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ORIGINAL RESEARCH

Atorvastatin Inhibits High-Fat Diet-Induced Lipid Metabolism Disorders in Rats by Inhibiting *Bacteroides* Reduction and Improving Metabolism

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Purpose: The prevalence of hyperlipidemia and related illnesses is on its rise, and atorvastatin is the frequently used hypolipidemic agent. However, there is still uncertainty about the mechanisms, especially the relationship between the lipid-lowering effect, intestinal microbiome, and metabolic profiles. We aim to intensively explain the mechanism of the hypolipidemic effect of atorvastatin through multi-omics perspective of intestinal microbiome and metabolomics.

Methods: Multi-omics methods play an increasingly important role in the analysis of intestinal triggers and evaluation of metabolic disorders such as obesity, hyperlipidemia, and diabetes. Therefore, we were prompted to explore intestinal triggers, underlying biomarkers, and potential intervention targets of atorvastatin in the treatment of dyslipidemia through multi-omics. To achieve this, SPF Wistar rats were fed a high-fat diet or normal diet for 8 weeks. Atorvastatin was then administered to high-fat diet-fed rats.

Results: By altering intestinal microbiome, a high-fat diet can affect feces and plasma metabolic profiles. Treatment with atorvastatin possibly increases the abundance of *Bacteroides*, thereby improving "propanoate metabolism" and "glycine, serine and threonine metabolism" in feces and plasma, and contributing to blood lipid reduction.

Conclusion: Our study elucidated the intestinal triggers and metabolites of high-fat diet-induced dyslipidemia from the perspective of intestinal microbiome and metabolomics. It equally identified potential intervention targets of atorvastatin. This further explains the mechanism of the hypolipidemic effect of atorvastatin from a multi-omics perspective.

Keywords: hyperlipidemia, 16S rRNA sequencing, intestinal microbiome, metabolomics, atorvastatin calcium trihydrate

Introduction

Long-term intake of a high-fat diet (HFD) can cause hyperlipidemia,[1] leading to a gradual increase in its incidence.[2] Hyperlipidemia can result in cardiovascular and cerebrovascular diseases,[3] thereby posing a major threat to human health.[4] Therefore, the prompt control of abnormal blood lipid elevations will significantly lower the incidence of cardiovascular and cerebrovascular diseases. Hyperlipidemia is a chronic condition with an insidious onset;[5] thus, requiring daily medication. Statins are a class of medications frequently used to reduce blood lipid levels by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase.[6–9] Atorvastatin can also reduce LDL-Cholesterol levels[10] and plasma cholesterol by inhibiting the major histocompatibility complex II (MHC II) on antigen-presenting cells stimulated by interferon-γ. Although the modes of action of atorvastatin have been intensively investigated, there is still uncertainty

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about the mechanisms, especially the relationship between the lipid-lowering effect, intestinal microbiome, and metabolic profiles.

Increasing evidence suggest a close relationship between HFD, intestinal microbiome and metabolic profiles. Consuming a HFD for an extended period of time alters the intestinal microbiome,[11] resulting in obesity, atherosclerosis, and other disorders.[12,13] Intestinal microbiome is closely related to lipid metabolism.[14,15] For example, *Bacteroides* has bile salt hydrolase activity that can improve cholesterol metabolism.[16–18] This plays an important role in hyperlipidemia.[19] Long-term intake of an HFD can also affect metabolites.[20] For example, an HFD can reduce 2-hydroxybutyric acid involved in "propanoate metabolism" in feces and plasma.[21] Fecal metabolites are strongly associated with the activity of intestinal microbiome, whereas plasma metabolomics characterizes all small metabolites in plasma, and is closely related to the overall physiology of the organism.[22] Metabolomics are closely related to lipid metabolism. For example, "propanoate metabolism" is critical for the tricarboxylic acid cycle, and disorders in propanoate metabolism can affect energy production.[23] Glycine, which is involved in "glycine, serine and threonine metabolism", can promote lipid metabolism.[24] We sought to identify potential targets and elucidate the hypolipidemic mechanism of atorvastatin calcium trihydrate through a combined analysis of 16s rRNA sequencing, fecal metabolomics, and plasma metabolomics. Some authors attempted to explain this mechanism. The combined analysis of 16s rRNA sequencing and serum cholesterol showed that HFD affects cholesterol levels by changing the intestinal microbiome. Changes in the microbiome have been linked to atorvastatin treatment in an HFD-induced hypercholesterolemia rat model.[9] Thus, the mechanism of action of atorvastatin was studied by changes in the intestinal microbiome[14] or by analyzing plasma metabolites.[25]

We used an HFD-induced rat model to assess the effect of atorvastatin. We performed the following laboratory investigations: blood biochemistry, liver function tests, and intestinal microbiome analysis through 16S rRNA sequencing. Fecal and plasma metabolomics were then performed using a triple quadrupole-linear ion trap combined with a SCIEX QTRAP® 6500+ mass spectrometer in the multiple-reaction monitoring mode. In addition, we analyzed and interpreted specific intestinal microbiome, fecal and plasma metabolites to understand the mechanism of high-fat diet-induced intestinal microbiome and metabolic disturbances, as well as the potential mechanism of atorvastatin. We found that an HFD altered the intestinal microbiome and atorvastatin reduced blood lipid levels by enhancing the intestinal microbiome and metabolic pathways.

Materials and Methods

Animals and Groups

We used 30 male SPF Wistar rats, weighing 200–220 g for this study. They were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd., Jinan, China. Furthermore, they were housed in an experimental environment with a relative humidity of approximately 50% and temperature of approximately 22 °C. After the animals were fed adaptively, they were randomly divided into two groups. There were 20 male Wistar rats in the model group and 10 in the control group. The control group received a maintenance feed while the model group received HFD. Maintenance feed contained the following ingredients: crude protein: ≥ 18%, crude fat ≥ 4%, crude fiber ≤ 5%, crude ash ≤ 8%, moisture ≤ 10%, calcium 1.0–1.8%, total phosphorus 0.6–1.2%, total energy 3.40 kCal/g, energy supply ratio: protein 23.07%, fat 11.85%, carbohydrate 65.08%. The feed was provided by Keao Xieli (Beijing) Feed Co., Ltd. The HFD contained the following ingredients: crude protein: 12.7%, crude fat 18.9%, crude fiber 3.8%, crude ash 4.0%, moisture 9.2%, calcium 0.82%, total phosphorus 0.69%, nitrogen-free extract 53.1%, total energy 4.34 kCal/g, energy supply ratio: protein 11.7%, fat 39.3%, carbohydrate 49%. The feed is provided by Xiao Shu You Tai (Beijing) Biotechnology Co., Ltd. After three weeks of feeding, the high-fat diet rats were randomly divided into two subgroups; 10 were included into the model group (HFD) while the other 10 were considered as the drug intervention group (HFD+A). The control group (Control) received an equal volume of corn oil by gavage and maintenance feed; the model group (HFD) was given an equal volume of corn oil by gavage and HFD; and the drug treatment group (HFD + A) was given HFD and atorvastatin calcium trihydrate 0.1667 mg/kg.bw. Atorvastatin was administered by gavage once every morning for five weeks. The rats were euthanized with 5% chloral hydrate and 12.5% urethane (mixed 1:1), and blood was collected from the

abdominal aorta after 5 weeks of intervention. Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine transaminase (ALT), aspartate transaminase (AST), liver coefficients, and liver pathology were measured in all three groups. All animals were grossly examined, and liver tissue was weighed, with the relative weight (organ/body ratio) calculated. Liver lobes were immersed in 10% formalin solution for fixation, dehydrated, embedded, sliced, and stained with hematoxylin-eosin (HE). The histomorphological and pathological changes in the liver were observed under a light microscope. Data were subjected to the Shapiro–Wilk test to determine their distribution. SPSS version 26 was used for statistical analysis. The data were normally distributed. So, TC, TG, HDL-C, LDL-C, ALT, AST, and liver coefficients were analyzed through the one-way ANOVA. We used GraphPad Prism 8 to present comparisons between the groups. This experiment was approved by the Shandong University Preventive Medicine Animal Experiment Ethics Committee (protocol code LL20200303), and the study protocols adhered to the Ethics and Welfare requirements of the National Institutes of Health (NIH).

16S rRNA Sequencing and Analysis of Intestinal Microbiome

After the experiment, the rats were moved and their feces were placed in a 1-mL sterile EP tube and kept at −80 °C as soon as possible until the intestinal microbiome was subjected to 16S rRNA sequencing. Cetyl trimethylammonium bromide (CTAB) method was used to extract whole-genome DNA and then barcode-specific primers 515F and 806R was used to amplify 16S rRNA genes in the V4 region. Subsequently, a PCR was performed. Furthermore, an equal volume of 1X loading buffer (containing SYB green) with the PCR products was mixed, and an electrophoresis on a 2% agarose gel was performed. The Qiagen Gel Extraction Kit was used to purify the PCR products after isocratic mixing. The TruSeq® DNA PCR-free Sample Preparation Kit (Illumina, San Diego, CA, USA) was used to generate sequencing libraries and sequenced them on the Illumina NovaSeq platform to generate 250 bp paired-end reads.

To obtain high-quality clean tags,[26] quality control was carried out in accordance with QIIME (version 1.9.1)[27] and the raw tags were filtered. After the tags were compared with the Silva138, chimeric sequences were detected and removed[28] using the UCHIME algorithm.[29] The Uparse software (Uparse v7.0.1001) was used to sequence valid tags.[30] For effective tags, OTU clustering was performed with 97% consistency. The Silva database[31] was equally used to annotate the classification between the sequences. According to the species' annotation results, the top 10 most abundant species at the phylum level in each group were selected to generate a column chart of the relative abundant number of species. After normalizing the OTU level, alpha diversity, beta diversity, Linear discriminant analysis (LDA) effect size (LEfSe), and Tax4Fun function predictions were analyzed. Alpha diversity included rarefaction curves, rank abundance, and species accumulation boxplots. Beta diversity analysis included PCA, which was used to show whether the community compositions of the three groups were similar. Lefse analysis compared multiple groups to identify species with significant differences in abundance. We predicted the impact of the intestinal microbiome on metabolic pathways using iPath visualization. After calculation with QIIME (version 1.9.1), we used the R-software (version 2.15.3) to display the analysis of sample complexity. Raw data for high-throughput sequencing supporting the findings of this study are publicly available at https://www.ncbi.nlm.nih.gov/sra (ref: PRJNA804702).

Fecal Metabolomics and Plasma Metabolomics

After the metabolites were extracted, they were quantitatively analyzed by product ions (Q3) and qualitatively analyzed by parent ion (Q1), daughter ion (Q3), retention time (RT), declustering potential (DP), and collision energy (CE). SCIEX OS Version 1.4 was used to open the mass spectrum file, and chromatographic peak integration and correction were carried out. The metabolites were interpreted using the KEGG database (http://www.genome.jp/kegg/), HMDB database (http://www.hmdb.ca/), and Lipid maps database (http://www.lipidmaps.org/). We carried out quality control using a quality control sample overlap diagram and correlation analysis. After data conversion using metaX,[32] we conducted principal component analysis (PCA) and partial least squares discrimination analysis (PLS-DA), then get the variable importance in the projection (VIP) value of each metabolite. The statistical significance (p value) of each metabolite and fold change (FC) value between the control group and HFD group, as well as HFD group and HFD+A group was calculated by applying the student's unpaired t-test. The default criteria for screening differential metabolites were VIP > 1, P < 0.05, and FC > 1.2, or VIP > 1, P < 0.05, and FC < 0.833. Ipath was used to analyze whether the

intestinal microbiome is related to metabolic pathways. Volcano plots were drawn using ggplot2 in the R-language, thereby screening the metabolites of interest between the control group and the HFD, HFD and HFD + A groups. The bubble diagram was drawn using ggplot2 in the R-language, and the KEGG database was used to analyze the functions and metabolic pathways of metabolites between the control, HFD and HFD + A groups. Novogene provides 16S rRNA sequencing and metabolomics services.

Correlation Analysis

P-values and rho between intestinal microbiome and fecal metabolites were determined using the Spearman statistical method. $P < 0.05$ and an absolute value of rho ≥ 0.8 were considered statistically significant between the Control group and HFD group.

Results

Atorvastatin Calcium Trihydrate Can Improve Disorders Caused by HFD

Figure 1A shows a diagram of the animal experimental plan. After a high-fat diet for three weeks, TC, TG, and LDL-C levels significantly different between the HFD and control groups ($p < 0.05$), and TC and TG were significantly different

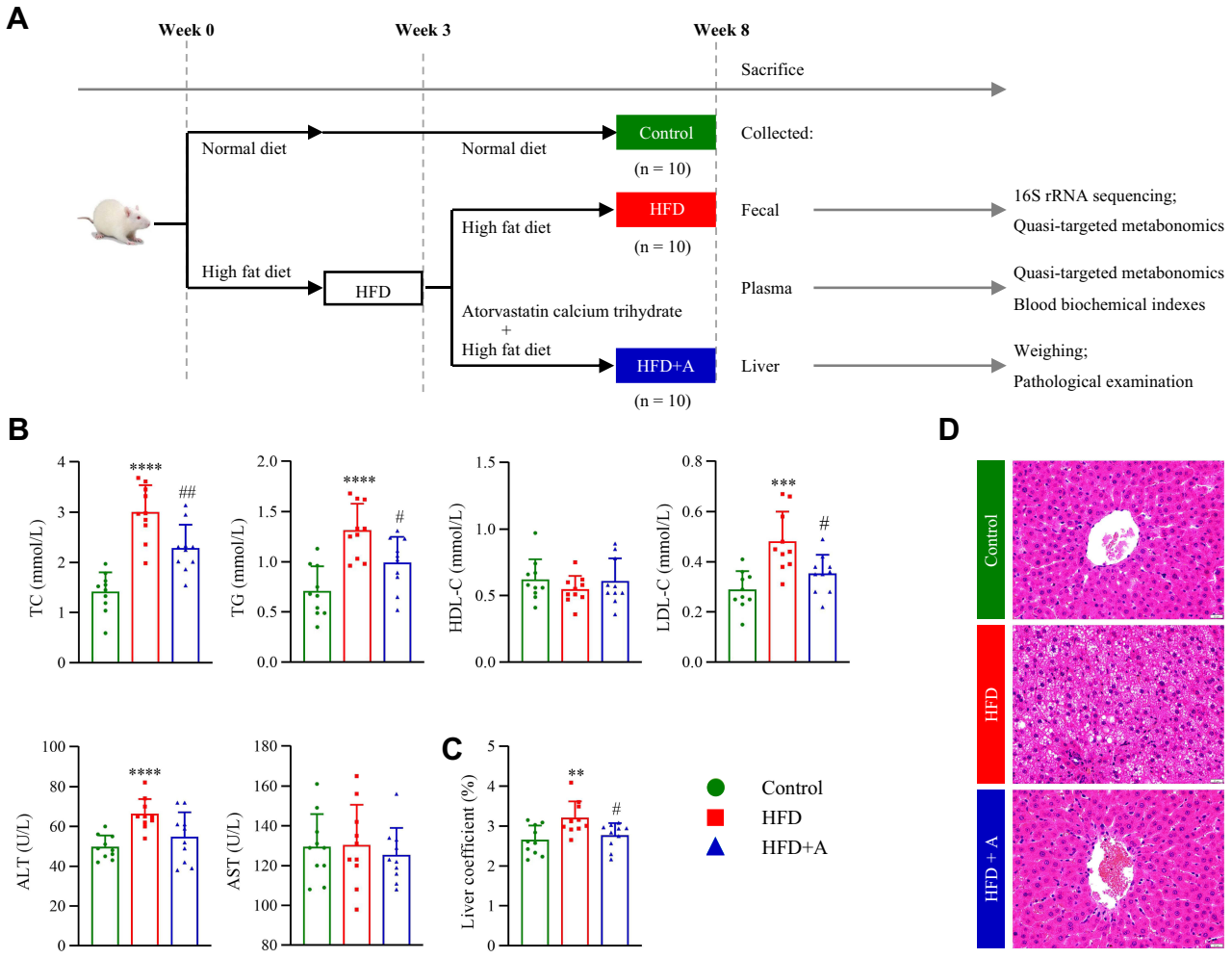

Figure 1 High fat diet induced hyperlipidemia of Wistar rats, atorvastatin significantly reduced blood lipid levels and improved liver steatosis. Animal experimental plan and changes of blood biochemical parameters and histology in rats. (**A**) Animal experiment plan. (**B**) Changes of blood biochemical parameters. (**C**) The ratio of liver to body weight in the three groups. (**D**) Liver pathological examination was performed in the three groups. All data is represented as Mean ± Standard Deviation. All data were accessed using One-way ANOVA. **p<0.01, *** p<0.001, ****p<0.0001 versus Control; #p<0.05, ##p<0.01 versus HFD.

Abbreviations: HFD, high fat diet; HFD+A, high fat diet and atorvastatin calcium trihydrate; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine transaminase, AST, aspartate transaminase.

between the HFD + A and control groups (Figure S1), implying that the model was successful. The level of TC, TG, LDL-C and ALT in serum of HFD group induced by high-fat diet were significantly different from the control group ($p < 0.05$) (Figure 1B). The liver-to-body weight ratio increased significantly ($p < 0.05$) (Figure 1C). HE staining revealed obvious steatosis in the hepatocytes (Figure 1D). After five weeks of drug treatment, the levels of TC, TG, and LDL-C significantly reduced in the experimental rats as compared to the HFD group ($p < 0.05$) (Figure 1B), as well as the ratio of liver-to-body weight ($p < 0.05$) (Figure 1C), and the pathological changes in the liver significantly improved (Figure 1D). This shows that a HFD significantly increases blood lipid levels and causes liver steatosis. Moreover, atorvastatin calcium trihydrate significantly reduced blood lipid levels and improved liver steatosis.

Quality control results revealed that the data were reliable. We compared the three groups to initially illustrate the effect of HFD and atorvastatin on the intestinal microbiome (Figure 2). We employed the LEfSe analysis and set LDA to 4 to identify potential biomarkers of intestinal microbiome in all groups (Figure 2A). Figure 2B illustrates the PCA of the three groups, indicating that the community composition of the HFD group was significantly different from that of the control group, while the HFD+A group had a tendency to return to normal after atorvastatin treatment.

The Effect of HFD on Intestinal Microbiome and Metabolomics

We used LEfSe analysis to identify potential biomarkers of the intestinal microbiome in the HFD and control groups, with an LDA threshold of 4 (Figure 3A). IPath (Figure 3B) showed that changes in the intestinal microbiome caused by an HFD affects metabolic pathways. We then used class-targeted metabolomics to study the changes in fecal and plasma metabolites. On this basis, we analyzed the correlation between nine intestinal microbiota markers at the genus level and 24 fecal metabolites involved in the KEGG pathway of the model and control groups. We observed that the intestinal microbiota has a strong correlation with the fecal metabolism pathway (Figure 3C). The intestinal microbiome that had the greatest impact on fecal metabolites included *Alloprevotella, Collinsella*, and *Romboutsia*. HFD may affect fecal metabolism by affecting *Alloprevotella, Collinsella* and *Romboutsia*. The level of some fecal metabolites changed in the model group than the control group (Figure 3D); 20 fecal metabolites increased, and 87 fecal metabolites decreased in the HFD group (Table S1). The KEGG bubble chart (Figure 3D) shows the top 20 metabolic pathways involved in different metabolites. "Propanoate metabolism" and "glycine, serine and threonine metabolism" were enriched. HFD reduces 2-hydroxybutyric acid involved

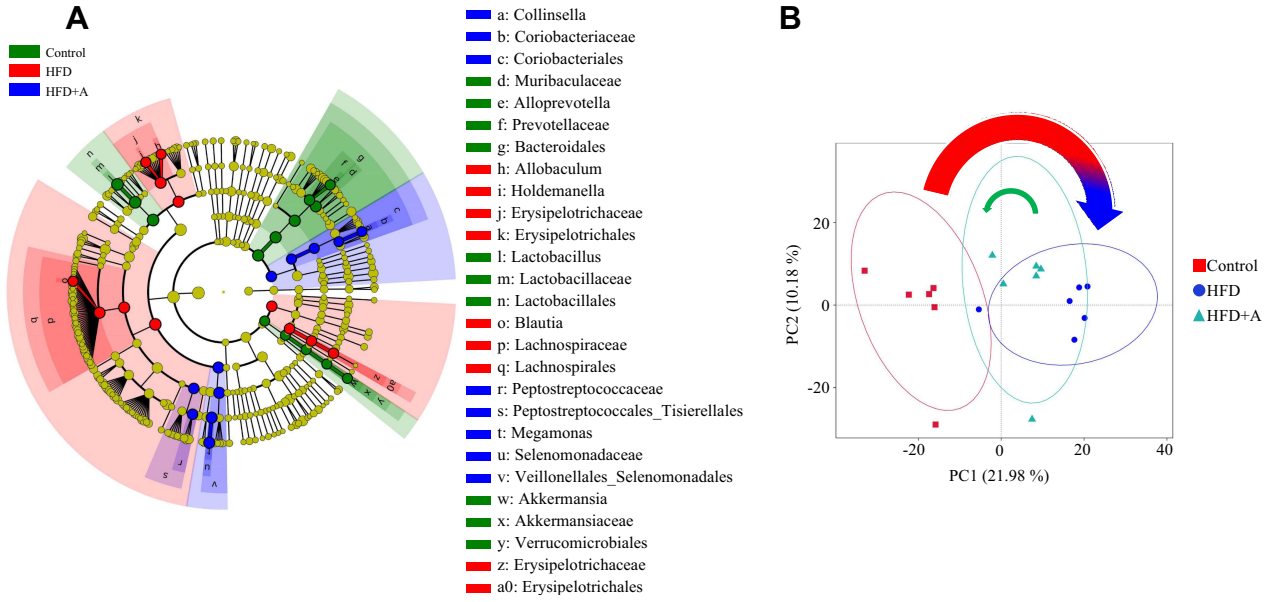

Figure 2 HFD alter the taxonomic composition of intestinal microbiome in Wistar rats, and atorvastatin can partially restore the intestinal microbiome changed with HFD. (**A**) LEfSe analysis of cladogram in three groups. The circle represents the classification level from phylum to genus. The diameter of the small circle is proportional to the relative abundance. (**B**) PCA Plot. The abscissa represents the first principal component (PC1), the ordinate represents the second principal component (PC2). The percentage represents the contribution value of PC1 or PC2 to the sample difference.
Abbreviations: HFD, high fat diet; HFD+A, high fat diet and atorvastatin calcium trihydrate.

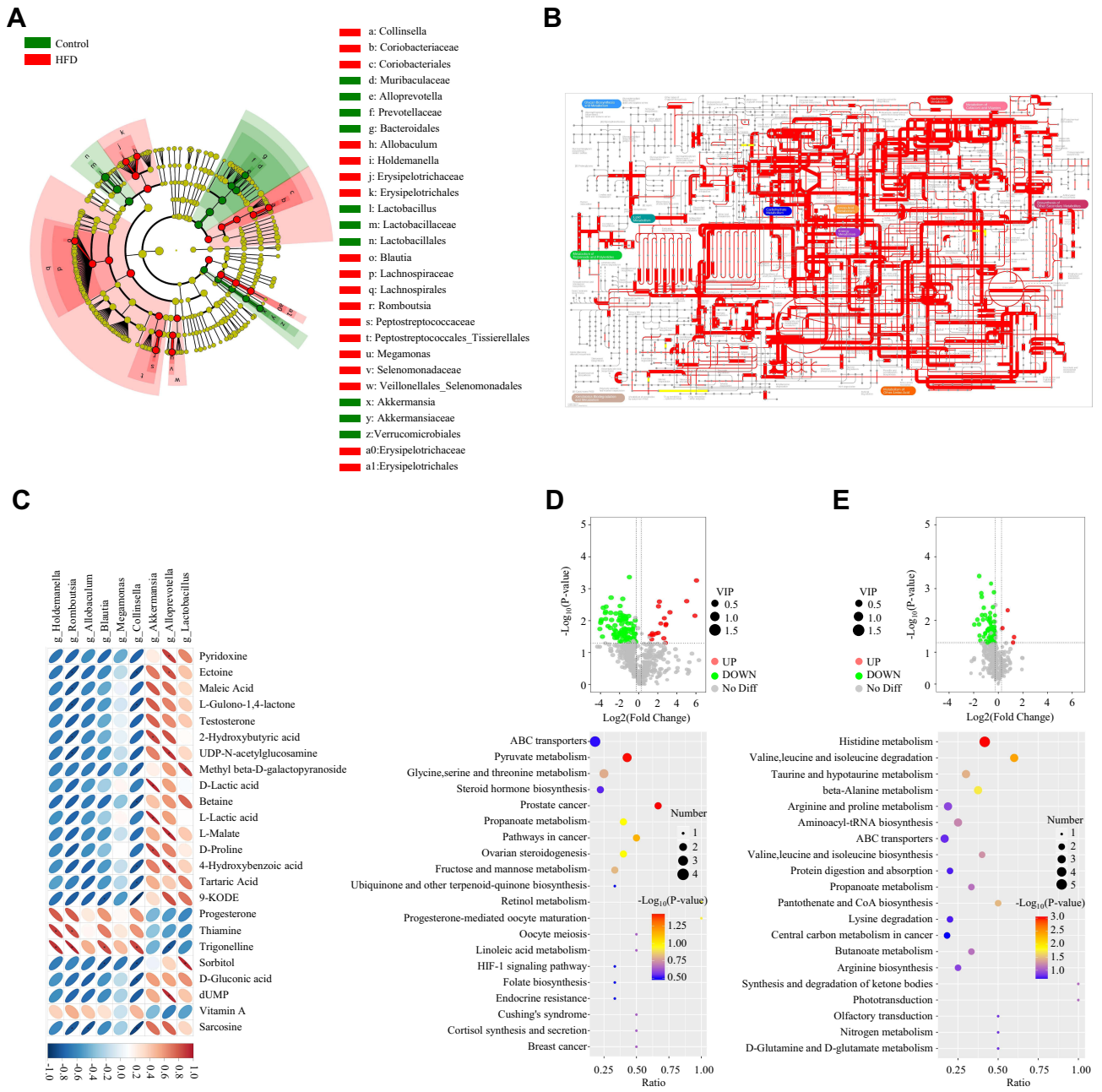

Figure 3 High fat diet induced intestinal microbiome dysbiosis and metabolic profiles disorders in Wistar rats. (**A**) LEfSe analysis of cladogram in Control group and HFD group. (**B**) Overview of the complete metabolism in Control group and HFD group. (**C**) The correlation graph shows the correlation between intestinal microbiome with fecal metabolites. (**D**) Above is the volcano map of fecal metabolites between the Control group and HFD group. Below is the KEGG bubble map of fecal metabonomics between the HFD group and the control group. The larger the Abscissa in the picture, the higher the enrichment of differential metabolites in the pathway. The color of the point represents the P-value value of the hypergeometric test, and the smaller the value is, the greater the reliability of the test is and the more statistically significant it is. The size of the point represents the number of differential metabolites in the corresponding pathway, and the larger the point, the more differential metabolites in the pathway. (**E**) Above is the volcano map of plasma metabolites between the Control group and HFD group. Below is the KEGG bubble map of plasma metabonomics between the HFD group and the control group.

Abbreviations: g, genus; HFD, high fat diet.

in "propanoate metabolism" in fecal metabolomics, as well as ectoine, sarcosine, and betaine, which are involved in "glycine, serine and threonine metabolism" (Table S1). Some plasma metabolites equally changed (Figure 3E); 4 plasma metabolites were higher in the HFD group, and 61 plasma metabolites were lesser in the same group (Table S2). Figure 3E shows a KEGG bubble chart. The bubble chart of plasma metabolomics was not enriched for "glycine, serine and threonine metabolism", but for "pantothenate and CoA biosynthesis" associated with this metabolism. HFD decreases 2-hydroxybutyric acid involved in

"propanoate metabolism" in plasma metabolomics, 3-methyl-2-oxobutanoic acid and L-valine involved in "pantothenate and CoA biosynthesis" (Table S2). The synthesis of L-valine involved in "valine, leucine and isoleucine biosynthesis" were also enriched.

Atorvastatin Calcium Trihydrate Can Improve Intestinal Microbiome and Metabolic Disorders Caused by HFD

Through 16S rRNA sequencing and metabolomic analysis, we compared the potential biomarkers of intestinal microbiome and differential metabolites in feces and plasma from the HFD + A and HFD groups to determine the mechanism of action of atorvastatin calcium trihydrate. Figure 4A shows the cladogram for the LEfSe analysis, with the LDA threshold set at 3. Among them, *Bacteroides* were potential biomarkers in the HFD + A group. Compared with the model

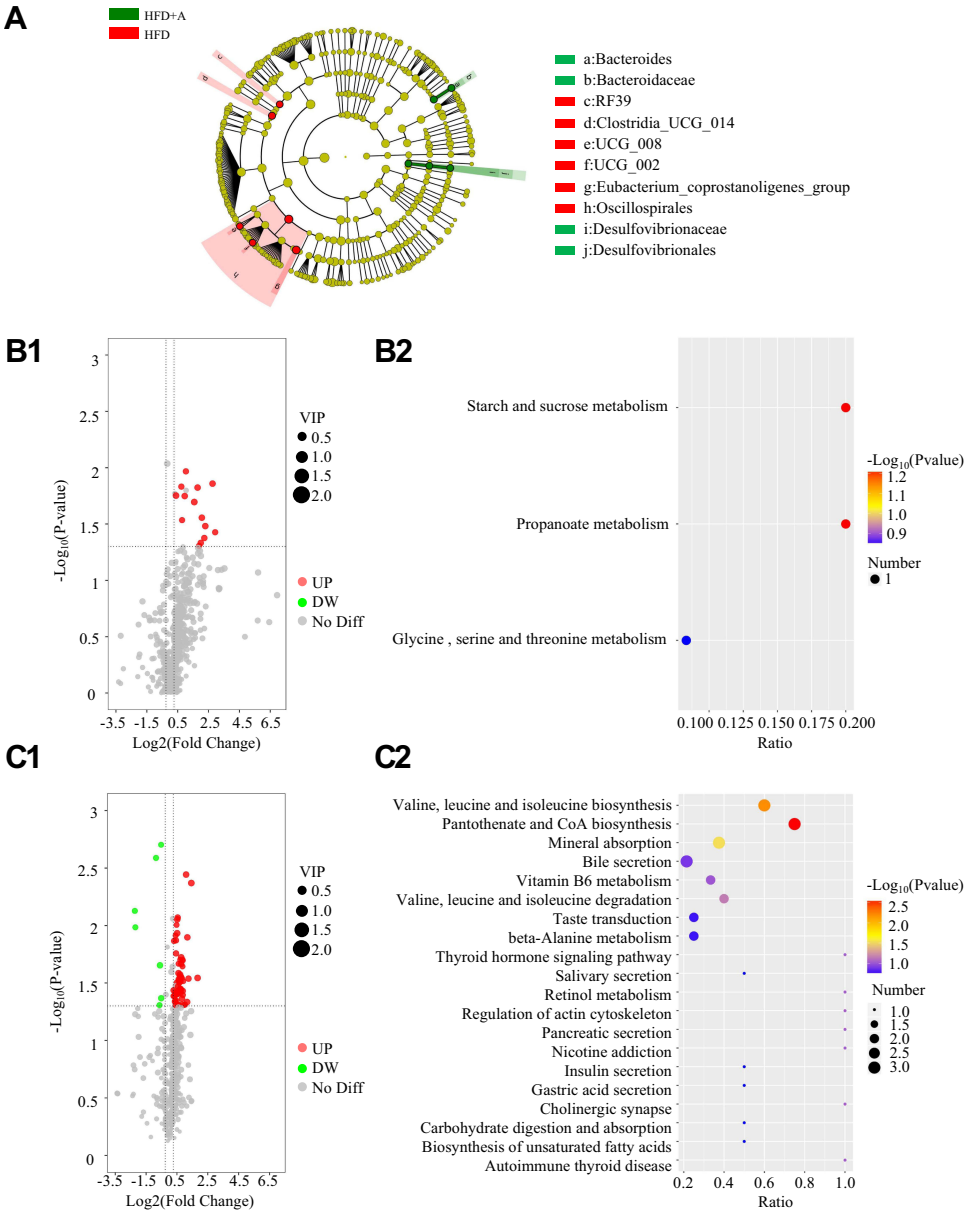

Figure 4 Atorvastatin calcium trihydrate improved intestinal microbiome and metabolic profiles disorders in rats' feces and plasma induced by high fat diet. (**A**) LEfSe analysis of cladogram in HFD group and HFD+A group with LDA Score larger than 3. (**B1**) The volcano map of fecal metabolites between the HFD+A group and HFD group. (**B2**) KEGG bubble map of fecal metabolomics between the HFD+A group and HFD group. (**C1**) The volcano map of plasma metabolites between the HFD+A group and HFD group. (**C2**) KEGG bubble map of plasma Metabolomics.

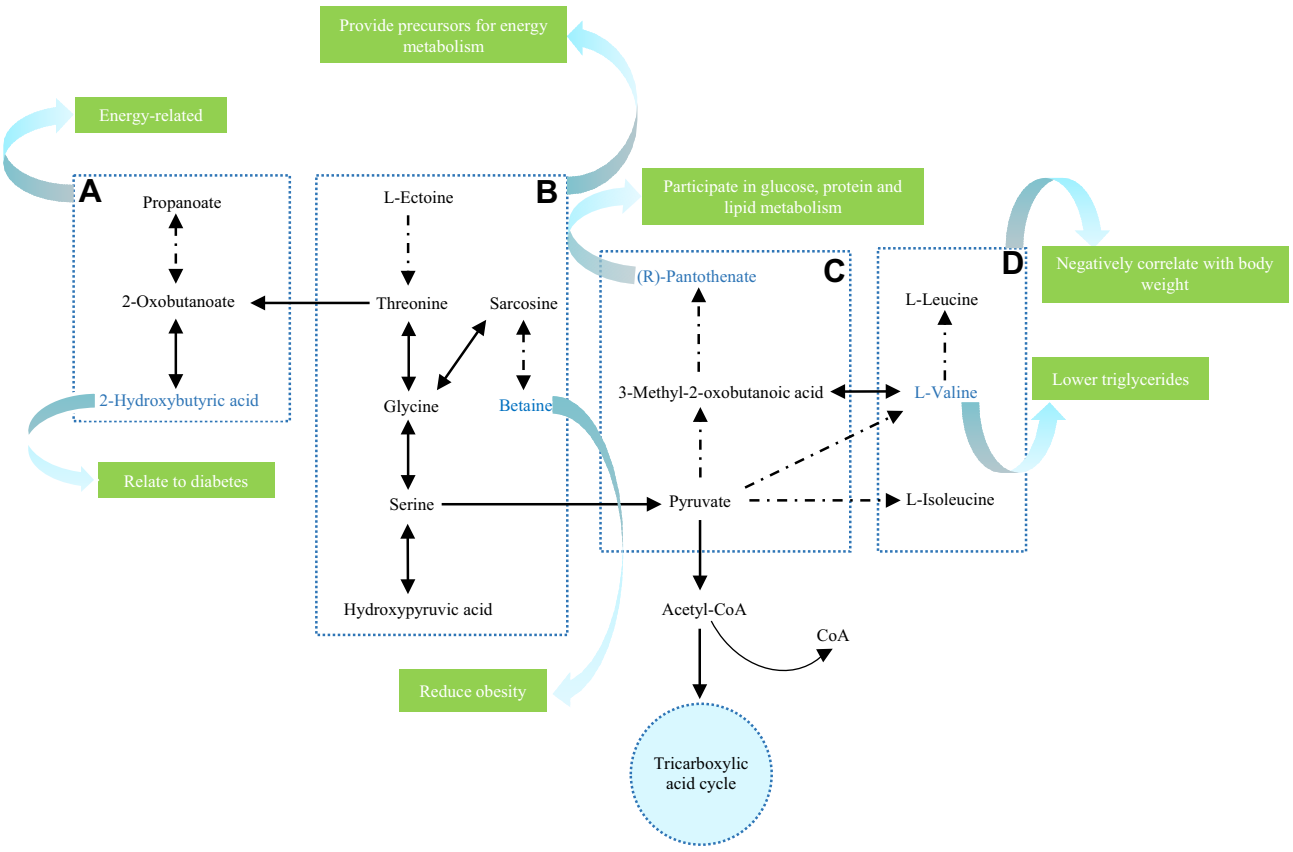

Figure 5 Metabolic pathway. (**A**) Propanoate metabolism; (**B**) Glycine, serine and threonine metabolism; (**C**) Pantothenate and CoA biosynthesis; (**D**) Valine, Leucine and Isoleucine biosynthesis.

group, some fecal metabolites differed in the HFD+A group (Figure 4B1); 14 fecal metabolites in the HFD+A group were higher than those in the HFD group (Table S3), and Figure 4B2 shows a KEGG bubble chart related to metabolic pathways. 2-Hydroxybutyric acid is an increased differential metabolite related to "propanoate metabolism", and ectoine is involved in "glycine, serine and threonine metabolism" (Table S3). Some plasma metabolites varied in the drug group (Figure 4C1); 61 plasma metabolites in the HFD+A group were higher than those in the HFD group, and 7 were lower than those in the HFD group (Table S4). The KEGG bubble chart shows the top 20 metabolic pathways in which different metabolites were involved (Figure 4C2). The increased 2-Hydroxybutyric acid in the plasma is involved in "propanoate metabolism", and L-threonine and hydroxypyruvic acid are involved in "glycine, serine, and threonine metabolism" (Table S4). In addition, atorvastatin calcium trihydrate also affects the "pantothenate and CoA biosynthesis", and "valine, leucine and isoleucine biosynthesis" in the plasma, as well as make L-valine involved in the two metabolic pathways cited above (Table S4). The metabolic pathways involved are shown in Figure 5.

Discussion

Consistent with previous findings, HFD raised blood lipids[9] and caused liver steatosis[33] in an animal model. Atorvastatin calcium trihydrate had a significant effect on lowering blood lipid levels and can reduce liver fat deformation. We investigated changes in the intestinal microbiome using 16s rRNA sequencing. We observed that an HFD has the potential to alter the structure and relative abundance of intestinal microbiome. The intestinal microbiome has a tendency to return to normal after treatment with atorvastatin calcium trihydrate. This reveals that HFD and the mechanism of action of atorvastatin are associated with intestinal microbiome.

Our findings also demonstrate a substantial relationship between intestinal microbiome and fecal metabolomics. We screened three intestinal microbiome that had the greatest impact on fecal metabolism under an HFD at the genus level,

namely *Alloprevotella, Collinsella*, and *Romboutsia. Collinsella* levels rose, as well as cholesterol and LDL levels.[34,35] *Collinsella* can oxidize bile acids,[35] thereby increasing cholesterol levels.[36] *Alloprevotella* plays a therapeutic role in inflammatory diseases[37] by producing short-chain fatty acids (SCFA).[38] *Romboutsia* is associated with weight loss.[39] Therefore, *Romboutsia* is a potential biomarker for forecasting obesity. Alterations in *Alloprevotella Collinsella*, and *Romboutsia* may be gut triggers of fecal metabolic disturbances caused by an HFD.

In our study, HFD affected "propanoate metabolism" and "glycine, serine and threonine metabolism" in the fecal metabolome; "propanoate metabolism", "valine, leucine and isoleucine biosynthesis", and "pantothenate and CoA biosynthesis" in the plasma metabolome. HFD reduces 2-hydroxybutyric acid involved in "propanoate metabolism" in feces and plasma,[21] and 2-hydroxybutyric acid is related to diabetes.[40] Moreover, a metabolic disorder of 2-hydroxybutyric acid reflects that of propanoate,[41] and "propanoate metabolism" is essential in the tricarboxylic acid cycle. Propanoate metabolic disorders can affect energy production.[23] Moreover, our findings indicate that an HFD decreases 2-hydroxybutyric acid, thereby influencing propanoate metabolism, lipid metabolism and the citric acid cycle. Glycine, which is involved in the "glycine, serine and threonine metabolism", is effective in reducing obesity and significantly affects lipid metabolism.[24] Studies have shown that glycine and serine possible analogues for predicting and treating obesity.[42] In addition, the glycine, serine and threonine metabolic pathways provide important energy metabolic precursors for entering the citric acid cycle. Our findings indicate that ectoine, sarcosine, and betaine are involved in "glycine, serine, and threonine metabolism". Betaine is a methyl donor that is involved in single-carbon metabolism and plays a vital role in methylation. Betaine promotes obesity and insulin resistance thereby causing metabolic disorders.[43] Thus, HFD affects "glycine, serine and threonine metabolism", and consequently, lipid and energy metabolism. "Pantothenate and CoA biosynthesis" is related to "propanoate metabolism". "Glycine, serine and threonine metabolism" through pyruvate can enter the tricarboxylic acid cycle through acetyl-CoA, which can be further degraded into coenzyme A. Our results illustrate that HFD affects "pantothenate and CoA biosynthesis". Valine reduces the accumulation of lipids and alter lipid metabolism.[44] HFD results in a decrease in of L-valine concentrations, which is involved in "pantothenate and CoA biosynthesis". HFD affects "propanoate metabolism" in feces and plasma, which indicates that HFD may affect fecal metabolism through intestinal disturbance, and may also affect plasma metabolism, then increase blood lipid levels.

In summary, HFD alters the composition of the intestinal microbiome, such as *Alloprevotella, Collinsella*, and *Romboutsia* resulting in abnormal "propanoate metabolism" and "glycine, serine and threonine metabolism" in feces. In our study, there was a strong correlation between fecal and plasma metabolite levels. HFD alter the "propanoate metabolism", "pantothenate and CoA biosynthesis", and "valine, leucine and isoleucine biosynthesis" in plasma. This in turn plays a vital role in raising blood lipids and causing liver steatosis in rats.

When the LDA threshold was set to 3, *Bacteroides* were identified as potential biomarkers of the HFD+A group. *Bacteroides* are potential targets for the improvement of atorvastatin-induced fecal metabolism. *Bacteroides* belongs to the family *Bacteroidaceae. Bacteroidaceae* have anti-inflammatory effects and maintain intestinal barrier function.[45] *Bacteroides* maintains the intestinal microecological balance,[46,47] provide energy to the host, and prevent obesity.[48] In addition, *Bacteroides* have bile salt hydrolase activity, and can improve cholesterol metabolism,[16,17] thereby playing an important role in hyperlipidemia.[19] *Bacteroides* can produce SCFA, and SCFA may play a key role in the improvement of hyperlipidemia metabolism.[49]

In this study, we discovered that atorvastatin calcium trihydrate can improve "propanoate metabolism" as well as "glycine, serine and threonine metabolism" in the feces and plasma, and can also improve "pantothenate and CoA biosynthesis" and "valine, leucine and isoleucine biosynthesis" in plasma. Atorvastatin calcium trihydrate therapy increases 2-hydroxybutyric acid, a metabolite of propanoate, in plasma and feces. This demonstrates that the drug improves "propanoate metabolism", thereby promoting fat oxidation and energy metabolism, reducing insulin resistance, lowering blood lipids, and reducing obesity. Atorvastatin calcium trihydrate therapy significantly increased ectoine (fecal metabolite), L-threonine (plasma metabolite), and hydroxypyruvic acid, correlating with "glycine, serine and threonine metabolism". This means that the drug improves "glycine, serine and threonine metabolism" induced by an HFD, thereby reducing liver fat and plasma cholesterol. In addition, atorvastatin calcium trihydrate significantly increases L-valine, which is involved in "pantothenate and CoA biosynthesis" and "valine, leucine and isoleucine biosynthesis". Pantothenate and CoA production are critical for fatty acid metabolism and the citric acid cycle. Increased intake of branched-chain amino acids has been shown to promote weight loss[50] and considerably improves hepatic steatosis.[51]

Moreover, valine decreases triglyceride levels and alter lipid metabolism.[44] L-valine levels significantly increased following atorvastatin calcium trihydrate therapy, indicating that the drug enhanced lipid metabolism.

In summary, atorvastatin calcium trihydrate improves the composition of the intestinal microbiome, such as *Bacteroides*, thereby affecting "propanoate metabolism" and "glycine, serine and threonine metabolism" in feces. On this basis, it affects "propanoate metabolism", "glycine, serine and threonine metabolism", "pantothenate and CoA biosynthesis", and "valine, leucine and isoleucine biosynthesis" in plasma. Thus, it lowers blood lipid levels and liver steatosis in rats.

Our study also has limitations, we cannot yet rule out the potential effects of unidentified confounders, nor can we infer causality from the observed associations. Although we speculate that atorvastatin improves the lipid metabolism disorder induced by HFD in rats by inhibiting the reduction of Bacteroides, the in-depth mechanism by requires further studies for confirmation.

Conclusion

Our study further demonstrated the lipid-lowering mechanism of atorvastatin from a multi-omics perspective. HFD and atorvastatin work through the intestinal microbiome and metabolic profiles; thus, altering the relative abundance of specific intestinal microbiome and associated metabolites through gut microbial manipulations could be considered as effective strategies for improving dyslipidemia.

Abbreviations

A, Atorvastatin calcium trihydrate; ALT, Alanine transaminase; AST, Aspartate transaminase; CTAB, Cetyl trimethylammonium bromide; FC, Foldchange; HDL-C, High-density lipoprotein cholesterol; HE, Hematoxylin-eosin; HFD, High-fat diet; LDL-C, Low-density lipoprotein cholesterol; LEfSe, Linear discriminant analysis effect size; MHC II, Major histocompatibility complex II; PCA, Principal component analysis; SCFA, Short-chain fatty acids; TC, Total cholesterol; TG, Triglyceride; VIP, Variable importance in the projection.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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