



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Integrative Transcriptomics and Metabolomics Reveal the Role of Novel Anti-Obesity Peptide YFRPR in Preventing High-Fat Diet-Induced Obesity by Modulating Hepatic Lipid Metabolism

Hao Yin ^{a,b}, Jiangxiong Zhu^a, Yu Zhong ^a, Ting Zhao ^b, Yun Deng ^{a,b*}^a Department of Food Science & Technology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China^b Shanghai Jiao Tong University Yunnan (Dali) Research Institute, Dali, Yunnan, 671000, China

ABSTRACT: Obesity has emerged as a global public health challenge, closely associated with various chronic diseases. Growing evidence suggests that bioactive peptides have the potential to alleviate obesity, although the molecular mechanisms behind their weight-loss effects remain not fully understood. This study evaluates YFRPR, an anti-obesity peptide derived from *Rosa roxburghii* seed protein, for its ability to prevent high-fat diet (HFD)-induced obesity in mice. The mechanisms of YFRPR in obesity prevention were further investigated through comprehensive transcriptomic and metabolomic analyses. The results showed that YFRPR administration effectively reduced weight gain induced by HFD and alleviated adipocyte hypertrophy and liver steatosis. Additionally, it improved dyslipidemia, insulin resistance, and oxidative stress, and reduced the secretion of inflammatory cytokines. Transcriptomic analysis of the liver identified several differential metabolic genes, including Cytochrome P450, *Ehhadh*, *Apoa4*, *Plb1*, *Acot1*, *Acs14*, and *Ptges*. Metabolomic analysis revealed that YFRPR treatment primarily influenced metabolites related to lipids and lipid-like molecules, organic acids, and their derivatives, and organic heterocyclic compounds. Integrated analysis of both transcriptomics and metabolomics confirmed that YFRPR prevents obesity by modulating lipid metabolism pathways, including the PPAR signaling pathway, fatty acid metabolism, arachidonic acid metabolism, glycerophospholipid metabolism, and cholesterol metabolism. Moreover, oxidative stress and inflammation-related pathways were improved following YFRPR administration. This study provides valuable insights into the beneficial effects of the novel anti-obesity peptide YFRPR and contributes to the development of functional foods with anti-obesity properties based on bioactive peptides.

Keywords: Obesity, transcriptomics, metabolomics, bioactive peptides, lipid metabolism

1. Introduction

Obesity, as a complex metabolic syndrome, has emerged as a major public health challenge worldwide due to its increasing prevalence. According to the latest World Health Organization (WHO) projections, the global population affected by overweight/obesity will exceed 4 billion by 2035, accounting for 51% of the total population^[1]. The pathophysiological basis of obesity involves multiple factors, including genetic susceptibility, energy homeostasis imbalances, and lifestyle factors^[2]. The comorbidities associated with obesity, such as insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease, and cardiovascular

*Corresponding author
y_deng@sjtu.edu.

Received 24 January 2025
Received in revised form 6 March 2025
Accepted 3 April 2025

diseases, significantly increase the public health burden^[3, 4]. Although existing pharmacological treatments (e.g., orlistat) can alleviate some obesity symptoms, their side effects, such as gastrointestinal disturbances, limit their clinical application^[5]. There is a pressing need for the creation of novel, safe, and effective intervention approaches.

In recent years, bioactive proteins and their enzymatic hydrolysis products have demonstrated unique advantages in metabolic regulation and are considered promising functional food factors. Proteins like whey protein, pea albumin, and phycobiliprotein have been shown to significantly attenuate high-fat diet-induced obesity by regulating fat metabolism, reducing liver steatosis, and improving glucose tolerance^[5-7]. Peptides derived from salmon, brewer's spent grain, and corn hydrolysates have also shown positive effects on metabolic syndrome by increasing energy expenditure, reducing visceral fat accumulation, and improving insulin sensitivity^[8-10]. Hydrolyzed milk peptides, such as CHM-273S, can alleviate body weight and visceral fat accumulation by upregulating leptin signaling^[11]. Furthermore, further identification and study of active peptides in protein hydrolysates have revealed their potential in preventing obesity. Egg-derived peptides (IRW and IQW) exert significant anti-obesity effects in obese mice by improving insulin tolerance and regulating lipid metabolism^[12]. Lunasin, a seed peptide, reduces chronic inflammation caused by obesity through immune modulation, demonstrating potential anti-obesity effects^[13]. Chickpea peptide VFVRN improves lipid profiles induced by a high-fat diet by inhibiting fatty acid synthase and cholesterol synthase^[14].

Although current studies have preliminarily confirmed the potential of proteins, their hydrolysates, and bioactive peptides in preventing and treating obesity, most studies focus on exploring known anti-obesity, oxidative stress, and anti-inflammatory targets. However, systematic molecular mechanism analyses remain insufficient. Transcriptomics, as an effective approach to studying the relationship between gene expression changes and physiological functions, has been widely applied to identify genes and metabolic pathways associated with various diseases and uncover their pathogenic mechanisms^[15, 16]. Metabolomics focuses on analyzing dynamic changes in endogenous metabolites within organisms, reflecting alterations in metabolic processes during disease^[17, 18]. The combined analysis of transcriptomics and metabolomics has become a key tool for identifying critical genes and metabolites in the study of diseases such as obesity, lipid metabolism disorders, and hyperlipidemia^[19-21].

Our previous research has isolated multiple anti-obesity peptides, including YFRPR, from *Rosa roxburghii* seed proteins. These peptides inhibit the activity of enzymes like lipid digestive enzymes, suppressing the digestion and absorption of lipids while regulating lipid metabolism in preadipocytes^[22-24]. YFRPR demonstrated the strongest activity in inhibiting lipid digestion and reducing lipid accumulation in 3T3-L1 cells. However, its anti-obesity effects have not yet been verified *in vivo*. This study aims to investigate whether the peptide YFRPR can prevent obesity *in vivo* and explore its potential mechanisms through the integration of multi-omics techniques. The findings of this study will contribute to understanding the role of YFRPR in preventing obesity and provide scientific evidence for the development of bioactive peptide-based weight loss products.

2. Materials and methods

2.1 Animal Grouping and Feeding Protocol

All animal experiments were approved by the Laboratory Animal Ethics Committee of Shanghai Jiao Tong University (A2023184). Male C57BL/6J mice, aged 4 weeks, were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China) and housed in a temperature-controlled environment ($24 \pm 2^\circ\text{C}$, $50\% \pm 5\%$ humidity, 12-hour light/dark cycle). Mice have free access to food and water. After a 1-week acclimatization period, the mice were randomly divided into four groups and subjected to different diets: normal fat diet (ND, 10% of energy from fat, XTCON50J, XieTong Pharmaceutical Biotech Co., Ltd., Jiangsu, China) or high-fat diet (HFD, 60% of energy from fat, XTHF60, XieTong Pharmaceutical Biotech Co., Ltd., Jiangsu, China). The diet composition is provided in Table S1. The experimental groupings were as follows: ND group ($n=8$), fed ND and orally administered PBS; HFD group, receiving oral PBS (HFD), 25 mg/kg body weight of YFRPR (HFD-L-Y), or 100 mg/kg body weight of YFRPR (HFD-H-Y). YFRPR (purity $> 98\%$) was synthesized by PeptideValley Biotechnology Co., Ltd. (Nanjing, China), and the feeding dose was determined by searching the literature, Caco-2 and 3T3-L1 cytotoxicity assays, and animal pre-tests. The peptide administration protocol is detailed in Fig. S1. The entire experiment lasted for 10 weeks, during which the mice's body weight and food intake were measured weekly, and body weight change curves and cumulative food intake were plotted. At the end of the experiment, fasting blood glucose levels were measured, and abdominal circumference (AC), body length, and Lee's index were determined according to the method described by Novelli et al. (2007)^[25].

2.2 Sample Collection

The experimental mice were euthanized under isoflurane anesthesia, followed by dissection to collect liver and white adipose tissue (WAT) samples, including epididymal WAT (epiWAT), inguinal WAT (ingWAT), and mesenteric WAT (mesWAT). Tissues were weighed and immediately frozen in liquid nitrogen, then stored at -80°C for later use. In addition, liver and adipose tissue samples were fixed in 4% paraformaldehyde solution for further histopathological analysis.

2.3 Serum Biochemical Analysis

Blood samples were collected in heparin-free tubes, then centrifuged at 3000 r/min for 10 minutes at 4°C to collect the serum. Serum levels of total triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and superoxide dismutase (SOD) were measured using commercial kits provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instructions. Serum levels of IL-1 α , IL-6, and TNF- α were detected using ELISA kits, with the procedure following the manufacturer's guidelines.

2.4 Liver Biochemical Analysis

Frozen liver samples were homogenized in saline (1:9, w/v) and centrifuged at 2500 r/min for 15 minutes. The supernatant was collected for further analysis. The levels of TC, TG, GSH-Px, MDA, SOD, and protein concentration in the liver were determined using commercial kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instructions.

2.5 Histological Analysis

Liver and epiWAT samples embedded in paraffin were sectioned into 4µm thick slices using a portable microtome (Thermo Fisher 456 Scientific, CA, USA) and stained with hematoxylin-eosin (HE) according to standard procedures. Images of the sections were captured using a microscope (Olympus IX50) and analyzed using NIS-ELEMENTS F3.2 software.

2.6 Liver Transcriptome Analysis

Liver tissue samples were processed by Shanghai Paishenno Biotechnology Co., Ltd. (Shanghai, China) for transcriptomic analysis^[26]. Total RNA was extracted using Trizol reagent (Invitrogen Life Technologies, USA) and its quality was assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA). RNA libraries were prepared from 3 µg RNA and sequenced using the Illumina platform (PE150 mode). Raw FASTQ data were filtered using fastp (v0.22.0), mapped to the reference genome using HISAT2 (v2.1.0), and gene quantification was performed with HTSeq (v0.9.1). Differential gene expression was analyzed using DESeq (v1.38.3), with $|\log_2\text{FoldChange}| > 1$ and a significance P -value < 0.05 as the filtering criteria. KEGG pathway enrichment analysis of differentially expressed genes was performed using ClusterProfiler (v4.6.0), with a P -value < 0.05 indicating significant enrichment. Gene set enrichment analysis (GSEA) was performed using GSEA (v4.1.0), and data analysis was conducted using the online platform Personalbio GenesCloud (<https://www.genescloud.cn>).

2.7 Validation of RNA-seq by quantitative PCR

To validate the accuracy of the RNA-seq results, eight differentially expressed genes associated with lipid metabolism were selected—*Ehhadh*, *Cyp4a31*, *Cyp4a12b*, *Gstm2*, *Cd36*, *Gpx8*, *Ppargc1a*, and *Apoa4*—for relative gene expression analysis. mRNA expression levels of the target genes were quantified using the ABI Prism 7500 Real-Time PCR System (Applied Biosystems, USA). Total RNA was extracted from liver tissue samples (n=3) using TRIzol reagent (Invitrogen, USA), and cDNA was synthesized using SuperScript II Reverse Transcriptase. Gene-specific primer sequences are detailed in Table S2, with β-Actin serving as the reference gene for normalization. Amplification reactions were performed using PowerUp™ SYBR Green Master Mix (ABI, USA). Each sample was analyzed in triplicate, and a no-template negative control was included. Relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method.

2.8 Liver Metabolomics Analysis

Liver metabolomics analysis followed the modified protocol from Jiang et al. (2022)^[27]. Briefly, liver samples (50 mg) were homogenized for 30 minutes in a mixture of methanol/acetonitrile/water (2:2:1, v/v), then centrifuged and concentrated. The samples were analyzed using a UPLC-QTOF-MSE system (Waters

Corporation, USA, Acquity UPLC I-class). After structural identification, data preprocessing, and quality evaluation, data analysis was performed using the R software package (v3.2.0). A t-test was used to evaluate significant differences between groups, with P -values < 0.05 and $VIP > 1$ considered as significant metabolites. Metabolic pathways were constructed based on KEGG (<http://www.genome.jp/kegg/pathway.html>), with pathways having P -values < 0.05 regarded as important.

2.9 Combined Analysis

For the integrated analysis of metabolomics and transcriptomics, quantitative detection results from both datasets were initially obtained, followed by correlation analysis and O2PLS analysis. Subsequently, differentially expressed metabolites and transcripts were extracted, and the correlation results were screened. Based on the compound information from the KEGG database (https://www.kegg.jp/dbget-bin/www_bfind?compound), enzyme-related transcripts were mapped, and the differential expression trends of the paired metabolites and transcripts were organized. Finally, the pathways enriched in both omics datasets were consolidated, and the commonly annotated pathways for the differentially expressed metabolites and genes were compiled.

2.10 Statistical Analysis

All experiments were independently repeated at least 6 times. Data are expressed as mean $\pm$ standard deviation (SD) unless otherwise noted. Data analysis and visualization were performed using SPSS software (version 27.0, SPSS Inc., USA) and GraphPad Prism software (version 9.0, GraphPad Software Inc., USA). Statistical significance was determined by one-way ANOVA followed by Duncan's test, with P -values < 0.05 indicating statistical significance.

3. Results

3.1 YFRPR Reduces High-Fat Diet (HFD)-Induced Obesity in Mice

To investigate the potential of YFRPR in preventing obesity, the peptide was synthesized with a purity of 98.37% using solid-phase synthesis (chromatogram and mass spectrometry data are provided in Fig. S2). Subsequently, a 10-week peptide intervention study was conducted on high-fat diet-induced mice. Mice were orally administered two doses of YFRPR (25 mg/mL and 100 mg/mL) daily (Fig. S1). Compared to the normal diet (ND) group, the body weight of HFD-fed mice significantly increased by week 2. At the end of the experiment, the average body weight of the ND group was (28.44 ± 1.71) g, whereas the HFD group weighed (46.55 ± 1.49) g (Fig. 1A). The YFRPR treatment groups showed significant weight differences compared to the HFD group starting from week 3. By week 10, the body weights of the low-dose and high-dose YFRPR treatment groups were (34.48 ± 3.03) g and (30.64 ± 1.74) g, respectively, significantly lower than the HFD group, suggesting that YFRPR effectively prevents HFD-induced obesity in mice with a clear dose-dependent effect (Fig. 1A). The effect size (Cohen's d) was calculated to assess the magnitude of differences between the groups. The analysis revealed a large effect size of 5.05 between the HFD group and HFD-L-Y, and an even more substantial effect size of 9.82 between the HFD group and HFD-H-Y, indicating a pronounced and

statistically significant impact of the experimental treatment on body weight. To evaluate the robustness of the experimental design, a power analysis was conducted. With 8 mice per group, the study achieved a statistical power of nearly 100%, significantly surpassing the conventional threshold of 80%. This high statistical power underscores the reliability of the experimental design in detecting true group differences. As a result, the findings of this study are not only statistically significant but also highly reproducible, reinforcing the validity of the observed outcomes. The typical appearance of the mice in each group is shown in Fig. 1B.

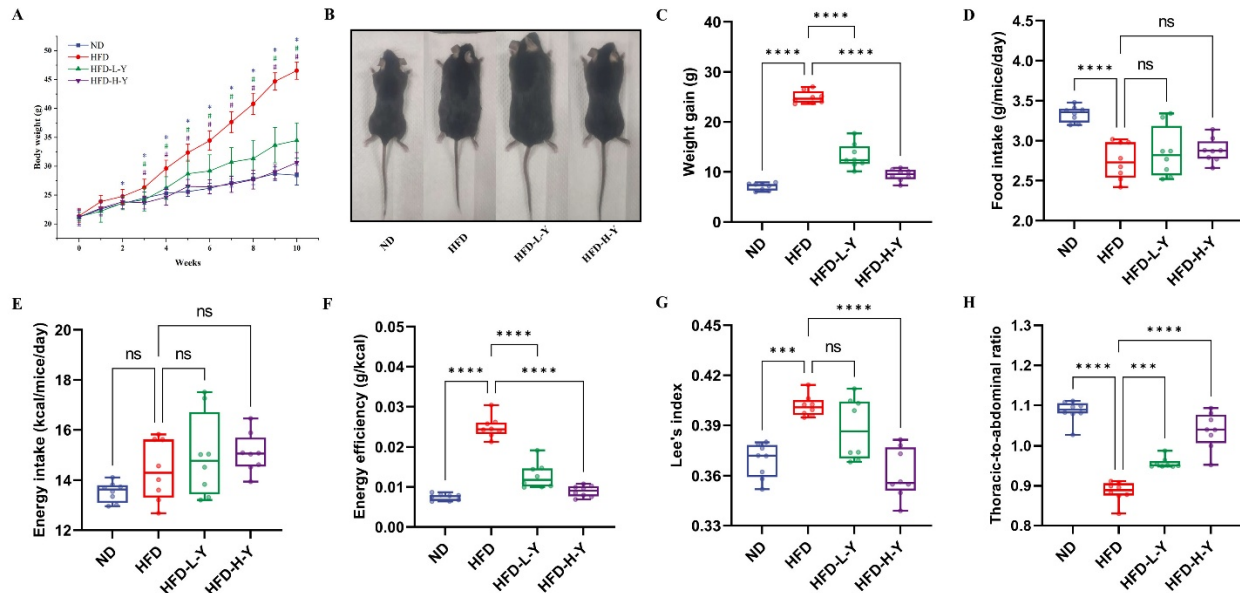


Fig. 1. YFRPR reduces high-fat diet (HFD)-induced obesity in mice. (A) Body weight change curve. $*P < 0.05$, HFD vs. ND; $\#P < 0.05$, HFD vs. HFD-L-Y or HFD-H-Y. (B) Representative photographs of mice. (C) Weight gain. (D) Food intake. (E) Energy intake. (F) Energy efficiency. (G) Lee's index. (H) Thoracic to abdominal ratio. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$, n.s. no significance. ND, normal fat diet; HFD, high-fat diet; HFD-L-Y, 25 mg/kg body weight of YFRPR; HFD-H-Y, 100 mg/kg body weight of YFRPR.

Regarding weight gain, the ND group gained (7.05 ± 0.72) g, significantly lower than the (24.91 ± 1.25) g gain observed in the HFD group ($P < 0.0001$). Mice receiving low-dose and high-dose YFRPR gained (13.16 ± 2.45) g and (9.40 ± 1.17) g, respectively, significantly less than the HFD group ($P < 0.0001$) (Fig. 1C). Although the ND group had a higher food intake than the other groups (Fig. 1D), there was no significant difference in total energy intake among the four groups due to the higher energy density of the high-fat diet (Fig. 1E). However, the energy efficiency of the HFD group was significantly higher than that of the ND group ($P < 0.0001$), and after YFRPR treatment, energy efficiency in the high-fat diet group significantly decreased ($P < 0.0001$) (Fig. 1F). This suggests that YFRPR effectively inhibited HFD-induced obesity and reduced the energy utilization efficiency of a high-fat diet.

Additionally, to assess the health status of the mice, the Lee's index and the thoracic-to-abdominal (TA) ratio were measured. The results showed significant changes in both Lee's index and TA ratio in mice fed a high-fat diet (HFD). However, after treatment with a high dose of YFRPR, these indices were restored to normal levels (Fig. 1G, H). In conclusion, the findings suggest that YFRPR can effectively reduce obesity induced by a high-fat diet in mice, with the higher dose showing more pronounced effects than the lower dose.

3.2 YFRPR Prevents HFD-Induced Adipocyte Hypertrophy and Liver Steatosis

Adipose and liver tissues from different groups of mice were dissected and analyzed to explore the effects of YFRPR on adipose tissue and liver of HFD mice (Fig. 2). After 10 weeks of HFD feeding, the weights of the mesenteric, inguinal, and epididymal fats were (1.37 ± 0.16) g, (0.73 ± 0.07) g, and (3.01 ± 0.15) g, respectively, accounting for $9.99\% \pm 0.47\%$ of the total body weight (Fig. 2A-D). However, after YFRPR intervention, the fat weights were significantly reduced. Mice in the high-dose YFRPR group showed reduced mesenteric, inguinal, and epididymal fat weights to (0.20 ± 0.03) g, (0.46 ± 0.05) g, and (1.52 ± 0.11) g, respectively, and the percentage of fat weight relative to body weight decreased to $7.00\% \pm 0.33\%$ ($P < 0.0001$). Notably, after high-dose YFRPR treatment, the mesenteric fat of the mice returned to levels similar to those in the ND group, while inguinal and epididymal fat, although decreased compared to the HFD group, remained significantly higher than those in the ND group. The typical morphology of abdominal fat in each group is shown in Fig. 2E. Furthermore, adipocyte morphology in epididymal fat was examined by HE staining (Fig. 2F). Compared to the ND group, HFD feeding significantly increased the size and diameter of epididymal adipocytes. Interestingly, following YFRPR treatment, the epididymal adipocytes of HFD mice exhibited a dose-dependent reduction in size.

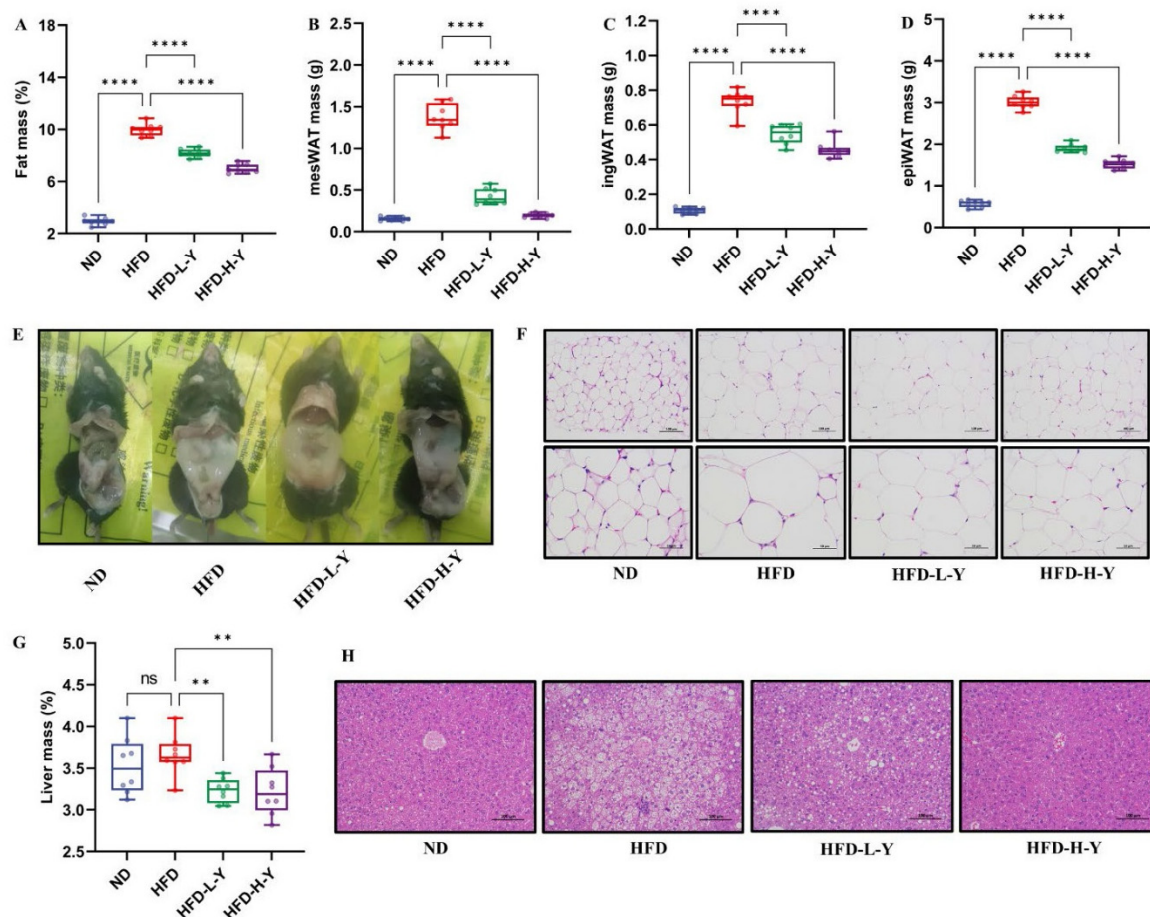


Fig. 2. YFRPR prevents HFD-induced adipocyte hypertrophy and hepatic steatosis. (A) Fat mass percentage of mice. (B) Weights of mesWAT. (C) Weights of ingWAT. (D) Weights of epiWAT. (E) Representative anatomical image of mice. (F) Representative images of epiWAT. (G) Liver mass percentage of mice. (H) Representative images of H&E staining of the liver section. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, n.s. no significance. ND, normal fat diet; HFD, high-fat diet; HFD-L-Y, 25 mg/kg body weight of YFRPR; HFD-H-Y, 100 mg/kg body weight of YFRPR.

The liver index (Fig. 2G) showed an increase in liver weight in the HFD-fed mice, although there was no significant difference in the liver index when compared to the ND group. After treatment with YFRPR, the liver index in the HFD group decreased significantly ($P < 0.01$), suggesting that YFRPR helped reduce the liver burden caused by the high-fat diet. To further evaluate the effect of YFRPR on liver steatosis, liver tissue was examined using HE staining (Fig. 2H). The results indicated significant fat accumulation in the livers of HFD-fed mice, a sign of liver steatosis. Following YFRPR treatment, liver fat accumulation showed marked improvement, and this effect was dose-dependent. Overall, these findings suggest that YFRPR effectively prevents HFD-induced adipocyte hypertrophy and liver steatosis, providing notable protective benefits.

3.3 YFRPR Improves Lipid Metabolism Dysregulation, Oxidative Stress, and Inflammatory Response Induced by HFD

To further explore the effects of the peptide YFRPR on lipid metabolism dysregulation, oxidative stress, and inflammation in HFD-induced mice, we analyzed the biochemical serum indices of the mice (Fig. 3). Compared to the normal diet (ND) group, the HFD group showed significantly elevated serum triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels ($P < 0.0001$), while high-density lipoprotein cholesterol (HDL-C) levels were significantly reduced ($P < 0.0001$), indicating that HFD feeding induced dyslipidemia in the mice (Fig. 3A-D). In the YFRPR groups, serum TG, TC, and LDL-C levels significantly decreased, and HDL-C levels significantly increased in a dose-dependent manner. The high-dose YFRPR treatment restored blood lipid indices to normal levels.

Regarding glucose metabolism, the fasting blood glucose levels of HFD-fed mice were (8.39 ± 0.38) mmol/L, significantly higher than the (5.13 ± 0.57) mmol/L in the ND group (Fig. 3E). Insulin levels showed similar changes, with significantly elevated insulin content in HFD-fed mice ($P < 0.0001$), indicating the development of insulin resistance. After YFRPR intake, both fasting blood glucose and insulin levels significantly decreased (Fig. 3E, F).

In terms of oxidative stress, the HFD group exhibited significantly reduced levels of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), while malondialdehyde (MDA) levels were significantly increased, indicating an oxidative stress response. After YFRPR intake, these oxidative stress markers were notably restored (Fig. 3I, J, K). Additionally, YFRPR treatment significantly reduced serum levels of pro-inflammatory cytokines, including interleukin-1 α (IL-1 α), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) in HFD-fed mice (Fig. 3L, M, N), further suggesting that YFRPR can suppress the inflammatory response in the mice. Furthermore, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly elevated in HFD-fed mice, and these levels exhibited a dose-dependent decrease following YFRPR treatment (Fig. 3G, H).

Lastly, the biochemical indices of the mice's livers were examined. Consistent with the serum changes, HFD feeding significantly increased liver TG, TC, and MDA levels, while GSH-Px and SOD levels were significantly reduced ($P < 0.0001$). After YFRPR intake, these liver indices were restored in a dose-dependent manner (Fig. 3O-S).

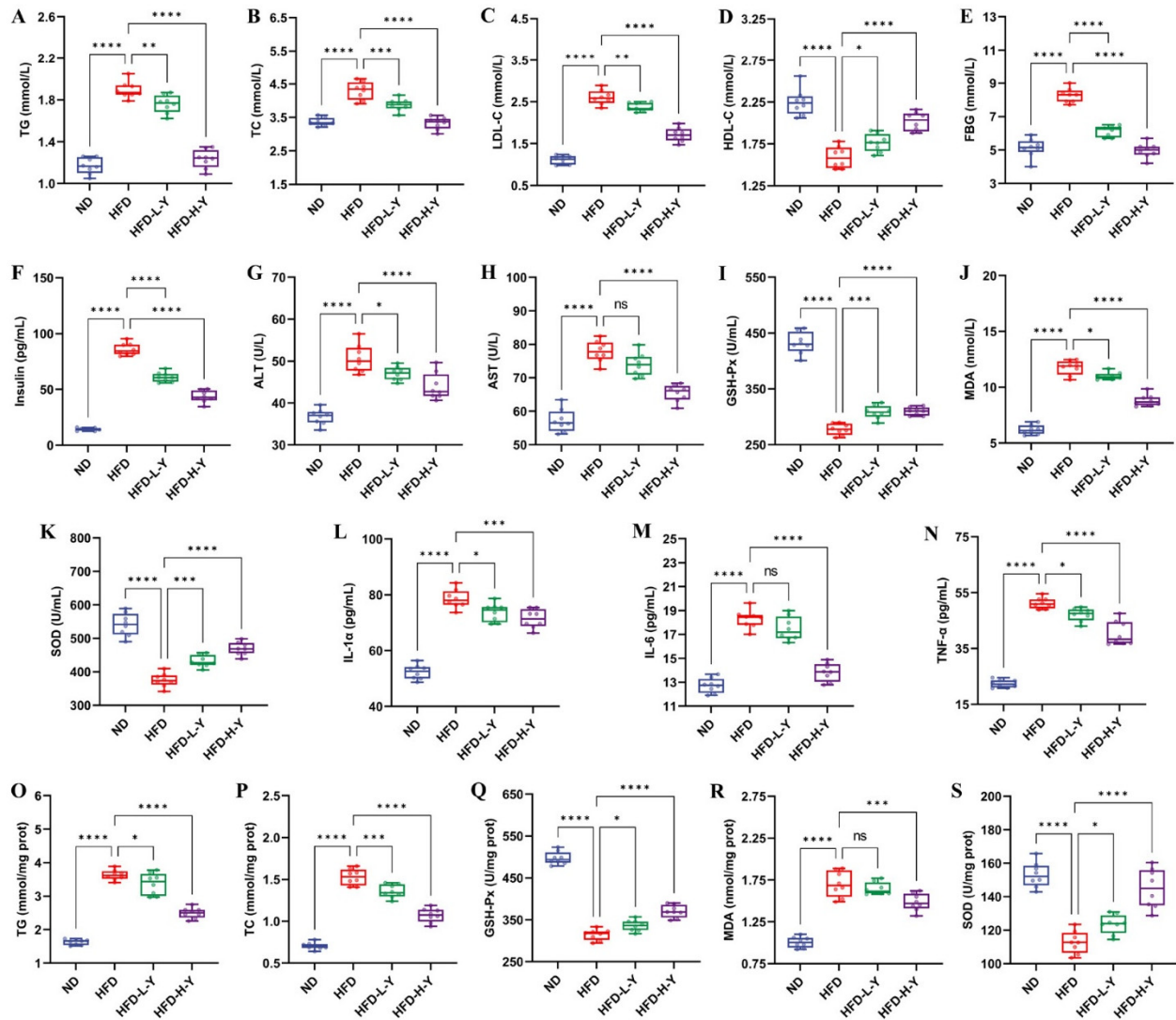


Fig. 3. YFRPR ameliorates HFD-induced lipid metabolism disorders, oxidative stress and inflammatory response. (A-N) Serum levels of TG, TC, LDL-C, HDL-C, FBG, Insulin, ALT, AST, GSH-Px, MDA, SOD, IL-1 α , IL-6, TNF- α . (O-S) Liver levels of TG, TC, GSH-Px, MDA, SOD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, n.s. no significance. ND, normal fat diet; HFD, high-fat diet; HFD-L-Y, 25 mg/kg body weight of YFRPR; HFD-H-Y, 100 mg/kg body weight of YFRPR. TG, Triglyceride; TC, Total cholesterol; LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; FBG, Fasting blood glucose; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GSH-Px, Glutathione peroxidase; MDA, Malondialdehyde; SOD, Superoxide dismutase; IL-1 α , Interleukin-1 α ; IL-6, Interleukin 6; TNF- α , Tumor necrosis factor- α .

3.4 Effects of YFRPR Intake on Liver Transcriptome in High-Fat Diet Fed Mice

To further investigate the potential molecular mechanisms through which YFRPR prevents HFD-induced obesity, transcriptome analysis was performed on liver samples collected at the end of the experiment. Principal component analysis (PCA) showed that PC1 and PC2 explained 40.4% and 22.9% of the variance, respectively (Fig. S3A), with distinct clustering observed between the different groups, indicating significant differences in the transcriptomes.

In pairwise comparisons between the ND, HFD, HFD-L-Y, and HFD-H-Y groups, differentially expressed genes (DEGs) were identified. As shown in Fig. 4A, a large number of DEGs were identified in the comparisons between the ND and HFD groups, HFD and HFD-L-Y groups, and HFD and HFD-H-Y groups

(481 upregulated genes and 349 downregulated genes between ND and HFD; 390 upregulated genes and 351 downregulated genes between HFD and HFD-L-Y; 465 upregulated genes and 548 downregulated genes between HFD and HFD-H-Y) (Fig. 4B, C, Table S3). In contrast, fewer DEGs were found between the HFD-L-Y and HFD-H-Y groups, with only 120 upregulated genes and 157 downregulated genes, indicating a minimal impact of dosage on gene expression.

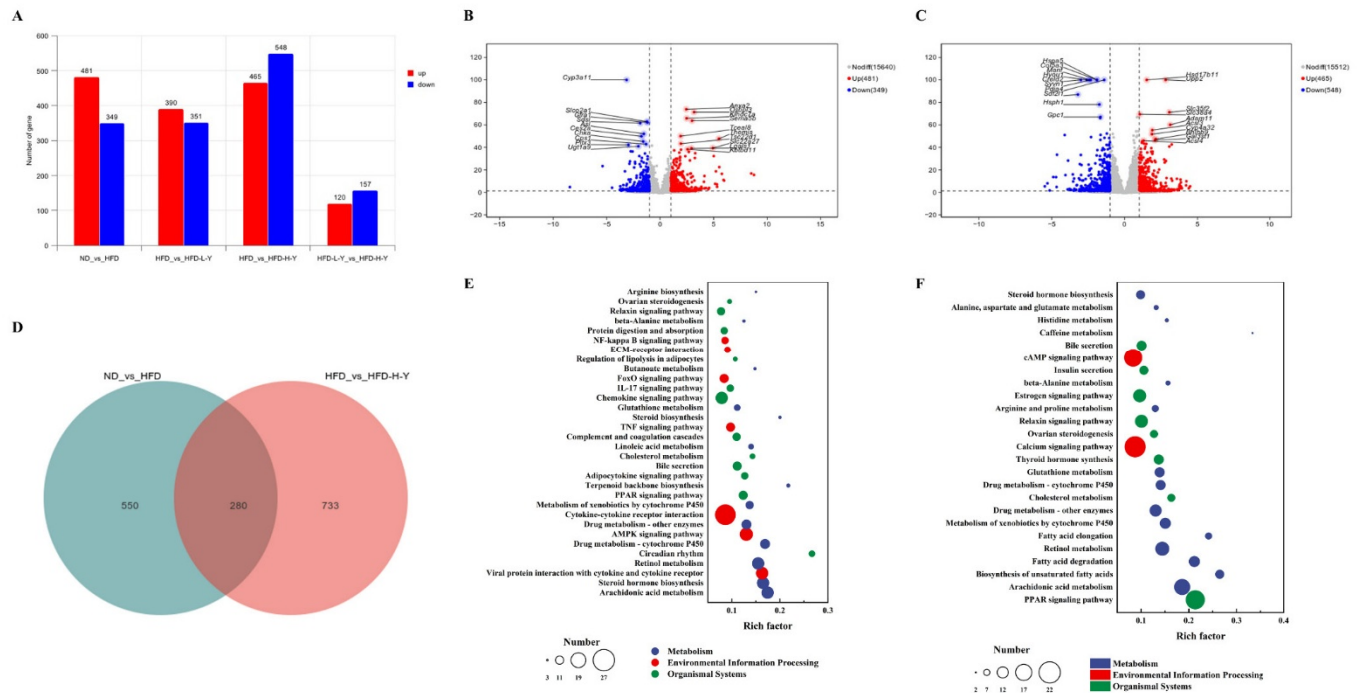


Fig. 4. Effect of YFRPR on gene expression in mouse liver. (A) Statistics of differentially expressed genes (DEGs). (B) Volcano plots of DEGs in ND vs. HFD. (C) Volcano plots of DEGs in HFD vs. HFD-H-Y. (D) Venn analysis. (E) Pathway analysis in ND vs. HFD. (F) Pathway analysis in HFD vs. HFD-H-Y. ND, normal fat diet; HFD, high-fat diet; HFD-L-Y, 25 mg/kg body weight of YFRPR; HFD-H-Y, 100 mg/kg body weight of YFRPR.

Hierarchical clustering and heatmap analysis (Fig. S3B) revealed that after HFD feeding, many DEGs were significantly upregulated and downregulated, and following YFRPR intervention, part of the DEGs were restored to normal levels. This result suggests that YFRPR intake can modulate the liver transcriptome, contributing to the prevention of obesity. Venn analysis of the DEGs between the ND and HFD groups, and between the HFD and HFD-H-Y groups, revealed 280 common DEGs (Fig. 4D), suggesting that YFRPR may regulate these genes to exert its anti-obesity effects.

The pathway enrichment analyses on these DEGs were conducted to explore the mechanisms of YFRPR in obesity prevention. GO analysis (Fig. S3C, D) indicated significant enrichment of GO terms related to lipid metabolism, including lipid metabolic process, fatty acid metabolic process, unsaturated fatty acid metabolic process, and long-chain fatty acid metabolic process, in comparisons between the ND and HFD groups, as well as between the HFD and HFD-H-Y groups. These results suggest that YFRPR treatment significantly affects lipid metabolism in HFD-fed mice.

KEGG pathway enrichment analysis (Fig. 4E, F) further revealed significant differences in multiple biological pathways between the ND and HFD groups, involving arachidonic acid metabolism, cholesterol metabolism, adipocytokine signaling pathway, inflammatory pathways (such as TNF, NF- κ B, IL-17), PPAR

signaling, and lipolysis regulation. After YFRPR intervention, the HFD-H-Y group showed significant differences from the HFD group in several important pathways, including PPAR signaling, arachidonic acid metabolism, fatty acid degradation and elongation, glutathione metabolism, insulin secretion and fat storage, steroid hormone biosynthesis, and cAMP signaling pathways.

To validate the accuracy of the RNA-seq results, eight differentially expressed genes associated with lipid metabolism were selected for qPCR analysis. The relative expression levels of these genes varied across different groups, and their expression patterns were consistent with the RNA-seq data (Fig. 5). These findings confirm the reliability of our RNA-seq results, providing robust data for investigating YFRPR-induced metabolic differences in mouse liver.

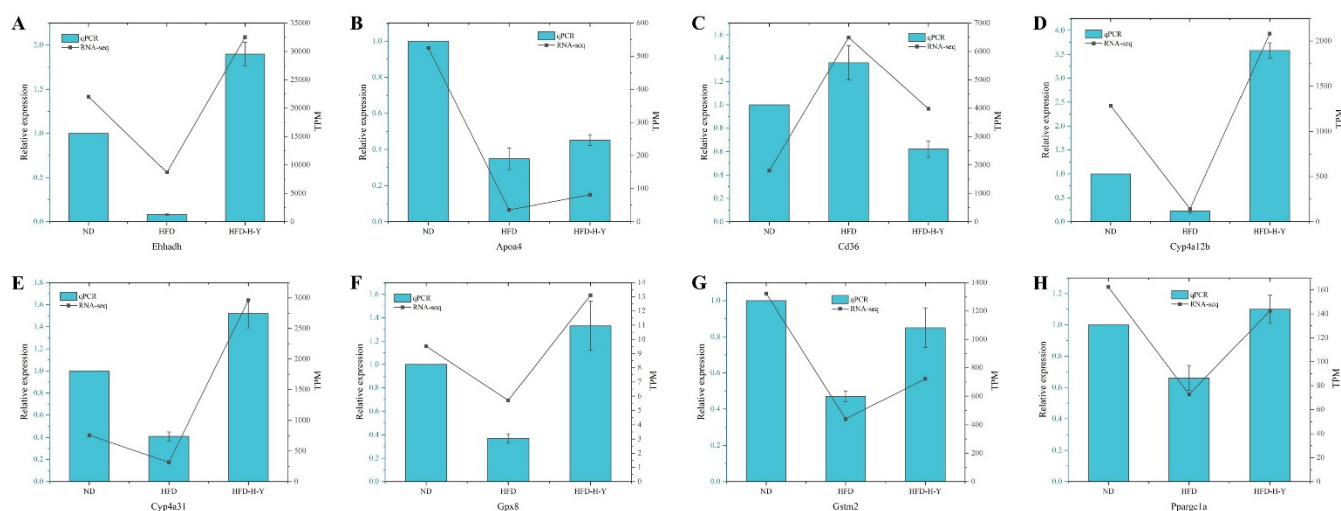
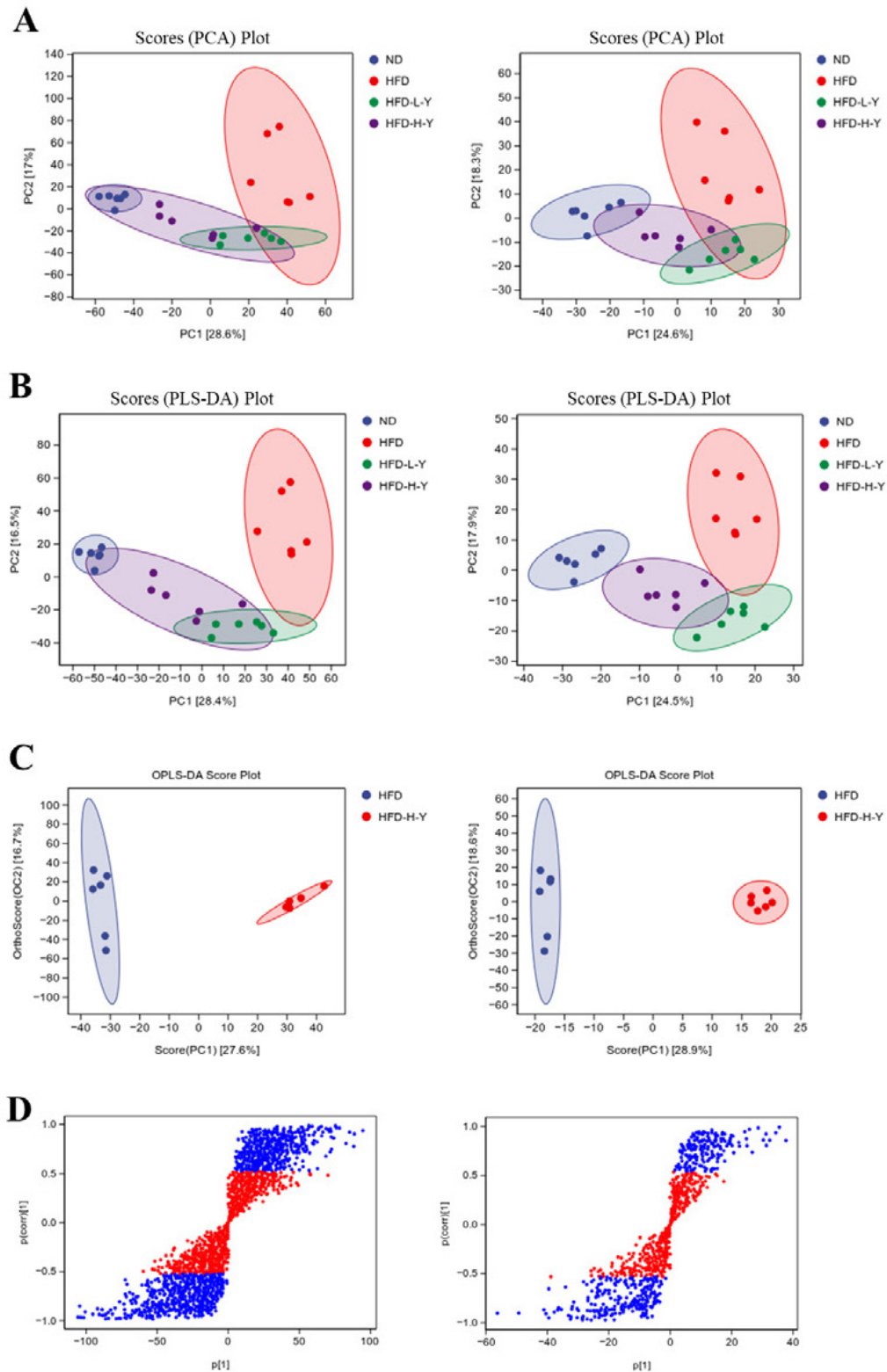


Fig. 5. Relative expression levels of eight key DEGs in ND, HFD and HFD-H-Y group mice. Values are presented as mean $\pm$ standard deviation. ND, normal fat diet; HFD, high-fat diet; HFD-H-Y, 100 mg/kg body weight of YFRPR.

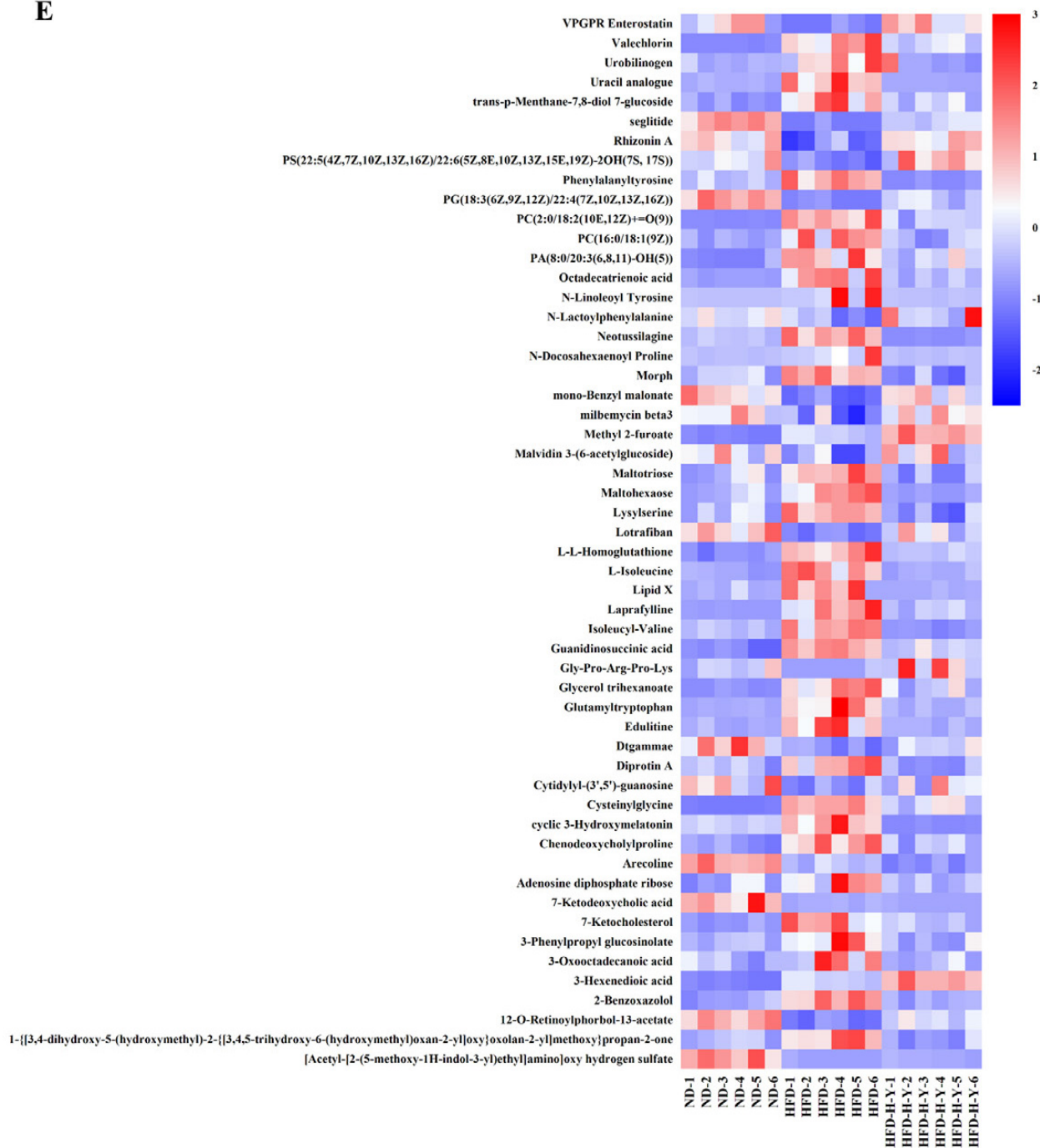
3.5 Effects of YFRPR Intake on the Liver Metabolome of High-Fat Diet (HFD)-Fed Mice

To further investigate the effects of YFRPR on the liver's physiological and metabolic responses in HFD-fed mice, this study performed liver metabolomics analysis using UHPLC-MS/MS on four groups of mice. A total of 6415 peaks were detected, with 5115 peaks in positive ion mode (ESI+) and 1300 peaks in negative ion mode (ESI⁻). Multivariate statistical analysis was employed to distinguish liver metabolites across the four groups of mice (Fig. 6 A-D, Fig. S4 A-B). As shown in Fig. 6A, PCA score plots demonstrated clear clustering of samples from different groups, indicating significant differences in liver metabolite profiles. The PLS-DA score plot (Fig. 6B) further assessed the data quality and revealed the effects of YFRPR intake. The distinct separation of the four groups indicated significant metabolic changes in the liver of HFD mice, with some degree of recovery following YFRPR intervention. OPLS-DA analysis showed significant metabolic variations between the ND-HFD and HFD-HFD-H-Y groups (Fig. S4A, Fig. 6C). The S-plot (Fig. S4B, Fig. 6D) revealed potential biomarkers in the ND-HFD and HFD-HFD-H-Y groups, with metabolites located at the two corners of the plot. The model's explanatory power ($R^2Y > 0.5$) and predictive ability ($Q^2 > 0.5$) indicated excellent reliability and prediction capacity. Specifically, the R^2Y and Q^2 values for ND vs. HFD were 0.998 and 0.970 (positive ion mode), 0.998 and 0.939 (negative ion mode), and for HFD vs.

HFD-H-Y, they were 0.989 and 0.925 (positive ion mode), 0.996 and 0.924 (negative ion mode), further confirming the good fit and predictive power of the model.



E



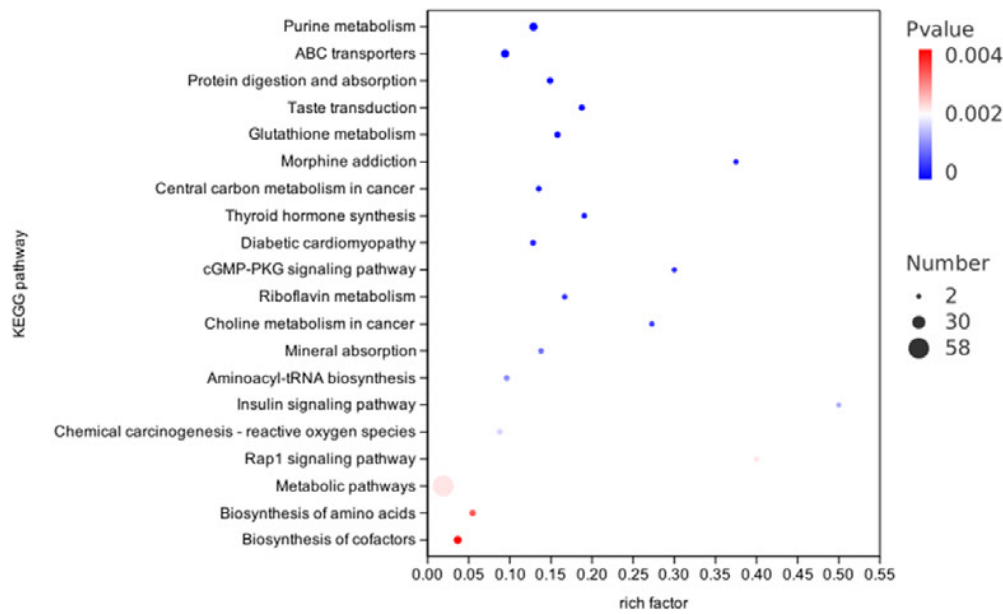
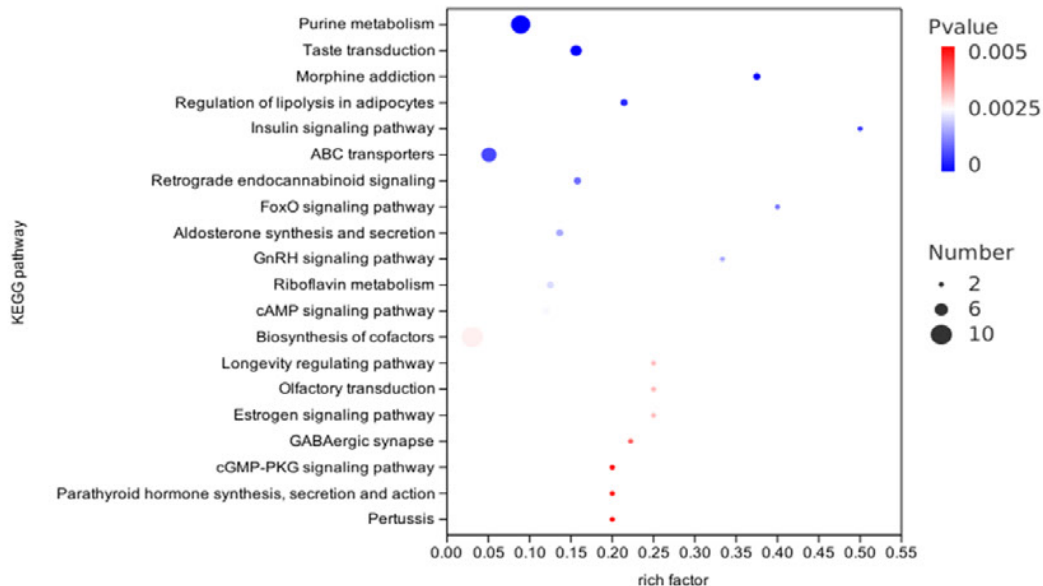
F**G**

Fig. 6. Effect of YFRPR on liver metabolites in mice. (A) PCA score plot of the different groups in the positive ion (left) and negative ion (right). (B) PLS-DA score plot of the different groups in the positive ion (left) and negative ion (right). (C) OPLS-DA score plot of the HFD and HFD-H-Y groups in the positive ion (left) and negative ion (right). (D) S-loading plot based on OPLS-DA analysis model of the HFD and HFD-H-Y groups in the positive ion (left) and negative ion (right). (E) Heatmap of the relative abundance of significantly different metabolites between different groups. (F) The metabolic pathway impact prediction between ND and HFD groups based on the KEGG online database. (G) The metabolic pathway impact prediction between HFD and HFD-H-Y groups based on the KEGG online database. ND, normal fat diet; HFD, high-fat diet; HFD-L-Y, 25 mg/kg body weight of YFRPR; HFD-H-Y, 100 mg/kg body weight of YFRPR.

To identify significant differential metabolites, criteria of $FC \geq 2$ or ≤ 0.5 , $VIP > 1$, and $P < 0.05$ were applied. The heatmap for all differential metabolites (DAMs) is shown in Fig. S4C. In the comparison between the HFD model and the ND group, a total of 172 DAMs were identified, with 89 upregulated and 83 downregulated (Table S4). In the comparison between the HFD-H-Y and HFD groups, 149 DAMs were identified (49 upregulated and 100 downregulated) (Table S4). These differential metabolites were related to lipids and lipophilic molecules, organic acids and their derivatives, alkaloids and their derivatives, phenolic compounds, nucleosides, nucleotides and analogs, organic oxygen compounds, organic heterocyclic compounds, as well as phenylacetones and polyphenolic compounds. To more accurately identify metabolites that were restored to normal levels after YFRPR intake, we analyzed the common DAMs between the ND-HFD and HFD-HFD-H-Y groups, resulting in the identification of 54 potential biomarkers (Fig. 6E). These metabolites included lipids and lipophilic molecules, organic acids and their derivatives, organic heterocyclic compounds, phenolic compounds, nucleosides and their analogs, among others. Among these metabolites, 37 DAMs were significantly upregulated and 17 DAMs were significantly downregulated in HFD-fed mice compared to the ND group. In the YFRPR intervention group, 37 DAMs were significantly downregulated and 17 DAMs were significantly upregulated.

To further understand the impact of high-dose YFRPR on liver metabolites, a KEGG-based metabolic pathway enrichment analysis was conducted. The results showed that the identified DAMs mapped to 122 KEGG pathways (Fig. 6F, G). In the comparison between the ND and HFD groups, the most significantly changed metabolic pathways included purine metabolism, ABC transporters, protein digestion and absorption, taste transduction, glutathione metabolism, morphine addiction, central carbon metabolism in cancer, thyroid hormone synthesis, diabetic cardiomyopathy, cGMP-PKG signaling pathway. In the comparison between the HFD and HFD-H-Y groups, the significantly altered metabolic pathways mainly included purine metabolism, taste transduction, morphine addiction, regulation of lipolysis in adipocytes, insulin signaling pathway, ABC transporters, Retrograde endocannabinoid signaling, FoxO signaling pathway, aldosterone synthesis and secretion, GnRH signaling pathway.

3.6 Integrated Analysis of Metabolomics and Transcriptomics in the Prevention of Obesity by YFRPR

To further explore the mechanisms by which YFRPR prevents obesity, this study integrated metabolomics and transcriptomics data and performed an analysis using iPath 3.0. Differential metabolites (DAMs) and differentially expressed genes (DEGs) were combined to further explore the metabolic pathways altered in response to YFRPR intake (Fig. 7). Comparisons between the ND and HFD groups, as well as between the HFD and HFD-H-Y groups, revealed that the changes in metabolic pathways were primarily enriched in lipid metabolism, energy metabolism, amino acid metabolism, and nucleotide metabolism. Further analysis of the enriched pathways between the HFD and HFD-H-Y groups identified that lipid metabolism pathways mainly involved linoleic acid metabolism, arachidonic acid metabolism, triglyceride metabolism, glycerophospholipid metabolism, and fatty acid elongation and degradation. Additionally, energy metabolism involves β -alanine metabolism, metabolism of valine, leucine, and isoleucine, metabolism of alanine,

aspartate, and glutamate, and the tricarboxylic acid (TCA) cycle. In carbohydrate metabolism, enriched pathways included glycolysis/gluconeogenesis and the TCA cycle (Fig. 7).

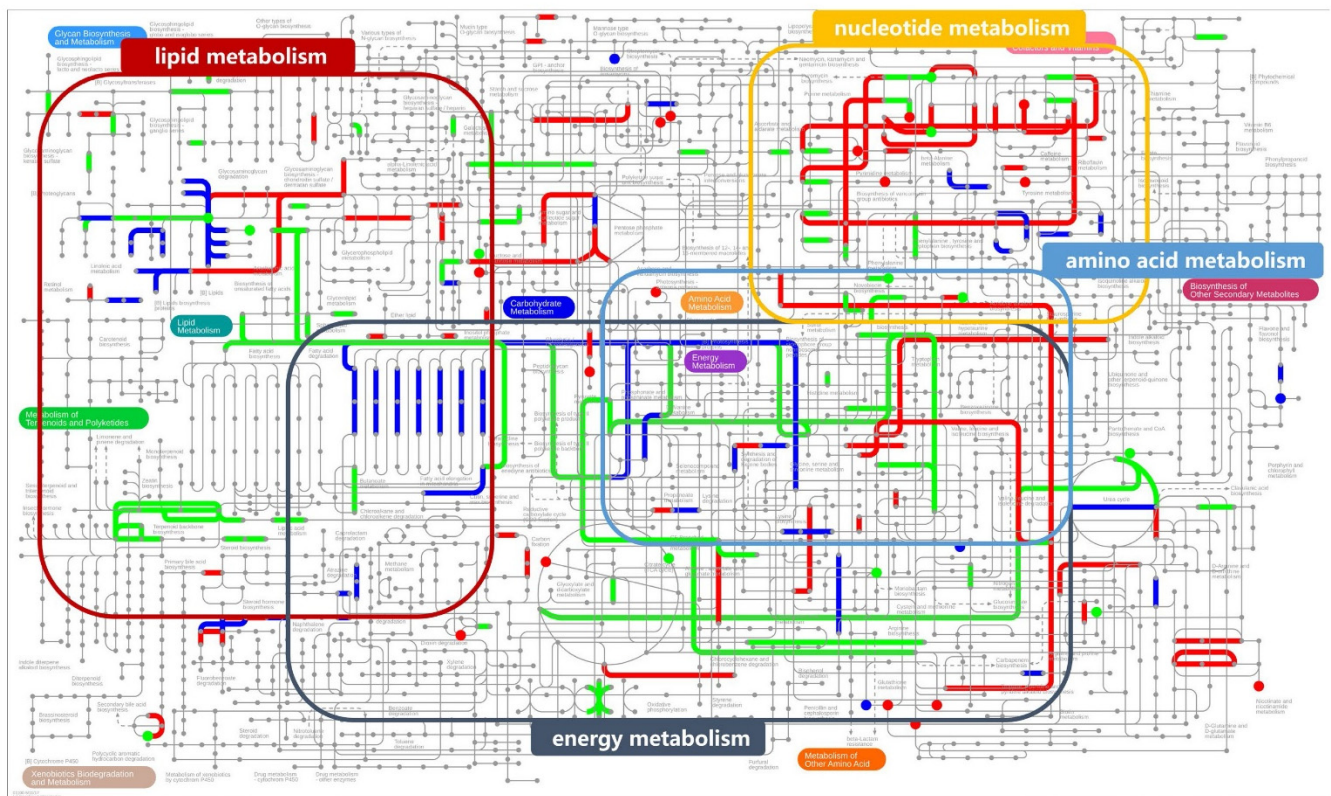


Fig. 7. Integrated analysis of transcriptome and metabolome in lipid metabolism. Dots and lines represent DAMs and DEGs, respectively. Red, ND vs. HFD; green, HFD vs. HFD-H-Y; blue, shared DAMs and DEGs. ND, normal fat diet; HFD, high-fat diet; HFD-H-Y, 100 mg/kg body weight of YFRPR.

The integrated analysis of transcriptomics and metabolomics suggested that YFRPR alleviates obesity by modulating key pathways involved in lipid metabolism. Furthermore, pathways associated with inflammation and oxidative stress, which are linked to obesity, were also improved. To further validate this hypothesis, several important metabolic pathways were analyzed, including PPAR signaling (map03320), arachidonic acid metabolism (map00590), cholesterol metabolism (map04979), fatty acid degradation (map00071), fatty acid elongation (map00062), and glycerophospholipid metabolism (map00564). Key DAMs and DEGs in these pathways were manually integrated to illustrate the potential mechanisms through which YFRPR prevents HFD-induced obesity by regulating lipid metabolism (Fig. 8).

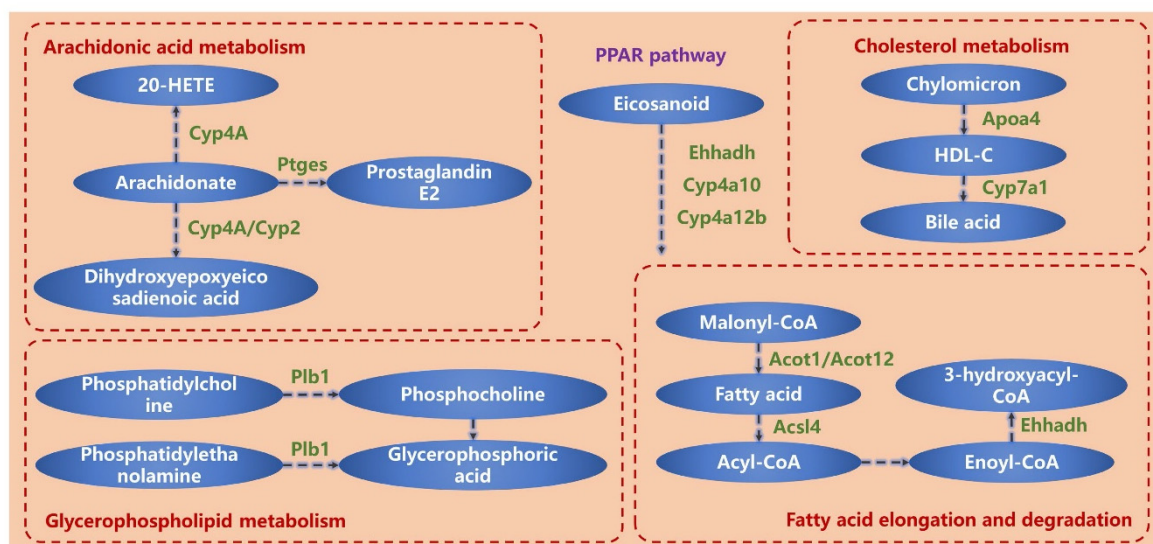


Fig. 8. Possible mechanisms of YFRPR to prevent HFD-induced disorders of hepatic lipid metabolism.

4. Discussion

YFRPR, identified as an inhibitor of pancreatic lipase and cholesterol esterase from *Rosa roxburghii* seed protein, was analyzed for its molecular mechanism. The findings indicate that it primarily competes with the enzyme's active site and substrate through hydrogen bonding and hydrophobic interactions, thereby inhibiting the enzyme's activity^[23]. Additionally, YFRPR was found to enhance lipid accumulation in 3T3-L1 cells, reduce LDL-C levels, and increase HDL-C levels, suggesting its potential to regulate lipid metabolism^[24]. Therefore, animal experiments are needed to validate its ability to prevent diet-induced obesity.

An increased Lee's index is often linked to weight gain, abdominal fat accumulation, enlarged adipocytes, and elevated blood lipid levels, making it an effective indicator for assessing obesity in adult mice^[28]. This study shows that, with the same energy intake, daily administration of 25 mg/kg and 100 mg/kg YFRPR significantly slowed weight gain in mice, reduced Lee's index, and decreased abdominal circumference. Anatomical analysis further demonstrated that YFRPR intake effectively reduced fat accumulation, adipocyte hypertrophy, and improved liver steatosis caused by a high-fat diet (HFD). Previous research has indicated that proteins or bioactive peptides, such as quinoa peptides, phycobiliproteins, and pea albumin, can significantly reduce adipocyte hypertrophy and liver steatosis^[5, 7, 29]. These studies suggest that bioactive peptides may prevent obesity by regulating lipid metabolism, oxidative stress, and inflammation. After feeding mice a high-fat diet, fasting blood glucose, serum and liver total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and insulin levels were significantly elevated, while high-density lipoprotein cholesterol (HDL-C) levels decreased. These changes are closely linked to metabolic disruptions in adipose tissue and the liver, insulin resistance, and fat accumulation. YFRPR intake significantly reversed the elevated blood lipid levels, suggesting that it may help prevent obesity by modulating lipid metabolism. Similarly, microencapsulated brewer's spent grain peptides have been shown to effectively improve blood lipid levels in hyperlipidemia model mice by lowering triglycerides, cholesterol, and free fatty acids, while reducing adipocyte size and fat accumulation^[8]. Oxidative stress is a key factor

contributing to obesity-related issues, with malondialdehyde (MDA) serving as a biomarker for lipid peroxidation^[18]. In HFD-fed mice, MDA levels in both serum and liver were significantly elevated. The body defends against oxidative stress through endogenous antioxidants like superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)^[30]. However, in HFD-fed mice, the activities of SOD and GSH-Px were significantly lower compared to the normal diet (ND) group, exacerbating oxidative damage. This may be due to the rapid depletion of these enzymes as they combat the excess free radicals generated by the high-fat diet^[31]. YFRPR treatment significantly reduced MDA levels and restored the activities of SOD and GSH-Px, indicating that YFRPR may alleviate oxidative stress through its antioxidant properties. Similarly, corn peptides have been shown to exhibit strong antioxidant effects in HFD-induced fatty liver, significantly reducing reactive oxygen species and slowing liver lipid accumulation^[10]. Furthermore, obesity is often associated with chronic low-grade inflammation, primarily driven by the secretion of pro-inflammatory factors like IL-1 α , IL-6, and TNF- α , which contribute to immune responses^[32]. The elevation of these pro-inflammatory cytokines is closely linked to obesity-induced insulin resistance, metabolic syndrome, and cardiovascular diseases. In HFD-fed mice, elevated levels of these pro-inflammatory cytokines in adipose tissue and the liver promoted inflammation, further exacerbating metabolic disorders associated with obesity. YFRPR significantly reduced the levels of these pro-inflammatory cytokines, suggesting that it may help reduce inflammation by regulating abnormal cell proliferation in the liver and adipose tissue, decreasing macrophage secretion of pro-inflammatory factors, and mitigating the body's inflammatory response. Similarly, pea albumin has also been shown to modulate the inflammatory response, reducing the metabolic inflammation associated with obesity^[5].

The liver, as a central metabolic organ in the human body, plays a crucial role in lipid metabolism and energy balance. To further investigate the molecular mechanisms by which YFRPR supplementation prevents obesity, this study assessed the transcriptomic and metabolomic changes in the livers of mice from different experimental groups. Through comprehensive analysis of transcriptomic and metabolomic data, we found that YFRPR intake modulates multiple metabolic pathways, thereby improving obesity induced by a high-fat diet. These regulatory effects include lipid metabolism (such as PPAR signaling pathway, arachidonic acid metabolism, and cholesterol metabolism), oxidative stress and antioxidant responses (such as glutathione metabolism), anti-inflammatory signaling pathways (such as PPAR signaling pathway and calcium signaling pathway), hormone and endocrine regulation (such as steroid hormone biosynthesis), amino acid metabolism (such as β -alanine metabolism), and bile secretion. These results suggest that YFRPR counteracts high-fat diet-induced obesity through multiple mechanisms, involving various physiological functions, including metabolism, oxidative stress and inflammation.

Key differentially expressed genes (DEGs) in lipid metabolism, oxidative stress and inflammation-related pathways were analyzed, as shown in Table S5. Lipid metabolism is a central process in obesity, involving the synthesis, storage, and energy balance of fatty acids. When lipid metabolism becomes dysregulated, abnormal fat accumulation in the body occurs, leading to the development of obesity^[21].

Compared to the HFD group, mice subjected to YFRPR intake exhibited significant differences in multiple pathways related to lipid metabolism, including the PPAR signaling pathway, arachidonic acid metabolism, and cholesterol metabolism. The PPAR pathway is a key regulator of fatty acid metabolism, adipocyte differentiation, and energy metabolism. Drug interventions that activate the PPAR pathway can promote fatty acid oxidation and energy expenditure, thus reducing fat storage and preventing obesity^[16]. Both corn protein hydrolysates and oyster protein hydrolysates have also been found to prevent obesity by modulating PPAR pathway-related genes, consistent with the findings of this study^[10, 33]. This suggests the presence of peptides in these hydrolysates that share similar physiological effects with YFRPR. In comparison to the normal diet (ND) group, HFD-fed mice showed overall suppression in the PPAR pathway. High-fat diet feeding resulted in the downregulation of genes involved in fatty acid oxidation (such as *Ehhadh*, *Cyp3a11*, *Cyp3a25*, *Cyp4a10*, *Cyp4a12b*, *Cyp4a31*, *Cyp4a32*, *Ppargc1a*, etc.), while YFRPR intake significantly upregulated the expression of these genes. Specifically, *Ehhadh* participates in the β -oxidation of fatty acids, catalyzing the hydration reaction of enol-CoA and promoting the conversion of intermediates in fatty acid metabolism. Its upregulation indicates a significant increase in fatty acid degradation and conversion^[34, 35]. Dietary fiber from quinoa bran has also been reported to treat obesity by inhibiting *Ehhadh*^[34]. However, the regulation of *Ehhadh* by peptides, as discovered in this study, is a novel finding. *Cyp4a31* and *Cyp4a12b* are primarily involved in ω -oxidation reactions, helping to remove excess free fatty acids from the body and generating energy in the mitochondria in the process. Reduced expression of the CYP family is closely linked to liver fat accumulation^[35]. Arachidonic acid metabolism is closely related to fatty acid metabolism, inflammation, and membrane fluidity. It plays a crucial role in obesity development by regulating inflammation and energy balance in adipose tissue^[20]. After HFD feeding, CYP genes (such as *Cyp4a31*, *Cyp2c40*, *Cyp4a12b*) in the liver of mice were significantly downregulated, while YFRPR intervention restored the expression of these genes to normal levels (e.g., *Cyp4a32*, *Cyp4a31*, *Cyp4a10*). Additionally, transcripts involved in fatty acid synthesis (such as *Acs14*) and fatty acid degradation (such as *Acot1*, *Acot12*) were suppressed in the HFD group but were upregulated following YFRPR intake. Genes involved in fatty acid transport (such as *Cd36*, *Pik3r3*) and storage (such as *Plin4*) were elevated in the HFD group, whereas YFRPR intervention inhibited their expression. *CD36* is a lipogenic gene in the PPAR signaling pathway and encodes a membrane receptor. Up-regulation of *CD36* promotes adipogenesis, and inhibition of *CD36* helps reduce visceral fat accumulation in obese mice^[36, 37]. The transcriptomic data align with metabolic features, as observed with significant upregulation of 3-oxooctadecanoic acid, 7-ketocholesterol, 3-Oo, glycerol trihexanoate, and octadecatrienoic acid in the HFD group, which returned to normal levels following YFRPR intake. 7-Ketocholesterol is considered a novel lipid factor that regulates the adipogenic potential of undifferentiated adipocyte precursors and accelerates liver lipid accumulation while downregulating fatty acid oxidation^[38]. 3-Oxooctadecanoic acid, an intermediate product of fatty acid biosynthesis, is significantly elevated in individuals with lipid metabolic disorders, and in this study, YFRPR intervention restored its levels to normal^[39, 40].

Octadecatrienoic acid, a key substrate for liver triglyceride biosynthesis and fatty acid oxidation, was significantly elevated after HFD feeding but returned to normal levels following YFRPR intake^[41].

Studies have shown that glycerolipid metabolism and glycerophospholipid metabolism are among the most significantly affected metabolic pathways under high-fat diet (HFD) conditions^[42]. In this study, HFD feeding led to a significant downregulation of genes associated with glycerophospholipid metabolism, such as *plbl1*, whereas after YFRPR intake, the expression of these genes was significantly restored^[43]. Metabolic profiling revealed that levels of glycerolipid and glycerophospholipid underwent significant alterations in the liver of HFD-fed mice. Glycerophospholipids, due to their role in reducing blood triglycerides and cholesterol, are considered beneficial lipids. In this study, the significant increase of phosphatidic acid (PA) and phosphatidylcholine (PC) in the HFD group was restored after YFRPR intervention (Fig. 6E), in agreement with the findings of Feng et al. (2023)^[17]. Obesity typically activates lipid biosynthesis pathways, leading to an increase in phosphatidic acid (PA) levels. As a phospholipid signaling molecule, PA plays a crucial role in cell signaling and is key to the biosynthesis of fatty acids. The elevated PA levels are associated with enhanced lipogenesis because PA serves as a precursor for diacylglycerol (DAG), which plays an essential role in fat cell development and lipid storage^[17]. PC, an essential component of plasma lipoproteins, is known to reduce triglycerides and cholesterol in the blood^[19]. In addition, in the HFD-fed group, phosphatidylglycerol (PG) and phosphatidylserine (PS) levels were significantly reduced, consistent with findings from other studies on the downregulation of glycerophospholipids in HFD-fed mice^[42]. PS and PG are important phospholipids that play key roles in lipid metabolism. In the context of obesity, reductions in PS and PG levels can disrupt lipid biosynthesis, impair cell membrane integrity, and alter cellular function, contributing to metabolic dysfunction and the progression of obesity-related diseases^[44]. YFRPR intake effectively reversed this trend, suggesting that increased glycerophospholipid levels may help alleviate obesity and improve lipid metabolism.

Furthermore, cholesterol metabolism-related genes (such as *Cyp2c55*, *Cyp3a11*, and *Cyp3a25*) were downregulated in the HFD group but were restored after YFRPR intake. Cholesterol metabolism directly affects lipid synthesis and breakdown, and abnormal cholesterol synthesis often leads to fat accumulation and related metabolic diseases. By regulating cholesterol metabolism, lipid metabolism, and weight management can be effectively improved^[45]. Additionally, *Apoa4*, a lipoprotein primarily synthesized in the intestine and liver and typically part of high-density lipoprotein (HDL), plays a role in promoting fatty acid oxidation, reducing fat storage, and improving insulin sensitivity, thus helping to mitigate chronic low-grade inflammation induced by obesity and improve lipid metabolism^[46, 47]. *Apoa4* expression was significantly downregulated in HFD-fed mice, but its level was restored after YFRPR intake, consistent with the findings from serum biochemical measurements. In the process of cholesterol metabolism, 7-Ketocholesterol, an oxidation product of cholesterol, is mainly catalyzed by members of the CYP450 enzyme family (such as *CYP3A* and *CYP2C* families) and is involved in lipid metabolism, atherosclerosis, and other cholesterol-related diseases^[48]. Similarly, Chenodeoxycholyproline, a conjugate of bile acid, is closely

related to cholesterol metabolism and its excretion^[49]. In this study, the significant increase in these cholesterol metabolites in the HFD group reflected disturbances in cholesterol metabolism, while YFRPR intervention effectively lowered their levels, restoring cholesterol metabolism to normal levels.

Metabolic pathways are not isolated but are intricately interconnected and closely related. Lipid metabolism pathways interact with various biological processes such as oxidative stress, antioxidant responses, and inflammation, particularly in the regulation of fatty acid metabolism, fat storage, and energy expenditure^[50]. Specifically, lipid metabolism directly influences oxidative stress levels by regulating fatty acid metabolism, thereby affecting the cell's antioxidant capacity. Oxidative stress during lipid metabolism not only impacts the response of the antioxidant system but also, in turn, changes in the antioxidant system may further modulate lipid metabolism. This interaction reveals the complex feedback mechanisms involved in metabolism. Transcriptomic analysis showed that genes related to antioxidant responses (such as *Gpx8*, *Gsta1*, *Gstm2*, *Gstp1*) and oxidative stress (such as *Fmo2*, *Fmo4*, *Nme4*, *Nos3*, *Dhrs9*) were significantly suppressed in the HFD group, while YFRPR intervention led to a significant upregulation of these genes. *Gpx8* is a member of the glutathione peroxidase family and primarily functions to eliminate intracellular hydrogen peroxide (H₂O₂) and lipid peroxides. Meanwhile, *Gsta1*, *Gstm2*, and *Gstp1* belong to the glutathione S-transferase family and are involved in detoxification metabolism and antioxidant responses^[51]. Prolonged oxidative stress impairs their functions, but intervention with YFRPR partially restores their activity, consistent with findings from Dharmaraj et al. (2018)^[52]. Similarly, *Nos3* and *Dhrs9* are directly associated with antioxidant functions, while *Fmo2*, *Fmo4*, and *Nme4* indirectly influence oxidative stress through metabolic regulation^[53]. Biotransformed bear bile powder, pyridoinole, and rutin have been shown to modulate these genes, thereby regulating lipid metabolism, which aligns with the findings of this study^[53, 54]. Moreover, YFRPR intervention improved the obesity-related inflammatory response induced by HFD by downregulating pro-inflammatory pathways (such as cytochrome P450 metabolism of exogenous substances and calcium signaling pathways) and upregulating anti-inflammatory pathways (such as PPAR signaling and fatty acid degradation)^[33, 35]. Additionally, β -alanine metabolism and bile secretion pathways are also essential in regulating fat digestion, absorption, and energy metabolism. The interactions between these pathways further indicate their synergistic role in metabolic regulation. The metabolomic analysis further revealed that HFD feeding led to disturbances in amino acid metabolism, with multiple amino acids and their derivatives (such as Cysteinylglycine, Glutamyltryptophan, Isoleucyl-Valine, L-Isoleucine, Lysylserine, N-Lactoylphenylalanine, N-Linoleoyl Tyrosine, etc.) being significantly upregulated in the HFD group. After YFRPR intake, the levels of these amino acids and their derivatives were restored. Serum studies also showed that in obese patients, concentrations of glutamate, aromatic amino acids, and branched-chain amino acids were significantly elevated, while the levels of glycine, glutamine, cysteine, asparagine, and homocysteine were notably decreased^[55]. Qin et al. (2023) and Feng et al. (2023) also found that a high-fat diet disrupts multiple amino acid-related metabolic pathways, primarily by modulating liver function and energy metabolism, which indirectly influences obesity^[18, 20]. These findings are consistent with the results of this

study. Notably, Cysteinylglycine, a key intermediate in the glutathione (GSH) metabolism pathway, is often elevated due to accelerated GSH degradation induced by oxidative stress. This suggests that YFRPR may alleviate obesity-related metabolic disturbances induced by HFD by modulating oxidative stress and amino acid metabolism.

5. Conclusion

In conclusion, this study explored the mechanisms by which the peptide YFRPR, derived from *Rosa roxburghii* seed protein, prevents high-fat diet (HFD)-induced obesity. The results of this study indicate that YFRPR intake significantly improved key features of obesity, including lipid abnormalities in the liver and serum, inflammation, oxidative stress levels, and liver steatosis, with no observed adverse effects. Through the integration of transcriptomic and metabolomic data, we conducted an in-depth analysis of the potential mechanisms underlying HFD-induced obesity. The study found that YFRPR primarily prevents obesity by regulating lipid metabolism and improving oxidative stress and inflammation. This research represents the first systematic exploration of the role of bioactive peptides in obesity prevention using multi-omics approaches. Future studies should combine molecular biology techniques and bioinformatics analysis to further validate and uncover the key target points of YFRPR, providing a theoretical basis for the development of bioactive peptide-based anti-obesity agents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Reference:

- [1] X. P. Li, T. Wei, J. F. Li, et al., Tyrosol ameliorates the symptoms of obesity, promotes adipose thermogenesis, and modulates the composition of gut microbiota in hfd fed mice, *Mol. Nutr. Food Res.* 66 (2022) 2101015.
- [2] Z. Z. Li, B. Zhang, N. Wang, et al., A novel peptide protects against diet-induced obesity by suppressing appetite and modulating the gut microbiota, *Gut.* 72 (2023) 686-698.
- [3] J. J. Lim, A. H. Jung, H. J. Suh, et al., K8-based paraprobiotics prevents obesity and obesity-induced inflammatory responses in high fat diet-fed mice, *Food Res. Int.* 155 (2022) 111066.
- [4] S. Wang, Z. Y. Lv, W. D. Zhao, et al., Collagen peptide from walleye pollock skin attenuated obesity and modulated gut microbiota in high-fat diet-fed mice, *J. Funct. Foods* 74 (2020) 104194.
- [5] N. Liu, Z. Song, W. H. Jin, et al., Pea albumin extracted from pea (*pisum sativum* l.) seed protects mice from high fat diet-induced obesity by modulating lipid metabolism and gut microbiota, *J. Funct. Foods* 97 (2022) 105234.
- [6] A. D. Ji, W. Chen, T. Y. Zhang, et al., Whey protein and soy protein prevent obesity by upregulating uncoupling protein 1 to activate brown adipose tissue and promote white adipose tissue browning in high-fat diet-fed mice, *Food Funct.* 13 (2022) 12836-12851.
- [7] J. Liu, D. Y. Zhen, C. B. Hu, et al., Reconfiguration of gut microbiota and reprogramming of liver metabolism with phycobiliproteins bioactive peptides to rehabilitate obese rats, *Nutrients* 14 (2022) 3635.
- [8] M. R. Ferreira, A. G. Garzón, M. E. Oliva, et al., Lipid-lowering effect of microencapsulated peptides from brewer's spent grain in high-sucrose diet-fed rats, *Food Biosci.* 49 (2022) 101981.
- [9] M. Valle, P. L. Mitchell, G. Pilon, et al., Salmon peptides limit obesity-associated metabolic disorders by modulating a gut-liver axis in vitamin d-deficient mice, *Obesity.* 29 (2021) 1635-1649.

- [10] K. Wei, Y. Wei, W. D. Xu, et al., Corn peptides improved obesity-induced non-alcoholic fatty liver disease through relieving lipid metabolism, insulin resistance and oxidative stress, *Food Funct.* 13 (2022) 5782-5793.
- [11] N. A. Mitkin, V. V. Pavshintcev, I. A. Sukhanova, et al., The novel peptide chm-273s has therapeutic potential for metabolic disorders: Evidence from in vitro studies and high-sucrose diet and high-fat diet rodent models, *Pharmaceutics*. 14 (2022) 2088.
- [12] Z. Z. Liu, S. J. Ding, H. M. Jiang, et al., Egg protein transferrin-derived peptides irw (Ile-arg-trp) and iqw (Ile-gln-trp) prevent obesity mouse model induced by a high-fat diet via reducing lipid deposition and reprogramming gut microbiota, *Int. J. Mol. Sci.* 23 (2022) 11227.
- [13] C. C. Hsieh, Y. F. Wang, P. Y. Lin, et al., Seed peptide lunasin ameliorates obesity-induced inflammation and regulates immune responses in c57bl/6j mice fed high-fat diet, *Food Chem. Toxicol.* 147 (2021) 111908.
- [14] W. Shi, T. Hou, D. J. Guo, et al., Evaluation of hypolipidemic peptide (val-phe-val-arg-asn) virtual screened from chickpea peptides by pharmacophore model in high-fat diet-induced obese rat, *J. Funct. Foods* 54 (2019) 136-145.
- [15] Y. H. Meng, W. L. Li, C. X. Hu, et al., Ginsenoside f1 administration promotes ucp1-dependent fat browning and ameliorates obesity-associated insulin resistance, *Food Sci. Hum. Well.* 12 (2023) 2061-2072.
- [16] X. J. Zhang, C. Z. Jin, H. Liu, et al., Polysaccharide extract from *rosa laevigata* fruit attenuates inflammatory obesity by targeting redox balance and gut interface in high-fat diet-fed rats, *Food Sci. Hum. Well.* 12 (2023) 442-453.
- [17] J. L. Feng, X. Chen, S. T. Wang, et al., Transcriptomics integrated with metabolomics reveals the ameliorating effect of mussel-derived plasmalogens on high-fat diet-induced hyperlipidemia in zebrafish, *Food Funct.* 14 (2023) 3641-3658.
- [18] X. D. Fu, Z. M. Liu, R. Li, et al., Amelioration of hydrolyzed guar gum on high-fat diet-induced obesity: Integrated hepatic transcriptome and metabolome, *Carbohydr. Polym.* 297 (2022) 120051.
- [19] C. Y. Fang, Y. F. Shen, F. Y. Wang, et al., Combined transcriptomics and metabolomics to elucidate the underlying beneficial mechanisms of l-theanine in mitigating obesity-induced cardiac injury in rats, *J. Funct. Foods* 113 (2024) 106037.
- [20] Y. Qin, R. Y. Fan, Y. X. Liu, et al., Exploring the potential mechanism of rubus corchorifolius l. Fruit polyphenol-rich extract in mitigating non-alcoholic fatty liver disease by integration of metabolomics and transcriptomics profiling, *Food Funct.* 14 (2023) 9295-9308.
- [21] K. X. Zhang, T. Q. Liu, Z. P. Sun, et al., Investigating transcriptomics and metabolomics differences offers insights into the mechanisms of muscular fat deposition in common carp (*cyprinus carpio*), *Food Biosci.* 62 (2024) 105082.
- [22] H. Yin, Y. L. Jiang, X. F. Zhou, et al., Effect of radio frequency, ultrasound, microwave-assisted papain, and alcalase hydrolysis on the structure, antioxidant activity, and peptidomic profile of *rosa roxburghii* tratt. Seed protein, *J. Food Sci.* 87 (2022) 4040-4055.
- [23] H. Yin, J. X. Zhu, Y. Zhong, et al., Kinetic and thermodynamic-based studies on the interaction mechanism of novel r. *Roxburghii* seed peptides against pancreatic lipase and cholesterol esterase, *Food Chem.* 447 (2024) 139006.
- [24] H. Yin, J. X. Zhu, Y. Zhong, et al., Insights into rapid screening and molecular mechanism of novel *rosa roxburghii* seeds pancreatic lipase/cholesterol esterase inhibitory peptides: A combined in silico and in vitro perspective, *Food Sci. Hum. Well.* (2024) 1-23.
- [25] E. L. B. Novelli, Y. S. Diniz, C. M. Galhardi, et al., Anthropometrical parameters and markers of obesity in rats, *Lab Anim-Uk.* 41 (2007) 111-119.
- [26] Y. L. Jiang, Y. L. Du, C. Y. Chen, et al., Integrative metabolomic and transcriptomic landscape during *akebia trifoliata* fruit ripening and cracking, *Int. J. Mol. Sci.* 24 (2023) 16732.
- [27] Y. L. Jiang, Y. Z. Wu, H. Yin, et al., Metabolic and bioactive comparative analyses reveal the mechanisms of color changes in *akebia trifoliata* (thunb.) koidz fruit, *Sci. Hortic-Amsterdam.* 295 (2022) 110819.
- [28] S. T. Liao, X. S. Long, Y. X. Zou, et al., Mulberry leaf phenolics and fiber exert anti-obesity through the gut microbiota-host metabolism pathway, *J. Food Sci.* 86 (2021) 1432-1447.
- [29] Y. J. Li, Z. Feng, T. L. Wu, et al., Quinoa peptides alleviate obesity in mice induced by a high-fat diet via regulating of the ppar-a signaling pathway and gut microbiota, *Mol. Nutr. Food Res.* 67 (2023) 2300258.
- [30] O. Blokhina, E. Virolainen, K. V. Fagerstedt, Antioxidants, oxidative damage and oxygen deprivation stress: A review, *Ann. Bot-London.* 91 (2003) 179-194.

- [31] Y. F. Liao, C. Wang, Z. P. Gao, et al., Anti-obesity mechanism of ganpu tea revealed by microbiome, metabolome and transcriptome analyses, *Food Chem.* 412 (2023) 135048.
- [32] M. B. Afonso, P. M. Rodrigues, M. Mateus-Pinheiro, et al., Ripk3 acts as a lipid metabolism regulator contributing to inflammation and carcinogenesis in non-alcoholic fatty liver disease, *Gut.* 70 (2021) 2359-2372.
- [33] H. Chen, X. T. Dong, M. Y. Zhou, et al., Modulation of lipid metabolism by oyster peptides in mice with high-fat diet-induced obesity, *Food Biosci.* 58 (2024) 103529.
- [34] Y. F. Ge, Y. Shi, C. H. Wei, et al., The effects of quinoa bran dietary fiber on glucose and lipid metabolism and hepatic transcriptome in obese rats, *J. Sci. Food Agr.* 104 (2024) 2692-2703.
- [35] H. Tang, J. Wang, Y. Fang, et al., Hepatic transcriptome discloses the potential targets of xuefu zhuyu decoction ameliorating non-alcoholic fatty liver disease induced by high-fat diet, *J. Tradit. Compl. Med.* 14 (2024) 135-147.
- [36] T. Su, C. H. Huang, C. F. Yang, et al., Apigenin inhibits stat3/cd36 signaling axis and reduces visceral obesity, *Pharmacol. Res.* 152 (2020) 106816.
- [37] H. L. Zeng, P. L. Chen, Z. Y. Wang, et al., *Porphyra haitanensis* polysaccharides attenuates blood lipid via gut-liver axis in diet-induced high-fat *mesocricetus auratus* through multiple integrated omics, *Mol. Nutr. Food Res.* 67 (2023) 2200638.
- [38] J. Y. Chang, M. Koseki, A. Saga, et al., Dietary oxysterol, 7-ketocholesterol accelerates hepatic lipid accumulation and macrophage infiltration in obese mice, *Front. Endocrinol.* 11 (2021) 614692.
- [39] H. Chen, L. Chen, D. Liu, et al., Combined clinical phenotype and lipidomic analysis reveals the impact of chronic kidney disease on lipid metabolism, *J. Proteome Res.* 16 (2017) 1566-1578.
- [40] L. Xiong, M. A. Azad, Y. Liu, et al., Intrauterine growth restriction affects colonic barrier function *via* regulating the nrf2/keap1 and tlr4-nf- κ b/erk pathways and altering colonic microbiome and metabolome homeostasis in growing-finishing pigs, *Antioxidants-Basel.* 13 (2024) 283.
- [41] T. Ide, M. Murata, M. Sugano, Octadecatrienoic acids as the substrates for the key enzymes in glycerolipid biosynthesis and fatty-acid oxidation in rat-liver, *Lipids.* 30 (1995) 755-762.
- [42] S. Dash, C. T. Xiao, C. Morgantini, et al., New insights into the regulation of chylomicron production, *Annu. Rev. Nutr.* 35 (2015) 265-294.
- [43] M. D. Xu, Y. Y. Zhang, Y. Zhang, et al., Integrated lipidomics and transcriptomics analyses reveal key regulators of fat deposition in different adipose tissues of geese (*anser cygnoides*), *Animals-Basel.* 14 (2024) 1990.
- [44] M. Yan, J. P. Hu, L. Wang, et al., Metabolic profiling of abdominal subcutaneous adipose tissue reveals effects of apple polyphenols for reversing high-fat diet induced obesity in c57bl/6 j mice, *Food Chem.* 473 (2025) 143055.
- [45] R. Kosir, K. Spaninger, D. Rozman, Circadian events in human diseases and in cytochrome p450-related drug metabolism and therapy, *Iubmb Life* 65 (2013) 487-496.
- [46] T. Rogi, N. Tomimori, Y. Ono, et al., The mechanism underlying the synergetic hypocholesterolemic effect of sesamin and α -tocopherol in rats fed a high-cholesterol diet, *J. Pharmacol. Sci.* 115 (2011) 408-416.
- [47] Q. Zheng, X. J. Y. Li, N. A. Huang, et al., Saikosaponins ameliorate hyperlipidemia in rats by enhancing hepatic lipid and cholesterol metabolism, *J. Ethnopharmacol.* 305 (2023) 116110.
- [48] M. L. Cheng, H. Y. Tang, P. T. Wu, et al., 7-ketocholesterol induces lipid metabolic reprogramming and enhances cholesterol ester accumulation in cardiac cells, *Cells-Basel.* 10 (2021) 3597.
- [49] J. Zhang, Y. Q. Yang, Z. P. Wang, et al., Integration of metabolomics, lipidomics, and proteomics reveals the metabolic characterization of nonalcoholic steatohepatitis, *J. Proteome Res.* 22 (2023) 2577-2592.
- [50] R. M. Wang, L. L. Yao, X. Lin, et al., Exploring the potential mechanism of *rhodomyrtus tomentosa* (ait.) hassk fruit phenolic rich extract on ameliorating nonalcoholic fatty liver disease by integration of transcriptomics and metabolomics profiling, *Food Res. Int.* 151 (2022) 110824.
- [51] M. J. Picklo, E. K. Long, E. E. Vomhof-Dekrey, Glutathionyl systems and metabolic dysfunction in obesity, *Nutr. Rev.* 73 (2015) 858-868.
- [52] S. Dharmaraj, U. S. M. Rao, M. Azzubaidi, et al., Identification and functional analysis of genes selected using different chemometric techniques on multiarray expression data of liver from high-fat diet treated mice, *Res. J. Pharm. Tech.* 17 (2024) 4043-4048.

- [53] S. Jiang, X. L. Wei, Y. Zhang, et al., Biotransformed bear bile powder ameliorates diet-induced nonalcoholic steatohepatitis in mice through modulating arginine biosynthesis via fxr/pxr-pi3k-akt-nos3 axis, *Biomed. Pharmacother.* 168 (2023) 115640.
- [54] L. Salvaras, T. Kovacic, P. Janega, et al., Synthetic pyridoindole and rutin affect upregulation of endothelial nitric oxide synthase and heart function in rats fed a high-fat-fructose diet, *Physiol. Res.* 70 (2021) 851-863.
- [55] K. R. Short, J. Q. Chadwick, A. M. Teague, et al., Effect of obesity and exercise training on plasma amino acids and amino metabolites in american indian adolescents, *J. Clin. Endocr. Metab.* 104 (2019) 3249-3261.