Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

Effects of High-Fat Diet on Cardiovascular Protein Expression in Mice Based on Proteomics

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Purpose: To investigate the effect of high-fat diet on protein expression in mouse heart and aorta using proteomic techniques.

Methods: A high-fat diet was used to construct an obese mouse model, and body weight was checked regularly. After the experiment, serum lipid and oxidative stress levels were measured. Proteomic detection of cardiac and aortic protein expression. Cardiac and aortic common differentially expressed proteins (Co-DEPs) were screened based on proteomic results. Subsequently, functional enrichment analysis and screening of key proteins were performed.

Results: A high-fat diet significantly increased body weight in mice. Obese mice had considerably higher levels of TC, TG, LDL-C, ROS, and MDA. In the heart and aorta, 17 Co-DEPs were discovered. The results of functional analysis of these proteins indicated that they were mainly related to lipid metabolism. Ech1, Decr1, Hsd17b4, Hsdl2 and Acadvl were screened as key proteins. In mice, a high-fat diet causes lipid metabolism to become disrupted, resulting in higher levels of oxidative stress and lipid peroxidation products.

Conclusion: Ech1, Decr1, Hsd17b4, Hsdl2 and Acadvl as cardiac and aortic Co-DEPs are closely related to lipid metabolism and may serve as potential diagnostic and therapeutic targets for obesity-induced cardiovascular disease.

Keywords: high-fat diet, obesity, proteomics, cardiovascular disease, lipid

Introduction

Obesity is a major disease worldwide and usually refers to the state of excess accumulation of adipose tissue in the body. The main cause of obesity is due to a chronic positive energy balance, which means that when energy expenditure is less than energy intake, adipose tissue increases, which then lead to the development of obesity.[1] The current increasing global incidence is due primarily to changes in dietary patterns, excessive high-fat, high-sugar and high-calorie intake and relatively little exercise, in addition to genetic factors and disease states that are responsible for its rising incidence.[2] Obesity itself can lead to soft tissue damage in joints, reduced reproductive capacity, and psychological disorders. In addition, obesity can increase the risk of cardiovascular disease, affect the function of the endocrine system and increase the risk of cancer.[3–5] Therefore, obesity is a disease syndrome that deserves our continuous attention.

Obesity induces multiple hemodynamic changes that may lead to changes in cardiac morphology, ventricular function, and vascular endothelial cells, which contribute to the development of various cardiovascular diseases, such as cardiac hypertrophy, heart failure, and atherosclerosis.[6,7] Furthermore, obesity-induced metabolic abnormalities in vivo play a key role in cardiovascular disease. Insulin resistance can contribute to elevated blood pressure and cardiovascular load by increasing vascular tone and blood volume.[8] Atherosclerosis is considerably increased by lipid metabolism disorders.[9] Elevated levels of oxidative stress can harm a variety of target organs, primarily affecting the function of cardiovascular endothelial cells, and triggering the atherosclerosis pathological process. Additionally, increased oxidative stress increases lipid peroxidation products, which can cause cellular damage.[10] There is mounting evidence that bioactive substances secreted by adipocytes

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can cause cardiovascular damage directly, as well as promote lipid metabolism disorders, inflammation, and increased levels of oxidative stress, all of which can lead to the development of various cardiovascular diseases indirectly.[11,12]

A number of serum markers, such as hypersensitive cardiac troponin T, pulse wave velocity, and central systolic blood pressure, have recently been utilized to diagnosis early heart injury and subclinical arteriosclerosis, and these markers have been shown to increase dramatically in obesity.[13] MicroRNAs have also been linked to cardiovascular disease in obese people.[14] Obesity has been found in proteomics studies to change the expression of a variety of cardiac proteins that can be exploited as diagnostic and therapeutic targets.[15,16] Proteomic research on the consequences of obesity on the aorta are lacking. The benefits of screening common indicators or treatment targets for obesity-induced heart and aortic damage can be increased because of the strong link between heart and vascular pathophysiology.

Our research used proteomics to investigate changes in cardiac and aortic protein expression caused by a high-fat diet, as well as the molecular pathways involved. This provides an experimental foundation for cardiovascular disease diagnosis and treatment.

Method and Materials

Construction of Obesity Model

Specific pathogen-free male C57BL/6 6-week-old mice were randomly divided into a normal chow diet group (NCD group) and a high-fat group (HFD group) after 1 week of adaptive feeding, with 8 mice in each group. The NCD group was given a normal diet containing 4% fat and the HFD group was given a high-fat diet containing 60% fat. All mice were housed in a standard sterile animal house. Bedding, food and water were changed regularly. The success of the model construction was assessed after 12 weeks of feeding on a high-fat diet, and then the intervention was continued for 12 weeks. This experiment was done with the approval of the Animal Ethics Association of the Hebei General Hospital. Animals used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publications No. 8023, revised 1978).

Serological Index Analysis

Mice were fully anesthetized and blood was taken from the internal canthal vein. The blood was centrifuged after resting and the supernatant was taken for the next step of the experiment. Automatic biochemical analyzer to analyze serum total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C). ELISA was used to detect serum superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels. All operations were performed according to the manufacturer's instructions, and the kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Sample Collection

At the end of the experiment, mice were anesthetized and then both groups of mice were sacrificed and placed on ice plates. The thoracic cavity of the mice was opened and the heart and aorta were harvested. After rinsing with saline and blotting on filter paper, the tissues were frozen in a −80°C refrigerator.

Proteomics Analysis

To explore the effect of high-fat diet on cardiac and aortic protein expression, we used proteomics to confirm protein expression in tissues. The process of mass spectrometry analysis includes protein extraction, peptide digestion, TMT labeling, chromatographic classification, liquid chromatography-tandem mass spectrometry (LC-MS/MS) data acquisition, and database retrieval.

Identification of DEPs

To analyze the proteins with differential expression between groups, the experimental data were further screened for differences. In the significant difference protein screening, the number of up-regulated and down-regulated proteins between the compared groups was obtained by expression fold change (FC) > 1.2-fold (up-regulation > 1.2-fold or down-regulation < 0.83-fold) and P value < 0.05 (*T*-test).

GO and KEGG Enrichment Analysis

Gene Ontology (GO) is an important bioinformatics tool for annotating genes and analyzing the biological processes of these genes. The String tool is able to provide not only the interactions between proteins, but also specific information about individual proteins. GO-enriched functional analysis of relating proteins is also available from this online tool.

Cellular activity is generally a dynamic process of changes in a series of proteins and metabolites, a process called pathway. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (https://www.kegg.jp/) were used for pathway enrichment analysis of DEPs.

PPI Network Construction and Modules Analysis

PPI network analyses can indicate the functional link between proteins and proteins, using string software (https://cn.string-db.org/) for PPI network analysis of differential genes. Subsequently, cytoscape software was applied to visualize and analyze PPI network. Molecular Complex Detection (MCODE) can find the interacting dense region in the PPI network, and the dense regions of interest can also be extracted and visualized. So we use MCODE to discover modules across the network.

Data Processing

Graphpda 8.0 software was used for statistical analysis. Statistical differences were taken into account in the P value was < 0.05. Count data were expressed as mean ± standard deviation. Student's t-test was used to statistically test the data of both groups.

Results

Body Weight Change of Mice

At the beginning of the experiment, there was no significant difference between the body weight of mice in the NCD and HFD groups (P > 0.05). The body weight of mice in the HFD group was significantly higher than that in the NCD group at 3 weeks after the high-fat diet intervention (P < 0.01). After that, the mice in the HFD group gained weight rapidly, while the mice in the NCD group gained weight slowly (Figure 1A). Figure 1B shows the representative body shapes of the two groups of mice at the end of the experiment.

Lipid, MDA and SOD Changes

A high-fat diet increases body weight in mice while also causing disruptions in lipid metabolism. Serum TC, TG, LDL-C and MDA were significantly increased in the HFD group compared to the NCD group (P < 0.01) (Figure 1C–F). The high-fat diet decreased SOD activity (P < 0.01) (Figure 1G), indicating a decrease in vivo resistance to oxidative stress, while the increase in MDA levels indicated increased production of lipid peroxidation products.

Identification of DEPs

Figure 2 illustrates a volcano map of the changes in cardiac and aortic protein expression induced by a high-fat diet. The results of principal component analysis (PCA) showed that the tissue proteomic characteristics of the NCD and HFD groups were significantly different (Figure 3A). A total of 64 DEPs were identified in cardiac tissue, 507 DEPs were found in the aorta, and there were 17 common differentially expressed proteins (Co-

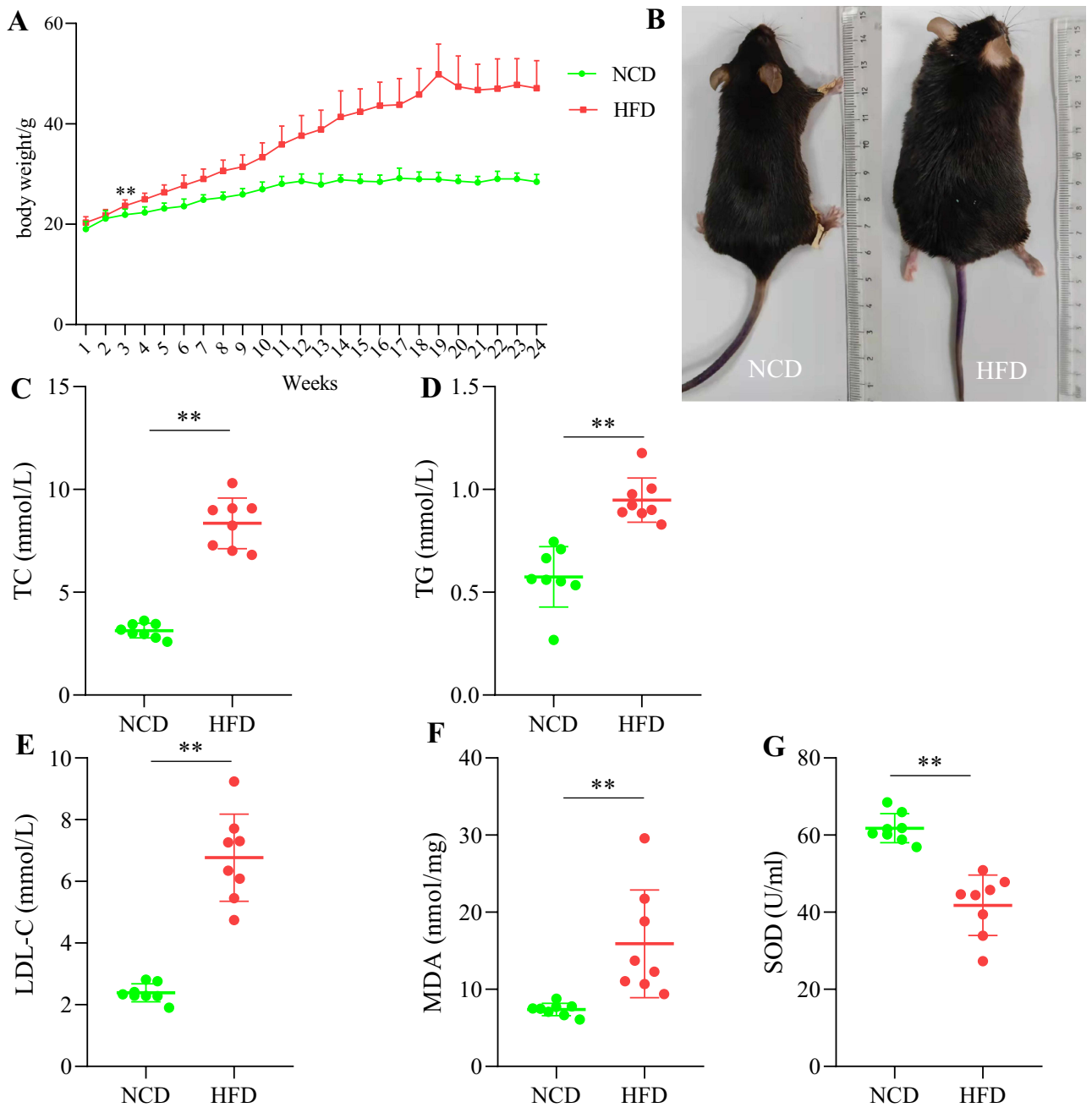

Figure 1 Effects of a high-fat diet on body weight and serological markers in mice. (**A**) Changes in mouse body weight over time. (**B**) Comparison of body shape of mice in the NCD and HFD groups. (**C**) TC. (**D**) TG. (**E**) LDL-C. (**F**) MDA. (**G**) SOD. N=8/group. **Means P < 0.01. Values are mean±SD.
Abbreviations: NCD, normal chow diet; HFD, high-fat diet; TC, total cholesterol; TG, total cholesterol; LDL-C, low-density lipoprotein cholesterol; MDA, malonic dialdehyde; SOD, superoxide dismutase.

DEPs) (Figure 3B). Figures 3C and D show the expression of all Co-DEPs in the heart and aorta. A high-fat diet resulted in downregulation of Col1a1 expression in the heart and aorta, while Wipi2 expression was down-regulated in the heart and upregulated in the aorta, and the rest of the co-DEPs were upregulated.

GO Enrichment Analysis of DEPs

To further understand the function of Co-DEPs, we performed functional enrichment analysis. The biological processes (BP) were mainly enriched in lipid catabolic process, lipid modification and fatty acid oxidation (Figure 4A). Cellular component (CC) was mainly enriched in peroxisome, microbody and very-low-density lipoprotein particle (Figure 4B).

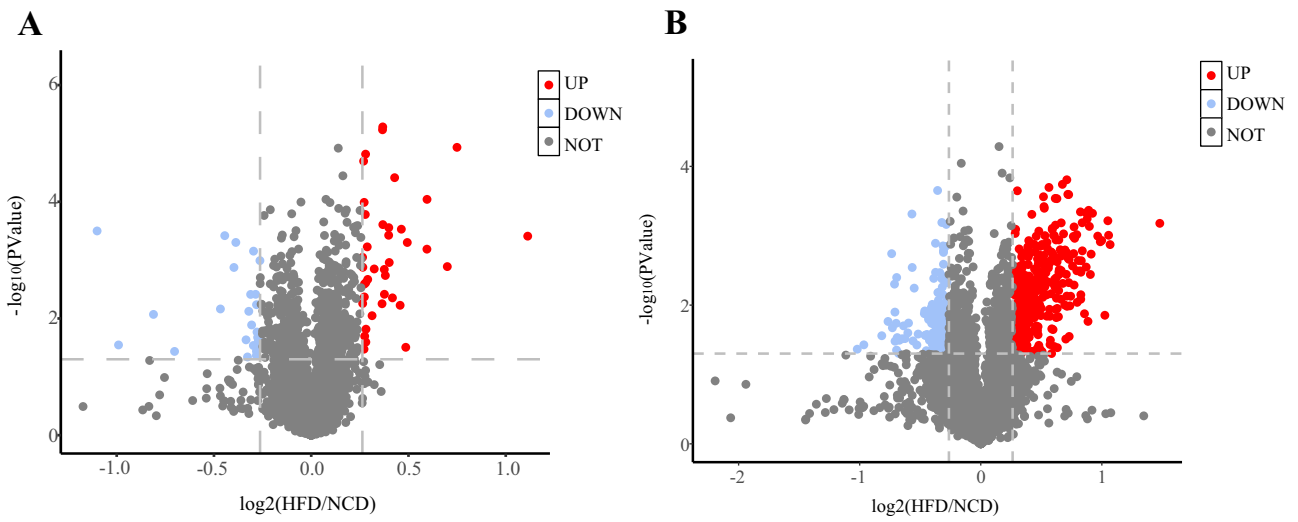

Figure 2 Volcano plots of all DEPs. (**A**) Volcano plot of all DEPs in cardiac tissue. (**B**) Volcano plot of all DEPs in the aorta.
Abbreviations: DEPs, differentially expressed proteins; NCD, normal chow diet; HFD, high-fat diet.

Molecular function (MF) is mainly focused on lipase inhibitor activity, fatty acid binding and oxidoreductase activity, acting on the CH-CH group of donors (Figure 4C).

KEGG Pathway Analysis of DEPs

KEGG pathway analysis revealed that DEPs were mainly enriched in Fatty acid metabolism, Cholesterol metabolism and Peroxisome (Figure 4D).

PPI Network and Module Analysis

We found 17 nodes and 19 edges of the PPI reciprocal network with an average node level of 2.24. We also found that Ech1, Decr1, Hsd17b4, Hsdl2 and Acadvl were more closely linked in this network (Figure 5). We then imported this network into Cytoscape and found that the above genes were more interlinked and were recognized as key modules.

Discussion

Obesity, metabolic syndrome, and atherosclerosis are among the most common diet-related metabolic illnesses.[17] Inflammation, oxidative stress, and mitochondrial dysfunction have all been implicated as key processes in the etiology of metabolic diseases, with a clear link between oxidative stress and metabolic disorders.[18] Increased LDL and reduced HDL are linked to oxidative stress, and oxidative stress causes a considerable rise in lipid peroxidation products as a result of enormous lipid oxidation.[19] Activation of numerous oxidative and oxygenating enzymes, as well as decreased activity of cellular antioxidant enzymes including Cu-Zn SOD, catalase, and GPx, can induce lipid peroxidation and increased reactive oxygen species (ROS), which can harm cells and tissues.[20,21] The accumulated adipose tissue in obesity stimulates the synthesis of proinflammatory factors, which increase the production of ROS and activate the oxidative stress process, leading to multi-organ damage.[22] It has also been shown that the GPx activity decreases in the obese state and the ability to scavenge ROS reduces, thus participating in the multi-organ damage process of obesity.[23]

We used a high-fat diet to construct an obese mouse model, and proteomic technology was used to examine the effect of the high-fat diet on cardiac and aortic protein expression in order to better understand the mechanism of obesity-induced cardiovascular disease. Serological markers revealed that a high-fat diet raised TC, TG, and LDL-C levels considerably. Obesity, on the other hand, increased MDA and ROS in mice, implying that the increased oxidative stress generated by a high-fat diet enhances lipid peroxidation, resulting in an increase in the lipid peroxidation product MDA. Increased MDA levels in the body can cause a wide range of cellular damage. The heart and aorta share 17 DEPs, according to the proteomic studies. The functional investigation of Co-DEPs revealed that they are mostly involved in lipid metabolism, with Ech1, Decr1, Hsd17b4, Hsdl2, and Acadvl being important proteins. To summarize, a high-fat diet

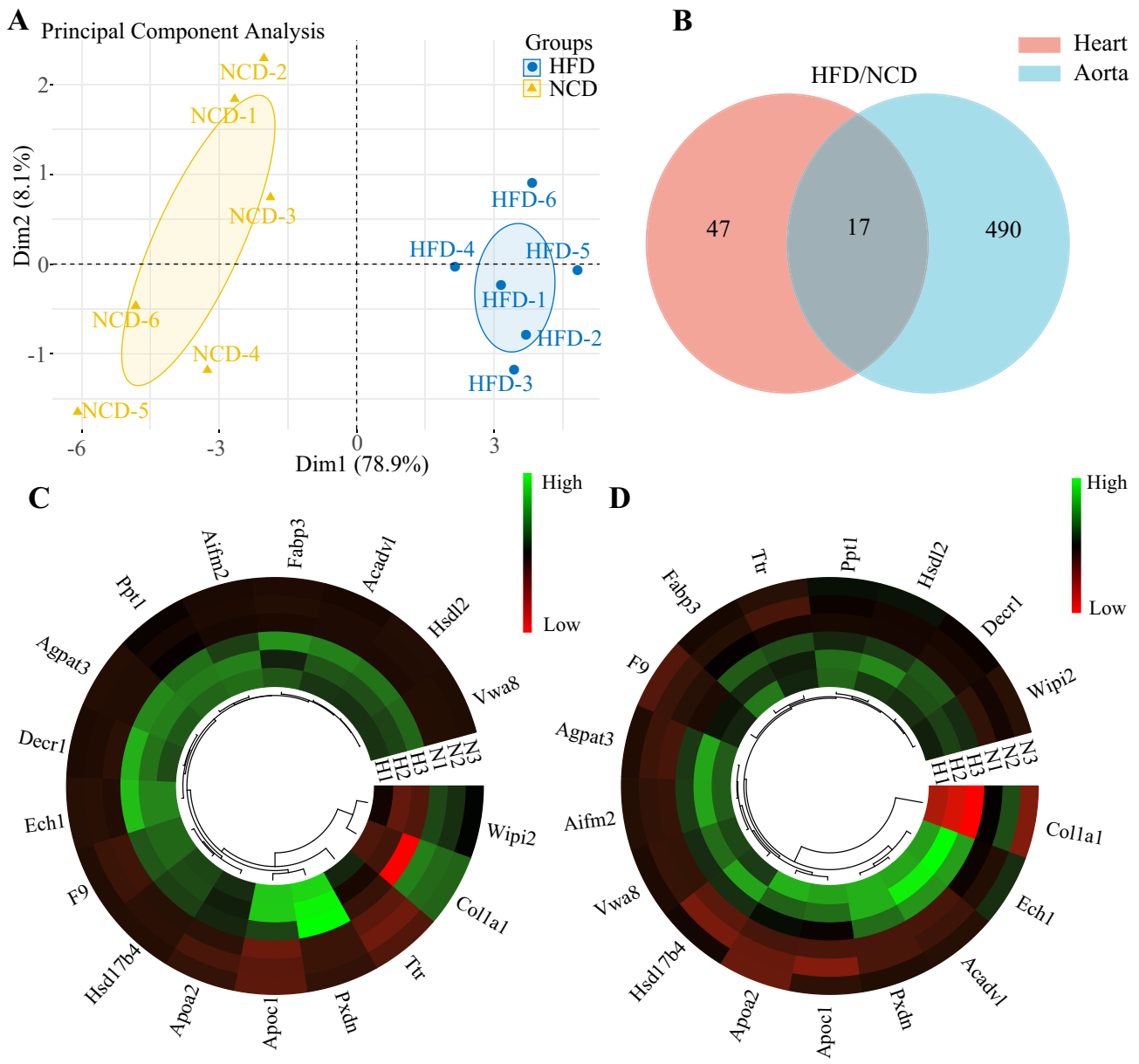

Figure 3 Common DEPs (Co-DEPs) in the heart and aorta. (**A**) Principal component analysis of individual samples. (**B**) Venn diagram of Co-DEPs in the heart and aorta. (**C**) Heatmap of Co-DEPs in cardiac tissue. (**D**) Heatmap of Co-DEPs in the aorta.
Abbreviations: DEPs, differentially expressed proteins; NCD, normal chow diet; HFD, high-fat diet.

raises lipid levels and oxidative stress in the body, leading in lipid peroxidation and increased MDA generation, which leads to cardiovascular disease. As Co-DEPs in the heart and aorta, Ech1, Decr1, Hsd17b4, Hsdl2, and Acadvl may become novel targets for the diagnosis and therapy of obese cardiovascular disease.

The enoyl-CoA hydratase 1 (Ech1) gene encodes a 290-amino-acid enzyme that catalyzes the hydration of medium- and short-chain enoyl-CoAs.[24] Ech1 is involved in fatty acid oxidation and lipid metabolism in mitochondria. Ech1 appears to play a role in cancer development by regulating cell proliferation and apoptosis, according to new research.[25] Overexpression of Ech1 can also help to alleviate metabolic abnormalities caused by a high-fat diet.[26] Ech1 can cause white adipose tissue to brown, lowering body weight, reducing insulin resistance, and improving lipid metabolism.[27] Decr1 is a 2,4-dienoyl-CoA reductase that belongs to the short-chain dehydrogenase/reductase family (SDR). Decr1 is a rate-limiting enzyme in the polyunsaturated fatty acid (PUFA) β-oxidation accessory pathway, and elevated expression of Decr1 enhances lipolysis.[28] Interestingly, Decr1 appears to play different roles in the pathology of different

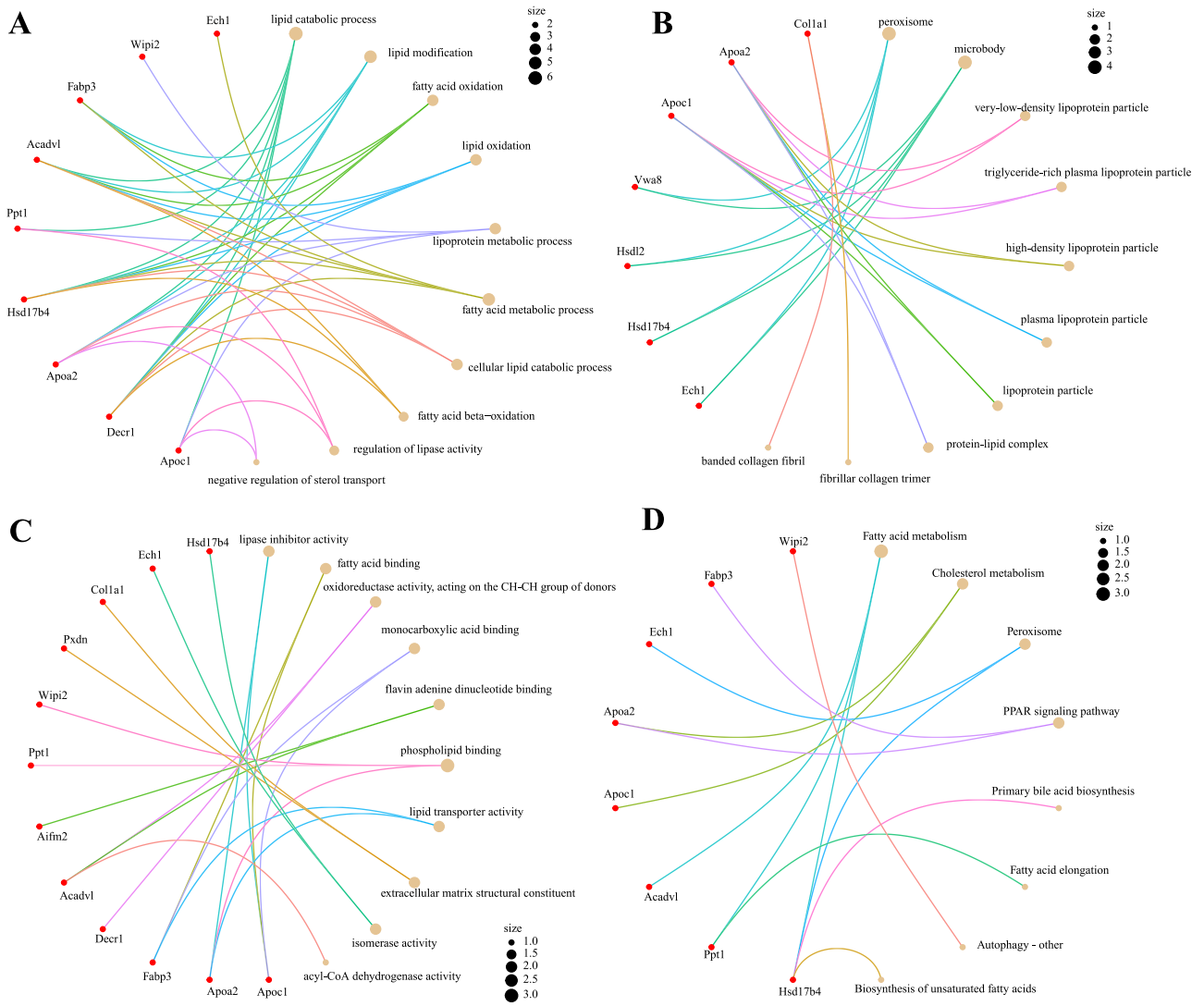

Figure 4 Bioinformatics analysis of DEPs. (**A**) BP. (**B**) CC. (**C**) MF. (**D**) KEGG enrichment analysis. Orange represents different items and red represents DEPs. The size of the dots indicates the number of DEPs enriched on the item.

Abbreviations: DEPs, differentially expressed proteins; BP, biological processes; CC, cell composition; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

tumors.[29,30] However, the increased expression of Decr1 in the heart can alleviate lipid metabolism disorders and play a cardioprotective role.[31] High-fat diet significantly increased cardiac and aortic Ech1 and Decr1 expression in the present study, possibly related to increased fat metabolism leading to decreased fat deposition and decreased lipid aggregation.

Peroxisomal D-bifunctional protein also known as 17beta-Hydroxysteroid dehydrogenase type 4 (Hsd17b4), or multifunctional protein 2, is a key enzyme in peroxisomal ultra-long-chain fatty acid β-oxidation and DHA production. Hsd17b4 protein deficiency can lead to accumulation of very long-chain fatty acids (VLCFAs) and induce endoplasmic reticulum stress, which in turn leads to cellular damage and mitochondria-mediated apoptosis leading to cellular damage.[32] Hydroxysteroid dehydrogenase like protein 2 (Hsdl2), a member of the steroid dehydrogenase family, is located in peroxisomes and mitochondria, and can not only bind to peroxisomes, but also interact with the coenzyme NADPH, which plays an important role in fatty acid metabolism.[33] Research on these two proteins has focused on finding that their levels are significantly elevated in various cancers and can be used as markers for cancer diagnosis and as therapeutic targets.[34,35] Our study showed that Hsd17b4 and Hsdl2 were up-regulated in the heart and aorta. We

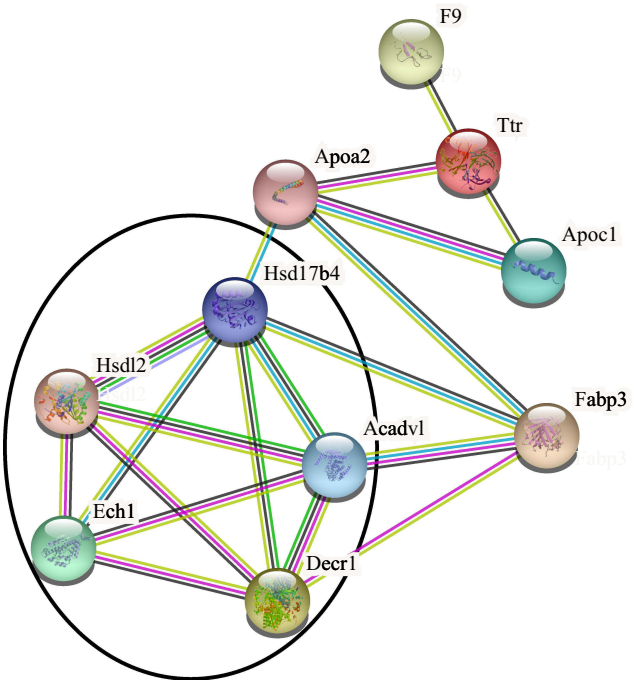

Figure 5 Protein-protein interaction network. Black circles represent key modules on this network.

hypothesize that Hsd17b4 and Hsdl2 are involved in regulating lipid metabolism and oxidative stress because they are both related to catalase activity and the peroxisome is vital in maintaining cellular redox homeostasis.

Acyl-CoA dehydrogenase very long chain (Acadvl) gene encodes very long-chain acyl-coenzyme A dehydrogenase (Vlcad), which is located in mitochondrial membrane and involved in fatty acid oxidation. Deficiency of Vlcad can lead to the accumulation of toxic long-chain acylcarnitines leading to dysfunction of organs such as the heart, skeletal muscle and liver.[36] Vlcad deficiency has been linked to cardiomyopathy, aortic valve calcification, and arrhythmias in studies.[37–39] The lack of Small heterodimer partner (SHP) can lower the expression of myocardial Vlcad, reducing lipid accumulation and protecting heart function in a high-fat diet animal model.[40] The proteome data revealed that Vlcad is highly expressed in the heart and aorta of obese mice, suggesting that a high-fat diet causes proteomic signatures in the early stages of cardiac and aortic diseases.

Conclusions

Increased oxidative stress, lipid peroxidation, and body weight are all consequences of high-fat diet in mice. With their connections to lipid metabolism, the proteins Ech1, Decr1, Hsd17b4, Hsdl2, and Acadvl could possibly be employed as potential targets for the detection and therapy of obesity-related cardiovascular disease, according to proteomics data.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The authors declare that they have no conflicts of interest.

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