A Calorically Restricted High-fat Diet Reverses High-fat Diet-induced Injurious Effects on Health and Gene Expression in Rats

Yuqing Wu
Harbin Medical University
Yue Guan
Harbin Medical University
Fan Ling
Harbin Medical University
Qiushuang Zhu
Harbin Medical University
Dandan Zhang
Harbin Medical University
Yuqiao Li
Harbin Medical University
Jinxiao Liu
Harbin Medical University
Yucun Niu (niuyucun@163.com)

Research

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Abstract

Background: A High-fat diet has been reported to produce excess lipid accumulation and increase inflammatory factors and oxidative stress in various metabolic diseases. Caloric restriction (CR) is one of the most valuable tools in reducing inflammation, enhancing anti-oxidative activity and ameliorating various metabolic diseases. However, excess CR may restrain growth, development and normal physiological processes. Our study was conducted to investigate the effects of a high-fat diet containing the same number of calories as a basic diet on the health and gene expression patterns of rats.

Methods: 30 Wistar male rats were randomly devided into a normal control (NC) group, an equicaloric high-fat (EHF) group as the NC group, and a high-fat (HF) ad libitum group. Food consumption and body weight were recorded once a week. Blood biochemistical and genomic assessments of the liver were carried out after intervention for 20 weeks.

Results: Compared with the NC group, serum triglycerides (TG), total cholesterol (TCHO), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST) and andalanine aminotransferase (ALT) levels were significantly increased in the HF group, and the serum levels of interleukin- 6 (IL-6), reactive oxygen species (ROS) and glutathione (GSH) were significantly decreased in the HF group. Compared with the HF group, serum TG,TCHO,LDL-C, AST, ALT, IL-6, ROS levels were significantly decreased in the EHF group, and the serum levels of GSH and superoxide dismutase (SOD) were also significantly increased. Histological studies showed decreased macrovesicular steatosis, inflammatory cell infiltration and structural damagein EHF group compared to the HF group. In addition, transcription analysis revealed that an EHF led tochanges in gene expression, including a reduction inToll-like receptor 4 (TRL4),which inhibited NF-kappa B signaling pathway and upregulatedglutathione S-transterases (GSTs) to increase antiocidative activity.

Conclusions: an EHF restored deleterious changes in the health and gene expression patterns induced by a high-fat diet ad libitum in rats via reduced inflammation and increased antioxidative activity.

Background

Obesity, one of the most common metabolic diseases worldwide, which has an important impact on public health and reduces quality of life. Reports carried out over long periods have shown that a more energy-dense diet result in greater weight gain, inducing obesity [1, 2]. A high-fat diet, the Western dietary pattern, which has a large supply of energy from fat, is typically energy-dense diet and can be found across the world due to continuous economic development. However, high-fat diets have been reported to produce excess lipid accumulation, increase inflammatory factors across all tissues and organs and stimulate the production of ROS, leading to inflammation and oxidative stress [3, 4]. In animal research, a high-fat diet leads to the accumulation of lipids in the liver and insulin resistance, finally resulting in nonalcoholic fatty liver disease (NFLD) and type 2 diabetes mellitus (T2DM) [5, 6]. Cardiovascular diseases are closely related to a high-fat diet as well [7]. Other studies have shown that a high-fat diet has

a profound impact on brain, behavior and cognition. In Alzheimer disease mice continuously fed a high-fat diet, neuropathological markers were upregulated while behavioral defects were increased [8]. Nevertheless, the above studies did not control for differences in energy, and it was impossible to determine whether the damage was induced by ingestion of a high-fat diet or excessive energy intake.

Caloric restriction (CR), the consumption of fewer calories than required while avoiding malnutrition, has been indicated to prolong various species including Caenorhabditis elegans, rodents and rhesus monkeys [9–11]. CR, one of the most valuable tools in aging research and an intervention proven to delay aging and age-related disease, has also been widely proven to reduce inflammation and improve oxidative stress [12, 13]. Interestingly, CR with a low fat content was shown to positively promote health while detailed studies of CR within a high-fat diet have been carried out by only few people.

In our project, we normalized the energy intake of the group fed a high-fat diet with CR (EHF group) to that of a normal control group (NC group) to ensure that rats in the EHF group were not subjected to malnutrition. We examined serum lipids, inflammation factors and oxidative stress markers in all experimental rats. In particular, we carried out a transcription analysis of rat liver tissues to better understand the mechanisms by which different dietary patterns induced their effects. Our purpose was to explore whether caloric restriction could restore injury produced by a high-fat diet fed ad libitum on rats in terms of health and gene expression patterns and to explain the mechanisms of this restoration.

Methods

Rat experiment and tissue collection

30 Four-week-old male Wistar rats, weighting 40–60 g, were obtained from Vital River Laboratory Animal Technology Company LTD (Beijing, China). The animals were individually housed in stainless steel cages in a room at 22 ± 2℃ on a 12 h light/dark cycle with free access to food and water. After an acclimatization period of one week, the animals were randomly divided into 3 groups, the NC group (normal control group, AIN-93M diet fed ad libitum, n = 10), the EHF group (high-fat diet under the same number of calories as the NC group, n = 10) and the HF group (high-fat diet fed ad libitum, n = 10). NC group received the AIN-93M diet ad libitum, the others received a high-fat diet (33.8% fat), the EHF group daily feed intake was adjusted to ensure the same energy based on the NC group, the HF group received a high-fat diet (33.8% fat) ad libitum. Diet formula is showed as followed in Supplement1. Food consumption and body weight were recorded once a week, food consumption is shown in Supplement1. All rats drank water ad libitum.

The project was completed at 20 weeks, all rats were sacrificed for blood, liver tissues and white fat (WAT) tissues collection. Blood was immediately collected from the aortaventralis using a blood taking needle and placed on ice for 30 min. Serum was obtained by centrifugation (4℃, 3800 r/min, 10 min) and stored at − 80℃, the liver tissues and the WAT tissues were immediately frozen in liquid nitrogen and stored at -80℃ for subsequent analysis.

Biochemical analysis

The levels of TG, TCHO, LDL-C, AST and ALT were examined by a ROCHE Modular P800 Automatic Biochemical Analyzer (Roche Diagnostics, Mannheim, Germany) with kits purchased from F.Hoffmann-La Roche Ltd (Mannheim, Germany). TNF-α and IL-6 were measured by an enzyme-linked immunosorbent assay (ELISA) with kits (Elisa Biotech, Shanghai, China). The levels of serum SOD, GSH, MDA and ROS were measured by enzymatic methods with kits (Beyotime Biotechnology, Shanghai, China).

Histological study

Fresh liver samples from the right lobe were fixed in 10% neutral-buffered formalin and embedded in paraffin and sectioned at 5 μm thickness, stained with hematoxylin and eosin (H□E).

RNA library construction and RNA-seq analysis

We used the Trizol reagent (Invitrogen, Carlsbad, CA, USA) to extract the total RNAs from 6 rats hepatic tissues (3 EHF samples, 3 HF samples) and libraryed RNA construction using Agilent 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit). Then, we eliminated the low quality reads, reads with adaptors and reads with unknown bases to get the clean reads. Finally, we mapped those clean reads onto reference genome using Bowtie2 and calculated gene expression level with RSEM [14, 15]. We excluded the genes if their average FPKM values were less than 1 or FPKM was 0 in any sample.

PCA and GSEA analysis

Principal component analysis (PCA) was used to analyze the gene expressions in different samples by OmicShare platform [16]. Then, we performed Gene Set Enrichment analysis (GSEA) anaylsis for the two groups with KEGG functional enrichment by WEB-based Gene Set Analysis Toolkit [17, 18].

DEG detection and Functional enrichment analysis

Based on gene expression levels, we identified the DEG in parameters (fold change > = 2.00 and a P value < = 0.05) [19]. We performed GO functional enrichment for DEGs by phyper, a function of R package, based on three aspects: biological processes (BP), cellular components (CC), and molecular functions (MF); and performed KEGG pathway functional enrichment by phyper based on seven branches: Cellular Processes, Environmental Information Processing, Genetic Information Processing, Human Disease (For animals only), Metabolism, Organismal Systems and Drug Development. After that, we calculated false discovery rate (FDR) for each Pvalue and established a cut-off at a FDR < = 0.05 as a significant point. These results of the functional enrichment analysis were visualized by OmicShare platform.

PPI Network and Module Analysis

In our project, STRING online database was used to get relationships between DEGs [20]. We provided an input file by STRING that could be imported directly into Cytoscape, which is a software for complex network analysis and visualization [21]. Module analysis was performed by plugin MCODE (Molecular Complex Detection) at Cytoscape in parameters (degree cut-off > = 2, node score cut-off > = 0.2, K-core > =

2, MAX depth = 100) to identify the most significant MCODE cluster according to clustering scores. Finally, the identified cluster network was also visualized by Cytoscape.

Measurement of mRNA Levels by Quantitative Real-time PCR

Total RNAs were isolated from the livers in rats using Trizol (Invitrogen, Carlsbad, CA, USA) and reversed into cDNAs using High-Capacity cDNA Reverse Transcription Kits (Invitrogen, USA) at reaction condition: 25℃, 10 min; 37℃, 120 min; 85℃, 5 min; one cycle, according to the manufacturer's instructions. The quantitative real-time PCR was performed with SYBR® Green PCR Kit (Applied Biosystems, Foster City, CA, USA) using a 7500 real-time PCR machine (Applied Biosystems, Foster City, CA, USA) by 20μL system according to the manufacturer's instructions, and three biologically independent replicates were analyzed for all reactions. The primer sequences used in quantitative real-time PCR were listed in Stable1.

Measurement of Protein Level by Western Blot

The protein expression levels ofnuclear factor kappa-B (NF-κB), toll like receptor 4 (TLR4), glutathione S-transferase (GST) and TNF-α were measured by Western blot analysis. The primary antibodies of NF-κB, TRL4 and TNF-α were purchased from Wanleibio(Shenyang, LN, China), the primary antibody of GST was purchased from Cell Signaling Technology (Beverly, MA, USA). Second antibody was goat anti-rabbit IgG purchased from Sangon Biotech(Shanghai, China). Proteins from the live tissues were extracted with a RIPA lysis buffer, and protein concentrations were determined by BCA Protein Assay Kit(Solarbio, Beijing, China). Equal amounts of protein were separated by SDS-PAGE, and electrotransferred onto polyvinylidene difluoride(PVDF) membranes. Representative blots were shown in figures and each test was performed at least three times.

Statistical analysis

The statistical differences were analyzed using the SPSS 21.0 (Beijing Stats Data Mining Co. Ltd., Beijing, China) by One-way ANVOA and independent-samples t-test. All data were shown as the means ± SD and a $P < 0.05$ was regarded as statistically significant.

Results

Body Weight and WAT Weight Determination

Compared with the body weight in the NC group, that in the HF group was significantly increased beginning at 4 weeks ($P < 0.05$), but no significant changes in body weight were observed in the EHF group during the entire experimental process. Simultaneously, a significant difference in body weight between the EHF and the HF groups was found at 6 weeks ($P < 0.01$). Compared with the NC group, the WAT weight was significantly increased in the HF group ($P < 0.01$). However, the WAT weight in the EHF group was significantly lower compared to the HF group ($P < 0.01$). Although the WAT weight in the EHF

group were much smaller than than in the NC group, this difference between the two groups was not significant (Fig. 1A-B).

Serum TCHO, TG, LDL-C and HDL-C Levels

At the end of the 20th week, serum TCHO, TG and LDL-C levels in the HF group were significantly increased compared with the NC group ($P < 0.05$), and the levels of these factors were significantly decreased in the EHF group compared with the HF group ($P < 0.01$). However, no significant difference in serum HDL-C level between the three intervention groups was observed (Fig. 1C-F). These data suggest that a calorically restricted high-fat diet improves some metabolic health parameters associated with lipid metabolism compared with their levels with a high-fat diet fed ad libitum.

Serum Inflammatory and Oxidative Stress Factor Levels

Compared to the NC group, the serum levels of TNF-α and MDA in the HF group were increased and the serum level of IL-6 and ROS were significantly increased ($P < 0.05$), while there was no significant differences in these factors between the NC and the EHF groups. Interestingly, compared with the HF group, the serum levels of IL-6 and ROS were significantly decreased in the EHF group ($P < 0.01$), but no significant difference in the serum levels of TNF-α and MDA was observed in the EHF group compared with the HF group (Fig. 1G-J). Additionally, a significant increase in the serum levels of GSH and SOD between the EHF and HF groups was observed ($P < 0.05$). However, there were no significant differences in the serum levels of GSH and SOD between the NC and HF groups, or the NC and the HF groups (Fig. 1K-L). This finding indicates that a calorically restricted high-fat diet ameliorated inflammation and oxidative stress compared to that under a high-fat diet ad libitum .

Changes to Liver Injury and Histological Analysis

Serum AST and ALT levels are critical markers of liver injury. Compared to the NC group, serum AST and ALT levels were significantly increased in the HF group and only the serum AST level was significantly decreased in the EHF group ($P < 0.05$). Compared to the HF group, serum AST and ALT levels were significantly decreased in the EHF group ($P < 0.05$) (Fig. 2A-B). Hematoxylin and eosin(H&E) staining illustrated that a high-fat diet fed ad libitum induced severe macrovesicular steatosis, increased inflammatory cell infiltration and caused serious structural damage to the liver, while the EHF group exhibited a dramatic decreased in liver steatosis and various injuries(Fig. 2C). Based on these results, liver steatosis and injury induced by a high-fat diet fed ad libitum were improved by a calorically restricted high-fat diet.

Transcriptional Data Preprocessing and Gene Expression Analysis

To further clarify the mechanism by which a calorically restricted high-fat diet with energy to that of a basic diet, we sequenced 6 samples from the EHF group and the HF group (3EHF group samples, 3HF

group samples) on the Illumina HiSeq Platform and generated approximately 6.37 Gb of data per sample, and we determined the gene expression levels in each sample with RSEM. Variations in the expression profiles between the examined groups were determined by principal component analysis (PCA). We observed two subgroups (EHF samples data were on the upper half of the plot, while HF sample data were on the lower half of the plot) by PCA (Fig. 3A), which confirmed that dietary modification changed the rat sample transcriptome protiles, and moderate interindividual variation between the expression profiles was also observed. Afterwards, we performed GSEA analysis to determine the functional enrichment of KEGG pathways for all genes with a significance cut-off of an FDR = < 0.05 (Stable2, Fig. 3B). A total of 139 pathways (94 pathways were downregulated, 45 pathways were upregulated) were enriched in differentially expressed genes in the EHF group versus the HF group.

DEG Selection and Functional Enrichment Analysis

We identified the differentially expressed genes between the two groups by DEseq2 algorithms (fold change > = 2.00 and a P value < = 0.05). There were 863 DEG (596 genes were downregulated, while 267 genes were upregulated) in the EHF group versus the HF group (Stable3). Scatter and volcano plots were generated to visualize the DEG, and a heatmap was used to visualize the levels of DEG expression (Fig. 3D-E).

Upon their classification, the 596 downregulated DEG were enriched in 26 biological processes, 16 cellular components and 13 molecular functions. A total of 288 pathways were enriched, and 48 pathways were enriched significantly (P value < 0.05) (Stable4). Most downregulated DEG were enriched in the “signal transduction”, “immune system”, “infectious diseases” and “cancers” patterns. The top20 most highly enriched pathways were listed, and the most highly enriched pathway was the “complement and coagulation cascades” pathway, an important pathway in the immune system process, while the “PI3K-Akt signaling pathway”, the key pathway in glycolipid metabolism, was also listed (Fig. 4A-C).

Upon their classification, 267 upregulated DEG were enriched in 25 biological processes, 15 cellular components and 12 molecular function classification. A total of 234 pathways were enriched, and 54 pathways were enriched significantly (P value < 0.05) (Stable5). Most upregulated DEG were enriched in the “signal transduction”, “immune system”, “lipid metabolism” and “endocrine system” patterns. The top 20 most highly enriched pathways were listed, and the most highly enriched pathways were the“drug metabolism-other enzymes” and “fatty acid metabolism” pathways, while the “peroxisome” and “glutathione metabolism” pathways, which participate in antioxidative activities were listed (Fig. 4D-F).

We compared the results in GSEA and DEG analysis, and found that the “NF-kappa B signaling pathway” was significantly enriched according to GSEA but not DEG analysis (P = 0.063). However, expression of the key factor NF-kappa B was significantly altered, as shown by Western blot analysis and quantitative RT-PCR (Fig. 5B and Fig. 6D), which also suggested that the use of functional enrichment anaysis alone leads to inaccurates.

PPI Analysis of DEG

We performed PPI (protein-protein interaction) networks analysis of the DEG by STRING with a medium confidence score threshold of 0.4. Then, the data were visualized with Cytoscape, and the resultant network contained 553 nodes and 3060 edges (Fig. 5A). We identified the top10 hub genes by node degree, Vwf, Tlr4, Tlr2, Pecam1, Mmp9, Itgb2, Itgam, Cybb, Csf1r and C5arl, which were strongly connected to other DEG and may play crucial roles in the changes induced by different dietary patterns (Fig. 5B, Stable6). Then, we detected significant modules in this PPI network with the MCODE plugin, and the top cluster was selected as a subnetwork (cluster score: 13.5) (Fig. 5C), this subnetwork was considered as a potential core regulatory subnetwork. Finally, genes both in the subnetwork and among the top 10 hub genes (Tlr4, Vwf and Tlr2) were selected and verified by quantitative RT-PCR.

Validation of Some DEG and Key Genes That Participate in Inflammation and Oxidative Stress by Quantitative RT-PCR

To validate the reliability of the transcription data, six DEGs between the two groups were selected, and their expression was measured by quantitative RT-PCR. The six DEGs comprised three upregulated DEG (GSTA2, GSTA3, GSTA5) and three downregulated DEG (TLR2, TLR4, VWF). Compared with the HF group, the mRNA levels of GSTA2, GSTA3 and GSTA5 were significantly increased in the EHF group, and the mRNA levels of TLR2, TLR4 and VWF were significantly decreased in the EHF group (Fig. 6A-F). These results show that the transcription data are credible. Then, we measured the mRNA levels of the inflammatory factors TNF-α, IL-6 and NF-κB and the antioxidants SOD1 (CuZnSOD) and SOD2 (MnSOD). The mRNA levels of TNF-α, IL-6 and NF-κB were significantly lower level in the EHF group, and the SOD2 mRNA expression was significantly higher in the EHF group compared to the HF group (Fig. 6G-K).

Protein Expression of NF-κB, TLR4, GST and TNF-α in the Rat Livers

The protein expression levels of NF-κB, TLR4 and TNF-α were significantly decreased, and the protein expression level of GST was significantly increased in the EHF group compared to the HF group (Fig. 7A-D). The mRNA expression levels of NF-κB, TLR4, TNF-α and GST were consistent with their proteins expression levels, indicating that an EHF can improve inflammatory and oxidative

damage by decreasing TLR4 and NF-κB expression and increasing GST expression, respectively.

Discussion

In our study, we found that a calorically restricted high-fat diet could protect against the adverse effects included by a high-fat diet fed ad libitum, including dyslipidemia, inflammation, oxidative stress and liver function damage in Wistar rats. Additionally, the transcription analysis revealed the major mechanisms for the improvements in inflammation and oxidative stress, which occurred via downregulation of the NF-kappa B signaling pathway and the upregulation of GSTs.

Over the last decade, the effects of a standard diet with CR on improving health and prolong life have been proven in various model species [22, 23]. However, CR has been found to have detrimental effects on bone health, wound healing, and certain immune responses, and excess CR may restrain growth, development and normal physiological processes [24]. To avoid excess CR, we calculated the number of calories based on food intake ingested by rats supplied a normal diet fed ad libitum (NC group) and normalized the number of calories between the NC and EHF groups. Some previous studies have discussed the effects of a high-fat diet with caloric restriction, but the high-fat diets in those studies contained 45% or 60% fat, which are extreme proportions of fat when compared to the Western dietary pattern [25, 26]. Our study aimed to mimic typical Westernized dietary habits and carry out long-term intervention in rats. Therefore, we chose high-fat diet containing 33.8% fat, which is similar in macronutrient composition to the standard AIN-93M diet. As a result, rats in the EHF group gained less weight, accumulated less body fat, and had lower serum TG, serum LDL-C and serum TCHO than rats in the HF group, which is similar to the pattern revealed in previous studies.

Inflammatory and oxidative stress are thought to be the key contributors to the pathogenesis of metabolic dysfunctions caused by a high-fat diet, while CR can delay the aging process via reducing inflammatory and oxidative stress [23, 27]. For example, rats under long-term CR are protected against inflammatory and oxidative stress in multiple tissues, including the brain, lung, liver, kidney and heart[28, 29]. Simultaneously, CR reduced the inflammatory factor levels in tissues and the circulatory system and decreased ROS, and animals under a CR modulated their antioxidant defenses to more effectively detoxify the generated radicals [30]. In the present study, compared with the NC group, the serum levels of ROS and IL-6 significantly increased in the HF group, and the serum levels of TNF-α and MDA were slightly increased. No significant differences in these factors were observed between the EHF and the NC groups. Compared with the HF group, the serum levels of IL-6 and ROS were significantly decreased, while the serum levels of TNF-α and MDA were slightly decreased. Moreover, the mRNA expression level of IL-6 and the mRNA level and protein expression level of TNF-α in the liver were significantly decreased in the EHF group. The activities of SOD and GSH were assayed as indices of antioxidant capacity. Rats in the EHF group expressed a higher level of both indices in the serum and the mRNAs in the liver in comparison with the HF group, while the serum levels of SOD and GSH were only slightly increased in the NC group compared to the HF group. These data indicate that an EHF maintained the oxidoreductive force in

tissues and organs, which was similar to, or even higher than that in the NC group and revealed reduced inflammatory and oxidative damage induced by a high-fat diet fed ad libitum.

The liver is the most important metabolic and secretory organ and participates in various physiological activities. A high-fat diet was reported to aggravate liver lipid accumulation, resulting in hepatic steatosis or pathological changes [5, 31]. In our study, the EHF group showed lower serum levels of AST and ALT in serum compared to the HF group. In addition, histological examination of liver tissues showed decreased macrovesicular steatosis, inflammatory cell infiltration and structural damage in the EHF group compared to the HF group. Therefore, these findings suggest that an EHF reduces liver lipid accumulation, relieves functional damage and improves inflammatory and oxidative stress.

Whole genome expression analysis was conducted in liver tissues, where the gene transcription level was distinctly higher compared with that in other organ types [32]. GSEA analysis and DEG enrichment analysis with KEGG pathway were both executed to avoid deviations due to single analysis. Members of Toll-like receptors (TLRs) family act as primary sensors that detect a wide variety of microbial components and elicit immune responses. All TLR signaling pathways culminate in activation of the transcription factor NF-κB [33]. NF-κB, a primary regulator of inflammatory responses, plays a critical role in a variety of physiological and pathologic processes, and the NF-kappa B signaling pathway is involved in both inflammation and oxidative stress and participates in the development of various metabolic diseases [34]. Recent studies have demonstrated that a high-fat diet can affect peripheral tissues, and cause systematic inflammation by directly or indirectly activating the TLR4 and nuclear factor-kappa B NF-κB pathways [35, 36]. Our transcription data obtained through KEGG pathway analysis indicated that the NF-kappa B signaling pathway was significantly inhibited by an EHF, and TLR4, the most pronounced hub gene according to both PPI and MDOCE analyses, was significantly downregulated in the EHF group. Furthermore, the mRNA and protein levels of TRL4 and NF-κB in liver tissues were significantly decreased in the EHF group versus the HF group in the present study. These data proved that a major component of the mechanism by which the EHF ameliorates inflammation and oxidative damage is via a reduction in TRL4 to inhibit the NF-kappa B signaling pathway. In addition, the Glutathione metabolism pathway was significantly upregulated in the EHF group, and the GSTA2, GSTA3 and GSTA5 genes were also significantly upregulated, as shown by RNA-seq analysis and verified by Q-PCR. Glutathione S-transferases (GSTs) are a major group of detoxification enzymes and the main line of defense against the spectrum of highly toxic substances produced by ROS-mediated oxidative reactions [37]. The increased levels of GSTs induced by an EHF probably participate in antioxidant processes and mitigate oxidative stress.

Conclusion

our results suggest that a calorically restricted high-fat diet can improve the harmful effects of a high-fat diet fed ad libitum on the health and gene expression patterns in rats. Serum lipids, inflammatory factors and oxidative stress factors were all improved by a calorically restricted high-fat diet. Moreover, hepatic steatosis, injury, inflammation and oxidative stress were partly ameliorated by this dietary pattern.

Simultaneously, the oberved transcriptional changes might explain the mechanisms by which a calorically restricted high-fat diet improves the inflammation and injurious oxidative effects by inhibiting TLR4 and NF-kappa B and activating GSTs. In addition, the WAT weight, serum TCHO, LDL-C, SOD and GSH in the EHF group trended to improve in comparison to the NC group. These data indicate that a calorically restricted diet with a moderate fat content may be more beneficial than a low-fat diet of equal energy, however, more experiments are required to prove these conclusions.

Declarations

Ethics approval

All procedures described involving animals were approved by the Harbin Medical University Institutional Animal Care Committee, and conducted in accordance with the guidelines of Harbin Medical University on the experimental use of laboratory animals.

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Availability of data and materials

All data supporting the conclusions of this paper are included in this

manuscript. The raw data are available from the authors upon request.

Authors' contributions

YW and YG contributed equally to this work.YW and YN designed the study; FL and YG conducted the experiments; DZ and QZ assisted with the animal experiments; YL and ZL analyzed the data; YW wrote the paper;All authors have read andapproved the final manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

Abbreviations

NC group: normal control group; EHF group: high-fat diet under the same number of calories as the NC group; HF group: high-fat diet fed *ad libitum*; CR: caloric restriction; TG: triglycerides; TCHO: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; AST:aspartate aminotransferase; ALT: andalanine aminotransferase; MDA: malondialdehyde; IL-6: interleukin- 6; ROS: reactive oxygen species; GSH: glutathione; SOD: superoxide dismutase; WAT: white fat; DEG: differentially expression genes; GSTA2: Glutathione S Transferase Alpha 2; GSTA3: Glutathione S Transferase Alpha 3; GSTA5: Glutathione S Transferase Alpha 5; TLR2: toll like receptor 2; TLR4: toll like receptor 4; VWF: von Willebrand Factor; NF-κB: nuclear factor kappa-B; GST: glutathione S-transferase; NFLD: nonalcoholic fatty liver disease; T2DM: type 2 diabetes mellitus; PCA: principal component analysis; GSEA: Gene Set Enrichment analysis; PPI: protein-protein interaction networks; MCODE: Molecular Complex Detection; PVDF: polyvinylidene difluoride.

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Figures

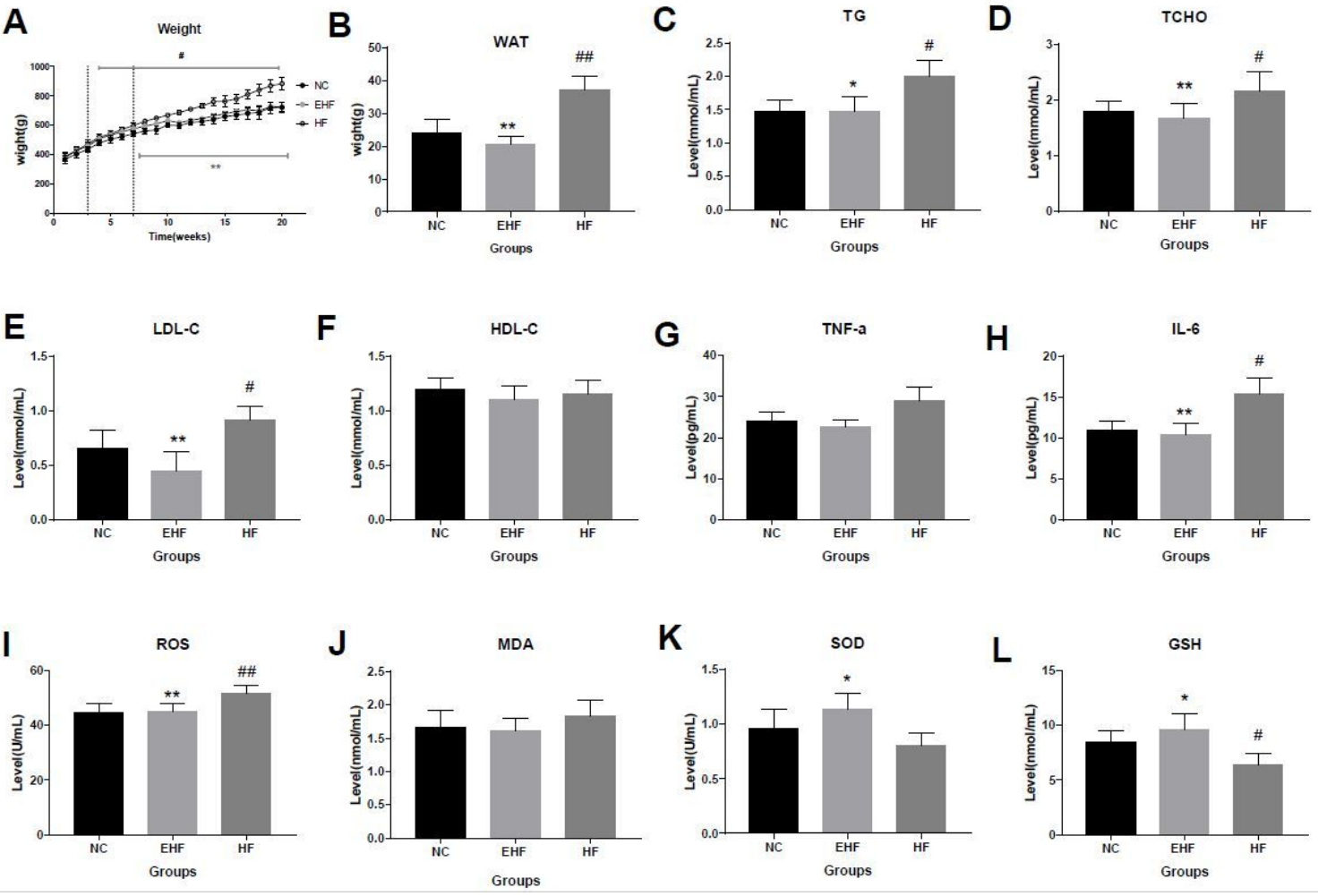

Figure 1

A Body weight. B WAT weight. C-F The serum levels of TG, TCHO, LDL-C and HDL-C. G-L The serum levels of TNF-α, iL-6, ROS, MDA, GSH and SOD. Data are presented as means±SD, p<0.05 was considered significant. #P < 0.05, ##P < 0.01 vs. the NC group. *P < 0.05, **P < 0.01 vs. HF group.

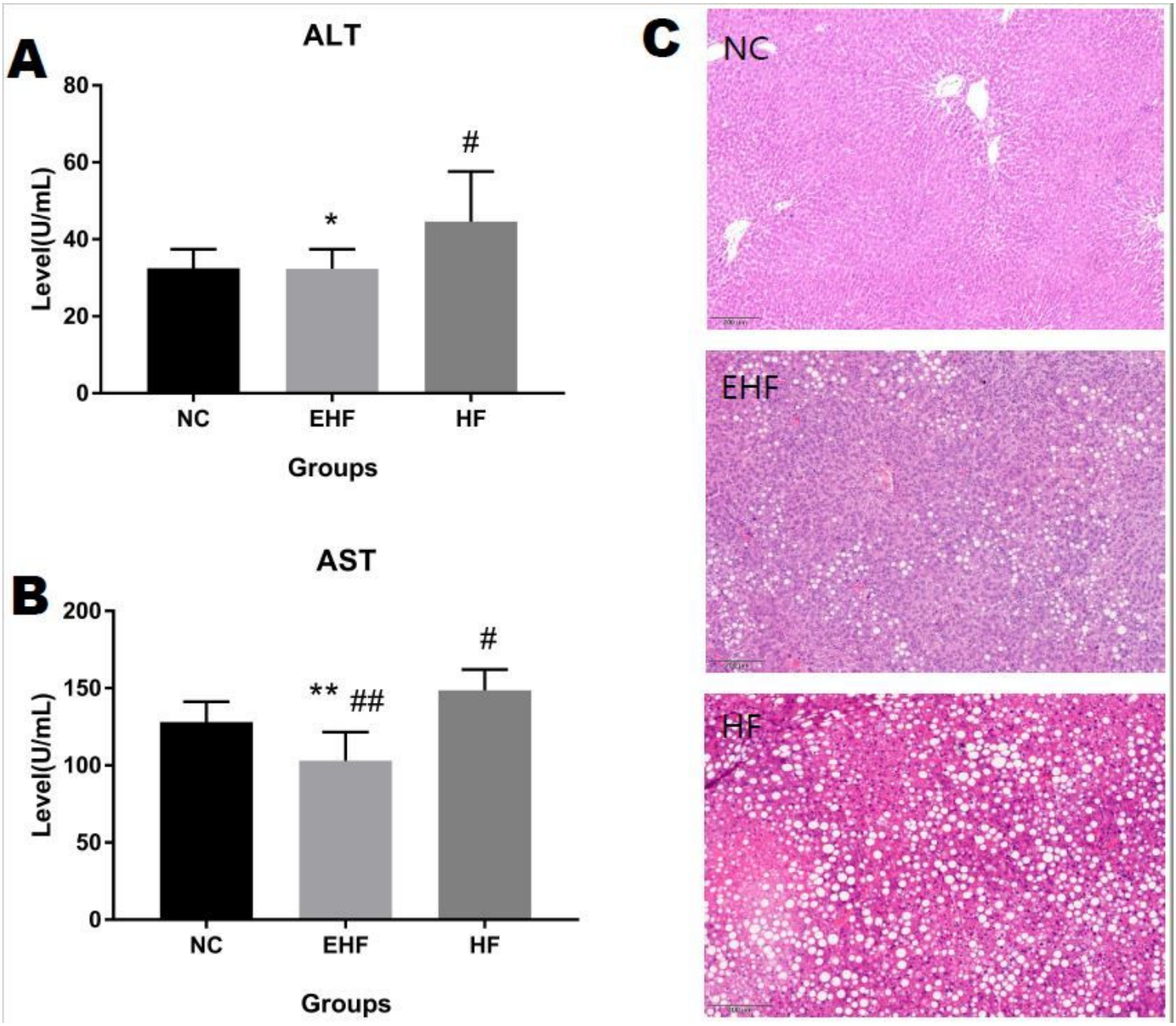

Figure 2

A The serum level of ALT. B The serum level of AST. C H&E straining(200X) on liver tissues. Data are presented as means±SD, p<0.05 was considered significant.#P < 0.05, ##P < 0.01 vs. the NC group. *P < 0.05, **P < 0.01 vs. HF group.

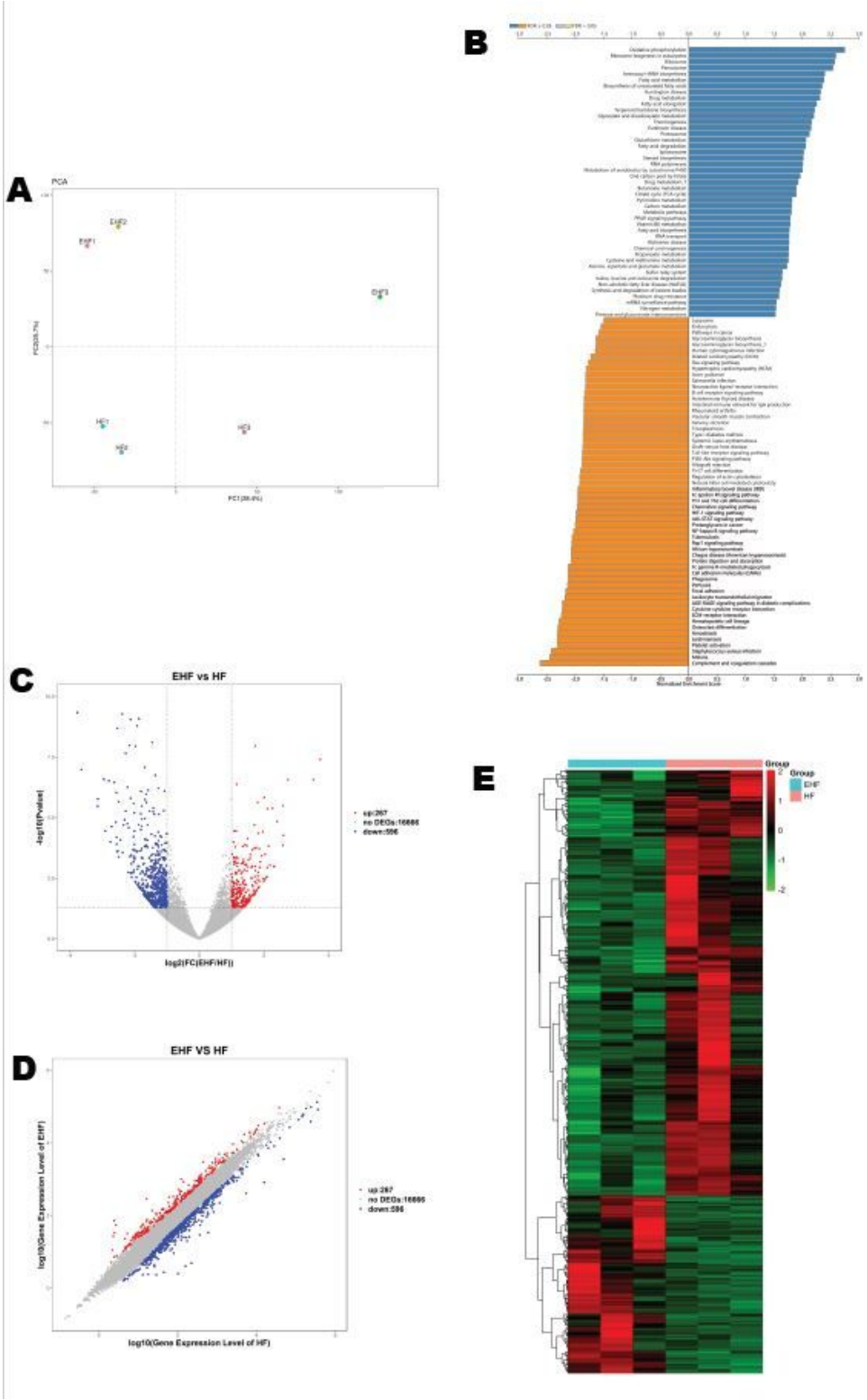

Figure 3

A PCA result (the PC1 is 28%, the PC2 is 25.7%). B GSEA result. Normalized Enrichment Score is the degree of enrichment. Blue one is the symbol of up-regulated in EHF group and yellow one is the symbol of down-regulated in EHF group vs. HF group. C DEGs by scatter plot.D DEGs by volcano plot. E DEGs expression levels by heatmap.

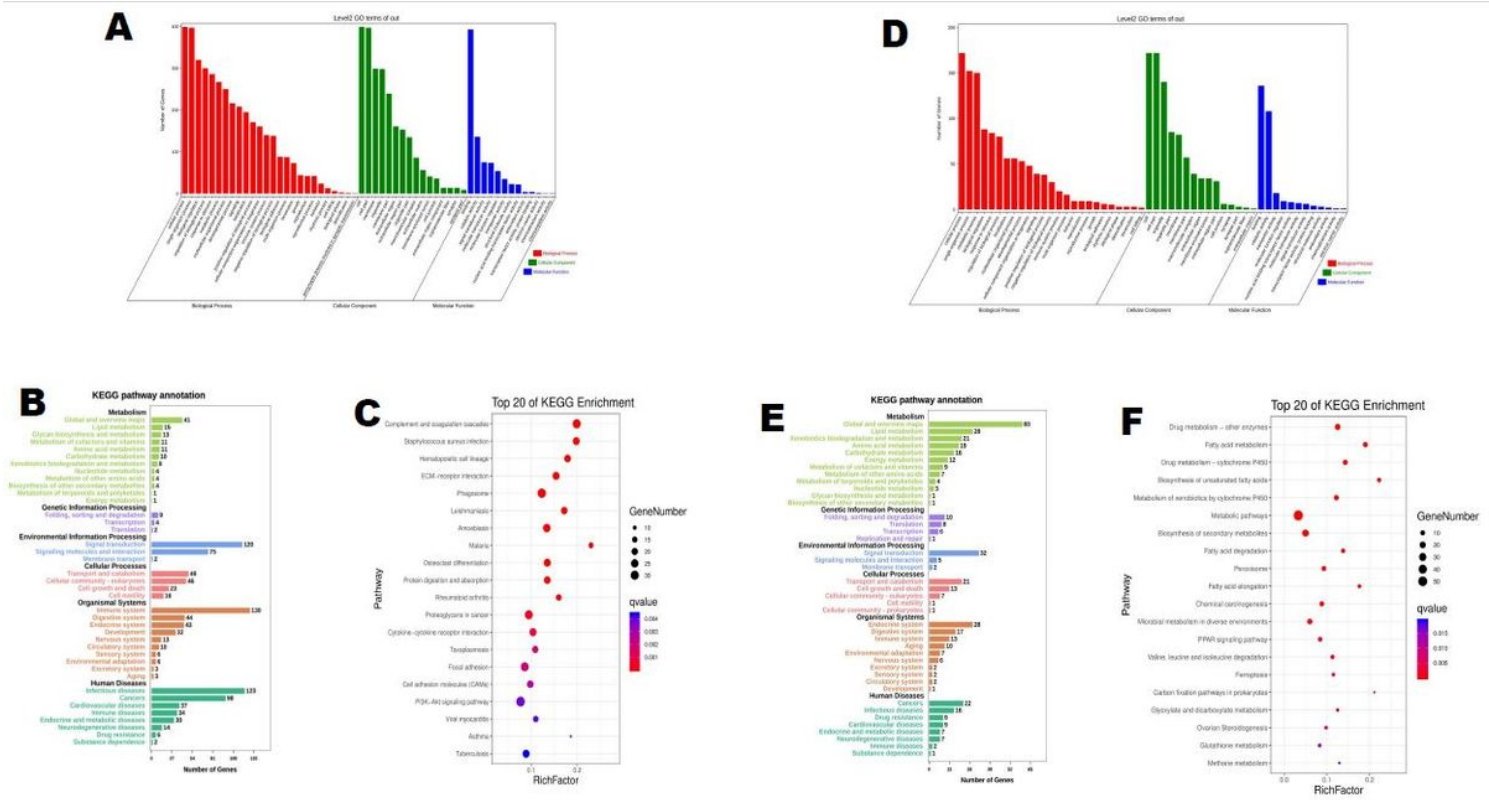

Figure 4

A-C Down-regulated DEGs GO enrichment result, KEGG enrichment result and TOP20 pathways enriched.
D-F Up-regulated DEGs GO enrichment result, KEGG enrichment result and TOP20 pathways enriched.

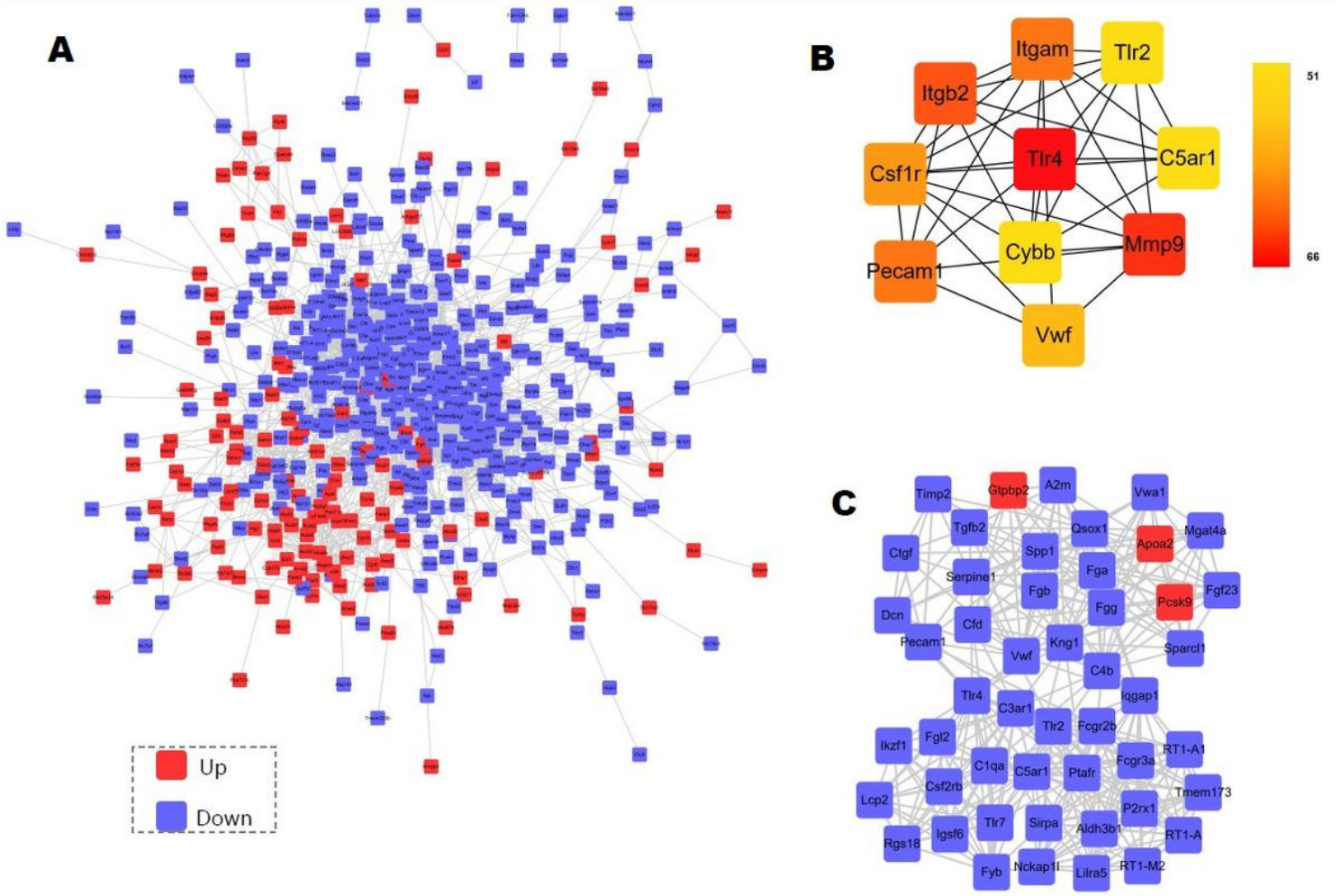

Figure 5

A The whole PPI visualized by Cytoscape.B TOP10 HUB genes. C TOP one cluster (cluster score : 13.5).

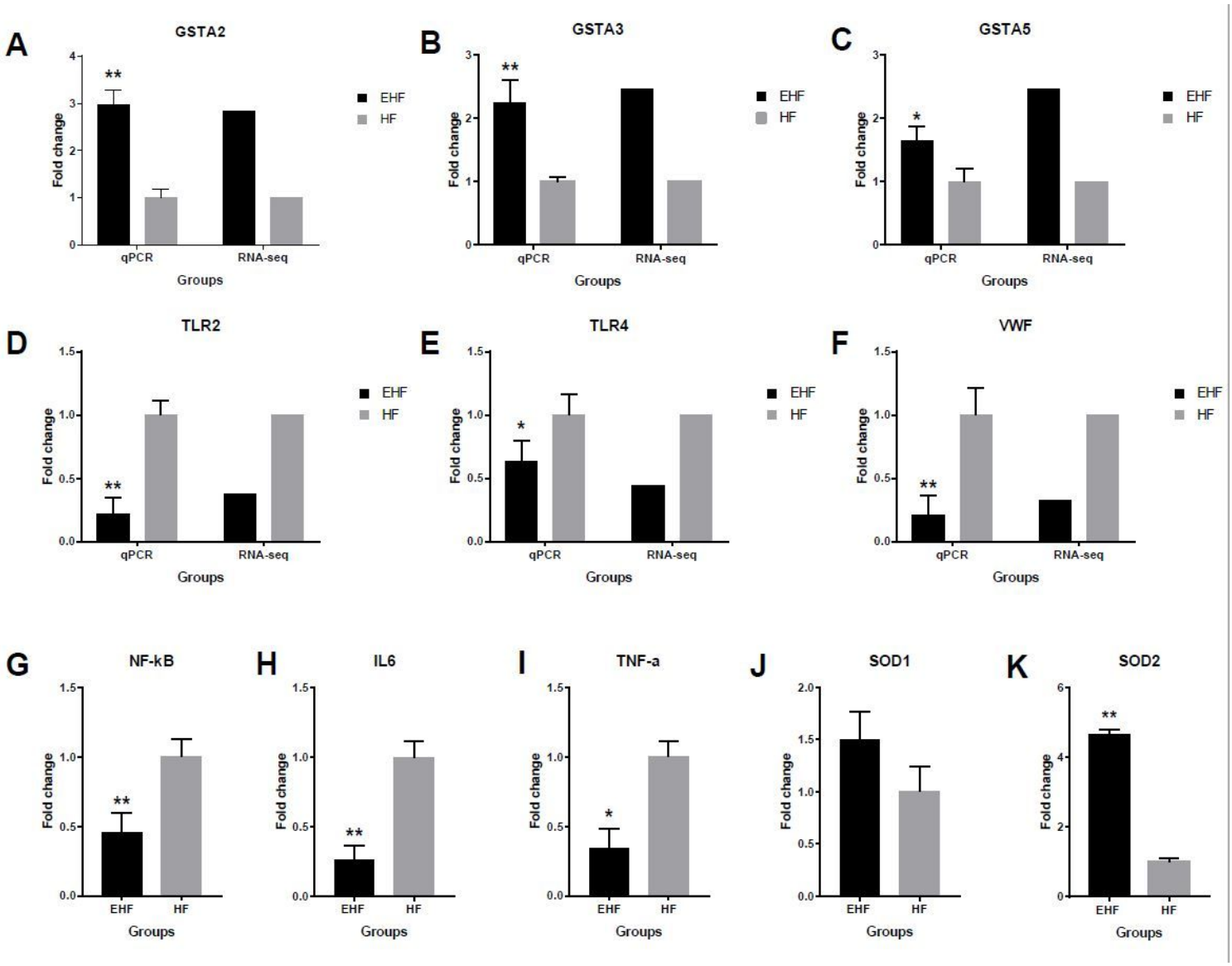

Figure 6

A-C The mRNA levels of three up-regulated DEGs (GSTA2, GSTA3, GSTA5) in EHF group vs. HF group (n= 5). D-F The mRNA levels of three down-regulated DEGs (TLR2, TLR4, VWF) in EHF group vs. HF group (n= 5). G-K The mRNA levels of inflammation factors(TNF-α, iL-6 and NF-κB) and antioxidants(SOD1 and SOD2) (n= 5). Data are presented as means±SD, p<0.05 was considered significant. *P < 0.05, **P < 0.01 vs. HF group.

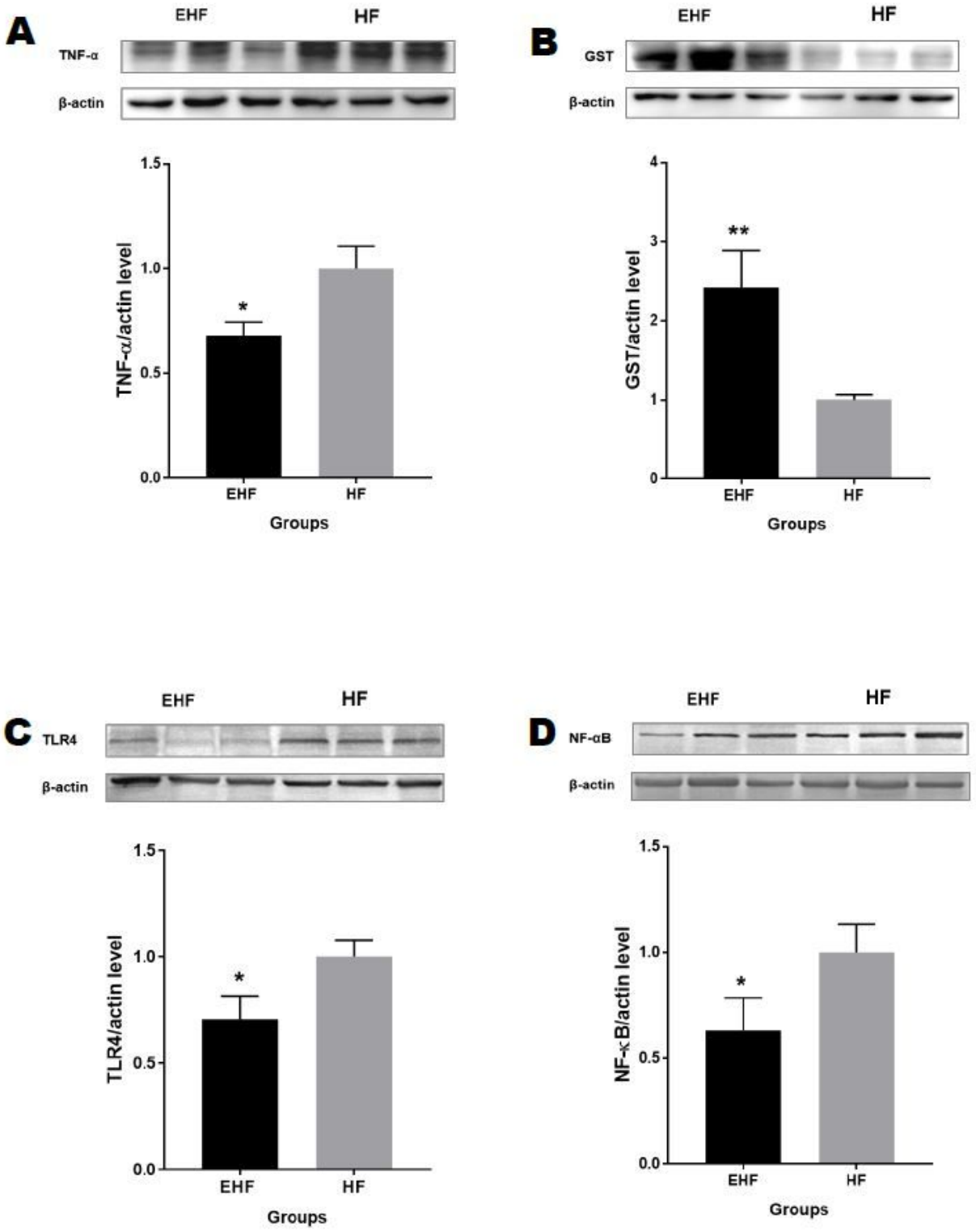

Figure 7

A The protein expression of TNF-α (n= 4). B The protein expression of GST (n= 4). C The protein expression of TLR4 (n= 4). D The protein expression of NF-κB (n= 4). Data are presented as means±SD, $p<0.05$ was considered significant. *P < 0.05, **P < 0.01 vs. HF group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.xls
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- SupplementaryTable2.xls
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