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Fenofibrate alleviates the composition and metabolic pathways of gut microbiota in high-fat diet treated hamsters

Qifeng Liu[1,2*]

Abstract

Background Fenofibrate is a compound with diverse biological properties that can be utilized to lower blood lipids. Understanding the impact of the gut microbiota in hyperlipidemia is vital for controlling systemic inflammation and improving serum lipid control. Nevertheless, the specific effects of fenofibrate on the phenotype and gene expression of resident gut bacteria, as well as its influence on the transformation of microbial metabolism into functional networks, remain unclear. In this study, our aimed to examine the gene and metabolic pathways of the gut microbiota in a hamster fed a high-fat diet (HFD) and administered fenofibrate.

Results In this study, we conducted metagenomic analyses on samples from HFD hamsters treated with fenofibrate. The results indicated that fenofibrate treatments significantly reduce the serum lipid levels in hyperlipidemia hamsters. And the group treated with fenofibrate exhibited higher levels of beneficial bacterial species associated with health, including *Bacteroides ovatus, Bifidobacterium animalis, Bacteroides intestinalis, Allobaculum stercoricanis, Lactobacillus reuteri, and Bacteroides acidifaciens*, in comparison to the HFD group. Additionally, analysis of metabolic pathways demonstrated that dietary fenofibrate significantly enhanced the biosynthesis of unsaturated fatty acids, glycerophospholipid metabolism, and pyrimidine metabolism, while reducing glyoxylate and dicarboxylate metabolism, tyrosine metabolism, tryptophan metabolism, and nonribosomal peptide structures. Furthermore, these metabolic pathway changes were associated with relative alterations in the abundance of genes from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, namely K01667, K11358, K13953, K04072, K06131, K00655, K04567, K02864, K06409, K05366, K01867, K21071, and K13292. Moreover, significant changes were observed in related to carbohydrate and antibiotic resistance, such as glycosyltransferase family 51 (GT51) as well as adeC, carA, and MexT.

Conclusions Dietary fenofibrate exerted significant effects on intestinal flora and genes related to lipid, energy, and amino acid metabolism, ultimately promoting a healthier colonic environment for the host. And these findings contribute to a better understanding of the mechanism of action of fenofibrate and provide a valuable foundation for future experimental and clinical studies, aiming to explore its practical applications.

Keywords Intestinal flora, Hyperlipidemic hamsters, Fenofibrate, Metagenomics

*Correspondence:
Qifeng Liu
liuqifeng@wchscu.cn
[1]Core Facility, West China Hospital, Sichuan University, Chengdu 610041, China
[2]State Key Laboratory for Bioactive Substances and Functions of Natural Medicines and Beijing Key Laboratory of New Drug Mechanisms and Pharmacological Evaluation Study, Institute of Materia Medica, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100050, China

Background

Globally, cardiovascular disease (CVD) remains a leading cause of death (Virani et al. 2020). According to statistics from the World Economic Forum, CVD is responsible for over 50% of noncommunicable disease deaths, and it is projected to cause over 22.2 million deaths by 2030. Furthermore, the American Heart Association estimates that by 2035, approximately 1.1 trillion dollars will be spent on managing CVD (Roth et al. 2020). Statins are commonly used as the first-line treatment for CVD, primarily for reducing LDL-C levels, and have been proven to reduce the incidence and mortality rates of CVD (Baigent et al. 2005). While reducing LDL-C levels is the primary treatment goal, other lipid parameters, such as elevated triglyceride (TG) levels, also contribute to the risk of coronary heart disease (Tall et al. 2022; Zhang et al. 2022). The coexistence of these lipid risk factors is referred to as dyslipidemia, which is a component of metabolic syndrome. Medications that target TG reduction have potential applications in addressing TG accumulation. Fenofibrate, a peroxisome proliferator-activated receptor α agonist, has shown great efficacy in reducing TG levels (McKeage & Keating 2011), particularly in human studies (Rosen et al. 1999).

In recent years, significant progress has been made in the study of drug-drug gene interactions, particularly in the realm of drug metabolism. Enhancing our understanding of the genetic mechanisms underlying drug therapy can help accurately pinpoint the therapeutic effects and adverse reactions of drugs. The structure and function of the gut microbiota have been recognized as important factors influencing drug efficacy (Lynch & Pedersen 2016). Diet plays a role in shaping the composition of the gut microbiota (Proctor et al. 2017), and maintaining a balanced gut microbiota is crucial for host health. Disruption of gut homeostasis can lead to metabolic disorders and contribute to the development of various diseases, including inflammatory bowel disease, CVD, and metabolic syndrome (Hanage 2014). The modulation of gut microbiota through probiotics, prebiotics, and medications has been shown to improve human health (Clements & Carding 2018), with examples like metformin (Sun et al. 2018) and berberine (Zhang et al. 2021). Fenofibrate, as a secondary preventive cardiovascular medication, has garnered increased attention regarding its functional characteristics and mechanisms of action in relation to the interaction with gut microbiota and its metabolites. Research has indicated that fenofibrate can regulate blood lipid profiles and markers of intestinal barrier function, thereby enhancing intestinal barrier function in in vivo and in vitro settings (Crakes et al. 2021). Fenofibrate has also been found to attenuate systemic and retinal inflammation induced by a HFD, along with the restoration of intestinal barrier integrity and modulation of gut microbiota/metabolites (Wang et al. 2022c). Understanding the impact of the gut microbiota in hyperlipidemia is vital for controlling systemic inflammation and improving serum lipid control. Nevertheless, the specific effects of fenofibrate on the phenotype and gene expression of resident gut bacteria, as well as its influence on the transformation of microbial metabolism into functional networks, remain unclear.

Metagenomic sequencing is a powerful technique that involves sequencing and analyzing the complete genetic material of a microbial specimen, in contrast to the targeted sequencing of specific regions like the 16 S rRNA gene. By utilizing metagenomics, researchers can gain more comprehensive insights into microbial taxonomy and gene content. This sequencing approach not only allows for the identification of bacteria at the species or even strain level but also provides information about the functional capabilities of the microbiome (Zou et al. 2020). In this study, metagenomic sequencing was employed to investigate the effects of fenofibrate on the metabolic pathways, composition, function, and genes recovery of the gut microbiota. Through this analysis, this study aimed to shed light on the mechanistic actions of fenofibrate on the gut microbiota and their implications for host health.

Materials and methods

Animal experiments

The Ethics Committee approved this study by the Chinese Academy of Medical Sciences and the Peking Union Medical College Ethics Committee. Acclimatization was carried out for 7 days with Syrian golden hamsters (5–6 weeks, males, 80–110 g) purchased from Vital River Laboratory Animal Technology (Beijing, China). Afterward, 18 hamsters were randomly separated into the control (C), HFD, fenofibrate-treated (F) ($n=6$ per group). A standard diet was fed to the control group, whereas the hyperlipidemic model was fed HFD for 8 weeks. And compositions and corresponding nutrient concentrations of diets used in the current study was shown in Supplementary Table 1. After 8 weeks, fenofibrate (50 mg/kg/day) were dissolved in CMC-Na solution and given once daily by oral gavage for 9 weeks, and C and HFD group hamsters were gavage the same solution. At 17 weeks, fresh feces were collected through anus, snap-frozen in liquid nitrogen, and then stored at -80 ℃ for further study.

Serum biochemistry analysis

At the age of 8, 14, and 17 weeks, blood samples were collected from hamsters. The serum levels of lipids and glucose were tested using the previous method (Li et al. 2018), including glucose, total cholesterol (TC),

triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C).

Metagenomic analysis of fecal microbiota

Genomic DNA was extracted from fresh feces extruded from the anus (n_C=6, n_{HFD}=6, n_F=6) at 17 weeks using a DNA extraction kit (PowerSoil® DNA Isolation kit). Total genomic DNA was extracted from fecal samples using the PowerSoil® DNA Isolation kit ((Mo Bio Laboratories) according to manufacturer's instructions. The quality and quantity of the extracted DNA were examined using Qubit dsDNA HS Assay Kit on a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and electrophoresis on a 1% agarose gel, respectively. Paired-end libraries (insert size, ~350 bp) were prepared using a VAHTS Universal Plus DNA Library Prep Kit for Illumina (Vazyme Biotech). The library was sequenced on an Illumina NovaSeq 6000 platform (Biomarker Technologies Co., Ltd., Beijing, China) using the 150-bp paired-end sequencing mode. The two paired FASTQ files were base called from the Illumina raw sequence read data. The quality of the raw sequence reads was assessed using Trimmomatic v0.33 (Bolger et al. 2014) was used to trim sequencing adapters, reads with a quality score < 20 over a sliding window size of 50 bp, and reads with a sequence length < 100 bp. After trimming the adaptors and filtering low-quality reads, the clean sequence data were used for further bioinformatics analyses.

Metagenomics data were assembled using MEGAHIT (Li et al. 2015) (https://github.com/voutcn/megahit), which makes use of succinct de Bruijn graphs. Assembly summary statistics were determined using QUAST software version 2.3 (Gurevich et al. 2013). Contigs with the length being or over 300 bp were selected as the final assembling result, and then the contigs were used for further gene prediction and annotation. Open reading frames (ORFs) from each assembled contig were predicted using MetaGeneMark (Zhu et al. 2010) (http://exon.gatech.edu/meta_gmhmmp.cgi, Version 3.26, default parameters). All predicted genes with a 95% sequence identity (90% coverage) were clustered using MMseqs2 software (Steinegger & Soding 2017) (https://github.com/soedinglab/mmseqs2, Version 12-113e3).

Representative sequences of non-redundant gene catalog were aligned to NCBI NR database with e-value cutoff of 1e-5 using Diamond software for taxonomic annotations. KEGG annotation was conducted using Diamond (version 0.9.29) against the Kyoto Encyclopedia of Genes and Genomes database(http://www.genome.jp/keeg/) with an e-value cutoff of 1e-5. If there are multiple alignment results (HIt), the best alignment result is selected as the annotation of the sequence. Antibiotic resistance annotation was conducted using rgi software (version 4.2.2) against the CARD database (https://card.mcmaster.ca/) with default parameters.

Data analysis

The lowest common ancestor (LCA) algorithm (applied using the MEGAN software system) was used to ensure the annotation significance by picking out the classified LCA for final display. Genes were predicted using MetaGeneMark, whereas the protein Basic Local Alignment Search Tool (BLASTP) was used to search the protein sequences of the predicted genes in the non-redundant (NR), Carbohydrate Active enzymes (CAZy), evolutionary genealogy of genes: Non-supervised Orthologous Groups (eggNOG), and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases with an e-value < 1e-5. To determine the similarity or difference of taxonomic and functional components between different samples, relative clustering analysis and principal component analysis (PCA), LEfSe were performed. Bioinformatic analysis was performed using the OECloud tools at https://cloud.oebiotech.cn.

Statistical analysis

The results of experiments conducted with two groups were analyzed using an unpaired Student's t-test. Data are presented as the means ± standard error of the mean (SEM) and results with a P-value < 0.05 were considered statistically significant.

Results

Measurement of serum lipids and glucose

The animal experiment procedure is illustrated in Fig. 1a. The serum levels of lipids and glucose at 8, 14, and 17 weeks of different treatment are displayed in Fig. 1b. Glucose, LDL-C, TC and TG in the HFD group are higher than those in the C group at 8, 14, and 17 weeks, which indicates that the hyperlipidemia hamster model was successfully established. Moreover, fenofibrate-treated could significantly reduce the serum level of lipid in hyperlipidemia hamsters.

Fenofibrate alters the gut microbiota composition

Study obtained an average of 36 million paired-end reads for each sample (from 30 to 52 million; Supplementary Table 2). MetaGeneMark software was used to distinguish coding regions in the genome. Cd-hit software was used to edit the nonredundant genome, the similarity threshold was set to 95%, and the coverage threshold was set to 90%. Fenofibrate treatment altered the gut microbiota composition of high-fat diet-treated hamsters, from phylum to species. The three groups were clearly separated in the PCA (Fig. 2a). At the genus level, *Clostridium, Oscillibacter, Bacteroidetes, Ruminococcus* were the predominant microbial divisions (Fig. 2b). At

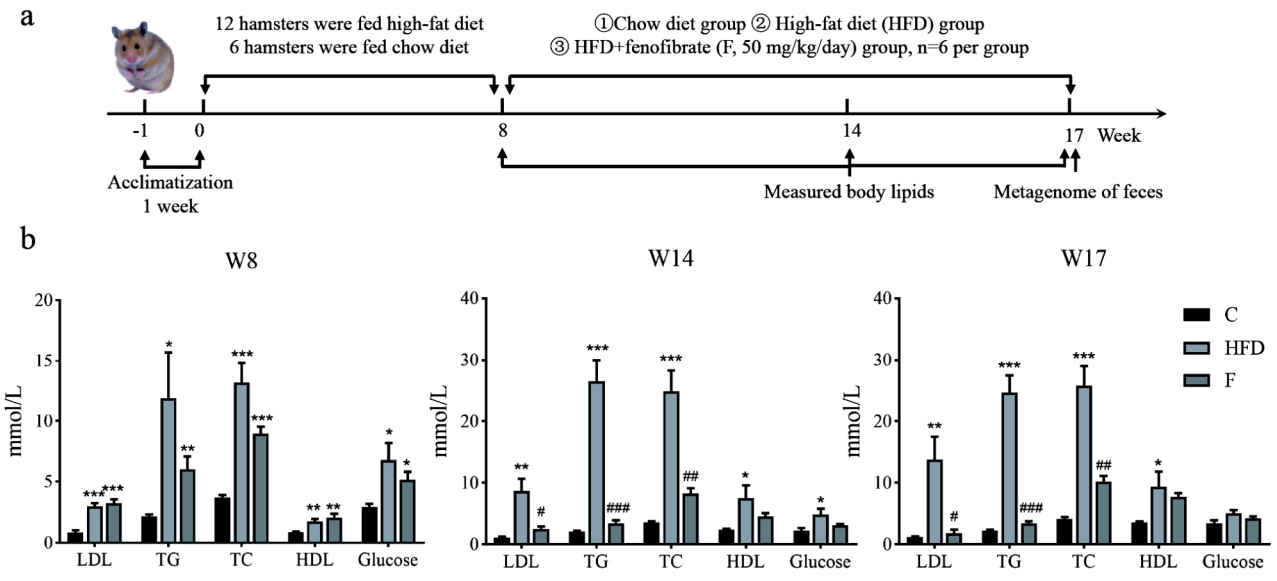

Fig. 1 (**a**) Time flow chart of animal treatments. (**b**) Boxplots for effects of fenofibrate and high-fat diet on serum TC, TG, LDL-C, HDL-C and glucose levels in hamsters at week 8, 14, 17. The data are presented as the mean ± SEM. *Notes*$^{\#}P<0.05$, $^{\#\#}P<0.01$, and $^{\#\#\#}P<0.001$ vs. the HFD group; $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs. C group. C: control group, HFD: high-fat diet group, F: fenofibrate treated group. $n=6$

the genus level, Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria were the predominant microbial divisions (Fig. 2c). Compared with C, HFD decreased the relative abundance of *Prevotella*. Compared with HFD, F increased the relative abundance of *Bacteroides, Parabacteroides, Alistipes* and *Mycoplasma* genera. The difference in the abundance of taxa within groups was also identified using the linear discriminant analysis (LDA) effect size (LEfSe) method using non-parametric factorial Kruskal-Wallis and Wilcoxon rank-sum tests. The histogram of the distribution of LDA values (>2) shows the typical microbiota of the groups (Supplementary Fig. 1a-b). The F group showed selective genus enrichment of *Tenericutes, Thermodesulfobacteria*, et al., and showed decreased genus of *Planctomycetes* and *Basidomycota* (Supplementary Fig. 1c).

The results showed that 14,450 bacterial species were annotated. Compared with the C group, the HFD group showed a significant increase ($P<0.05$) in the levels of 853 bacteria species including *Bacteroides acidifaciens*, *Faecalibaculum rodentium*, and *Listeria monocytogenes*, whereas levels of 380 bacterial species were decreased including *Bacteroides ovatus*, *Prevotella veroralis*, and *Prevotella saccharolytica* (Supplementary Table 3). However, compared with the HFD group, the F group showed a significantly ($P<0.05$) higher relative abundance of 695 bacterial species ($P<0.05$) including *Bacteroides ovatus*, *Bifidobacterium animalis* and *Bacteroides intestinalis* et al. Furthermore, there was a significant reduction in the abundance of 276 bacteria species ($P<0.05$), such as *Lactobacillus johnsonii*, *Lachnoclostridium* sp. An14 et al. (Fig. 2e and Supplementary Table 4). Random forest regression showed that *Firmicutes bacterium CAG:582, Flavobacteriales bacterium, Bifidobacterium animalis* et al. were the main biomarkers for HFD, and F (Fig. 2d). And the relative abundance of these species was showed as Fig. 2e. Furthermore, the study performed a correlation analysis between these species and serum biochemical indicators. The findings revealed a notable negative correlation between indicators such as TC, TG, and LDL, which increased with HFD, and beneficial bacteria. Conversely, HDL, which decreased with HFD, exhibited a positive correlation trend with beneficial bacteria (Fig. 2f, Supplementary Table 5).

Fenofibrate changed metabolic pathway of gut microbiota

PCA based on KEGG modules revealed differences in microbial functions within groups (Fig. 3a). The number of carbohydrate metabolism-related genes was highest at KEGG level 1. Based on the KEGG level 2 analysis, the HFD group demonstrated a higher level of carbohydrate metabolism and signal transduction, but lower metabolism of cofactors and vitamins than the C group. However, the F group showed higher levels of replication and repair than the HFD group (Supplementary Fig. 2). At KEGG level 3, The one-way ANOVA showed that 44 pathways were significantly different within groups (Fig. 3c). Compared with the C group, the HFD group showed significantly lower levels ($P<0.05$) in the activity of 24 metabolic pathways, including glycosphingolipid biosynthesis-ganglio series, biosynthesis of siderophore group nonribosomal peptides, atrazine degradation,

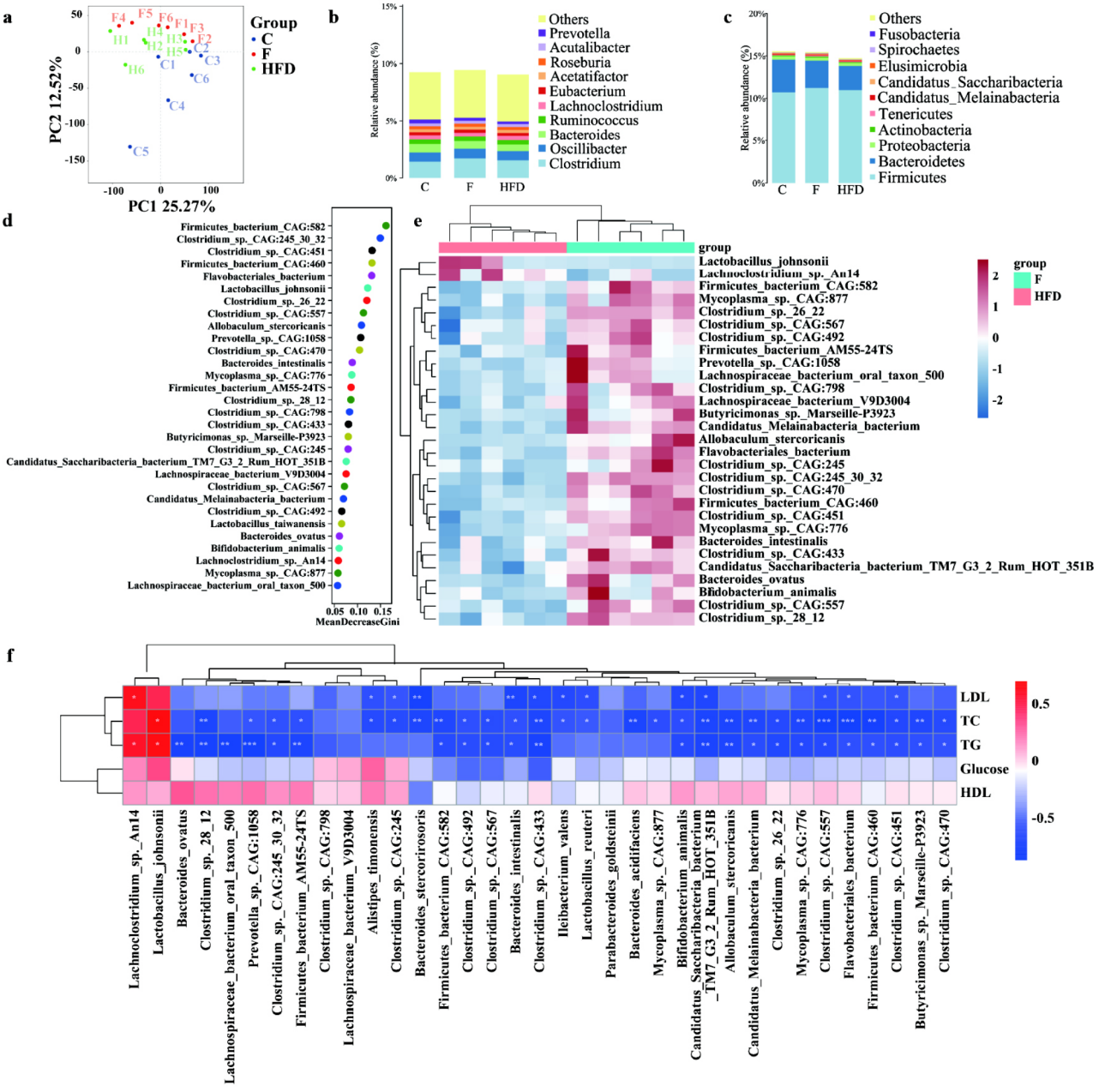

Fig. 2 (**a**) PCA of species abundance between the C, HFD and F groups. (**b**) Relative abundance of the gut microbiome at the genus level. Level taxonomy is presented as a percentage of total sequences. (**c**) Relative abundance of the gut microbiome at the phylum level. (**d**) Random forest analysis of the HFD and F groups at the species level. (**e**) Heatmap of the relative abundance of the species with a significant change after fenofibrate administration. (**f**) Spearman correlation network analysis of species and serum biochemistry, different colors indicate different correlation, red indicate positive correlation, blue indicate negative correlation. ***$p < 0.001$ **$p < 0.01$, *$p < 0.05$. $n = 6$

limonene and pinene degradation, naphthalene degradation. In contrast, 12 metabolic pathways showed higher activity in the HFD group than in the C group, including valine, leucine and isoleucine degradation, taurine and hypotaurine metabolism, beta-alanine metabolism, lipopolysaccharide biosynthesis, riboflavin metabolism. However, compared with the HFD group, the F group showed a significantly higher level ($P < 0.05$) of activity in five pathways, including biosynthesis of unsaturated fatty acids, base excision repair, glycerophospholipid metabolism, pyrimidine metabolism, mismatch repair, decreased 5 kinds pathways including glyoxylate and dicarboxylate metabolism, tyrosine metabolism, tryptophan metabolism, nonribosomal peptide structures, biosynthesis of siderophore group nonribosomal peptides (Fig. 3c). The LDA score analysis determined that the most effective

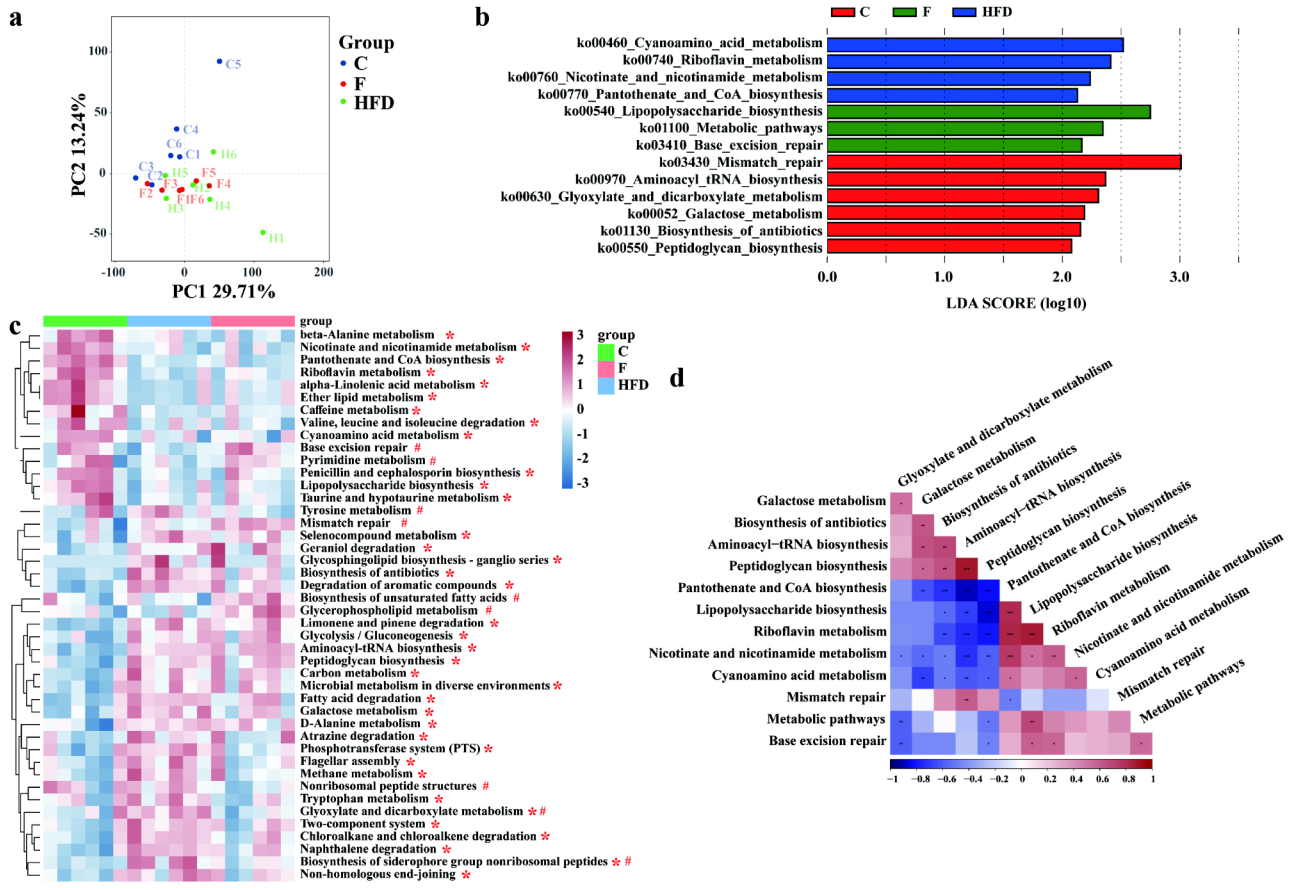

Fig. 3 (**a**) PCA of KEGG metabolic pathway between the C, F and HFD groups. (**b**) LDA analysis of KEGG metabolic pathway; (**c**) KEGG level 3 differences within groups, ∗Significantly different between C-HFD, #significantly different between F-HFD; (**d**) Spearman correlation network analysis of LDA score higher than 2 pathways, different colors indicate different correlation, red indicate positive correlation, blue indicate negative correlation. ***$p < 0.001$ **$p < 0.01$, *$p < 0.05$. $n = 6$

pathways for C, HFD, and F were peptidoglycan biosynthesis, lipopolysaccharide biosynthesis, and riboflavin metabolism and pantothenate and CoA biosynthesis, respectively (Fig. 3b). The LDA biomarker pathways correlation analysis showed that lipopolysaccharide biosynthesis was positively correlated to pantothenate and CoA biosynthesis but negatively related to peptidoglycan biosynthesis and biosynthesis of antibiotics (Fig. 3d, Supplementary Table 6).

Fenofibrate changed the abundance of gut microbiota genes

Based on the KEGG annotation analysis, the research identified 6,572 genes from 18 samples, 3 groups shared 6135 genes, and the one-way ANOVA showed that 1347 genes were significantly different within groups. The LDA showed that k06147 (ABCB-BAC, ATP-binding cassette), k07667 (kdpE, two-component system, KDP operon response regulator KdpE), k03088 (rpoE, RNA polymerase sigma-70 factor) were the predominant genes of the HFD, F, and C groups (Fig. 4a). Furthermore, the Welch's T-test of genes showed that compared with the C group, in the HFD group, the relative abundance of 24 genes was upregulated, whereas this was downregulated for 5 others (Fig. 4b). However, compared with the HFD group, the relative abundance of the following 6 genes in the F group k00655 (plsC), k06131 (clsA_B), k01667 (tnaA), k06409 (spoVB), k04567(KARS, lysS), k02864(RP-L10, MRPL10, rplJ) was upregulated. Furthermore, the abundance of 7 genes k04072 (adhE), k13953 (adhP), k11358 (yhdR), k05366 (mrcA), k21071 (pfk, pfp), k13292 (lgt, umpA), k01867 (WARS, trpS) was downregulated (Fig. 4b). Moreover, the results of the Spearman correlation analysis of 13 genes and significantly different gut microbial species determined 7 genes negatively and 6 genes positively related to *Bacteroides ovatus*, *Bifidobacterium animalis* and *Bacteroides intestinalis*, the bacteria identified as the most predominant species between the HFD and F group (Fig. 4c, Supplementary Table 7).

Further comparative analysis of the CAZy database also found that murein polymerase (GT51), sucrose synthase (GT4), β-xylosidase (GH43) were dominant enzymes

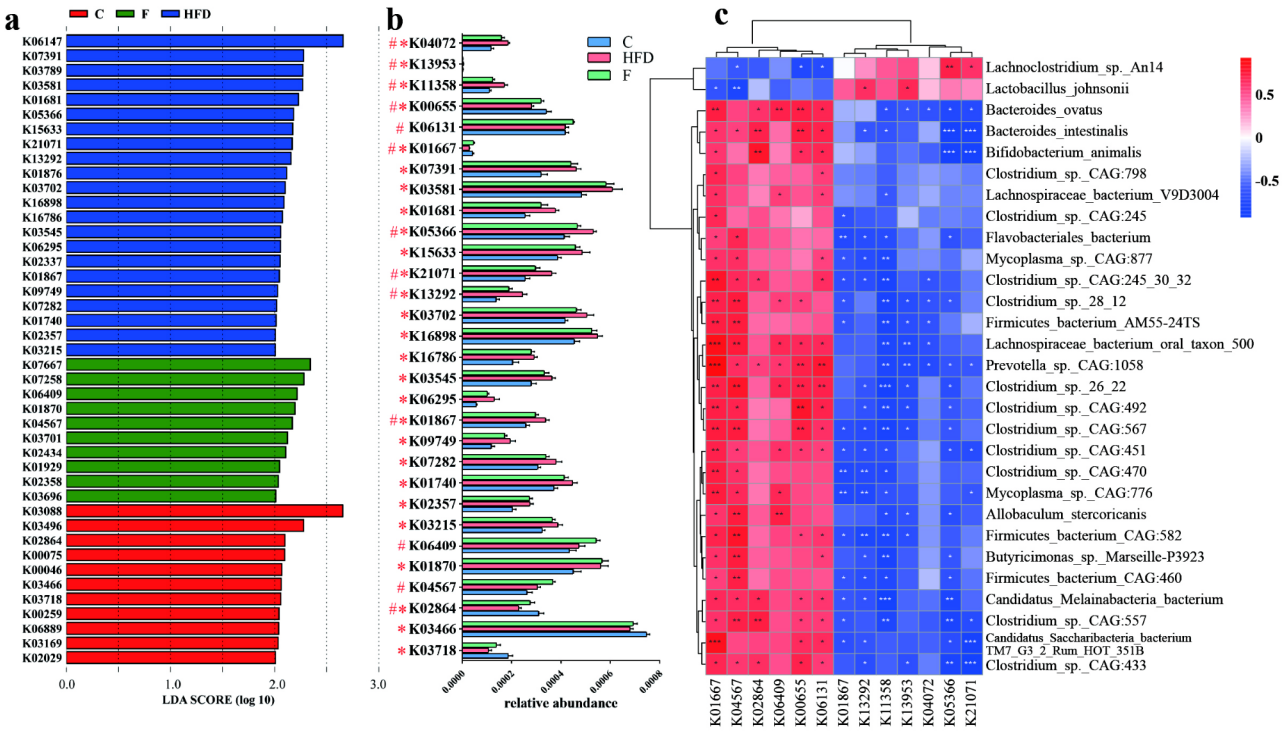

Fig. 4 Fenofibrate effects on genes of intestinal flora. (**a**) LDA analysis of genes which annotated on significantly different metabolic pathways; (**b**) Significantly different genes between C, HFD and F group; (**c**) Spearman correlation analysis of 7 genes and significantly different bacteria species between HFD and F group. ***$p < 0.001$ **$p < 0.01$, *$p < 0.05$. $n = 6$

in the HFD, F, and C groups the after LDA analysis, respectively (Fig. 5a). After the Welch's T-test, the HFD group showed higher PL21, GT51, CBM51, GT5 expression levels than those of the C group, whereas levels of the other 8 enzymes were lower. However, the F group showed relatively lower levels of the 6 enzymes, including GT51, GT5, GH96, GH77, GT8, GH140 than those of the HFD group (Fig. 5b). Furthermore, the distribution of eggNOG functions was also compared among the three groups, which were clearly separated based on the PCA (Fig. 5c). According to the LDA analysis, the dominant eggNOG pathway of C, F and HFD are transcription, replication recombination and repair, and defense mechanisms pathway (Fig. 5d)

The study also analyzed the distribution of antibiotic-resistant genes (ARGs) in the experimental groups. The ARG annotation in the CARD database results showed that 779 antibiotic resistant organisms (ARO) were annotated. The Venn diagram illustrates that the significant changed ARO between C and HFD, F and HFD groups had 71 and 48, respectively, and 23 common ARO (Fig. 6a). The LDA showed that ARO:3,002,368 (PER-6), ARO:3,002,651 (APH(3')-Vc), ARO:3,002,817 (carA) were the predominant genes of the HFD, F, and C groups (Fig. 6b). The HFD group demonstrated higher levels of APH (3')-VIIa, AAC (2')-Ia, chrB, fexA, vanTC, TriC, baeR, baeS, amrB, AAC (6')-Iih, tet (J), CMY-38, lsaE and Bacillus clausii chloramphenicol acetyltransferase, and lower levels of carA and adeC, compared with C group. However, the F group showed significantly higher levels of vgbA, MexT, vanD, clbA, carA and adeC than those in the HFD group (Fig. 6c). The spearmen correlation analysis demonstrated that the above mentioned 6 ARO were positively related with decreased bacteria in HFD group, and negatively related with increased bacteria species in HFD group (Fig. 6d, Supplementary Table 8).

Overall, the analysis of the microbiome-associated genes revealed that the HFD group displayed a dysfunction in several pathways of the KEGG, CAZy, and eggNOG databases. The metabolism of energy, carbohydrates, lipids, vitamins, and nucleotides was shown to be more disrupted in the HFD group than the C group. However, these metabolic pathways and their related genes were regulated in the F group, which established a healthy intestinal condition

Discussion

Fenofibrate reverse HFD-induced lipid disorders by regulating the structure of intestinal microbiota

In current study, metagenomic analysis revealed the beneficial impact of fenofibrate treatment on the structure and function of the gut microbiota in hamsters with an HFD. Specifically, there was a significant increase in the abundance of *Bacteroides*, a genus of bacteria widely

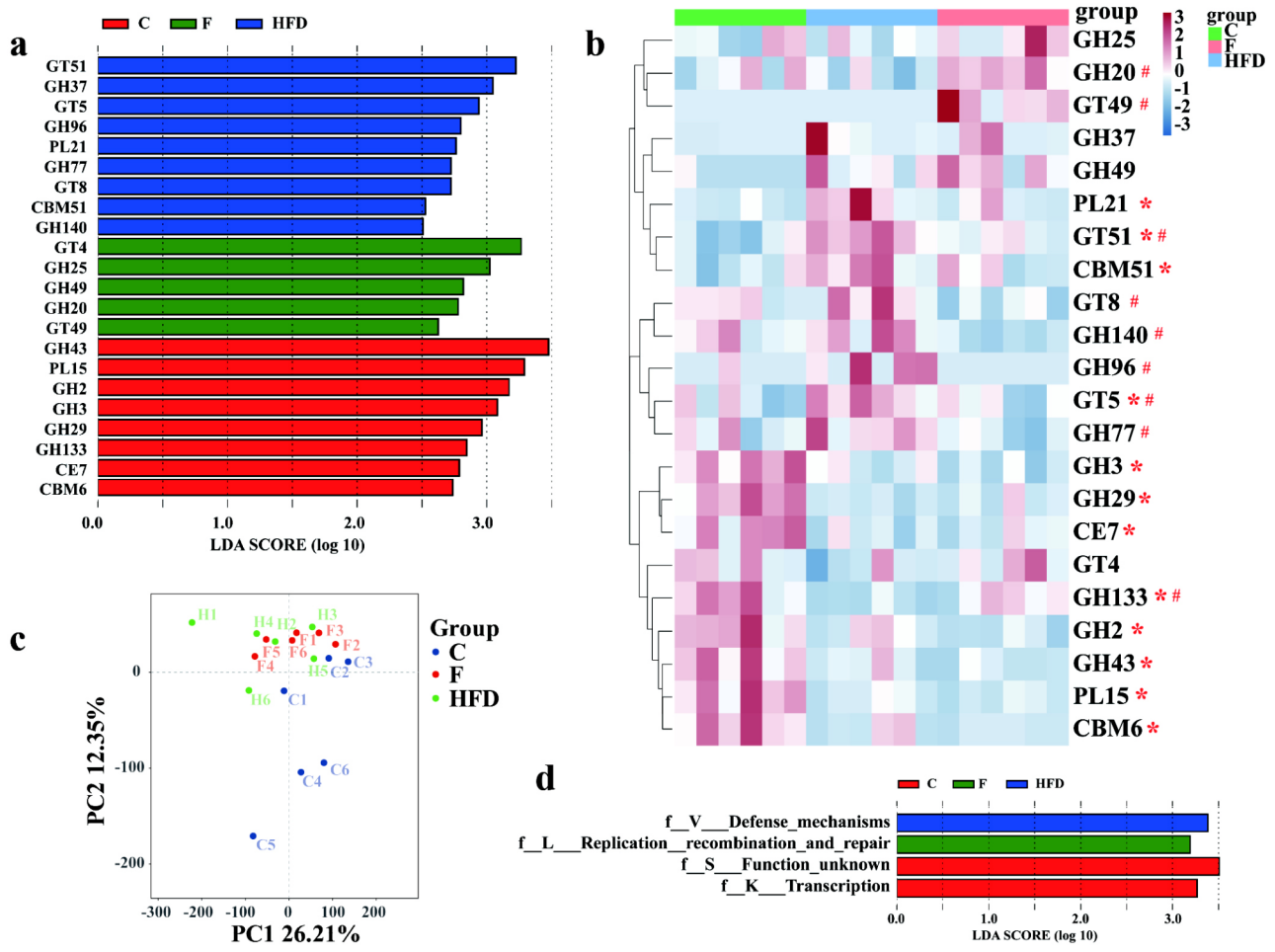

Fig. 5 Functional analysis based on CAZy orthologs and egg NOG Orthologous. (**a**) LDA analysis of significantly different enzymes; (**b**) Significantly different enzymes within groups; (**c**) PCA analysis of eggNOGs function was clearly separated within groups; (**d**) LDA analysis of significantly different eggNOG class

recognized as beneficial for the body. Additionally, I noticed an enrichment of specific species within the *Bacteroides* genus, including *Bacteroides ovatus, Bacteroides intestinalis*, and *Bifidobacterium animalis*, all of which have been known to exert health-promoting effects (Fig. 2e, Supplementary Tables 3– 4).

Previous research reports have shown that *Bacteroides, Parabacteroides* and *Bifidobacterium* genus produces short chain fatty acids and some beneficial metabolites that affect intestinal function, playing an important role in lipid homeostasis and reducing inflammation (Pan et al. 2023). The HFD group showed decreased of the species *Bacteroides ovatus* ($p<0.05$) and *Lactobacillus reuteri* ($p<0.05$), *Bacteroides intestinalis, Parabacteroides goldsteinii*, moreover, fenofibrate treatment reversed this trend. And the species *Bifidobacterium animalis* ($p<0.05$), and *Bacteroides acidifaciens* ($p<0.05$) were increased after fenofibrate treatment (Fig. 2e, Supplementary Tables 3– 4). *Bacteroides ovatus* can produce a beneficial metabolite called *N*-methylserotonin, which has been shown to promote weight loss, reduce fat accumulation, and enhance intestinal transit (Han et al. 2022). Moreover, *Bacteroides ovatus* has been found to alleviate inflammation induced by lipopolysaccharides in mice, exhibiting its potential in reducing intestinal inflammation and gastrointestinal disorders (Ihekweazu et al. 2021; Li et al. 2022; Hayase et al. 2023). *Bacteroides intestinalis* is known to degrade complex arabinoxylans and xylan from dietary fibers, and these degradation products, including butyrate, have been shown to have a protective role in the intestinal mucosa (Martens et al. 2011; Hong et al. 2014; Yasuma et al. 2021). *Bacteroides acidifaciens* is considered one of the prevalent species within the intestinal commensal bacterial population. Research has shown that *Bacteroides acidifaciens* can prevent obesity and improve insulin sensitivity (Wang et al. 2022a; Yang et al. 2017; Proctor et al. 2017). *Bifidobacterium animalis* is one of the beneficial strains that has been used in

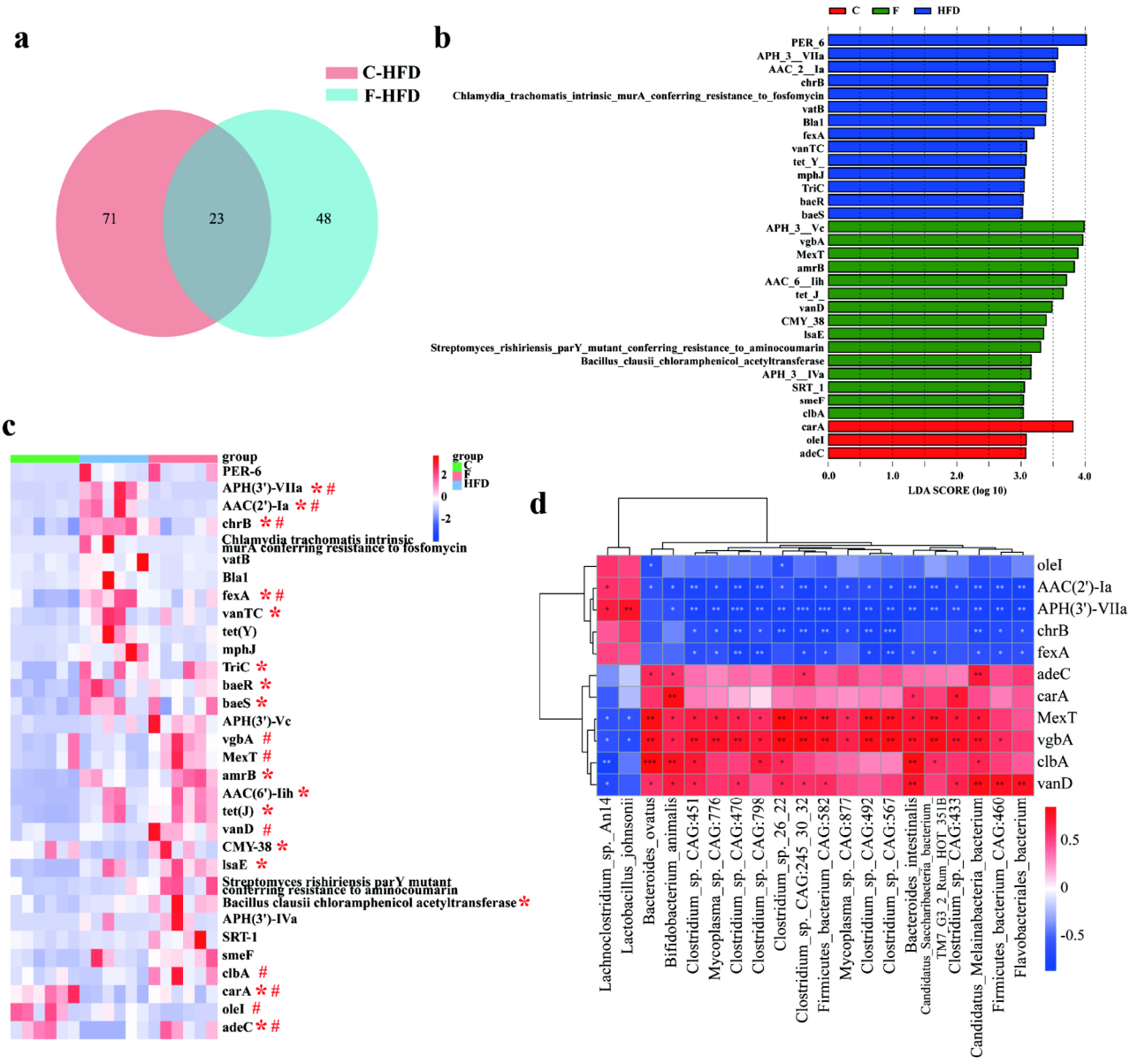

Fig. 6 Antibiotic resistance gene annotation on CARD database. (**a**) Venn diagram of ARO within three groups; (**b**) LDA analysis of significantly different ARO; (**c**) Heatmap of significantly different ARO within groups; (**d**) Spearman correlation analysis of 11 AROs and significantly different bacteria species between HFD and F group. ***$p < 0.001$ **$p < 0.01$, *$p < 0.05$. $n = 6$

clinical practice for many years (Chouraqui et al. 2004) and is widely employed in various health products (Tsai et al. 2022). It has been shown to effectively reduce cholesterol levels, possess anti-cancer properties, contribute to anti-aging effects, and exhibit a range of other benefits (Araujo et al. 2022). Moreover, it activates the TGR5 pathway in brown adipose tissue by increasing the production of secondary bile acids, thereby increasing energy expenditure in the body (Wang et al. 2022b). The mechanism of increased SCFAs, secondary bile acids, and other beneficial metabolites in HFD hamsters after fenofibrate supplementation needs further investigation. *Parabacteroides goldsteinii* can reduce intestinal inflammation and decreased obesity (Lai et al. 2022). This is often accompanied by increased heat production in adipose tissue, improved intestinal integrity, and reduced levels of inflammation and insulin resistance (Wu et al. 2019). Overall, the results show that fenofibrate significantly boosted the growth of bacteria linked to beneficial metabolites in HFD hamsters. This study implies that the rise in beneficial bacteria from fenofibrate treatment enhanced the production of beneficial metabolites, improving hyperlipidemia and reducing inflammation.

Furthermore, there are some beneficial bacteria that have shown notable increases in abundance following intervention with fenofibrate. *Lactobacillus reuteri*, a probiotic strain capable of colonizing various mammalian hosts, possesses antibacterial properties that can inhibit the colonization of pathogenic microorganisms and reshape the composition of symbiotic microbial communities. Additionally, *Lactobacillus reuteri* is beneficial for the host immune system by reducing the production of pro-inflammatory cytokines and promoting the development and functionality of regulatory T cells. Moreover, this probiotic has the ability to enhance intestinal barrier function, potentially reducing the translocation of microorganisms from the intestinal lumen to the tissues (Mu et al. 2018). *Allobaculum stercoricanis* is a Gram-positive bacterium belonging to the *Erysipelotrichidae* family. Studies have demonstrated that treatment with *Allobaculum stercoricanis* can provide protection against hepatic fat accumulation under metabolic stress, thus alleviating the progression of non-alcoholic fatty liver disease (Li et al. 2023). The HFD group showed decreased of the species *Lactobacillus reuteri* ($p<0.05$), and fenofibrate treatment reversed this trend. And the species *Allobaculum stercoricanis* ($p<0.01$) was increased after fenofibrate treatment (Fig. 2e, Supplementary Tables 3– 4). This indicates that fenofibrate effectively increased the abundance of beneficial bacteria in the intestines of hyperlipidemic hamsters, alleviating the intestinal environment disorder caused by an HFD. Moreover, through correlation analysis, it was observed that there is a negative correlation between beneficial bacteria *Bacteroides ovatus, Bacteroides intestinalis*, *Allobaculum stercoricanis*, and *Bifidobacterium animalis* and LDL, TC, TG, and a positive correlation with HDL (Fig. 2f, Supplementary Table 5), which indirectly verifies the effect of fenofibrate on improving the structure of gut microbiota.

Fenofibrate improve the functional characteristics of the intestinal microbiota in hyperlipidemic hamsters

Alistipes timonensis is a bacterium that was found to be significantly enriched after fenofibrate treatment. It was originally isolated from the fecal microbiota of healthy individuals and has the ability to metabolize tryptophan into indole (Parker et al. 2020). Interestingly, the enzyme K01667 (tnaA; tryptophanase), which is involved in the conversion of tryptophan to indole, was also enriched following fenofibrate administration. Indole and its derivatives are endogenous tryptophan metabolites produced by the gut microbiota, and they exhibit a range of biological activities. Research has shown that endogenous indole can help maintain the intestinal barrier, significantly improve intestinal health (including conditions like inflammatory bowel disease, hemorrhagic colitis, and colorectal cancer), and have a beneficial role in alleviating atherosclerosis (Tennoune et al. 2022; Lu et al. 2023). However, excessive production of indole may have adverse effects on emotions and behavior and could potentially lead to cardiovascular toxicity (Ye et al. 2022). Further research is necessary to determine whether fenofibrate treatment has beneficial or harmful effects on indole production and its subsequent impact on health.

In addition to taxonomic variations, the functional capacity of the gut microbiota also demonstrates significant differences in related metabolic pathways, genes, and correlations. In this study, pathway significance analysis revealed that the biosynthesis of unsaturated fatty acids was the dominant pathway in the F group. This suggests that fenofibrate treatment acted through this pathway to effectively reduce levels of cholesterol and triglycerides in the blood circulation. This reduction is beneficial for improving blood circulation, enhancing brain cell activity, and preventing the occurrence of cardiovascular and cerebrovascular diseases. Another enriched pathway was glycerophospholipid metabolism, with fenofibrate significantly increasing the abundance of genes such as K06131 (clsA/B; cardiolipin synthesis A/B) and K00655 (plsC). The clsA/B genes encode a synthase that converts phosphatidylglycerol into phosphatidylglycerol (PG). PG, a vital phospholipid for human nutrition, can lower cholesterol levels, protect the liver, strengthen the immune system, and combat fatty liver. On the other hand, plsC is an enzyme involved in the transformation of glycerol phospholipid into phosphatidic acid. Phosphatidic acid, an important physiological substance, exhibits various functions and effects in the human body. It primarily plays a role in liver protection, reducing blood lipid levels, mitigating the occurrence of atherosclerosis, strengthening the barrier function of the intestinal mucosa, inhibiting the growth of harmful bacteria, promoting the proliferation of beneficial bacteria, maintaining intestinal health, and facilitating the proliferation and differentiation of immune cells, thus enhancing immunity.

The abundance of genes such as K11358 (yhdR; aspartate aminotransferase), K13953 (adhP; alcohol dehydrogenase, propanol-preferring), and K04072 (adhE; acetaldehyde dehydrogenase/alcohol dehydrogenase) significantly decreased following fenofibrate treatment. These genes are involved in the degradation of tyrosine and norepinephrine, which are associated with tyrosine metabolism and have implications for increased dopamine, thyroxine, and energy metabolism. Research has demonstrated that low levels of tyrosine and norepinephrine can result in a slow metabolism, which is not favorable for liver metabolism, growth, and development. Fenofibrate usage has been shown to enhance norepinephrine sensitivity (Campo et al. 2011), thereby improving metabolism and accelerating energy consumption in the human body. Our research also supports this

notion, but the specific regulatory mechanism and precise relationship between these factors require further verification.

Furthermore, fenofibrate treatment led to a reduction in GT51 (glycosyltransferase family 51), GT5 (glycosyltransferase family 5), GT8 (glycosyltransferase family 8), as well as GH96, GH77, and GH140 (glycoside hydrolase family 96, 77, and 140), in the high-fat diet (HFD) group. GT51, as a key enzyme in bacterial cell wall synthesis, has long been considered a promising but challenging target for antibiotic development (Goossens et al. 2020). Additionally, changes in antibiotic resistance genes were also observed. To gain further functional insights, a spearman correlation analysis was conducted, revealing that health-promoting bacterial species were negatively correlated with certain genes involved in antibiotic resistance, such as oleI, AAC (2')-Ia, APH (3')-VIIa, chrB, fexA. Conversely, positive correlations were observed with six genes that exhibited an increase, namely adeC, carA, MexT, vgbA, clbA, and vanD (Fig. 6d). The changes in genes activity was dependent on the composition of bacterial species.

There are some limitations in the present study. Firstly, this study did not set up a separate control group for the administration of fenofibrate. It will be more rigorous if gut microbiota composition of a separate group receiving fenofibrate was also analyzed at the outset of the study. Secondly, additional metabolomics and genetic-level testing would be needed to further confirm and validate the effects of fenofibrate on the gut microbiota of hyperlipidemic hamsters.

In summary, the administration of fenofibrate resulted in the promotion of beneficial bacterial species (such as *Bacteroides ovatus, Bifidobacterium animalis, Bacteroides intestinalis, Allobaculum stercoricanis*, and *Lactobacillus reuteri*), along with the upregulation of genes involved in lipid and energy metabolism. Pathways associated with tryptophan metabolism, biosynthesis of unsaturated fatty acids, glycerophospholipid metabolism, and tyrosine metabolism were positively influenced, leading to increased production of unsaturated fatty acids and indole. Analysis using the KEGG database indicated significant effects of fenofibrate on genes and pathways involved in lipid, energy, and amino acid metabolism. These findings contribute to a better understanding of the mechanism of action of fenofibrate and provide a valuable foundation for future experimental and clinical studies, aiming to explore its practical applications. Further investigations that assess gut microbial composition, fenofibrate metabolites, and gene levels are warranted to validate and expand upon our findings.

Abbreviations

HDL	High-density lipoprotein
LDL	Small dense low-density lipoprotein
LPS	Lipopolysaccharide
SCFAs	Short-chain fatty acids
HFD	High-fat diet
C	Control
TC	Total cholesterol
TG	Triglyceride
LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
PCA	Principal component analysis
KEGG	Kyoto Encyclopedia of Genes and Genomes
KOs	Orthologs
BCAAs	Branched-chain amino acids
CVD	Cardiovascular disease

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Data availability
All additional datasets and materials are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
All animal studies were performed following the guidelines of the Beijing Municipal Ethics Committee for the Care and Use of Laboratory Animals and were approved by the Animal Care & Welfare Committee, Institute of Materia Medica, CAMS & PUMC on January 4, 2019 (No. 00003635) (Beijing, China).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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