

Lacticaseibacillus paracasei CCFM1224-derived lipids alleviate high-fat diet- induced obesity in mice via integrated metabolic modulation

Botao Wang

Jiangnan University

Xin Tang

Jiangnan University

Yongli Lu

Jiangnan University

Fei Liu

Jiangnan University

Bingyong Mao

Jiangnan University

Qiuxiang Zhang

Jiangnan University

Jianxin Zhao

Jiangnan University

Wei Chen

Jiangnan University

Hai Ning Shi

Jiangnan University

Ruiyan Wang

Bloomage Biotechnology Co., Ltd

Shumao Cui

cuishumao@jiangnan.edu.cn

Jiangnan University

Research Article

Keywords: Obesity, Lacticaseibacillus paracasei, Postbiotics, Lipid fraction, Lipid metabolism

Posted Date: October 14th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11034279/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

***Lactacaseibacillus paracasei* CCFM1224-derived lipids alleviate high-fat diet-induced obesity in mice via integrated metabolic modulation**

Botao Wang^{a, b, e, f}, Xin Tang^{a, b}, Yongli Lu^{a, b}, Fei Liu^{a, b}, Bingyong Mao^{a, b}, Qiuxiang Zhang^{a, b}, Jianxin Zhao^{a, b}, Wei Chen^{a, b, c}, Hai Ning Shi^{b, e, f}, Ruiyan Wang^{e, *}, Shumao Cui^{a, b, d, *}

^aState Key Laboratory of Food Science and Resources, Jiangnan University, Wuxi 214122, Jiangsu, China

^bSchool of Food Science and Technology, Jiangnan University, Wuxi 214122, Jiangsu, China

^cNational Engineering Research Center for Functional Food, Jiangnan University, Wuxi 214122, Jiangsu, China

^dWuxi Institute for Specialized nutrition and Health Co., Ltd., Wuxi 214122, Jiangsu, China

^eBloomage Biotechnology Co., Ltd., Jinan 250000, China

^fNutrition Science Research and Development Center; Bloomage Health Corporation Limited, Hangzhou 310000, China

***Corresponding author:**

Shumao Cui, State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China.

E-mail address: cuishumao@jiangnan.edu.cn.

Ruiyan Wang, Bloomage Biotechnology Co., Ltd., Jinan 250000, China.

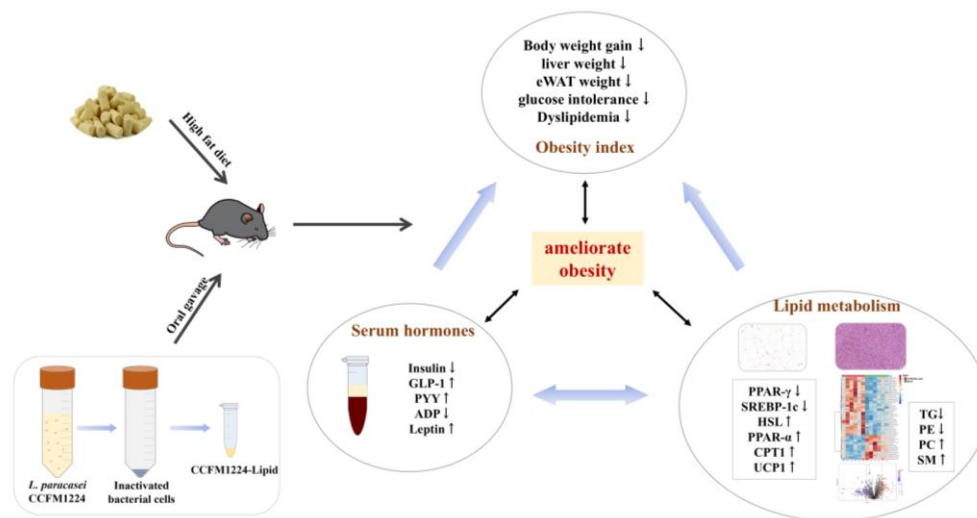
E-mail address: wangry@bloomagebiotech.com.

Abstract

Obesity is a global health challenge associated with metabolic disorders. Postbiotics, as non-viable microbial components, have shown potential in obesity intervention. This study aimed to identify the active anti-obesity components from *Lacticaseibacillus paracasei* CCFM1224 postbiotics and elucidate their mechanisms. Research findings demonstrate that the lipid fraction derived from *L. paracasei* CCFM1224 bacterial cells is the principal bioactive component mediating its anti-obesity effects. In a high-fat diet (HFD)-induced obesity model, oral administration of this lipid fraction significantly ameliorated metabolic disturbances. Compared to the model group, the treatment markedly reduced body weight gain by 23.88% ($p < 0.001$), liver weight by 37.67% ($p < 0.001$), and epididymal white adipose tissue mass by 21.23% ($p < 0.05$). It also improved glucose tolerance, alleviated dyslipidemia, attenuated hepatic steatosis, and suppressed pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) in the liver. Hormonally, the lipid fraction elevated circulating levels of the satiety hormones GLP-1 and PYY while reducing concentrations of leptin and insulin. In adipose tissue, it downregulated the expression of lipogenic genes (*PPAR- γ* , *SREBP-1c*) and upregulated genes involved in lipid catabolism and thermogenesis (*PPAR- α* , *HSL*, *CPT1*, *UCPI*). Furthermore, hepatic lipidomics analysis revealed that CCFM1224 lipids significantly remodeled the hepatic lipid profile, characterized by a distinct decrease in triglycerides and a concurrent increase in sphingomyelins. These findings demonstrate that lipids from *L. paracasei* CCFM1224 alleviate HFD-induced obesity through multiple pathways involving lipid metabolism modulation, inflammation suppression, and hormonal regulation, providing a scientific basis for postbiotic-based precision nutrition strategies.

Key words: Obesity; *Lacticaseibacillus paracasei*; Postbiotics; Lipid fraction; Lipid metabolism

Graphical abstract



Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province.

Appendix A. Supplementary data

The following is the Supplementary data to this article.

Data availability

Data will be made available on request

1.Introduction

Obesity is a complex chronic metabolic disorder characterized by excessive accumulation of adipose tissue, typically defined as a body mass index (BMI) ≥ 30 kg/m²[1], and has reached epidemic proportions globally[2]. It is a major risk factor for numerous comorbidities, including type 2 diabetes, cardiovascular diseases, non-alcoholic fatty liver disease, and certain cancers, posing a severe threat to public health and imposing substantial socioeconomic burdens[3-5]. Consequently, developing safe and effective obesity intervention strategies has become an urgent scientific and societal imperative.

In recent years, postbiotics, defined as formulations of inactivated microorganisms and/or their components that confer health benefits, have emerged as promising bioactive agents. Compared to live probiotics, postbiotics offer advantages in safety, stability, and standardized production[6]. Evidence suggests that various postbiotic components, such as cell wall fragments, proteins, and metabolites, can mitigate obesity through mechanisms including gut microbiota modulation, enhancement of intestinal barrier function, reduction of metabolic inflammation, and regulation of energy homeostasis[7, 8]. For instance, inactivated *L. plantarum* FRT4 was shown to reshape gut microbiota structure, increase short-chain fatty acid levels, inhibit pathogenic bacteria, and concurrently reduce body weight gain, improve blood lipids, and alleviate hepatic steatosis and adipose tissue hypertrophy[9]. Similarly, oral administration of inactivated *Akkermansia muciniphila* downregulated the lipid droplet regulator perilipin 2 in both brown and white adipose tissue of diet-induced obese mice, contributing to reduced adiposity[10]. Furthermore, fermentation products of *L. rhamnosus* HF01 were found to ameliorate obesity-associated hepatic lipid deposition by modulating the “gut microbiota–butyrate–liver lipid metabolism” axis[11]. Despite these promising findings, the specific bioactive constituents responsible for such effects are often inadequately characterized, which limits their precise application.

Our previous study demonstrated that postbiotics derived from *Lactocaseibacillus paracasei* CCFM1224 could attenuate weight gain, ameliorate dyslipidemia, and

prevent hepatic steatosis in HFD-fed mice, potentially via gut microbiota and lipid metabolism regulation[12]. Despite these promising findings, the precise molecular identity of the key anti-obesity components within this postbiotic mixture remained elusive, hindering a mechanistic understanding and further development.

To address this limitation, we fractionated *L. paracasei* CCFM1224 postbiotics into the fermentation supernatant and inactivated bacterial cells. The inactivated bacterial cell fraction was further separated into subfractions including cell walls, cell wall proteins, total lipids, and protoplasts, which were systematically evaluated in a murine HFD-induced obesity model. Furthermore, we investigated the underlying mechanisms through comprehensive analyses of metabolic phenotypes, serum hormones, inflammatory profiles, adipose tissue gene expression and non-targeted lipidomics. This study aims to elucidate key bioactive substances and their mechanisms, thereby providing a scientific foundation for developing precision nutrition interventions based on postbiotic lipids.

2. Materials and methods

2.1 Bacterial culture and postbiotic fractionation

2.1.1 Bacterial strains and cultivation

L. paracasei CCFM1224 was obtained from the Culture Collection of Food Microorganisms (CCFM), Jiangnan University. The strain was activated on solid MRS medium for 48 h, then a single colony was picked and inoculated into a simplified liquid MRS medium (containing per liter: glucose 15.0 g, peptone 5.0 g, yeast extract 5.0 g, disodium hydrogen phosphate 3.0 g, dipotassium hydrogen phosphate 3.0 g, diammonium hydrogen citrate 3.0 g, magnesium sulfate heptahydrate 0.1 g, manganese sulfate monohydrate 0.05 g, and Tween-80 1 mL) and cultured at 37°C for 14 h. This process was repeated for three consecutive generations to ensure culture stability before experimental use.

2.1.2 Preparation of inactivated bacterial components and fermentation supernatant

Bacterial cells from the fermented broth were harvested by centrifugation (8,000

× g, 20 min, 4°C). The pellet was washed 2-3 times with PBS buffer, then pasteurized (85°C, 30 min). Inactivation was confirmed by plate counting (<10³ CFU/mL). The inactivated bacterial cells were lyophilized and stored at -80°C. The fermentation supernatant was concentrated by rotary evaporation, filtered through 0.22 µm membrane, and stored at -80°C.

2.1.3 Preparation of cell walls

The cell wall isolation and extraction method was adapted from the protocol reported by Schwarzer et al. with appropriate modifications[13]. Briefly, bacterial pellets were washed and resuspended in deionized water, then heated in a boiling water bath for 10 min. The pellets were collected and treated with 5% (w/v) SDS in 50 mM MES buffer (pH 5.5) at 100°C to remove membrane components. After repeated washing to remove SDS, the crude cell wall fraction was sequentially digested with streptavidin (2 mg/mL, 60°C, 90 min), trypsin (200 µg/mL, 37°C, 2 h), and DNase/RNase (50 µg/mL each, 37°C, 1 h) to remove associated proteins and nucleic acids. The purified cell wall material was washed extensively, lyophilized, and stored at -80°C.

2.1.4 Preparation of cell wall proteins

Cell wall-associated proteins were extracted using a lysozyme-based protoplast formation method[14]. Bacterial pellets were resuspended in a protoplast-stabilizing buffer (0.02 M HEPES, 1 mM MgCl₂, 0.5 M lactose, pH 6.4) containing lysozyme (10 mg/L) and incubated at 37°C until microscopic observation confirmed complete morphological transition from rods to spheres. The mixture was centrifuged at low speed (1,500 × g, 15 min) to remove intact cells and debris. Proteins in the supernatant were precipitated with ice-cold trichloroacetic acid (12% w/v, final concentration), collected by centrifugation, dialyzed (14 kDa cutoff) against distilled water at 4°C for 24 h, and finally lyophilized.

2.1.5 Preparation of bacterial lipid

The method for isolating and extracting bacterial lipids was adapted from the protocol reported by Monnot et al[15]. Bacterial pellets were washed with 20 mM sodium acetate buffer (pH 4.5) and resuspended in a chloroform/methanol mixture (1:2,

v/v). After high-pressure homogenization and stirring, the mixture was centrifuged ($8,000 \times g$, 20 min). The supernatant was collected and allowed to phase-separate; the lower chloroform layer containing the lipids was carefully recovered. The solvent was evaporated under a gentle stream of nitrogen gas, and the dried lipid extract was stored at -80°C .

2.1.6 Preparation of protoplasts

Protoplasts were generated using the method described above for cell wall protein extraction[16]. Following lysozyme treatment and low-speed centrifugation, the pellet containing protoplasts was collected, washed, lyophilized, and stored at -80°C for subsequent animal experiments.

2.2 *In vivo* experiment

2.2.1 Animal experimental design

All animal experiment protocols were approved by the Experimental Animal Ethics Committee of Jiangnan University and were in compliance with institutional guidelines (JN.No20240630c1381018[335]). Male C57BL/6N mice were purchased from Vital River Co., Ltd. (Beijing, China). After one week of acclimatization under specific pathogen-free (SPF) conditions, the mice were randomly assigned to experimental groups ($n = 6$ per group), all groups except the control group were fed a high-fat diet (HFD, TP23300, TROPHIC, Nantong, China), while the control group received normal-fat diet (NFD, TP23302, TROPHIC, Nantong, China).

Batch 1 Experiment: Mice were divided into five groups: (1) Control group; (2) Model group; (3) Medium group: sterile simplified MRS culture medium (80 mg/kg/day); (4) CCFM1224-FS group: fermentation supernatant (FS, equivalent to $5 \times 10^9 \text{ CFU/mouse/day}$); (5) CCFM1224-IB group: inactivated bacterial cells (IB, $5 \times 10^9 \text{ CFU/mouse/day}$).

Batch 2 Experiment: Mice were divided into five groups: (1) Control group; (2) Model group; (3) CCFM1224-CWP group: cell wall proteins (CWP, extracted from $1 \times 10^{10} \text{ CFU bacterial mass/day}$); (4) CCFM1224-CW group: cell wall (CW, extracted from $1 \times 10^{10} \text{ CFU/day}$); (5) CCFM1224-Lipid group: total lipids (extracted from 1×10^{10}

CFU/day); (6) CCFM1224-Pt group: protoplasts (Pt, extracted from 1×10^{10} CFU/day).

All treatments were administered once daily via oral gavage in a 0.2 mL saline vehicle for 12 consecutive weeks. The control and model groups received an equal volume of saline. Body weight was recorded weekly.

2.2.2 Sample collection

At the end of the 12-week intervention, mice were fasted overnight and anesthetized. Blood was collected from the orbital sinus and centrifuged to obtain serum, which was stored at -80°C for subsequent analysis. Liver and epididymal white adipose tissue (eWAT) were promptly excised, weighed, and either fixed for histology or snap-frozen in liquid nitrogen and stored at -80°C for molecular analysis.

2.2.3 Oral glucose tolerance test (OGTT)

OGTT was conducted on all animals at week 12 of the intervention. After a 12 h fast, mice received an oral administration of 40% glucose solution at a dose of 2 mg/g body weight. Blood glucose levels were measured from the tail tip at 0, 15, 30, 60, 90, and 120 min post-administration using a glucometer. The area under the curve (AUC) for glucose was calculated.

2.2.4 Serum biochemical index assays

Serum biochemical markers were measured using the Mindray fully automated biochemical analyzer (Mindray, Shenzhen, China), including TC, TG, LDL-C, HDL-C, ALT, AST. Serum hormone levels of GLP-1 (SBJ-M0568), ADP (SBJ-M0862), PYY (SBJ-M0511), INS (SBJ-M0417), and leptin (SBJ-M0679) were quantified using specific commercial ELISA kits according to the manufacturers' instructions (Nanjing SenBeiJia Biological Technology Co., Ltd. , Nanjing, China).

2.2.5 Histopathological analysis

Liver and eWAT samples were fixed in 4% paraformaldehyde. After paraffin embedding, tissue sections (5 μm) were stained with hematoxylin and eosin (H&E). For hepatic lipid visualization, frozen liver sections were stained with Oil Red O. All stained sections were scanned using a digital slide scanner (Panoramic MIDI II, 3DHISTECH, Hungary) and evaluated.

2.2.6 Hepatic inflammatory marker assay

Liver tissues were homogenized in ice-cold PBS (1:9, w/v). The homogenates were centrifuged ($2,500 \times g$, 10 min, 4°C), and the supernatants were collected. Levels of IL-6(SBJ-M0657), TNF- α (SBJ-M0030), and IL-1 β (SBJ-M0027) were measured using ELISA kits according to the manufacturers' protocols(Nanjing SenBeiJia Biological Technology Co., Ltd. , Nanjing, China).

2.2.7 Real-time quantitative reverse transcription PCR (qRT-PCR)

RNA was extracted from adipose tissue using TRIzol® (Vazyme Biotech Co., Ltd., Nanjing, China). Reverse transcription was performed using a commercial kit (R334-01, Vazyme Biotech Co., Ltd., Nanjing, China). cDNA was synthesized with HiScript® III RT SuperMix for qPCR (+gDNA wiper) (R323, Vazyme Biotech Co., Ltd., Nanjing, China). Quantitative real-time polymerase chain reaction (qPCR) was performed using ChamQ Universal SYBR qPCR Master Mix (Q711, Vazyme Biotech Co., Ltd., Nanjing, China) on a BioRad-CFX384 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). Gene expression levels were normalized to β -actin and calculated using the $2^{-\Delta\Delta Ct}$ method. The specific primers used are listed in Supplementary Table S1.

2.3 Non-targeted lipid metabolomics analysis of the liver

A portion of the frozen liver tissue was used for lipidomic profiling. Lipids were extracted using a methyl tert-butyl ether (MTBE)/methanol method. Lipid separation was performed using ultra-high-performance liquid chromatography (UHPLC) on a reversed-phase column. Mass spectrometric analysis was conducted on an Orbitrap Exploris 120 mass spectrometer in both positive and negative ionization modes. Data processing, including peak alignment, identification, and quantification, was performed using XCMS software and an in-house lipid database.

2.4 Statistical analysis

Experimental data are expressed as mean $\pm$ standard deviation (SD). Data visualization and statistical analysis were performed using GraphPad Prism 9.0 software, with the Shapiro-Wilk normality test employed to assess sample normality. Statistical analysis employed one-way analysis of variance (ANOVA). Tukey's multiple comparison test was used to determine the significance of intergroup differences. For

the assessment of intergroup significance, statistically significant differences were defined as $p < 0.05$, with $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ indicating higher levels of significance.

3. Results

3.1 *L. paracasei* CCFM1224 inactivated bacterial cells improve obesity, glucose and lipid metabolism, and organ coefficients

To determine whether the anti-obesity bioactivity resided in the bacterial cells or the metabolites secreted into the medium, we separated the postbiotics into fermentation supernatant (CCFM1224-FS) and inactivated bacterial cells (CCFM1224-IB) components. These were administered to HFD-induced obese mice for a 12-week intervention.

As expected, HFD induced a significant increase in body weight compared to the control group (93.79% higher weight gain, $p < 0.001$; Fig. 1a, b). CCFM1224-IB significantly suppressed this weight gain, resulting in a 25.88% reduction compared to the model group ($p < 0.001$). In contrast, intervention with either the medium or CCFM1224-FS had no significant effect on body weight (Fig. 1a, b). Consistent with body weight changes, the model group exhibited significant increases in both liver weight and eWAT mass ($p < 0.001$; Fig. 1c, f). CCFM1224-IB markedly reduced liver weight by 23.92% ($p < 0.01$) and eWAT mass by 33.51% ($p < 0.001$) compared to the model group (Fig. 1c, f). Neither the medium nor CCFM1224-FS treatments affected these organ weights.

Oral glucose tolerance tests (OGTT) revealed that HFD mice developed impaired glucose tolerance. Treatment with CCFM1224-IB significantly improved glucose clearance (Fig. 1d), which was corroborated by a reduced AUC (Fig. 1e).

Serum lipid profiling showed that HFD elevated levels of LDL-C, TC, and TG. CCFM1224-IB effectively reversed these changes, significantly lowering serum LDL-C (by 47.96%, $p < 0.001$), TC (by 18.69%, $p < 0.05$), and TG (by 26.87%, $p < 0.01$) (Fig. 1h-j). No significant changes were observed in high-density lipoprotein cholesterol (HDL-C) among groups (Fig. 1g). The medium and CCFM1224-FS groups

showed no improvement in the serum lipid profile.

In summary, the anti-obesity and metabolic-improving effects of *L. paracasei* CCFM1224 postbiotics are primarily attributed to components within the inactivated bacterial cells, not the fermentation supernatant. These findings guided the subsequent fractionation of the bacterial cells to identify the specific active substance.

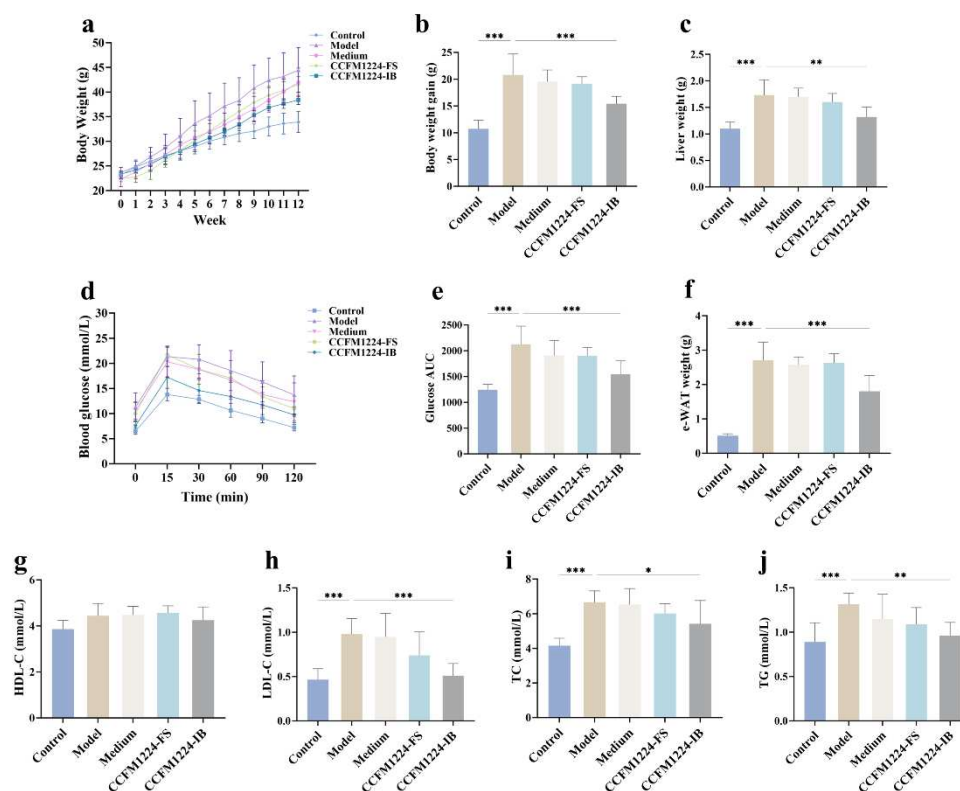


Fig. 1 Effects of *L. paracasei* CCFM1224 fermentation supernatant and bacterial cells on body weight, organ index, fasting blood glucose, and blood lipids. (a) Body weight changes over 12 weeks. (b) Total weight gain. (c) Liver weight. (d) OGTT showed changes in blood glucose at different time points after oral glucose. (e) The AUC according to the OGTT curve. (f) e-WAT weight. (g-j) Concentrations of serum lipids indices, namely TG, TC, LDL-C and HDL-C. significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. model group

3.2 *L. paracasei* CCFM1224 inactivated bacterial cells alleviate hepatic steatosis, adipose hypertrophy, and associated inflammation

We next investigated the effects of CCFM1224-IB on tissue-level pathology and

inflammation associated with HFD-induced obesity. Histological analysis revealed that HFD feeding caused severe hepatic steatosis, as evidenced by extensive lipid vacuolation in H&E-stained liver sections (Fig. 2a). Oil Red O staining confirmed a marked accumulation of neutral lipid droplets in the livers of HFD-fed mice (Fig. 2b). Intervention with CCFM1224-IB significantly attenuated this hepatic lipid accumulation, reducing both the number and size of lipid vacuoles and lipid droplets (Fig. 2a, b).

Hepatic steatosis was accompanied by liver injury, indicated by significantly elevated serum levels of the liver enzymes ALT and AST in the Model group. Treatment with CCFM1224-IB significantly reduced serum ALT by 40.03% ($p < 0.001$) and AST by 24.74% ($p < 0.05$) compared to the model group, whereas CCFM1224-FS only modestly reduced ALT levels ($p < 0.05$) (Fig. 2d, e).

Given the established link between lipid accumulation and chronic inflammation, we measured the levels of key pro-inflammatory cytokines in liver tissue. The model group exhibited a significant upregulation of IL-1 β , IL-6, and TNF- α ($p < 0.001$). CCFM1224-IB intervention potently suppressed this inflammatory response, reducing hepatic IL-1 β by 20.73% ($p < 0.01$), IL-6 by 26.66% ($p < 0.001$), and TNF- α by 26.80% ($p < 0.001$). Neither the medium nor CCFM1224-FS treatments showed significant anti-inflammatory effects (Fig. 2f-h).

Analysis of eWAT morphology further supported the anti-obesity effects. H&E staining showed that adipocytes in the control group were uniformly sized and regularly arranged. In contrast, the model group exhibited pronounced adipocyte hypertrophy. CCFM1224-IB intervention effectively reduced adipocyte size, indicating suppression of fat storage and tissue expansion (Fig. 2c).

Collectively, these results demonstrate that *L. paracasei* CCFM1224-IB not only reduces systemic and tissue-level fat accumulation but also ameliorates the concomitant liver injury and low-grade inflammatory state, highlighting its multifaceted protective role against HFD-induced metabolic dysfunction.

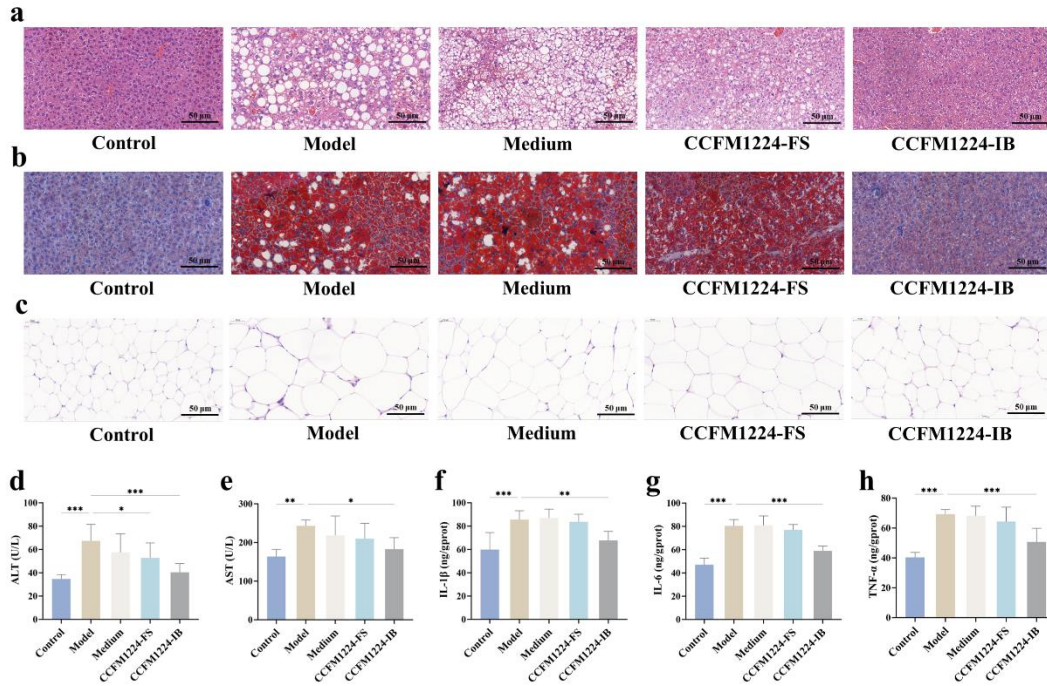


Fig. 2 Effects of *L. paracasei* CCFM1224 fermentation supernatant and bacterial cells on hepatic steatosis, lipid accumulation, liver function, hepatic inflammation. (a) H&E staining of the liver (magnification 200 ×). (b) Oil Red O staining of the liver (magnification 200 ×). (c) H&E staining of e-WAT (magnification 200 ×). (d-e) Serum content of ALT, AST. (f) IL-1β. (g) IL-6. (h) TNF-α. significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. model group

3.3 The lipid fraction is identified as the primary active component within *L. paracasei* CCFM1224 bacterial cells

Following the identification of anti-obesity activity in CCFM1224-IB, we sought to isolate the specific bioactive component. The CCFM1224-IB fraction was further separated into cell wall proteins (CWP), cell wall (CW), total lipids (Lipid), and protoplasts (Pt). These subfractions were administered to HFD-fed mice in a second 12-week intervention study.

HFD feeding again induced significant obesity, with the model group showing an 89.92% greater weight gain than the control group ($p < 0.001$; Fig. 3a, b). Among all subfractions tested, only the CCFM1224-Lipid intervention significantly attenuated HFD-induced weight gain, resulting in a 23.88% reduction compared to the model

group ($p < 0.001$; Fig. 3a, b). Interventions with CWP, CW, or Pt showed no significant effect on body weight. Consistent with systemic weight changes, HFD feeding markedly increased liver and epididymal white adipose tissue (eWAT) weights. The CCFM1224-Lipid intervention significantly reduced liver weight by 37.67% ($p < 0.001$) and eWAT mass by 21.23% ($p < 0.05$) relative to the model group (Fig. 3c, f). No other bacterial subfraction significantly affected these organ weights.

In the OGTT, the model group exhibited impaired glucose clearance, which was significantly improved by CCFM1224-Lipid treatment (Fig. 3d). This improvement was confirmed by a 30.22% reduction in the glucose AUC ($p < 0.001$; Fig. 3e). Other subfractions did not improve glucose tolerance.

Serum lipid profiling revealed that the CCFM1224-Lipid intervention effectively counteracted HFD-induced dyslipidemia. It significantly lowered serum levels of LDL-C by 36.31% ($p < 0.01$), TC by 25.59% ($p < 0.05$), and TG by 22.38% ($p < 0.05$) compared to the model group (Fig. 3h-j). No significant changes were observed in HDL-C across groups (Fig. 3g). The CWP, CW, and Pt subfractions failed to modulate the serum lipid profile.

In conclusion, a systematic bioactivity-guided fractionation revealed that the total lipids extract from *L. paracasei* CCFM1224 is the principal subfraction responsible for the previously observed anti-obesity and metabolic benefits of the inactivated bacterial cells. This finding directs subsequent mechanistic investigations towards the lipid component.

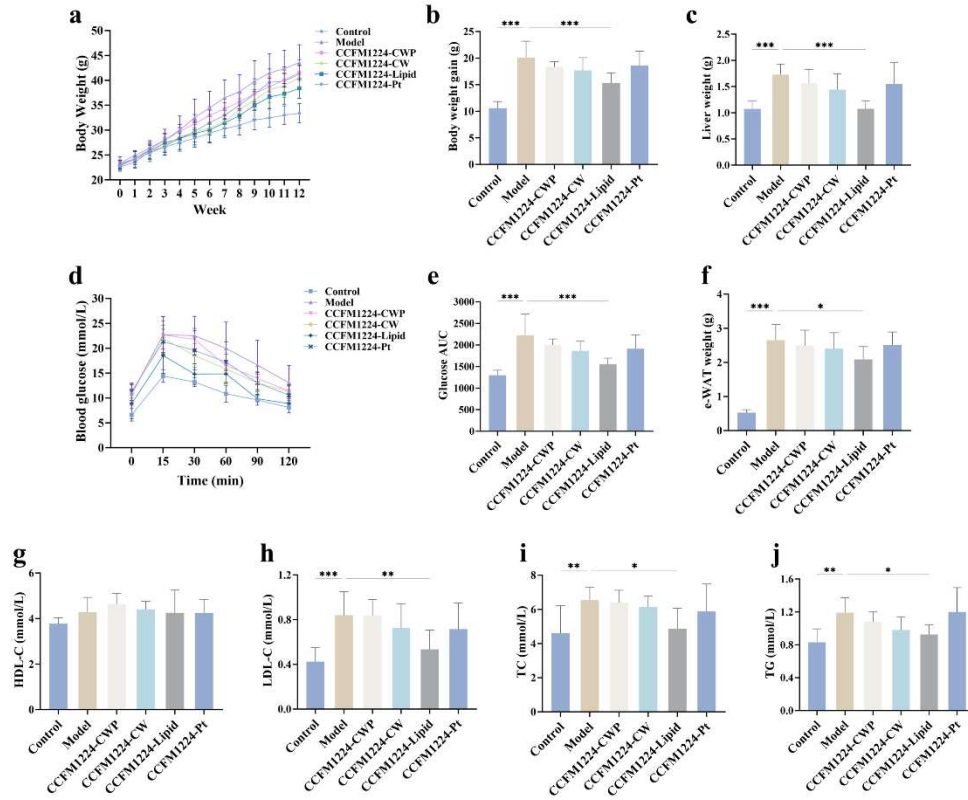


Fig. 3 Effects of individual components of *L. paracasei* CCFM1224 bacterial cells on body weight, organ index, fasting blood glucose, and blood lipids. (a) Body weight changes over 12 weeks. (b) Total weight gain. (c) Liver weight. (d) OGTT showed changes in blood glucose at different time points after oral glucose. (e) The AUC according to the OGTT curve. (f) e-WAT weight. (g-j) Concentrations of serum lipids indices, namely TG, TC, LDL-C and HDL-C. significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. model group

3.4 *L. paracasei* CCFM1224 lipids alleviate hepatic steatosis, adipose hypertrophy, and associated inflammation

To substantiate the systemic benefits of the lipid fraction, we examined its effects on tissue pathology, hepatic function, and local inflammatory status. Histological assessment of liver tissue confirmed that HFD feeding induced severe steatosis, characterized by extensive lipid vacuolation in H&E-stained sections (Fig. 4a) and pronounced neutral lipid accumulation in Oil Red O-stained sections (Fig. 4b). Treatment with CCFM1224-Lipid markedly attenuated these morphological alterations, demonstrating a clear reduction in hepatic lipid deposition (Fig. 4a, b). In contrast,

interventions with other bacterial components (CWP, CW, Pt) failed to significantly ameliorate hepatic steatosis.

Consistent with the observed lipid accumulation, serum levels of the liver injury markers ALT and AST were significantly elevated in the model group. CCFM1224-Lipid intervention effectively reversed this hepatocyte damage, significantly reducing serum ALT by 36.46% ($p < 0.001$) and AST by 19.51% ($p < 0.05$) compared to the model group (Fig. 4d, e). No other subfraction showed comparable hepatoprotective effects.

Given the established link between obesity-associated steatosis and chronic inflammation, we quantified key pro-inflammatory cytokines in liver tissue. The model group exhibited a significant upregulation of IL-1 β , IL-6, and TNF- α . Importantly, CCFM1224-Lipid administration potently suppressed this local inflammatory response, reducing hepatic IL-1 β by 18.65% ($p < 0.05$), IL-6 by 28.17% ($p < 0.001$), and TNF- α by 26.41% ($p < 0.001$) (Fig. 4f-h). The other bacterial component groups did not exhibit significant anti-inflammatory activity.

Further examination of eWAT morphology reinforced the tissue-specific benefits. H&E staining revealed that adipocytes in the control group were uniformly sized and neatly arranged, whereas the model group displayed significant adipocyte hypertrophy. Administration of CCFM1224-Lipid effectively reduced adipocyte size, indicating a suppression of lipid storage and adipose tissue expansion (Fig. 4c).

These results demonstrate that the *L. paracasei* CCFM1224 lipid component is uniquely effective in mitigating HFD-induced pathologies at the tissue level. It not only reduces ectopic lipid accumulation in the liver and adipose tissue but also alleviates associated hepatocellular injury and local inflammatory signaling, confirming its role as the key bioactive substance within the bacterial cell.

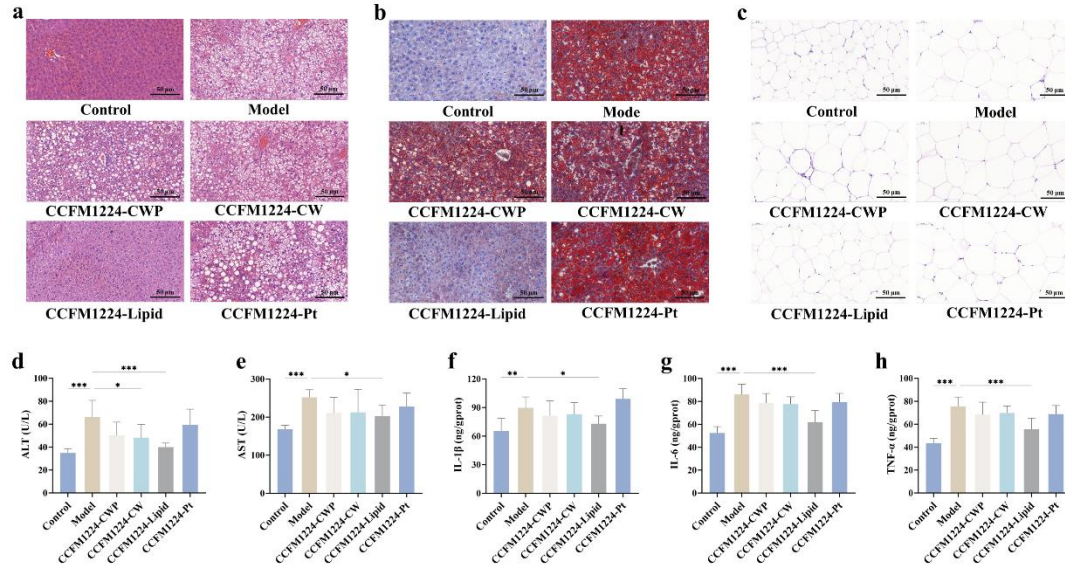


Fig. 4 Effects of individual components of *L. paracasei* CCFM1224 bacterial cells on hepatic steatosis, lipid accumulation, liver function, hepatic inflammation. (a) H&E staining of the liver (magnification 200 $\times$). (b) Oil Red O staining of the liver (magnification 200 $\times$). (c) H&E staining of e-WAT (magnification 200 $\times$). (d-e) Serum content of ALT, AST. (f) IL-1 β . (g) IL-6. (h) TNF- α . significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. model group

3.5 *L. paracasei* CCFM1224 lipids regulating key metabolic hormones

To explore the potential endocrine mechanisms underlying the anti-obesity effects of CCFM1224 lipids, we measured serum levels of Insulin (INS), glucagon-like peptide-1 (GLP-1), peptide YY (PYY), leptin, and adiponectin (ADP).

HFD-fed mice developed hyperinsulinemia ($p < 0.001$ vs. control), indicative of insulin resistance. Intervention with CCFM1224-Lipid significantly ameliorated this condition, reducing serum INS levels by 25.50% compared to the model group ($p < 0.001$; Fig. 5a). A similar but less pronounced effect was observed with the cell wall fraction ($p < 0.05$), aligning with the improved glucose tolerance observed in the OGTT.

Serum levels of the gut-derived satiety hormones GLP-1 and PYY were significantly suppressed in the model group ($p < 0.01$ and $p < 0.001$, respectively). Treatment with CCFM1224-Lipid effectively restored their circulating concentrations, increasing GLP-1 by 14.76% ($p < 0.05$) and PYY by 36.21% ($p < 0.001$) relative to the

model group (Fig. 5b, c). This suggests that CCFM1224 lipids may suppress food intake and weight gain by enhancing satiety signaling.

Furthermore, HFD feeding induced a profound dysregulation of adipokine profiles, characterized by a significant elevation in serum leptin ($p < 0.001$) and a marked reduction in ADP ($p < 0.001$). CCFM1224-Lipid intervention counteracted these alterations, lowering leptin by 26.84% ($p < 0.001$) and elevating ADP by 28.45% ($p < 0.001$) (Fig. 5d, e). The CCFM1224-Pt also reduced leptin and increased ADP, while the CCFM1224-CW fraction elevated ADP levels.

In summary, *L. paracasei* CCFM1224 lipids intervention orchestrates a favorable shift in key metabolic hormones: it enhances gut satiety signals (GLP-1, PYY) and the insulin-sensitizing adipokine ADP, while reducing the levels of insulin and the pro-obesity hormone leptin. These multi-hormonal adjustments provide a plausible systemic mechanism contributing to its anti-obesity and metabolic-improving effects.

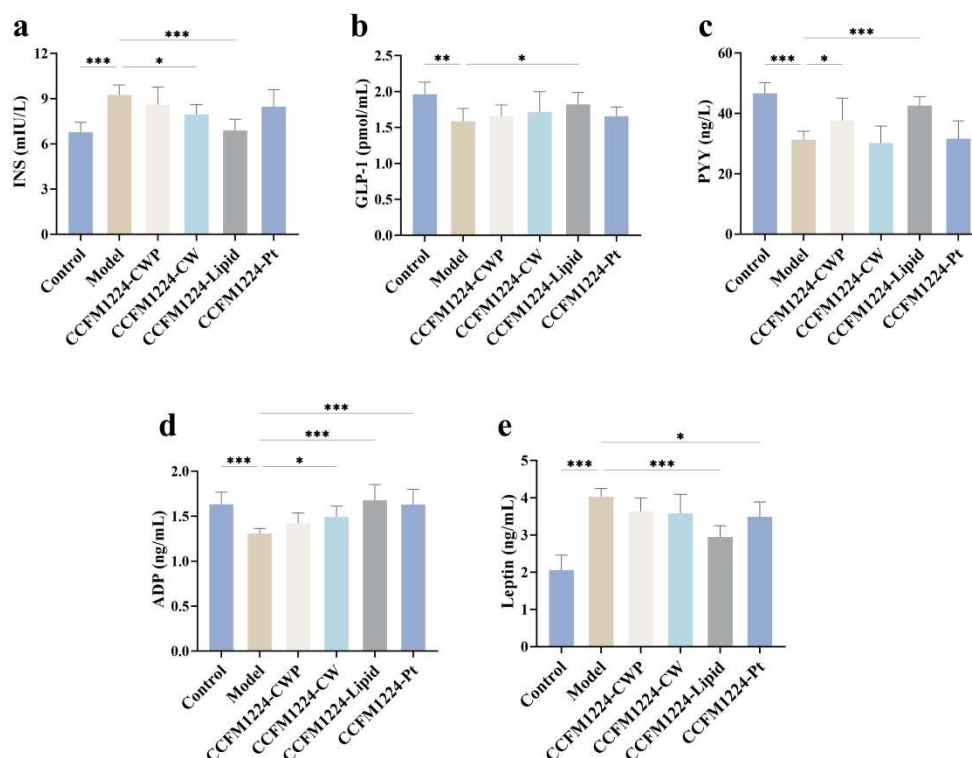


Fig. 5 Effects of individual components of *L. paracasei* CCFM1224 bacterial cells on serum-related hormones. (a) Serum level of INS. (b) Serum level of GLP-1. (c) Serum level of PYY. (d) Serum level of ADP. (e) Serum level of Leptin. significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

0.05, ** $p < 0.01$, *** $p < 0.001$ vs. model group

3.6 *L. paracasei* CCFM1224 lipids regulate adipose tissue gene expression toward a favorable metabolic phenotype

To elucidate the mechanisms underlying the reduction in fat mass, we analyzed the expression of key genes involved in lipid metabolism and energy expenditure in eWAT.

HFD significantly altered the transcriptional profile in eWAT, promoting a lipogenic state. Specifically, mRNA levels of the lipogenic transcription factors PPAR- γ and SREBP-1c were markedly upregulated in the model group compared to the control group (Fig. 6a, b). Conversely, the expression of genes associated with lipolysis and fatty acid oxidation—PPAR- α and HSL—was significantly suppressed (Fig. 6c, d). No significant changes were observed in the expression of CPT1 or UCP1 at this point (Fig. 6e, f).

Treatment with CCFM1224-Lipid effectively reversed these HFD-induced transcriptional alterations. It significantly downregulated the expression of lipogenic genes, reducing PPAR- γ by 35.50% ($p < 0.01$) and SREBP-1c by 25.78% ($p < 0.01$) compared to the model group (Fig. 6a, b). Concurrently, CCFM1224-Lipid intervention upregulated genes involved in lipid catabolism and energy dissipation: PPAR- α increased by 63.26% ($p < 0.05$), HSL by 63.53% ($p < 0.05$), and CPT1 by 53.27% ($p < 0.01$) (Fig. 6c-e). Notably, a profound induction of the thermogenic gene UCP1 was observed, with mRNA levels increasing by 172.75% ($p < 0.01$) in the CCFM1224-Lipid group relative to the model group (Fig. 6f).

In summary, *L. paracasei* CCFM1224 lipids remodel adipose tissue metabolism by coordinately suppressing lipogenic pathways and activating those for lipid breakdown, fatty acid oxidation, and thermogenesis. This transcriptional shift from a lipogenic to a catabolic/thermogenic phenotype underpins the observed reduction in adiposity and systemic metabolic improvements.

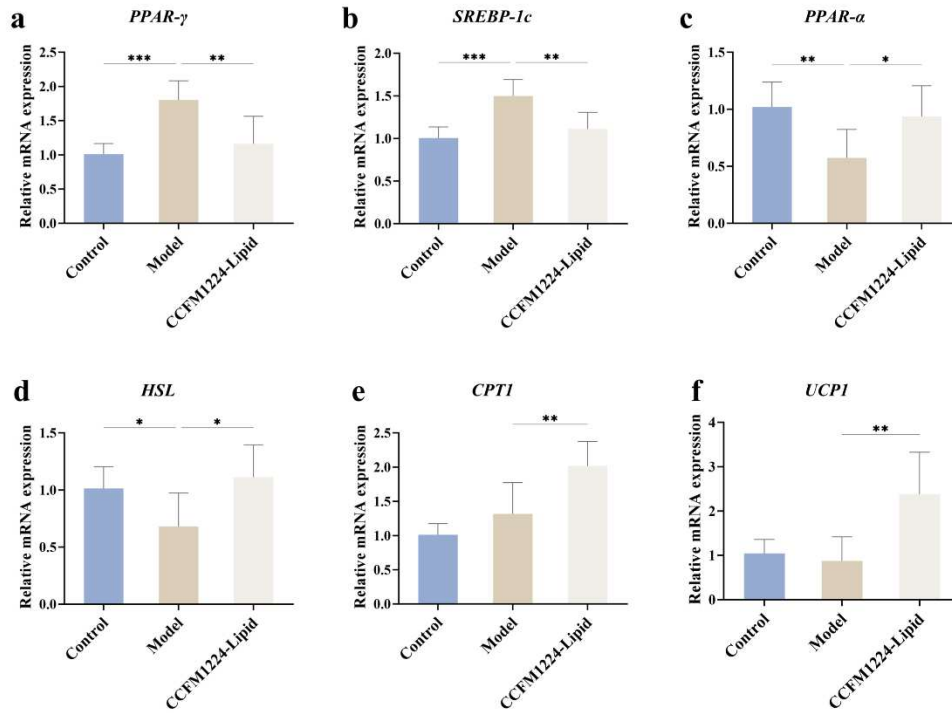


Fig. 6 CCFM1224-Lipid effects on gene expression related to lipid metabolism

(a)Relative mRNA expression of *PPAR- γ* . (b)Relative mRNA expression of *SREBP-1c*. (c)Relative mRNA expression of *PPAR- α* . (d)Relative mRNA expression of *HSL*. (e)Relative mRNA expression of *CPT1*. (f)Relative mRNA expression of *UCPI*. significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. model group

3.7 Effects of *L. paracasei* CCFM1224 lipids on liver lipidome

To investigate the metabolic alterations underlying the hepatoprotective effects of CCFM1224-Lipid, we performed non-targeted lipidomics on liver tissues from HFD-fed mice in the model and CCFM1224-Lipid groups. Multivariate analysis using OPLS-DA revealed a clear separation between the two groups along the predictive component (Fig. 7a), indicating that CCFM1224-Lipid intervention significantly reshaped the hepatic lipidome. Volcano plot analysis (fold change > 2 , VIP > 1 , $p < 0.05$) identified 61 significantly differential lipid metabolites. Compared to the model group, the CCFM1224-Lipid group exhibited 48 upregulated and 13 downregulated metabolites (Fig. 7b). A hierarchical clustering heatmap of these differential metabolites further illustrated distinct lipid profiles between the groups (Fig. 7c). Notably, CCFM1224-

Lipid intervention induced a broad downregulation of TG species, aligning with the observed reduction in hepatic steatosis. Conversely, several glycerophospholipid classes were upregulated, including sphingomyelins (SM), phosphatidylcholines (PC), and phosphatidylethanolamines (PE). Further analysis of the top ten most upregulated lipid species (Fig. 7d) revealed that SM lipids constituted the highest proportion among these significantly elevated metabolites. This suggests that modulation of sphingomyelin metabolism may be a prominent feature of the CCFM1224-Lipid intervention, potentially contributing to its metabolic benefits by influencing membrane integrity, lipid signaling, or inflammatory pathways.

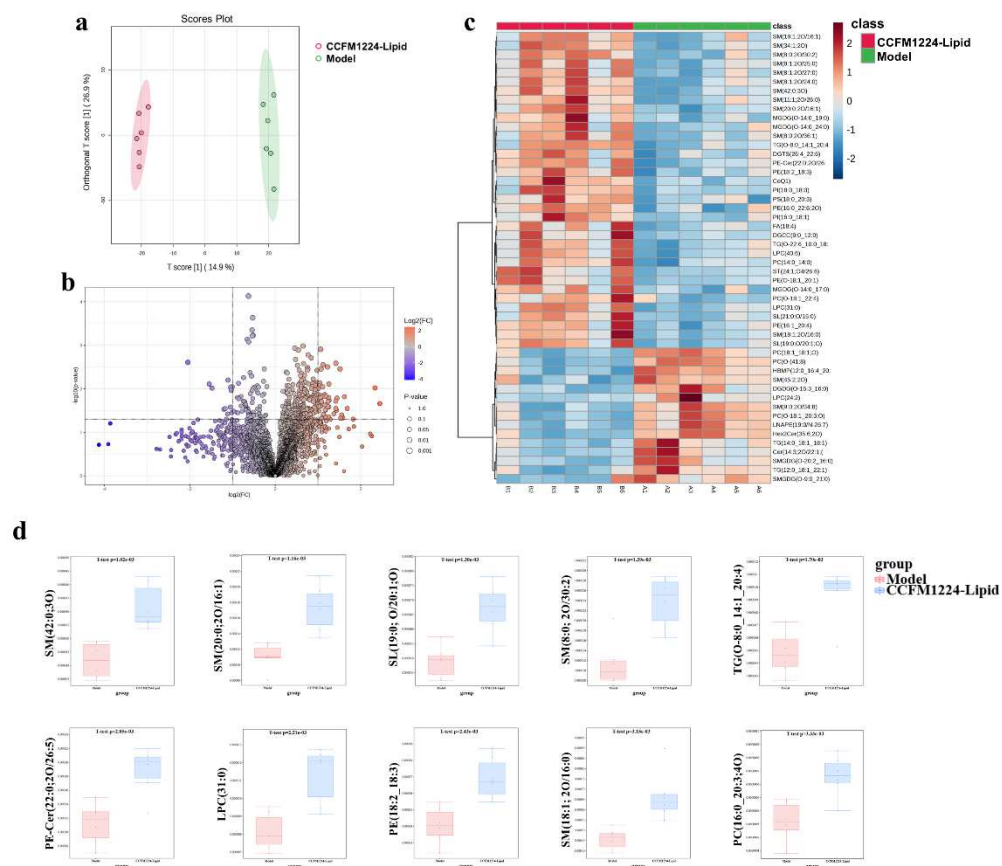


Fig. 7 CCFM1224-Lipid effects on hepatic lipid metabolism. (a) OPLS-DA Score Plot. (b) Volcano plots showed the differential metabolites between CCFM1224-Lipid and model group ($FC > 2$, $VIP > 1$, FDR-adjusted p -value < 0.05). (c) Heatmap of relative abundance of differential metabolites in liver between model and CCFM1224-Lipid. (d) Boxplot analysis for group control vs CCFM1224-Lipid (The top 10 lipid species with

higher content in CCFM1224-Lipid compared to model)

4. Discussion

The growing global epidemic of obesity necessitates innovative and effective intervention strategies[17]. Postbiotics have emerged as promising candidates due to their enhanced stability, safety profile, and defined composition compared to live probiotics[18]. While accumulating evidence supports the health benefits of various postbiotics, the precise bioactive constituents responsible for specific effects, such as anti-obesity activity, often remain uncharacterized, limiting mechanistic understanding and translational application[19-21]. Our previous work demonstrated that postbiotics from *L. paracasei* CCFM1224 ameliorated HFD-induced obesity in mice[12]. The present study builds upon this foundation by employing a systematic, bioactivity-guided fractionation approach to identify the key active component and elucidate its multifaceted mechanisms of action.

Postbiotics primarily comprise bacterial components and metabolites [8]. Our initial comparative study revealed that the anti-obesity efficacy resided not in the fermentation supernatant (FS) but within the inactivated bacterial cells (IB) of *L. paracasei* CCFM1224. The IB fraction effectively counteracted hallmark features of HFD-induced metabolic syndrome, including weight gain, visceral adiposity, dyslipidemia, glucose intolerance, hepatic steatosis, and associated inflammation. This finding directed our subsequent investigation toward the cellular components.

Further fractionation of the IB into cell wall proteins, cell wall, total lipids, and protoplasts unequivocally identified the total lipid extract as the principal bioactive fraction. The CCFM1224-Lipid intervention recapitulated the full spectrum of beneficial effects observed with the whole IB, whereas other subfractions showed minimal or no activity. Microbial-derived lipids can be directly sensed by the host, modulating innate and adaptive immune pathways and regulating metabolic pathways, thereby influencing the progression of chronic inflammation, autoimmune diseases, cardiovascular diseases, and metabolic syndrome[22]. For instance, phospholipids (a15:0-i15:0 PE) isolated from AKK selectively induce proinflammatory cytokines via

the TLR2-TLR1 heterodimer signaling pathway to maintain immune homeostasis[23]; Turicibacter lipids suppress weight gain, lower blood glucose, and reduce fat accumulation in mice[24]; Bacterial sphingolipids regulate host immune and inflammatory responses while participating in host metabolic pathways, altering hepatic metabolism in mice[25-27]. Our research findings also confirm these phenomena and demonstrate excellent weight loss effects.

To decipher the mechanisms by which CCFM1224-Lipid exerts its effects, we investigated its impact on systemic hormone regulation. Obesity is frequently associated with hormonal dysregulation, including hyperinsulinemia, leptin resistance, and diminished secretion of satiety hormones like GLP-1 and PYY. Our results demonstrate that CCFM1224-Lipid orchestrates a favorable hormonal recalibration: it significantly reduced circulating insulin and leptin levels while elevating GLP-1, PYY, and adiponectin[28, 29]. The enhancement of gut-derived satiety signals (GLP-1, PYY) suggests a potential mechanism for reduced food intake and weight gain, while the amelioration of hyperinsulinemia and the increase in insulin-sensitizing adiponectin point to improved systemic metabolic homeostasis[10, 30]. Among these, leptin acts on the hypothalamus to regulate hunger and energy balance, with obese individuals typically exhibiting elevated leptin levels[31]. Adiponectin (ADP) exerts anti-inflammatory, anti-atherosclerotic effects and enhances insulin sensitivity, its levels inversely correlating with fat accumulation[32]. CCFM1224-Lipid intervention significantly reduced serum leptin levels and elevated serum ADP levels. *L. paracasei* CCFM1224 lipids alleviates obesity by synergistically regulating multiple hormone secretions.

Beyond systemic endocrine modulation, we investigated how CCFM1224-Lipid remodels white adipose tissue (WAT) function at the molecular level. Obesity is characterized by excessive fat accumulation and is closely associated with lipid metabolism, including lipogenesis, lipolysis, and lipidation[33]. Therefore, we quantified the mRNA expression levels of genes related to lipid metabolism in epididymal adipose tissue to explore potential molecular mechanisms. At the tissue level, we found that CCFM1224-Lipid profoundly reprogrammed the gene expression

profile in WAT toward a metabolically favorable phenotype. It downregulated key lipogenic transcription factors (*PPAR-γ* and *SREBP-1c*) and concurrently upregulated genes governing lipolysis (*HSL*), fatty acid oxidation (*PPAR-α*, *CPT1*), and thermogenesis (*UCP1*). *PPAR-γ* and *SREBP-1c* are key transcription factors regulating lipogenesis; their excessive activation drives lipid synthesis and storage[34, 35]. *HSL* is the key enzyme catalyzing the hydrolysis of stored triglycerides into free fatty acids[36], *PPAR-α* is a critical regulator of the fatty acid oxidation pathway[37], and *CPT1* is the rate-limiting enzyme for fatty acid β -oxidation[38]. *UCP1* is a key regulator of non-shivering thermogenesis in brown and beige adipocytes, promoting energy expenditure in adipose tissue[39]. This coordinated shift from a lipid-storing to a lipid-oxidizing and energy-dissipating state in WAT provides a compelling molecular explanation for the reduced adiposity and improved lipid metabolism.

Long-term high-fat diets lead to abnormal accumulation of liver triglycerides[40]. The integration of non-targeted liver lipidomics provided a deeper, metabolite-level understanding of the hepatoprotective effects. The OPLS-DA model confirmed a significant restructuring of the hepatic lipidome by CCFM1224-Lipid. The most prominent change was a broad reduction in TG species, which correlates directly with the observed attenuation of hepatic steatosis in histological analyses. Concurrently, we observed an upregulation of specific glycerophospholipids, including PC and PE, which are crucial for maintaining membrane integrity and facilitating hepatic lipid export[41]. PC serves as a key precursor for lipoprotein synthesis, promoting hepatic fat transport to extrahepatic tissues and thereby reducing hepatic lipid deposition[42]. Meanwhile, increased PE abundance—a crucial cell membrane constituent—helps protect hepatocytes from structural damage induced by high-fat diets[43]. Therefore, CCFM1224 may enhance hepatic lipid efflux capacity and strengthen hepatocyte membrane stability by upregulating PC and PE in the liver, ultimately benefiting hepatic lipid metabolism. Most strikingly, SM constituted the highest proportion among the top upregulated lipid species. SM is a structural component widely present in cell membranes and serves as a crucial signaling molecule involved in regulating inflammation, immunity, autophagy, and cell growth and survival[25]. The increase in

hepatic SM could reflect enhanced membrane synthesis or, intriguingly, a "substrate diversion" that potentially limits the production of lipotoxic ceramides, thereby alleviating metabolic inflammation and dysfunction. Excessive accumulation of sphingomyelin's hydrolysis product, ceramide, is a key driver of metabolic dysfunction-associated steatohepatitis (MASH) progression[44]. Studies indicate that inhibiting sphingomyelin-degrading enzymes (e.g., sphingomyelin phosphodiesterase 3, SMPD3) reduces ceramide accumulation, thereby alleviating hepatic inflammation and fibrosis[44]. This lipidomic signature suggests that CCFM1224-Lipid improves hepatic health not only by reducing neutral fat storage but also by enhancing membrane phospholipid pools and modulating bioactive sphingolipid pathways.

While this study demonstrates the anti-obesity potential of the CCFM1224 lipid fraction, several limitations warrant consideration and future investigation. First, the precise molecular structure(s) of the key bioactive lipid species within this complex mixture remain unidentified. Second, the mechanistic understanding is currently limited to correlative observations at the phenotypic and certain transcriptional/epigenetic levels. The specific molecular targets (e.g., receptors), downstream signaling cascades, and functional alterations at the cellular level that mediate these effects have not been elucidated.

5. Conclusion

This study delineates that the anti-obesity efficacy of *L. paracasei* CCFM1224 postbiotics is primarily mediated by the lipid fraction of its inactivated bacterial cells. The lipid components exerted multi-faceted protective effects across multiple levels. At the phenotypic level, they significantly reduce body weight gain, ectopic lipid deposition in the liver and adipose tissue, and improve glucose intolerance, dyslipidemia, and hepatic inflammation. At the endocrine level, they enhance satiety signaling through upregulation of gut hormones GLP-1 and PYY, while concurrently improving systemic insulin sensitivity and counteracting leptin resistance. At the molecular level, they reprogram white adipose tissue metabolism by downregulating key lipogenic genes (*PPAR- γ* and *SREBP-1c*) and upregulating genes involved in

lipolysis (*HSL*), fatty acid oxidation (*PPAR- α* , *CPT1*), and thermogenesis (*UCP1*). At the metabolomic level, they induce a favorable remodeling of the hepatic lipidome, characterized by a marked reduction in triglycerides and an elevation in membrane-stabilizing phospholipids (PC, PE) and sphingomyelins. Collectively, these findings systematically elucidate the multi-target mode of action underlying the anti-obesity effects of CCFM1224 lipids, providing a pivotal scientific foundation for developing precision nutrition strategies centered on microbial-derived lipid bioactives.

CRedit authorship contribution statement

Botao Wang: Writing – review & editing, Experimental operation, Formal analysis. **Xin Tang:** Writing – original draft, Investigation, Experimental design. **Yongli Lu:** Writing – original draft, Experimental operation, Data analysis. **Fei Liu:** Writing – review & editing, Data curation. **Bingyong Mao:** Methodology, Software. **Qiuxiang Zhang:** Investigation. **Jianxin Zhao:** Visualization, Supervision. **Wei Chen:** Supervision. **Haining Shi:** Project administration. **Ruiyan Wang:** Project administration. **Shumao Cui:** Resources, Project administration.

References

- [1] X. Wen, B. Zhang, B. Wu, H. Xiao, Z. Li, R. Li, X. Xu, T. Li, Signaling pathways in obesity: mechanisms and therapeutic interventions, *Sig Transduct Target Ther*, 7 (2022) 298.
- [2] X. Jin, T. Qiu, L. Li, R. Yu, X. Chen, C. Li, C.G. Proud, T. Jiang, Pathophysiology of obesity and its associated diseases, *Acta Pharmaceutica Sinica B*, 13 (2023) 2403-2424.
- [3] S.P. Bapat, C. Whitty, C.T. Mowery, Y. Liang, A. Yoo, Z. Jiang, M.C. Peters, L.-j. Zhang, I. Vogel, C. Zhou, V.Q. Nguyen, Z. Li, C. Chang, W.S. Zhu, A.T. Hastie, H. He, X. Ren, W. Qiu, S.G. Gayer, C. Liu, E.J. Choi, M. Fassett, J.N. Cohen, J.L. Sturgill, L.E. Crotty Alexander, J.M. Suh, C. Liddle, A.R. Atkins, R.T. Yu, M. Downes, S. Liu, B.S. Nikolajczyk, I.-K. Lee, E. Guttman-Yassky, K.M. Ansel, P.G. Woodruff, J.V. Fahy, D. Sheppard, R.L. Gallo, C.J. Ye, R.M. Evans, Y. Zheng, A. Marson, Obesity alters pathology and treatment response in inflammatory disease, *Nature*, 604 (2022) 337-342.
- [4] J. Font-Burgada, B. Sun, M. Karin, Obesity and Cancer: The Oil that Feeds the Flame, *Cell Metabolism*, 23 (2016) 48-62.
- [5] I. Lingvay, P. Sumithran, R.V. Cohen, C.W. le Roux, Obesity management as a primary treatment goal for type 2 diabetes: time to reframe the conversation, *The Lancet*, 399 (2022) 394-405.
- [6] Y. Zhong, T. Wang, R. Luo, J. Liu, R. Jin, X. Peng, Recent advances and potentiality of postbiotics in the food industry: Composition, inactivation methods, current applications in metabolic syndrome, and future trends, *Critical Reviews in Food Science and Nutrition*, 64 (2024) 5768-5792.
- [7] J.E. Aguilar-Toalá, R. Garcia-Varela, H.S. Garcia, V. Mata-Haro, A.F. González-Córdova, B. Vallejo-Cordoba, A. Hernández-Mendoza, Postbiotics: An evolving term within the functional foods field, *Trends in Food Science & Technology*, 75 (2018) 105-114.
- [8] G. Vinderola, M.E. Sanders, S. Salminen, The Concept of Postbiotics, *Foods*, 11 (2022) 1077.
- [9] Y. Huang, Q. Wang, X. Han, K. Meng, G. Liu, H. Zhang, R. Zhang, H. Cai, P. Yang, Heat-Inactivated *Lactiplantibacillus plantarum* FRT4 Alleviates Diet-Induced Obesity via Gut–Liver Axis Reprogramming, *Foods*, 14 (2025) 2799.
- [10] C. Depommier, M. Van Hul, A. Everard, N.M. Delzenne, W.M. De Vos, P.D. Cani, Pasteurized *Akkermansia muciniphila* increases whole-body energy expenditure and fecal energy excretion in diet-induced obese mice, *Gut Microbes*, 11 (2020) 1231-1245.
- [11] Y. Sun, X. Liu, R. Wang, R. Liu, X. Lv, Y. Ma, Q. Li, *Lactocaseibacillus rhamnosus* HF01 fermented yogurt alleviated high-fat diet-induced obesity and hepatic steatosis via the gut microbiota–butyric acid–hepatic lipid metabolism axis, *Food & Function*, 15 (2024) 4475-4489.
- [12] Z. Pan, B. Mao, Q. Zhang, X. Tang, B. Yang, J. Zhao, S. Cui, H. Zhang, Postbiotics Prepared Using *Lactobacillus paracasei* CCFM1224 Prevent Nonalcoholic Fatty Liver Disease by Modulating the Gut Microbiota and Liver Metabolism,

International Journal of Molecular Sciences, 23 (2022) 13522.

[13] M. Schwarzer, U.K. Gautam, K. Makki, A. Lambert, T. Brabec, A. Joly, D. Šrůtková, P. Poinot, T. Novotná, S. Geoffroy, P. Courtin, P.P. Hermanová, R.C. Matos, J.J.M. Landry, C. Gérard, A.-L. Bulteau, T. Hudcovic, H. Kozáková, D. Filipp, M.-P. Chapot-Chartier, M. Šinkora, N. Peretti, I.G. Boneca, M. Chamaillard, H. Vidal, F. De Vadder, F. Leulier, Microbe-mediated intestinal NOD2 stimulation improves linear growth of undernourished infant mice, *Science*, 379 (2023) 826-833.

[14] J.N. Cole, S.P. Djordjevic, M.J. Walker, Isolation and Solubilization of Gram-Positive Bacterial Cell Wall-Associated Proteins, in: A. Posch (Ed.) 2D PAGE: Sample Preparation and Fractionation, Humana Press, Totowa, NJ, 2008, pp. 295-311.

[15] G.C. Monnot, M. Wegrecki, T.-Y. Cheng, Y.-L. Chen, B.N. Sallee, R. Chakravarthy, I.M. Karantza, S.Y. Tin, A.E. Khaleel, I. Monga, L.N. Uwakwe, A. Tillman, B. Cheng, S. Youssef, S.W. Ng, A. Shahine, J.A. Garcia-Vilas, A.-C. Uhlemann, L.A. Bordone, A. Han, C.H. Rohde, G. Ogg, D.B. Moody, J. Rossjohn, A. de Jong, Staphylococcal phosphatidylglycerol antigens activate human T cells via CD1a, *Nat Immunol*, 24 (2023) 110-122.

[16] M.G. Roth, M.I. Chilvers, A protoplast generation and transformation method for soybean sudden death syndrome causal agents *Fusarium virguliforme* and *F. brasiliense*, *Fungal Biol Biotechnol*, 6 (2019) 7.

[17] S.-J. Park, A. Sharma, H.-J. Lee, Postbiotics against Obesity: Perception and Overview Based on Pre-Clinical and Clinical Studies, *International Journal of Molecular Sciences*, 24 (2023) 6414.

[18] S. Gezgin, M. Dikme, E. Akan, Exploring Paraprobiotics and Postbiotics: A New Frontier in Obesity Treatment via Gut Microbiota Modulation, *Curr Nutr Rep*, 14 (2026) 129.

[19] E. Hijová, I. Bertková, J. Štofilová, Incorporating Postbiotics into Intervention for Managing Obesity, *International Journal of Molecular Sciences*, 26 (2025) 5362.

[20] B. Liang, D. Xing, The Current and Future Perspectives of Postbiotics, Probiotics and Antimicrobial Proteins, 15 (2023) 1626-1643.

[21] C. Tang, L. Kong, M. Shan, Z. Lu, Y. Lu, Protective and ameliorating effects of probiotics against diet-induced obesity: A review, *Food Research International*, 147 (2021) 110490.

[22] E.M. Brown, J. Clardy, R.J. Xavier, Gut microbiome lipid metabolism and its impact on host physiology, *Cell Host & Microbe*, 31 (2023) 173-186.

[23] M. Bae, C.D. Cassilly, X. Liu, S.-M. Park, B.K. Tusi, X. Chen, J. Kwon, P. Filipčík, A.S. Bolze, Z. Liu, H. Vlamakis, D.B. Graham, S.J. Buhrlage, R.J. Xavier, J. Clardy, *Akkermansia muciniphila* phospholipid induces homeostatic immune responses, *Nature*, 608 (2022) 168-173.

[24] K. Klag, D. Ott, T.S. Tippetts, R.J. Nicolson, S.M. Tatum, K.M. Bauer, E. Stephen-Victor, A.M. Weis, R. Bell, J. Weagley, J.A. Maschek, D.L. Vu, S. Heaver, R. Ley, R. O'Connell, W.L. Holland, S.A. Summers, W.Z. Stephens, J.L. Round, Dietary fat disrupts a commensal-host lipid network that promotes metabolic health, *Cell Metabolism*, 38 (2026) 157-173.e159.

- [25] E.M. Brown, X. Ke, D. Hitchcock, S. Jeanfavre, J. Avila-Pacheco, T. Nakata, T.D. Arthur, N. Fornelos, C. Heim, E.A. Franzosa, N. Watson, C. Huttenhower, H.J. Haiser, G. Dillow, D.B. Graham, B.B. Finlay, A.D. Kostic, J.A. Porter, H. Vlamakis, C.B. Clish, R.J. Xavier, Bacteroides-derived sphingolipids are critical for maintaining intestinal homeostasis and symbiosis, *Cell Host & Microbe*, 25 (2019) 668-680.e667.
- [26] E.L. Johnson, S.L. Heaver, J.L. Waters, B.I. Kim, A. Bretin, A.L. Goodman, A.T. Gewirtz, T.S. Worgall, R.E. Ley, Sphingolipids produced by gut bacteria enter host metabolic pathways impacting ceramide levels, *Nature Communications*, 11 (2020) 2471.
- [27] H.H. Le, M.-T. Lee, K.R. Besler, E.L. Johnson, Host hepatic metabolism is modulated by gut microbiota-derived sphingolipids, *Cell Host & Microbe*, 30 (2022) 798-808.e797.
- [28] M.M. Page, J.D. Johnson, Mild Suppression of Hyperinsulinemia to Treat Obesity and Insulin Resistance, *Trends in Endocrinology & Metabolism*, 29 (2018) 389-399.
- [29] Y. Tong, S. Xu, L. Huang, C. Chen, Obesity and insulin resistance: Pathophysiology and treatment, *Drug Discovery Today*, 27 (2022) 822-830.
- [30] H. Alhabeeb, A. AlFaiz, E. Kutbi, D. AlShahrani, A. Alsuhail, S. AlRajhi, N. Alotaibi, K. Alotaibi, S. AlAmri, S. Alghamdi, N. AlJohani, Gut Hormones in Health and Obesity: The Upcoming Role of Short Chain Fatty Acids, *Nutrients*, 13 (2021) 481.
- [31] A. Engin, The mechanism of leptin resistance in obesity and therapeutic perspective, in: A.B. Engin, A. Engin (Eds.) *Obesity and Lipotoxicity*, Springer International Publishing, Cham, 2024, pp. 463-487.
- [32] L. Luo, M. Liu, Adiponectin: friend or foe in obesity and inflammation, *Medical Review*, 2 (2022) 349-362.
- [33] Q. Fan, F. Xu, B. Liang, X. Zou, The Anti-Obesity effect of traditional chinese medicine on lipid metabolism, *Front. Pharmacol.*, 12 (2021) 696603.
- [34] J.V. Jester, E. Potma, D.J. Brown, PPAR γ Regulates Mouse Meibocyte Differentiation and Lipid Synthesis, *The Ocular Surface*, 14 (2016) 484-494.
- [35] E.-H. Lee, J.-H. Lee, D.-Y. Kim, Y.-S. Lee, Y. Jo, T. Dao, K.E. Kim, D.-K. Song, J.H. Seo, Y.-K. Seo, J.K. Seong, C. Moon, E. Han, M.K. Kim, S. Ryu, M. Shin, G.S. Roh, H.R. Jung, T.F. Osborne, D. Ryu, T.-I. Jeon, S.-S. Im, Loss of SREBP-1c ameliorates iron-induced liver fibrosis by decreasing lipocalin-2, *Exp Mol Med*, 56 (2024) 1001-1012.
- [36] X. Fang, Z. Zhao, P. Jiang, H. Yu, H. Xiao, R. Yang, Identification of the bovine HSL gene expression profiles and its association with fatty acid composition and fat deposition traits, *Meat Science*, 131 (2017) 107-118.
- [37] M. Pawlak, P. Lefebvre, B. Staels, Molecular mechanism of PPAR α action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease, *Journal of Hepatology*, 62 (2015) 720-733.
- [38] N. Casals, V. Zammit, L. Herrero, R. Fadó, R. Rodríguez-Rodríguez, D. Serra, Carnitine palmitoyltransferase 1C: From cognition to cancer, *Progress in Lipid Research*, 61 (2016) 134-148.
- [39] L. Wang, Y. Qiu, H. Gu, M. Gan, Y. Zhu, K. Zhu, L. Chen, Y. Zhao, L. Niu,

S. Zhang, X. Li, L. Zhu, L. Shen, Regulation of Adipose Thermogenesis and its Critical Role in Glucose and Lipid Metabolism, *Int. J. Biol. Sci.*, 18 (2022) 4950-4962.

[40] S.A. Willis, S.J. Bawden, S. Malaikah, J.A. Sargeant, D.J. Stensel, G.P. Aithal, J.A. King, The role of hepatic lipid composition in obesity - related metabolic disease, *Liver International*, 41 (2021) 2819-2835.

[41] R. Jain, R. Rajendran, S. Rajakumari, Diet-induced obesity dampens the temporal oscillation of hepatic mitochondrial lipids, *Journal of Lipid Research*, 66 (2025) 100790.

[42] J.E. Vance, Phospholipid Synthesis and Transport in Mammalian Cells, *Traffic*, 16 (2015) 1-18.

[43] S. Schweizer, G. Liebisch, J. Oeckl, M. Hoering, C. Seeliger, C. Schiebel, M. Klingenspor, J. Ecker, The lipidome of primary murine white, brite, and brown adipocytes—Impact of beta-adrenergic stimulation, *PLoS Biol*, 17 (2019) e3000412.

[44] J. Jiang, Y. Gao, J. Wang, Y. Huang, R. Yang, Y. Zhang, Y. Ma, Y. Wen, G. Luo, S. Zhang, Y. Cao, M. Yu, Q. Wang, S. Hu, K. Wang, X. Guo, F.J. Gonzalez, Y. Liu, H. Liu, Q. Xie, C. Xie, Hepatic sphingomyelin phosphodiesterase 3 promotes steatohepatitis by disrupting membrane sphingolipid metabolism, *Cell Metabolism*, 37 (2025) 1119-1136.e1113.

Supplementary Files

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

- [SupplementaryMaterial.docx](#)