NAFLD.

PCA showed that long-term high-fat diets significantly affected the transcriptional expression profile and protein expression profile of NAFLD, and volcano plots also showed that high-fat diets significantly altered the expression of 234 NAFLD-related genes and 37 NAFLD-related proteins, that were significantly enriched in fatty acid metabolism and organic acid biosynthesis.

Subsequently, we identified 18 genes were significantly regulated at both transcriptional and protein levels by a long-term high-fat diet, and most of them were regulated by a 16-week high-fat diet in a different way than a 38-week high-fat diet, with only CYP3A11 and CYP4A12A significantly down-regulated at both transcriptional levels and protein levels by both 16-week and 38-week high-fat diets. This suggests that the effect of high-fat diet on NAFLD is likely to be aging-dependent style, and that CYP3A11 and CYP4A12A may be markers for the early diagnosis of NAFLD.

The cytochrome P-450 monooxygenase 3A4 (CYP3A4) is designated as CYP3A11 in mice, responsible for the oxidative metabolism of a wide variety of xenobiotics including an estimated 60% of all clinically used drugs (Ren et al., 2021). An experiment demonstrated that 12 weeks of HFD feeding decreased the mRNA expression level of Cyp3a11 by approximately 8-fold (Wahlang et al., 2014). Subsequent studies have shown that hepatic CYP3A11 mRNA and protein expression was significantly decreased in both mice fed a high-fat diet for 8 weeks and palmitate (PA)-treated mouse primary hepatocytes (Zeng et al., 2019). In the current study, protein expression of CYP3A11 was downregulated approximately 11-fold in mice on a long-term high-fat diet.

Lipid ω-hydroxylation of medium- and long-chain fatty acid metabolized by the cytochrome P450 4A (CYP4A) family is an alternative pathway for fatty acid metabolism (Yang et al., 2021). In the present study, chronic high-fat diet downregulated the protein expression of CYP4A12A more than 2-fold, CYP4A14 more than 8-fold, and CYP4A10 nearly 5-fold.

Pyruvate dehydrogenase kinase 4 (PDK4) is a sensitive indicator of mitochondrial fatty acid oxidation, and its overexpression leads to increased fatty acid oxidation (Pettersen et al., 2019). L-bifunctional enzyme (Ehhadh) is part of the peroxisomal fatty acid β-oxidation pathway (Chen et al., 2018). In the present study, chronic high-fat diet downregulated PDK4 protein expression more than 20-fold and EHHADH protein expression more than 2-fold.

In addition, Acyl-CoA thioesterase 3 (ACOT3) acts as an auxiliary coenzyme in the β-oxidation of various lipids in the peroxisome, hydrolyses long-medium chain fatty acyl-CoA esters to FFA, thereby facilitating their transport into the peroxisome, from which FFA can then be transported to the mitochondria for further β-oxidation (Shen et al., 2022). The protein expression of acot3 was downregulated more than 2-fold

in our study. A long-term high-fat diet may increase hepatic fatty acid accumulation by reducing the expression of CYP4A10, CYP4A14, CYP4A12A, PDK4, EHHADH and ACOT3, thereby promoting the development of NAFLD.

Accumulation of liver cholesterol plays an important role during the pathogenesis of NAFLD. Excess hepatic cholesterol preferentially accumulates in hepatocyte lipid droplets, which may crystallize and contribute to the development of NASH (Bashiri et al., 2016; Ioannou et al., 2019). Mevalonate decarboxylase (MVD) and methylsterol monooxygenase 1 (MSMO1) contribute to cholesterol biosynthesis (Ershov et al., 2021). In the present study, a long-term high-fat diet upregulated MVD protein expression by more than 3-fold and MSMOL protein expression by 7-fold. This implies that a long-term high-fat diet may promote the development of NAFLD by increasing the expression of MVD and MSMOL.

Synovialin (SYVN1), an E3 ubiquitin ligase, also known as HMG-CoA reductase degradation protein 1 (HRD1), has been shown to be a favorable target for the treatment of arthropathies (Colbert et al., 2014). Subsequently, SYVN1 has been reported to be involved in fibrosis and to inhibit Nrf2-mediated cytoprotective effects during liver cirrhosis (Fujita et al., 2018). The present study showed that a chronic high-fat diet upregulated the protein expression of SYVN1 more than 3-fold. Therefore, a long-term high-fat diet may promote the development of liver fibrosis and cirrhosis by upregulating the expression of SYVN1.

Ethanolamine-phosphate phospho-lyase (ETNPPL), also called alanine-glyoxylate aminotransferase 2-like 1 (AGXT2L1), is a phosphorylase whose expression is significantly reduced in hepatocellular carcinoma tissues. Down-regulation of AGXT2L1 expression can also promote adipogenesis in cancer cells (Ding et al., 2016). Human transmembrane protein 176A (TMEM176A) is upregulated in several tumors. Growing evidence has suggested the high clinical value of TMEM176A as a biomarker for early tumor diagnosis (Liu et al., 2018). The data showed that long-term high-fat diet down-regulated protein expression of ETNPPL by more than 2-fold and up-regulated protein expression of TMEM176A by more than 5-fold, suggesting that long-term high-fat diet may promote the development of hepatocellular carcinoma in mice by down-regulating ETNPPL and up-regulating TMEM176A expression.

Deiodinase type 1 (Dio1) is a hepatic enzyme responsible for the conversion of pro-thyroxine (T4) to triiodothyronine (T3), thereby regulating thyroid hormones in hepatocytes. Currently, thyroid hormones are proven to be an effective treatment for NAFLD, low T4 levels lead to an increased incidence of NAFLD, and Dio1 expression has been shown to increase in the early stages of NAFLD (Bruinstroop et al., 2021; Harrison et al., 2019; Sinha et al., 2019). The protein expression of DIO1 was upregulated more than 5-fold by the long-term high-fat diet in this study, which also suggests that DIO1 may be a potential marker and therapeutic target for NAFLD.

Retinol saturase (RetSat) is an NADH/NADPH- or FADH-dependent oxidoreductase. In HS / HFD fed mice, acute depletion of RetSat decreases hepatic TG content. Inhibition of RetSat expression may have a therapeutic effect on hepatic steatosis (Heidenreich et al., 2017). However, in the mice with NAFLD in this study, the protein expression of RETSAT was downregulated more than 6-fold.

Through the above analysis, we identified a number of genes associated with fatty acid oxidation, cholesterol accumulation, liver fibrosis, and even hepatocellular carcinoma that are closely related to the development of NAFLD. This study shows for the first time the changes in the expression of these genes and their encoded proteins in the development of NAFLD, providing evidence for further screening of biomarkers of NAFLD. In addition, other substances not reported to be associated with NAFLD were identified in this study, and their expression was significantly altered in mice with NAFLD. CSAD (cysteine sulfonate decarboxylase) is considered to be the rate-limiting enzyme in taurine biosynthesisy (Park et al., 2017). ST3 beta-galactoside alpha-2,3-sialyltransferase 5 (ST3GAL5) catalyzes the formation of ganglioside (GM3) (Gordon-Lipkin et al., 2018). LPIN2 (lipoprotein 2), a phosphatidate phosphatase rich in liver, is expressed in mice in a body weight-dependent manner (Watahiki et al., 2020). Chronic high-fat diet downregulated the protein expression of CSAD, ST3GAL5, and LPIN2 by about 4-fold, 6-fold, and 10-fold, respectively. In addition, DHX58 is a RIG-I-like receptor involved in cytoplasmic RNA recognition and antiviral signaling (Bruns & Horvath, 2015). DHX58 protein expression is more than 3-fold upregulated by a high-fat diet. It suggests that a long-term high-fat diet may also promote the development of NAFLD by altering the expression of these genes, and that these genes, which are sensitively regulated in NAFLD mice, may be potential biomarkers and therapeutic targets for NAFLD.

5 Conclusion

In this study, a number of potential NAFLD-associated genes and their proteins regulated by high-fat diet were identified, including MSMO1, MVD, DIO1, SYVN1, DHX58, TMEM176A, EHHADH, CYP4A14, CYP4A10, RETSAT, ETNPPL, ACOT3, ST3GAL5, PDK4, LPIN2, CSAD, CYP3A11, CYP4A12A. Interestingly, the expression of most of these genes and proteins showed opposite trends in the effects of medium- and long-term high-fat diets, which provides clues for further studies on the development of NAFLD.

Conflict of interest

There are no conflicts of interest declared by the authors.

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Author contributions

Yuqi He and Daopeng Tan conceived and designed the research. Yuqi He, Daopeng Tan, Lin Qin and Yanliu Lu performed the experiments. Tingting Zhou and Ligang Cao analyzed the data and prepared the figures. Tingting Zhou drafted the manuscript. Yuqi He, Daopeng Tan and Lin Qin edited and revised the manuscript. The author (s) read and approved the final manuscript.

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