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Static magnetic field exposure modulates gut microbiota and host metabolism to alleviate high-fat diet-induced metabolic dysfunction-associated fatty liver disease

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ABSTRACT

Static magnetic field (SMF) exposure exerts notable regulatory effects on metabolic disorders, yet its influence on metabolic dysfunction-associated fatty liver disease (MAFLD) and gut microbiota during disease progression remains unclear. In this study, MAFLD was induced in mice via a high-fat diet (HFD), followed by exposure to a 0.2 T SMF for 12 h per day over a 10 week period. SMF treatment significantly attenuated body weight gain, alleviated hepatic lipid accumulation, and improved liver function. Sequencing analysis of intestinal contents revealed a significant increase in microbial diversity and enrichment of beneficial bacterial taxa under SMF exposure. Integrated multi-omics analysis and Spearman correlation further demonstrated that SMF significantly reduced the expression of genes involved in fatty acid synthesis and modulated pathways related to polyunsaturated fatty acid and glutamate metabolism, in close association with shifts in beneficial gut microbiota. Furthermore, transcriptomic profiling of liver tissue indicated that SMF inhibited fatty acid synthesis and elongation by regulating the expression of peroxisome proliferator-activated receptor γ (*PPARγ*), thereby contributing to reduced hepatic burden. These findings highlight SMF as a promising non-invasive strategy for MAFLD intervention and provide insights into the microbiota-mediated metabolic axis underlying its therapeutic effects.

Keywords: Static magnetic fields; Metabolic dysfunction-associated fatty liver disease; Gut microbiota; Metabolites; Mice

INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) has rapidly become the most common chronic liver disorder worldwide, affecting approximately 38% of the global adult population (Targher et al., 2024). As a multisystemic condition, MAFLD not only increases the risk of liver-specific complications, including nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma, but also elevates susceptibility to extrahepatic diseases such as type 2 diabetes mellitus (T2D) and chronic kidney disease (Thomas et al., 2024). Despite intensive clinical research over the past two decades, no pharmacological agents have been approved for MAFLD treatment (Tilg et al., 2023).

Recent studies have highlighted static magnetic fields (SMFs) as a promising non-invasive physical modality with therapeutic potential across a range of pathological conditions, including tumor suppression, neurological disorders, and metabolic dysfunction. For instance, Tian et al. (2023) found that exposure to a 9 T SMF alleviated imatinib-induced anxiety and depression in mice while inhibiting gastrointestinal mesenchymal tumor growth. Yu et al. (2021) reported that SMF exposure improved glucose homeostasis in T2D mice by regulating gut microbial iron metabolism and reducing reactive oxygen species (ROS) levels. In addition, homeostatic magnetic fields themselves have shown positive effects in

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improving fatty liver and obesity. Song et al. (2023) demonstrated that 0.1 to 0.2 T SMF significantly ameliorated alcoholic liver disease by decreasing inflammation and oxidative stress, while Chen et al. (2024) reported that SMF not only significantly reduced acetaminophen-induced oxidative stress and free radical levels but also increased DNA synthesis and hepatocyte proliferation, thereby alleviating liver injury. Additional evidence has also indicated that SMF exposure attenuates liver damage and functional abnormalities in obese and diabetic mice (Lv et al., 2023).

The pathogenesis of MAFLD is closely connected with gut microbial dysbiosis, characterized by compositional shifts in gut microbial communities, impaired intestinal barrier integrity, and altered host-microbe interactions (Behary et al., 2021; Li et al., 2024a; Yin et al., 2023). Microbial imbalances contribute to systemic inflammation, metabolic derangements, and progression of hepatic steatosis (Tilg et al., 2022; Zhang et al., 2024). Gut microbial metabolites also influence host lipid, glucose, and amino acid metabolism, contributing to disease pathogenesis (de Vos et al., 2022; Li et al., 2024b; Suárez-Zamorano et al., 2015; Wang et al., 2023). Therapeutic interventions targeting gut microbiota, such as probiotics, prebiotics, and fecal microbiota transplantation, have demonstrated potential in ameliorating MAFLD (Lee et al., 2021; Xue et al., 2022). Notably, SMFs have also been reported to modulate gut microbial composition. Yu et al. (2021) observed that SMF exposure restored Bacteroidetes abundance in T2D mice, while Tai et al. (2020) found that a low-intensity pulsed electromagnetic field (PEMF) reduced the Firmicutes/Bacteroidetes ratio.

Given these findings, a more comprehensive understanding of the interplay between SMF, gut microbiota, and hepatic metabolism is essential for advancing SMF-based therapies for MAFLD. In this study, the effects of a 0.2 T SMF on high-fat diet-induced MAFLD were systematically investigated using an integrated multi-omics approach. Results indicated that SMF exposure significantly ameliorated hepatic lipid accumulation and dyslipidemia in MAFLD mice, while also modulating gut microbial composition and liver metabolite profiles. These findings suggest that SMF may exert therapeutic effects on MAFLD through microbiota-associated metabolic reprogramming, providing a theoretical basis for disease intervention and prevention.

MATERIALS AND METHODS

Static magnetic field setup

The SMF device (Zhongxin Permanent Magnet Materials Co., Ltd. China) used for animal exposure was adapted from a previously validated experimental setup (Song et al., 2023). A downward-oriented, quasi-uniform SMF was generated using a permanent magnetic plate (250 mm long×160 mm wide×45 mm high) embedded with 12 neodymium magnet cubes (60 mm long×50 mm wide×30 mm high). Distribution of the magnetic field was determined using a magnet analyzer (FE-2100RD, Forever Elegance, China) and a Hall sensor (HG-106A, Asahi Kasei, Japan). Each horizontal plane displays a reasonably consistent SMF produced by the magnetic plate, with a reading of about 0.2 T (16 mm above the plate). Given that the biological effects may be associated with SMF intensity, the impact of higher SMF on MAFLD was explored in our study. However, due to the limitation of permanent magnet production technology, the maximum magnetic field intensity of the magnetic plate used in our study is about 0.2 T. Mice in the SMF treatment group were exposed to this downward-directed field for 12 h per day over a 10-week period to evaluate the biological effects of moderate-intensity SMF exposure on MAFLD.

Mouse model

Eighteen six-week-old male C57BL/6J mice were procured from GemPharmatech Co., Ltd. (China) and randomly assigned into three groups (n=6 per group): (1) normal control diet (NCD) group; (2) HFD group (Research Diets D12492 60 kcal%); and (3) SMF group (Research Diets D12492 60 kcal%). All mice were housed under barrier conditions maintained at 22–24°C and 56%–60% relative humidity and a 12-h light-dark cycle. Prior to the experiments, mice underwent a one-week acclimatization period with unrestricted access to food and water. SMF exposure in the treatment group was administered for 12 h daily over 10 weeks. This exposure regimen was selected to ensure sufficient SMF contact time without affecting normal circadian rhythm. The 10 week treatment window was designed to explore the effects of SMF on early-stage MAFLD development. All animal experiments were conducted in accordance with the guidelines provided by the Ethical and Humane Committee of Hefei Institutes of Physical Science, Chinese Academy of Sciences. All protocols were approved by the Hefei Institutes of Physical Science, Chinese Academy of Sciences Laboratory Animal Center (permit number DWLL(E)-2024-24).

Hematoxylin and eosin (H&E) staining

Liver samples were preserved for 24 h in 4% paraformaldehyde (G1101, Servicebio, China). Tissues were processed and paraffin blocks were prepared according to standard histopathological techniques. The tissues were cut into 5 μm slices and stained with H&E (G1076, Servicebio, China). Lipid droplet area was quantitatively analyzed by ImageJ software (v.1.54f, National Institutes of Health, USA).

Oil Red O staining

Oil Red O staining solution (G1261, Solarbio, China) was employed to detect lipid accumulation in liver slices. Frozen liver slices were preserved for 10 min in 10% formalin, then submerged in 100% isopropanolfor 5 min. After incubation for 6–10 min in a heated Oil Red O solution (60°C), the sections were moved to an 85% propylene glycol solution and washed for 1 min with deionized water. The Oil Red O-stained area was evaluated using ImageJ software.

Measurement of serum physiological indices

Total cholesterol (TC), total triglycerides (TG), alanine transaminase (ALT), aspartate aminotransferase (AST), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) were determined in accordance with the instructions provided by the commercial kits (Nanjing Jiancheng, China).

Indirect calorimetry test

Metabolic activity of mice was monitored using the Comprehensive Lab Animal Monitoring System (Columbus Instruments, USA). Mice underwent a 24 h acclimatization period before measurements were taken. Oxygen consumption (VO_2), carbon dioxide exhalation (VCO_2), and physical activity were measured and recorded over a 48 h cycle for each mouse subjected to a 12 h light-dark cycle.

16S rRNA gene sequencing analysis

Bacterial DNA was obtained from cecum contents using a Rapid-e E.Z.N.A. ®Stool DNA Kit (D4015, Omega, USA). The V3–V4 regions of the bacterial 16S rRNA gene were amplified using 341F-805R primers (341F: 5′-CCTACGGGGNGGCWGCAG-3′ & 805R: 5′-GACTACHVGGGGTATCTAATCC-3′). The PCR products were validated, purified, and quantified. Amplified libraries were prepared and sequenced on the NovaSeq PE250 platform (Illumina, USA). Under particular filter conditions, high-quality clean labels were obtained according to fqtrim (v.0.9.4). Vsearch (v.2.3.4) was used to search for chimeric sequences. BLAST comparisons and taxonomic annotations were performed using the SILVA database (https://www.arb-silva.de/).

Transcriptomic analysis

Total RNA was extracted from liver tissue, and RNA quality was assessed for purity, integrity, and concentration. Equivalent RNA volumes were used to generate sequencing libraries. Differentially expressed genes (DEGs) were defined by adjusted *P*-values≤0.05 and absolute fold change (FC)≥ 1.5. Functional enrichment of DEGs was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp/) to identify biologically relevant pathways (Xie et al., 2024).

Reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA was extracted from liver tissue using TRIzol reagent (Thermo Fisher, USA). RNA reverse transcription was performed using NovoScript Plus All-in-One First Strand cDNA Synthesis Supermix (E047-01A, Novoprotein, China). Target genes were amplified using NovoStart SYBR qPCR Supermix Plus (E096-01A, Novoprotein, China) with gene-specific primers (listed in Supplementary Table S1).

Untargeted liver metabolomic analysis using liquid chromatography-mass spectrometry (LC-MS)

Approximately 50 mg of thawed liver tissue was homogenized in 500 μL of pre-cooled methanol/water (1:1, v/v) by sonication for 30 min at 4°C. Acetonitrile was subsequently added to the solution and incubated at −20°C for 1 h. Samples were then centrifuged (12000 r/min, 4°C, 15 min), and the resulting supernatant was collected and freeze-dried. Pre-treated, blank, and quality control (QC) samples were stored at −80°C prior to analysis. Chromatographic separation was performed using a Thermo Scientific UltiMate 3000 HPLC system (USA), and mass spectrometric data were acquired on a Q Exactive mass spectrometer (Thermo Fisher, USA). Compound Discoverer (v.3.1.0) was employed to process the data, and metabolites were annotated against the KEGG database and Human Metabolome Database (HMDB).

Statistical analysis

Data were analyzed using GraphPad Prism (v.8.0), with results presented as mean±standard error of the mean (SEM). Statistical significance was evaluated using two-tailed Student's *t*-tests, with $P<0.05$ considered statistically significant. Spearman correlation coefficients and their significance were computed using the stats package in R (v.3.6.3). Heatmaps displaying hierarchically clustered correlations and significance levels were created using OmicStudio tools (https://www.omicstudio.cn).

RESULTS

SMF attenuates body weight gain, liver hypertrophy, and hepatic steatosis in MAFLD mice

To assess the potential protective effects of SMF exposure on MAFLD, mice were treated with a 0.2 T downward-oriented neodymium-iron-boron (NdFeB) magnetic field (Figure 1A), while control mice were exposed to an iron block of equivalent size. The average magnetic flux density applied to the abdominal region was quantified (Figure 1B). Body weight, food intake, and water consumption were monitored throughout the experiment (Figure 1C). Compared to the HFD group, SMF exposure significantly reduced both body weight and liver weight (Figure 1D, E), without significant differences in food and water consumption (Figure 1F, G).

To determine the effects of SMF on hepatic lipid accumulation, liver sections were examined using H&E and Oil Red O staining. The H&E staining results revealed disorganized hepatic architecture and prominent steatosis in the HFD group. Oil Red O staining further confirmed a substantial reduction in lipid droplet accumulation in the livers of SMF-exposed mice relative to those in the HFD group (Figure 1H–J).

SMF improves hepatic function and dyslipidemia in MAFLD mice

To evaluate whether SMF modulates metabolic homeostasis in mouse livers, liver function and lipid indices were examined. Compared with the HFD group, SMF exposure substantially reduced the serum levels of ALT (Figure 2A, B), indicating a protective effect against HFD-induced hepatic injury. Both HDL-c and LDL-c levels were significantly increased in the HFD group compared with the control group, while SMF exposure partially restored LDL-c levels (Figure 2C, D). Furthermore, SMF significantly reduced serum levels of TC and TG in MAFLD mice (Figure 2E, F), suggesting a significant ameliorative effect on HFD-induced dyslipidemia. Metabolic cage analysis revealed no significant differences in food intake, physical activity, or energy expenditure among groups (Figure 2G, H). However, SMF treatment led to significantly elevated O_2 consumption, CO_2 production, and total energy expenditure across both dark and light phases (Figure 2I–K). These findings suggest that SMF enhances energy expenditure in the context of a HFD, potentially contributing to its metabolic benefits.

SMF modulates gut microbiota composition and function in MAFLD mice

Given the established link between gut microbial dysbiosis and the pathogenesis of MAFLD, 16S rRNA sequencing was conducted to determine whether SMF exposure altered the intestinal microbiota. Sparsity and species accumulation curves plateaued with increasing sequence depth, confirming adequate sequencing coverage and comprehensive representation of microbial diversity across all samples (Figure 3A). Alpha diversity metrics revealed that HFD feeding significantly reduced Chao1 and Shannon indices relative to the NCD group, whereas SMF exposure partially restored these parameters, indicating improved microbial richness and evenness (Figure 3B–D).

Beta diversity analyses based on principal coordinates analysis (PCoA) and intergroup distance measurements demonstrated distinct alterations in gut microbiome composition between the SMF and HFD groups (Figure 3E).

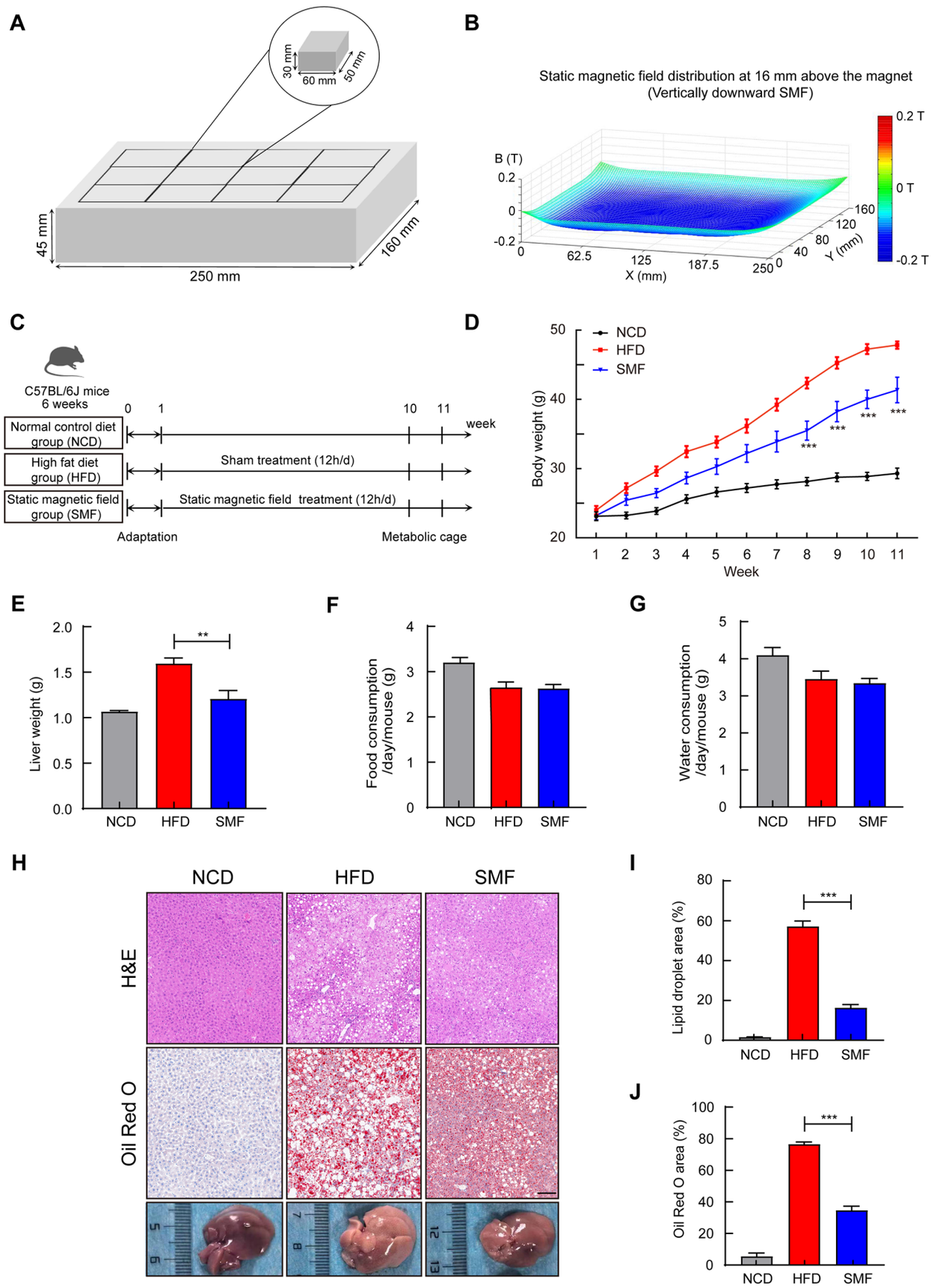

Figure 1 Static magnetic field (SMF) exposure ameliorates weight gain, liver damage, and lipid accumulation in MAFLD mice

A: SMF devices. B: Magnetic field distribution in mouse exposure area, 16 mm above magnetic plates. C: Schematic of experimental schedule. D: Body weight. E: Liver weight. F: Food consumption. G: Water consumption. H: Liver sections were subjected to H&E and Oil Red O staining. Scale bar: 50 μm. I: Quantification of liver H&E staining. J: Quantification of liver Oil Red O staining. Values represent means±SEM, n=6 per group. NCD: Normal control diet group; HFD: High-fat diet group; SMF: Static magnetic field group. **: P<0.01; ***: P<0.001.

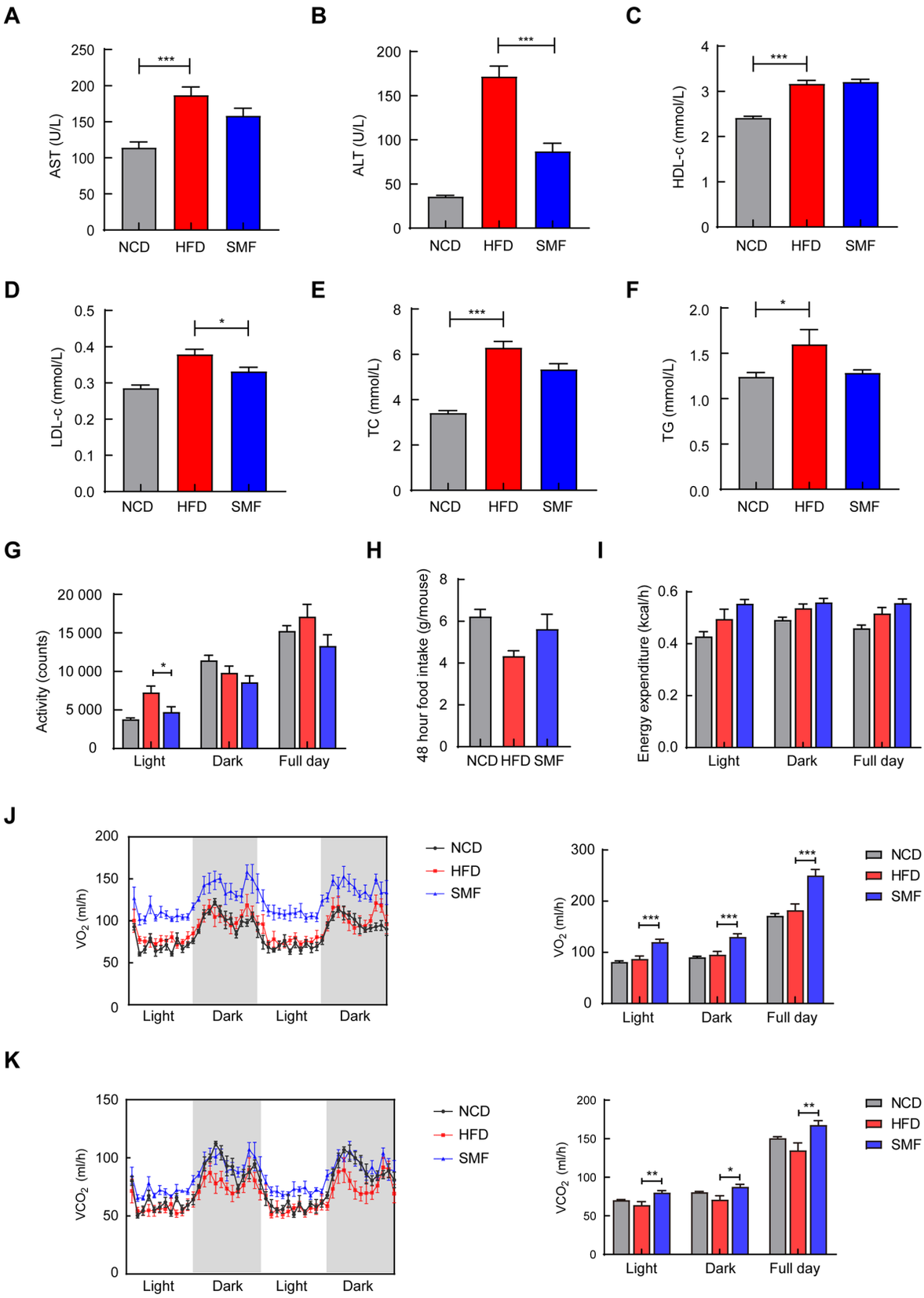

Figure 2 Static magnetic field (SMF) exposure improves liver function and dyslipidemia and increases energy expenditure in MAFLD mice

A–F: Liver function and serum lipid parameters were measured, including aspartate transaminase (A), alanine transaminase (B), high-density lipoprotein cholesterol (C), low-density lipoprotein cholesterol (D), total cholesterol (E), and total triglycerides (F). G–K: Activity levels and energy expenditure were also measured, including physical activity (G), food intake (H), energy expenditure (I), oxygen consumption (J), and carbon dioxide exhalation (K). Values represent means±SEM, n=6 per group. NCD: Normal control diet group; HFD: High-fat diet group; SMF: Static magnetic field group. *: P<0.05; **: P<0.01; ***: P<0.001.

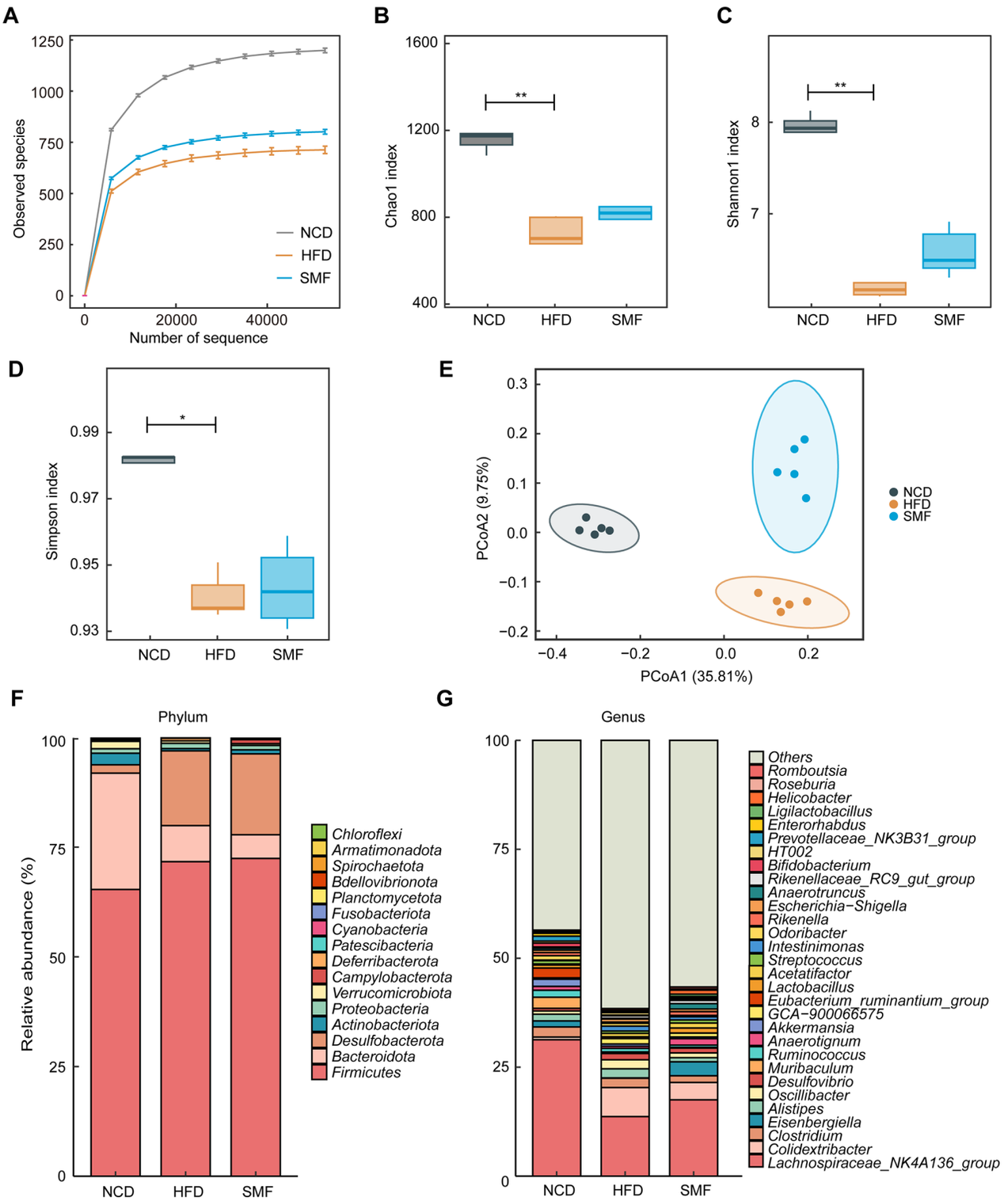

Figure 3 Static magnetic field (SMF) exposure significantly alters gut microbiota composition in MAFLD mice

A: Rarefaction curve showing observed species richness. B–D: Comparison of Chao1, Shannon, and Simpson indices among three groups. E: Principal component analysis of weighted UniFrac distances based on 16S rDNA profiling (ASV analysis) of gut microbiota in each group. F: Relative abundance of gut microbiota at the phylum level. G: Relative abundance of gut microbiota at the genus level. Values represent means±SEM, n=5 per group. NCD: Normal control diet group; HFD: High-fat diet group; SMF: Static magnetic field group. *: P<0.05; **: P<0.01.

Taxonomic profiling at the phylum level revealed that HFD feeding elevated the relative abundance of *Proteobacteria* and *Deferribacterota*, both of which were suppressed by SMF exposure. In contrast, *Actinobacteriota*, which was reduced in the HFD group, was enriched in SMF-treated mice (Figure 3F). At the genus level, SMF treatment increased the relative abundance of *Lachnospiraceae_NK4A136_group*, *Eisenbergiella*, and *Lactobacillus* (Figure 3G). Linear discriminant analysis (LDA) effect size (LEfSe) identified differentially enriched gut microbial taxa between groups (LDA score>3), highlighting distinct microbial signatures associated with SMF treatment. Functional prediction using PICRUSt2

further indicated that SMF modulated gut microbial metabolic potential, with secondary KEGG pathway enrichment analysis showing significant differences in amino acid metabolism, energy metabolism, and lipid metabolism relative to the HFD group. These findings suggest that SMF exerts protective effects against MAFLD by modulating the gut microbiota and restoring metabolic pathways (Supplementary Figure S1A, B).

SMF reshapes hepatic metabolite profiles in MAFLD mice

To investigate the metabolic impact of SMF on MAFLD, untargeted metabolomic profiling of liver tissue was conducted, given the central role of metabolites in mediating host-microbiota interactions. Principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) showed significant differences among the three groups, indicating distinct metabolic signatures (Figure 4A, B; Supplementary Figure S2A, B). OPLS-DA regression modeling further confirmed the high reliability of the data (Figure 4C; Supplementary Figure S2C). To identify key metabolites altered by SMF exposure, OPLS-DA was integrated with *t*-tests. Among the top 30 metabolites (VIP>1 and P<0.05), the SMF group showed significant enrichment in polyunsaturated fatty acids and amino acid metabolites compared to the HFD group (Figure 4D; Supplementary Figure S2D). Notable metabolites included linoleic acid, eicosapentaenoic acid, D-proline, and isoleucine. Subsequent KEGG pathway analysis revealed significant enrichment in amino acid metabolism, oxidative phosphorylation, linoleic acid metabolism, and glycerophospholipid metabolism pathways following SMF treatment (Figure 4E; Supplementary Figure S2E). These findings suggest that SMF modulates hepatic metabolic pathways linked to lipid handling and energy homeostasis in MAFLD.

SMF alters hepatic transcriptomic profiles

To analyze the potential molecular mechanisms underlying the protective effects of SMF in MAFLD, transcriptome profiling of liver tissue was performed using RNA sequencing (RNA-seq). Differential gene expression analysis was conducted using DESeq2, with thresholds set to P<0.05 and FC>1.5. A total of

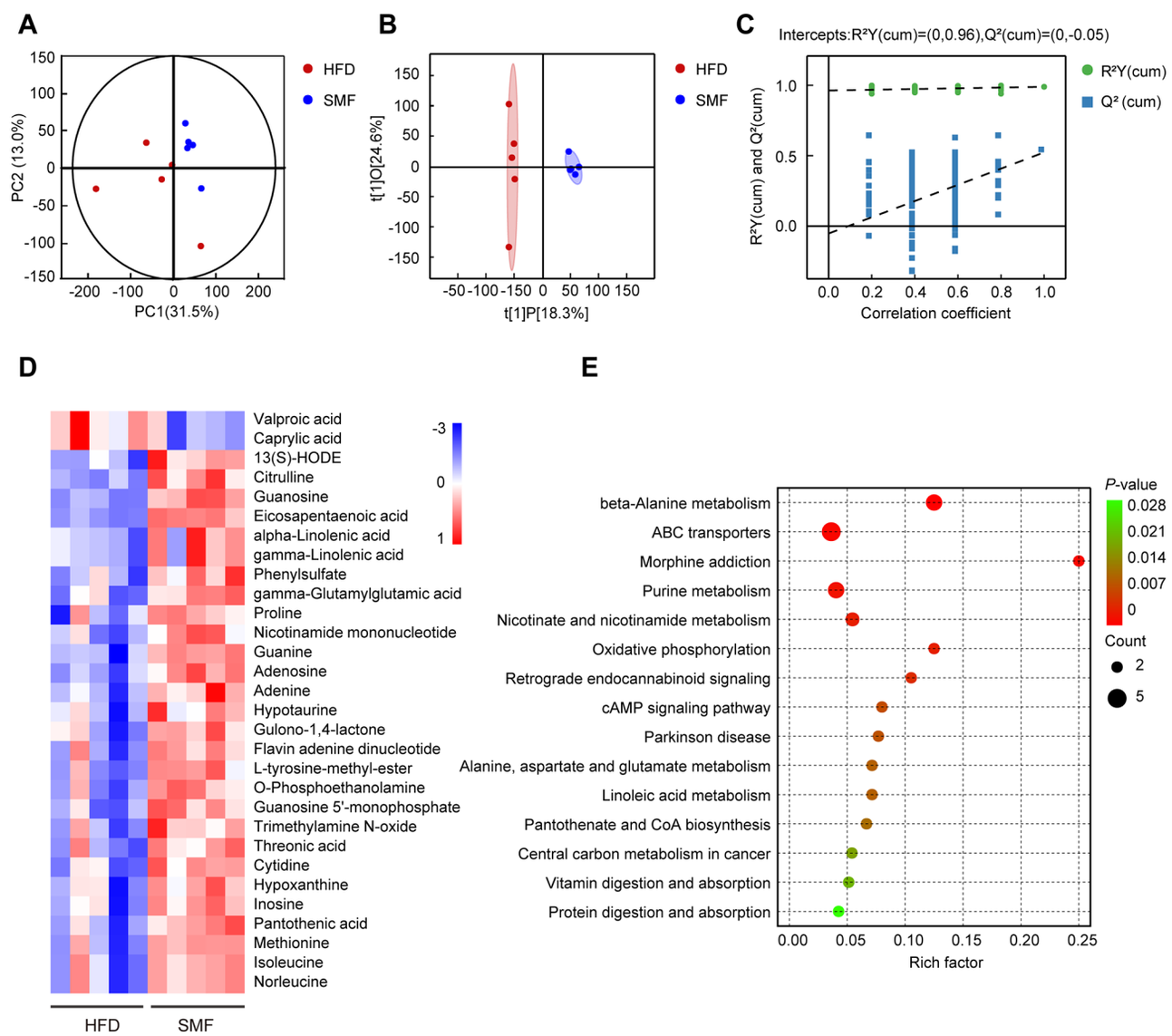

Figure 4 Static magnetic field (SMF) exposure significantly ameliorates hepatic metabolic disorders in MAFLD mice

A: PCA score plots for HFD group vs. SMF group. B: Orthogonal partial least squares discriminant analysis. C: Validation with the 200 substitution test model. D: Top 30 differential metabolites identified between HFD and SMF groups. E: KEGG enrichment pathway analysis. Values represent means±SEM, n=5 per group. HFD: High-fat diet group; SMF: Static magnetic field group.

199 DEGs were identified between the HFD and SMF groups, including 31 up-regulated and 168 down-regulated (Figure 5A, B). To further explore the impact of SMF treatment on the biological functions of the DEGs in HFD-induced MAFLD mice, GO and KEGG enrichment analysis was carried out. KEGG pathway analysis revealed significant enrichment of genes involved in metabolic pathways, including lipid metabolism, cofactor and vitamin metabolism, and immune response pathways in the HFD group compared to the SMF group (Figure 5C). GO enrichment analysis further showed enrichment in key biological processes such as the cytochrome P450-mediated oxidation, fatty acid biosynthesis, and lipid metabolic pathways (Figure 5D). Notably, KEGG analysis indicated significant enrichment in genes related to linoleic acid metabolism, peroxisome proliferator-activated receptor (PPAR) signaling, and arachidonic acid metabolism in both HFD and SMF groups (Figure 5E). Inspection of PPAR signaling and associated lipid metabolism-related genes

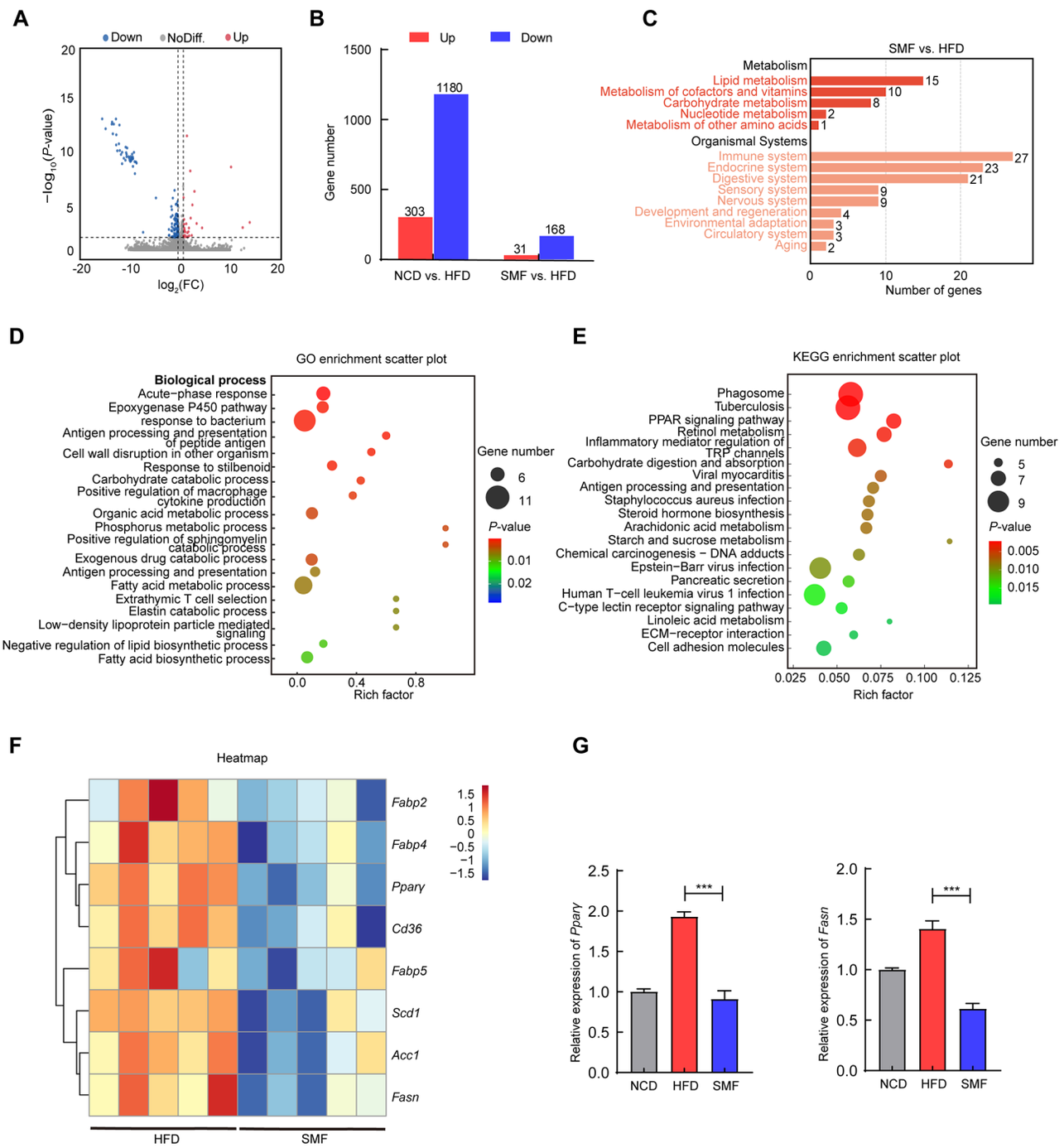

Figure 5 Transcriptomic analysis between HFD and SMF groups

A: Volcano map of differentially expressed genes (DEGs). B: Statistics of up-regulated and down-regulated genes. C: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway classification of DEGs. D, E: Scatter plot of DEG enrichment for Gene Ontology (GO) and KEGG. Circle size represents gene count and circle color represents *P*-value. F: Heatmaps of lipid metabolism mRNA expression based on mRNA-sequencing data. G: Gene expression related to lipid metabolism pathway in liver tissue. Values represent means±SEM, *n*=5 per group. NCD: Normal control diet group; HFD: High-fat diet group; SMF: Static magnetic field group. ***: *P*<0.001.

revealed a coordinated down-regulation in the SMF-treated group. Heatmap analysis showed that *Fabp2*, *Fabp4*, *Fabp5*, *PPARγ*, *Cd36*, *Scd1*, *Acc1*, and *Fasn* were down-regulated in the SMF-treated mice compared to the HFD group (Figure 5F). To validate these findings, *PPARγ* and *Fasn* were selected as candidate genes for RT-qPCR detection, which confirmed their significant down-regulation following SMF treatment, consistent with the RNA-seq results (Figure 5G). Collectively, these results suggest that SMF may exert its lipid-lowering effects in MAFLD through transcriptional repression of key lipogenic genes regulated by the *PPARγ* pathway, thereby ameliorating lipid accumulation in the liver.

Correlation analysis of key pathological indicators, transcriptome, metabolites, and gut microbiota

To further elucidate the interplay between gut microbiota, hepatic metabolites, transcriptomic alterations, and MAFLD, Spearman correlation analysis of key altered microbial communities and pathological indicators associated with MAFLD mice were assessed. Following SMF exposure, the relative abundances of *g_Lactobacillus*, *g_Lachnoclostridium*, *g_Lachnospiraceae_FCS020_group*, and *g_Eisenbergiella* were significantly increased and negatively correlated with body weight, ALT, LDL-c, and TG. In contrast, *g_Bilophila* and *g_Alloprevotella* exhibited strong positive correlations with body weight, ALT, and LDL-c (Figure 6A). To investigate the association between gut microbial shifts and hepatic gene expression, SMF-altered bacterial genera were correlated with differentially expressed genes involved in lipogenesis. Results indicated that *g_Lachnoclostridium*, *g_Rikenella*, and *g_Lachnospiraceae_FCS020_group* were significantly negatively correlated with *Fabp2*, *Fasn*, *Scd1*, *PPARγ*, *Cd36*, and *Fabp4* (Figure 6B). Correlations between gut microbial changes and hepatic metabolites also revealed that *g_Lachnoclostridium*, *g_Lachnospiraceae_FCS020_group*, and *g_Eisenbergiella* were significantly positively correlated with pantothenic acid, inosine, citrulline, and eicosapentaenoic

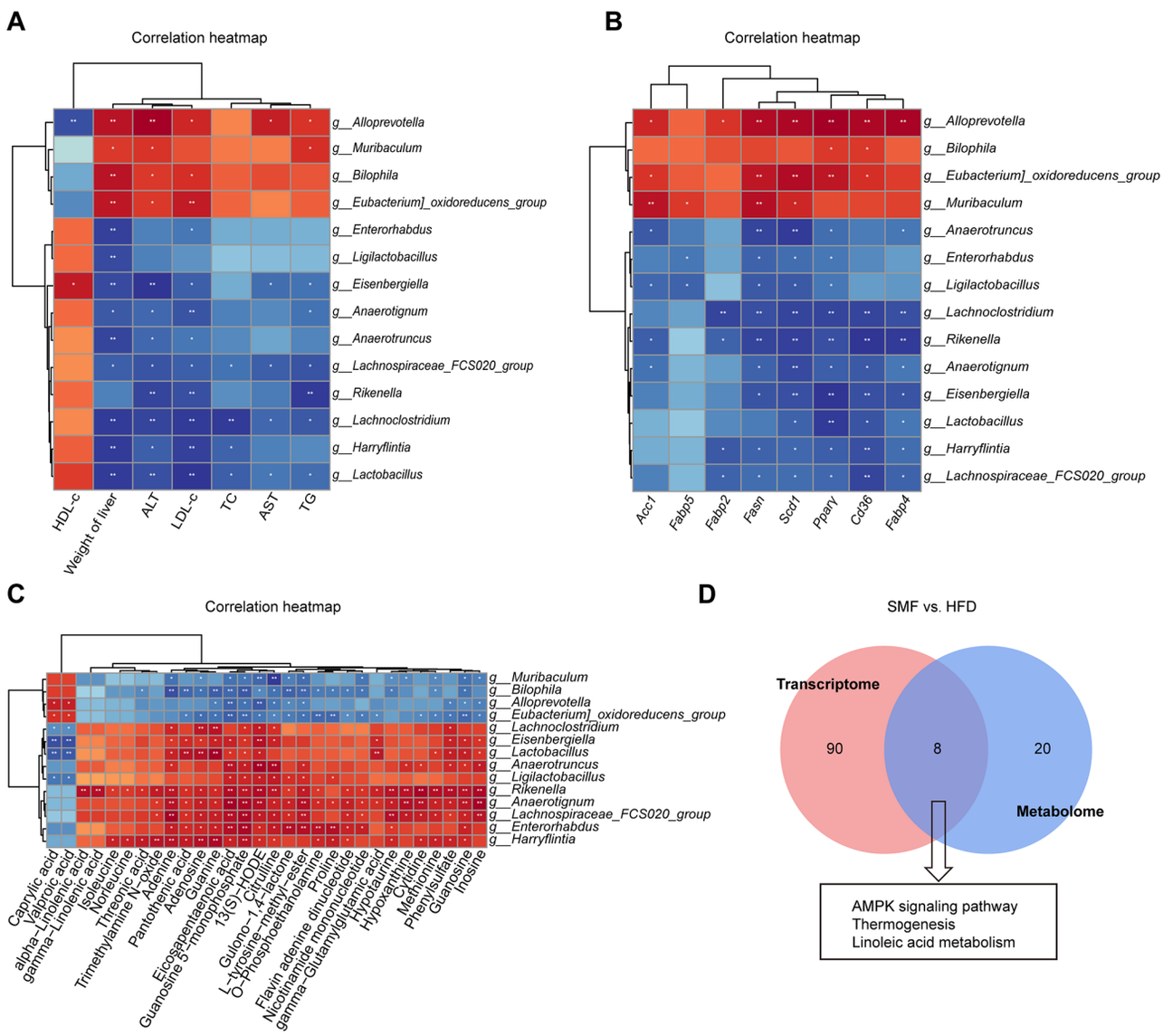

Figure 6 Correlation analysis of key pathological indicators, transcriptome, metabolites, and gut microbiota

A: Spearman correlation between genus-level gut microbiota and key pathological indicators. B: Spearman correlation between genus-level gut microbiota and DEGs involved in lipid metabolism. C: Spearman correlation between genus-level gut microbiota and metabolome. D: Integrated metabolomic and transcriptomic analysis. Red and blue indicate positive and negative correlations, respectively. Values represent means±SEM, n=5 per group. *: P<0.05; **: P<0.01; ***: P<0.001.

acid-metabolites elevated following SMF exposure. In addition, methionine, eicosapentaenoic acid, and 13-hydroxyoctadecadienoic acid were positively correlated with *g_Lactobacillus* but negatively correlated with *g_Bilophila*, *g_Eubacterium_oxidoreducens_group* and *g_Muribaculum* (Figure 6C). To ascertain the relationship between DEGs and metabolites, combined transcriptomic-metabolomic analysis was performed, identifying 28 significantly altered KEGG pathways at the metabolomic level and 98 significantly altered KEGG pathways at the transcriptomic level. Eight pathways overlapped between the two datasets, among which AMPK signaling, thermogenesis, and linoleic acid metabolism were directly associated with MAFLD progression and SMF-mediated metabolic modulation (Figure 6D).

DISCUSSION

MAFLD is characterized by excessive hepatic lipid accumulation, commonly arising in the context of obesity and insulin resistance, and frequently coexists with T2D, dyslipidemia, and hypertension (Kasahara et al., 2023). Emerging evidence highlights the therapeutic potential of physical field-based interventions in hepatic disorders. Previous studies have demonstrated that downward-oriented SMF can alleviate alcohol-induced liver injury, reduce lipid accumulation, and improve liver function (Song et al., 2023). Building on these findings, the present study demonstrated that exposure to a 0.2 T downward SMF significantly ameliorated HFD-induced MAFLD in mice. Notably, SMF treatment led to reductions in final body weight, liver weight, serum lipid levels, and circulating ALT, accompanied by reductions in liver damage and lipid accumulation. SMF treatment resulted in increased energy metabolism and exercise levels in mice, but did not affect food intake. These results suggest that SMF exposure increased oxygen consumption, carbon dioxide production, and overall energy expenditure, implicating elevated metabolic rate as a mechanistic contributor to the observed phenotype.

In recent years, mounting evidence has confirmed the therapeutic function of gut microbiota in the prevention and treatment of MAFLD (Liu et al., 2020). Previous studies have shown that directional downward SMF can partially prevent the development of HFD-induced diabetes via modulation of gut microbiota, reduction of unstable iron, and reduced ROS in pancreatic cells (Yu et al., 2021). Low-intensity pulsed electromagnetic fields have also been reported to alter gut microbial composition, notably by decreasing the proportion of thick-walled bacterial phyla in the gut microbiota of *Anopheles* mosquitoes (Tai et al., 2020). In this study, SMF exposure significantly increased microbial α-diversity and altered β-diversity, suggesting a shift in overall gut community structure. At the genus level, SMF-treated mice exhibited increased abundances of *g_Lactobacillus*, *g_Lachnoclostridium*, and *g_Lachnospiraceae_FCS020_group*, all of which were negatively correlated with pathological indices, including serum lipid, cholesterol, liver injury, and inflammatory factors. *Lactobacillus* species are widely recognized for their probiotic properties, including the suppression of hepatic fat accumulation and restoration of liver function (Liu et al., 2016). *Lachnoclostridium* is a producer of short-chain fatty acids, which promote lipolysis, enhance fatty acid oxidation, increase insulin sensitivity, and alleviate host obesity (Zhao et al., 2021). Members of the *Lachnospiraceae* family are associated with improved lipid metabolism and hepatic steatosis (Zhang et al., 2023). Conversely, genera such as *g_Mucispirillum* and *g_Bilophila*, which are typically enriched under HFD conditions (Natividad et al., 2018), were significantly reduced following SMF exposure. SMF also significantly reduced the relative abundance of *g_Eubacterium_oxidoreducens_group*, which is also typically up-regulated by HFD (Zhang et al., 2020). These findings suggest that SMF-mediated modulation of gut microbiota may play a contributory role in mitigating MAFLD-associated pathophysiological features.

Untargeted metabolomic profiling was conducted to further elucidate the metabolic mechanisms underlying SMF-mediated improvements in MAFLD. Results showed that SMF exposure induced significant changes in hepatic metabolite composition, with marked enrichment in pathways related to polyunsaturated fatty acids, amino acids, and vitamin metabolism. These findings suggest that SMF may reprogram hepatic metabolism and counteract lipid dysregulation associated with HFD feeding in MAFLD mice. Several metabolites with established hepatoprotective effects were elevated following SMF treatment. Among them, niacin plays an essential role in cellular metabolic reactions and may reverse hepatic steatosis (Meyer-Ficca & Kirkland, 2016; Pan et al., 2024). Eicosapentaenoic acid, a bioactive omega-3 polyunsaturated fatty acid, is known to prevent the development of metabolic disorders, possibly by modulating adipocytokine secretion and reducing inflammation (Oh et al., 2010; Pinel et al., 2016; Zhang et al., 2021). Notably, several differentially accumulated compounds associated with the regulation of these metabolic pathways may also depend on changes in the gut microbiota. Comparative KEGG pathway enrichment analysis revealed that that both microbiota-associated and metabolite-associated pathways converged on linoleic acid, glutamate, nicotinic acid, and nicotinamide metabolism, suggesting a correlation between shifts in the gut microbiota and improvements in liver function. Spearman correlation analyses also revealed that *g_Lactobacillus*, *g_Lachnoclostridium*, *g_Lachnospiraceae_FCS020_group*, and *g_Eisenbergiella* were positively correlated with hepatic eicosapentaenoic acid levels.

RNA-seq was employed to investigate the molecular mechanisms underlying the therapeutic effects of SMF in MAFLD. Transcriptomic profiling revealed that SMF exerted its protective action, at least in part, through modulation of the PPAR signaling pathway. PPARs are a class of ligand-activated nuclear receptors that orchestrate diverse aspects of lipid and glucose metabolism across multiple tissues (Montaigne et al., 2021). Among the three isoforms—PPARα, PPARδ, and PPARγ—PPARα is predominantly involved in β-oxidation and fatty acid transport, maintaining lipid homeostasis (Gross et al., 2017), while PPARδ regulates mitochondrial function and fatty acid β-oxidation (Mackenzie & Lione, 2013). PPARγ regulates the expression of genes involved in *de novo* lipogenesis, fatty acid re-esterification, and lipid storage (Lee et al., 2023; Wolf et al., 2017). Notably, hepatic *PPARγ* expression is significantly elevated in individuals with MAFLD (de Conti et al., 2020; Lee et al., 2021), and drives *FABP4*-mediated free fatty acid uptake and *FASN* expression, contributing to excessive lipid accumulation in hepatocytes (Chen et al., 2023). In the present study, SMF exposure down-regulated *PPARγ* and associated lipogenic genes in the liver, implicating suppression of this pathway as a key mechanism underlying SMF-mediated metabolic reprogramming in MAFLD.

In conclusion, sustained exposure to a 0.2 T downward-oriented SMF for 12 h per day over a 10 week period significantly improved HFD-induced MAFLD. Sequencing analysis of intestinal contents revealed that SMF significantly improved gut microbial abundance, resulting in an increase in beneficial bacterial taxa. Spearman correlation analysis demonstrated significant associations between gut microbial shifts and hepatic metabolites, particularly polyunsaturated fatty acids and glutamate, which were elevated following SMF treatment. In addition, liver RNA-seq indicated that SMF may inhibit fatty acid synthesis and liver elongation by down-regulating hepatic *PPARγ* signaling, resulting in reduced lipid accumulation in the liver. Overall, these findings suggest that the therapeutic efficacy of SMF on HFD-induced MAFLD may be attributed to the increasing abundance of beneficial bacteria, which were negatively associated with obesity. These results imply that SMF may act as a modulator of gut microbiota to ameliorate MAFLD.

DATA AVAILABILITY

The raw RNA-seq data reported in this study are available from the NCBI database (BioProjectID PRJNA1228996), China National Center for Bioinformation database (GSA: CRA037951), and Science Data Bank database (DOI: 10.57760/sciencedb.j00139.00177).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.F.T. and G.F.C. designed the experiment. G.F.C. drafted the manuscript. X.F.T. and X.Z. reviewed and edited the manuscript. G.F.C. and J.J.L. completed the main experiments. J.M.F. and C.L.F. participated in the collection of samples and methodological design. G.F.C. and Z.M.F. participated in data analysis and visualization. All authors read and approved the final version of the manuscript.

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