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Metabolic dysfunction-associated fatty liver disease (MAFLD): innovative management strategies using herbal medicines

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Metabolic dysfunction-associated fatty liver disease (MAFLD): innovative management strategies using herbal medicines

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Table of contents

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Editorial: Metabolic dysfunction-associated fatty liver disease (MAFLD): innovative management strategies using herbal medicines

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KEYWORDS

AMPK, herbal medicines, MAFLD, metabolic dysfunction-associated fatty liver disease, NAFLD, non-alcoholic fatty liver disease

Editorial on the Research Topic
Metabolic dysfunction-associated fatty liver disease (MAFLD): innovative management strategies using herbal medicines

Metabolic dysfunction-associated fatty liver disease (MAFLD), also known as non-alcoholic fatty liver disease (NAFLD), constitutes a substantial and growing global health burden, affecting approximately one-third of the adult population (https://doi:10.1136/gutjnl-2023-330595). Despite its high prevalence and progressive nature, current therapeutic strategies remain markedly limited, highlighting a critical demand for innovative treatment approaches. Within this context, herbal medicines have emerged as a promising direction for exploration. These natural interventions benefit from centuries of well-documented traditional application and exhibit multi-target therapeutic potential that aligns with the complex pathophysiology of MAFLD. This Research Topic, "Metabolic dysfunction-associated fatty liver disease (MAFLD): Innovative Management Strategies using Herbal Medicines," presents ten high-quality research articles and reviews that systematically investigate how botanical preparations and natural metabolites influence crucial disease mechanisms. These encompass the AMP-activated protein kinase (AMPK) signaling pathway, autophagy regulation, pyroptosis, ferroptosis, and gut-liver axis communication, thereby collectively advancing our understanding of herbal interventions and inspiring innovative strategies for MAFLD management.

The original research article by Lv et al. examined the hepatoprotective effects of total flavones from Abelmoschus manihot (TFA) using a high-fat diet-induced MAFLD mouse model. Through integrated transcriptomic and metabolomic analytical approaches, the authors demonstrated that TFA administration significantly ameliorated hepatic steatosis, inflammatory response, and oxidative stress. Their research further identified that the underlying mechanism involved autophagy enhancement via inhibition of the PI3K/AKT/mTOR signaling pathway. In another experimental study, Yu et al. explored the therapeutic potential of a purified polysaccharide (BSP-1) from Bletilla striata in both *in vivo* and *in vitro*

MAFLD models. Their results revealed that BSP-1 markedly improved serum lipid profiles and liver histopathology and exerted protective effects by suppressing the activation of the NLRP3/caspase-1/GSDMD pathway, consequently attenuating pyroptosis and hepatic inflammation. In addition, Chen et al. focused on quercetin, a bioactive compound isolated from *Zanthoxylum bungeanum* Maxim. Utilizing a combination of lipidomics and transcriptomics, their work elucidated that quercetin alleviated hepatic lipid accumulation and cellular damage by modulating the glycerophospholipid metabolism pathway and, significantly, inhibited ferroptosis through the p38 MAPK/ERK signaling axis.

Further, this Research Topic incorporates a series of insightful and timely reviews that systematically outline and interconnect emerging mechanistic frameworks and therapeutic strategies for MAFLD. Hao et al. comprehensively summarized how flavonoids modulate the gut-liver axis to ameliorate MAFLD, emphasizing multi-target regulation of PPARs, Nrf2, NF-κB, and FXR signaling, alongside innovative delivery systems and individualized nutritional strategies. Building upon the gut-liver dialogue, Zhang et al. elaborated on how botanical drugs and their metabolites target gut microbiota and hepatic immune responses, establishing a foundation for employing natural adjuvants to rectify immune dysfunction in MAFLD. Wen et al. highlighted the crosstalk between gut microbiota and mitochondrial function in obesity, elucidating how traditional Chinese medicine formulas and botanical metabolites may restore metabolic homeostasis by regulating this bidirectional axis. Wang et al. systematically reviewed natural active botanical metabolites that activate the AMPK signaling pathway, a central regulator of cellular energy, and provided a mechanistic foundation for their efficacy in improving lipid metabolic abnormalities in MAFLD. Zooming into specific botanicals, Xiao et al. illustrated the multi-target, multi-pathway mode of action of ginseng and its functional components, including saponins and non-saponins, in addressing NAFLD/MAFLD by regulating lipid metabolism, inflammation, and gut flora. Similarly, Chen et al. focused on *Scutellaria baicalensis* Georgi, detailing how its flavonoids exert anti-inflammatory, antioxidant, and lipid-regulating effects, thereby positioning it as a promising candidate for MAFLD intervention. Concluding this thematic thread, another review by Zhang et al. synthesized evidence on how natural products ameliorate NAFLD by targeting gut microbiota and lipid metabolism, underscoring their multi-target advantage in reducing hepatic steatosis and inflammation.

The findings and evidence yielded in this Research Topic underscore the therapeutic potential of herbal medicines in managing MAFLD through multi-target mechanisms, including AMPK activation, autophagy induction, pyroptosis and ferroptosis inhibition, and gut-liver-immune modulation. Nevertheless, certain aspects require more thorough investigation. Future studies should prioritize the conversion of preclinical efficacy into clinical validation via rigorously designed trials, intensify the exploration of synergistic effects among herbal components, and systematically elucidate the dose-response relationships and pharmacokinetic profiles of crucial bioactive metabolites. Moreover, the incorporation of emerging technologies, such as spatial multi-omics, single-cell sequencing, artificial intelligence, and organoid-based disease modeling, will further accelerate the interpretation of herb-host interactions and facilitate the development of standardized, evidence-based herbal formulations. By addressing these challenges, the field may effectively bridge traditional knowledge with modern precision medicine, paving the way for innovative, safe, and effective herbal-based interventions for MAFLD.

Author contributions

YC: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review and editing. YL: Data curation, Writing – review and editing. QZ: Formal Analysis, Writing – review and editing. WP: Conceptualization, Writing – original draft, Writing – review and editing.

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The mechanism study of quercetin isolated from *Zanthoxylum bungeanum* maxim. inhibiting ferroptosis and alleviating MAFLD through p38 MAPK/ERK signaling pathway based on lipidomics and transcriptomics

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Background: As a resource with a variety of medicinal and edible values, *Zanthoxylum bungeanum* Maxim has been found to improve high-fat diet-induced metabolic-associated fatty liver disease (MAFLD).

Aim of the study: The aim of this study was to predict the main active metabolites in *Z. bungeanum* Maxim. Based on network analysis, and to explore and validate their potential mechanisms of action through lipidomics and transcriptomic techniques.

Materials and Methods: MAFLD mouse model and cell model were established to evaluate the effect of active components in *Z. bungeanum* Maxim. on MAFLD. Serum biochemical indexes, pathological staining observation, lipid group and transcriptome were used to verify the mechanism of action of active components in *Z. bungeanum* Maxim. on MAFLD.

Results: Quercetin can regulate the liver lipid metabolites of MAFLD mice through the Glycerophospholipid metabolic pathway, thereby improving liver lipid accumulation and liver injury. At the same time, quercetin can also improve MAFLD by reducing oleic acid-induced lipid accumulation in HepG2 cells, and inhibit ferroptosis through the p38 MAPK/ERK signaling pathway, thereby alleviating the progression of MAFLD.

Conclusion: Quercetin isolated from *Z. bungeanum* Maxim. has ameliorative effects on MAFLD, probably mainly by affecting lipid metabolic pathways and MAPK signaling pathways.

KEYWORDS

***Zanthoxylum bungeanum* maxim., metabolic-associated fatty liver disease, quercetin, lipidomics, transcriptomics**

1 Introduction

Metabolically associated fatty liver disease (MAFLD) is a new definition proposed by an international panel of experts based on non-alcoholic fatty liver disease (NAFLD), a type of metabolic stress-related liver damage strongly associated with insulin resistance and genetic predisposition (Cai et al., 2025). MAFLD is a common chronic metabolic disease with a global prevalence of 25%–30% (Shi et al., 2025). According to the severity of the disease and symptoms, MAFLD can divided into simple fatty liver disease, non-alcoholic steatohepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma (Mansour et al., 2025). The pathogenesis of MAFLD is related to many factors such as diet, obesity, insulin resistance, inflammatory factors and adipose tissue dysfunction (Wan et al., 2025). Due to its complex pathogenesis, the research and development of drugs for the treatment of MAFLD has always been a great challenge. Currently, there is still a lack of effective therapeutic medications. Therefore, it is urgently needed to explore the pathological mechanisms of MAFLD and search for new treatment drugs and targets in order to prevent and slow down the progression of MAFLD at an early stage.

Natural extracts are the main way of drug development, accounting for 30% of clinical drugs for the treatment of diseases (Cai et al., 2025). Due to their rich pharmacological activity and low side effects, natural extracts have been widely used to treat MAFLD (Ma et al., 2023). In the early stage of MAFLD, dietary control and treatment can be utilized for management. Therefore, natural extracts, as a valuable resource, hold significant importance in the prevention and treatment of MAFLD. *Zanthoxylum bungeanum* Maxim. Is a plant of the genus Zanthoxylum in the rutaceae family, which is widely distributed in Japan, India, Korea, China and other places (Zhang T. et al., 2024). Modern scientific research found that *Z. bungeanum* Maxim. Contains rich chemical components, mainly including volatile oil, alkaloids, flavonoids and free fatty acids, with analgesic, anti-inflammatory, antibacterial, antioxidant, anti-tumor and other extensive biological activities (Li et al., 2025). In traditional folk medicine, the peel, stem and seeds of *Z. bungeanum* Maxim. Have been used to treat tuberculosis, malaria, tonsillitis, arthritis, fever and abdominal pain (Yuan et al., 2021; Ye et al., 2023). Studies have found that *Z. bungeanum* Maxim. Can improve high-fat diet-induced MAFLD by regulating fatty acid and cholesterol metabolism, intestinal microflora and metabolic characteristics (Huang X. et al., 2023). However, the specific components of *Z. bungeanum* Maxim. In the treatment of MAFLD are still unclear. Therefore, we further explored the therapeutic effect of active components in *Z. bungeanum* Maxim. On MAFLD. Quercetin is a flavonoid substance in *Z. bungeanum* Maxim., which is widely found in various plants and foods in daily life. Studies have found that quercetin can promote insulin secretion, improve insulin resistance, reduce blood lipid levels, inhibit inflammation and oxidative stress, alleviate liver lipid accumulation, and regulate intestinal microflora disorders to improve MAFLD (Katsaros et al., 2024; Markowska et al., 2024). Therefore, we speculated that quercetin may be an important active ingredient of *Z. bungeanum* Maxim. In the treatment of MAFLD.

As a resource with a variety of medicinal and edible values, the effect of *Z. bungeanum* Maxim. On MAFLD deserves further discussion. In recent years, the development of emerging technologies such as network analysis and molecular docking has shown great potential in revealing the active metabolites of *Z. bungeanum* Maxim. For the treatment of MAFLD and their mechanisms of action (Chandran et al., 2017; Dong et al., 2021). Therefore, the purpose of this study was to screen the main active components and potential mechanisms of *Z. bungeanum* Maxim. In the treatment of MAFLD by network analysis and molecular docking. MAFLD mouse model and cell model were established to evaluate the effect of active components in *Z. bungeanum* Maxim. On MAFLD. Serum biochemical indexes, pathological staining observation, lipid group and transcriptome were used to verify the mechanism of action of active components in *Z. bungeanum* Maxim. On MAFLD, so as to provide theoretical reference for clinical application of *Z. bungeanum* Maxim. In the treatment of MAFLD.

2 Materials and Methods

2.1 Reagents and antibodies

Quercetin (≥98% purity, SQ8030/(218P021)) purchased from Beijing Solarbio Technology Co. Ltd (Beijing, China). Tetracycline (20220225) purchased from National Pharmaceutical Group Chemical Reagents Co., Ltd (Shanghai, China). Oleic acid (#0000115688), purchased from Sigma Co., Ltd (St Louis, MO, United States). CCK-8 Kit (BS350B) purchased from Biosharp Biotechnology Co., Ltd. (Hefei, China). Aspartate aminotransferase (ALT), alanine aminotransferase (AST), LDL-C, HDL-C, TC and TG assay kits were obtained from Nanjing Jiancheng Biological Engineering (Nanjing, China). Paraformaldehyde 4% solution and hematoxylin and eosin dye were obtained from Biosharp Technology Co., Ltd (Anhui,

Abbreviations: MAFLD metabolic-associated fatty liver disease; ALT aspartate aminotransferase; AST alanine aminotransferase; LDL-C low-density lipoprotein cholesterol; HDL-C high-density lipoprotein cholesterol; TC cholesterol; TG triglyceride; MF molecular function; BP biological process; CC cell composition; CCK-8 Cell Counting Kit-8; IL-6 interleukin-6; TNF tumor necrosis factor; p38 MAPK p38 mitogen-activated protein kinase.

China). GAPDH was obtained from Servicebio Biotechnology Co., Ltd. (Wuhan, China). Primary antibodies against P-p38 MAPK and p38 MAPK were acquired from Cell Signaling Tech (Danvers, MA, United States). Primary antibodies against p-ERK1/2, ERK1/2, xCT and GPX4 were acquired from Abmart Shanghai Co., Ltd. (Shanghai, China). Primary antibodies against SLC3A2/CD98hc were acquired from ABclonal Technology Co., Ltd. (Wuhan, China).

2.2 Network analysis

2.2.1 Screening the active metabolites and target genes of *Zanthoxylum bungeanum* maxim

The active metabolites in *Z. bungeanum* Maxim. Were searched by using Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php). Oral bioavailability (OB)≥30% and drug similarity (DL)≥0.18 were used as screening conditions to obtain the main active metabolites and their action targets. The molecular structure of active metabolites was obtained through literature search and Pubchem platform, and the pharmacokinetic absorption, distribution, metabolism and excretion (ADME) characteristics of Swiss ADME (http://www.swiss adme. ch/) (Daina et al., 2017) were used to screen active metabolites. Potential targets of active metabolites were predicted by Swiss Target Prediction (http://www.swisstargetprediction.ch/) (Daina et al., 2019). The name of the targets are normalized by using UnitProt database (UniProt Consortium, 2021).

2.2.2 MAFLD related target retrieval

The 'metabolic associated fatty liver disease' was used as the key word to search and screen in the OMIM (https://omim.org/), Dis Genet (https://www.disgenet.org/), TTD (https://db.idrblab.net/ttd/) and Drug Bank (https://www.drugbank.com/), and remove duplicate targets to obtain related disease targets. The active components screened in 2.2.1 were crossed with MAFLD-related targets to obtain the main targets of *Z. bungeanum* Maxim. In the treatment of MAFLD, and the Venn diagram was drawn (Wang et al., 2024).

2.2.3 Construct protein-protein interaction network (PPI) and screen key target

The potential gene targets of *Z. bungeanum* Maxim. For treating MAFLD were obtained by intersecting the predicted target of *Z. bungeanum* Maxim. Active metabolites and the targets of MAFLD. The obtained potential gene targets were added to the STRING database, the species were set as "*Homo sapiens*", and the PPI network map was established. Import the result into Cytoscape 3.8.2 for visualization processing, use Analyze Network to analyze network topological features, and select core targets according to Degree value (Luo et al., 2024).

2.2.4 GO functional enrichment and KEGG pathway enrichment analysis

GO gene enrichment analysis and KEGG pathway enrichment analysis were performed using Metascape platform (https://metascape.org/gp/index.html). The relevant data of molecular function (MF), biological process (BP), cell composition (CC) and KEGG pathway were established, and the results were visualized using Bioinformatics online tool (http://www.bioinformatics.com.cn) (Chen et al., 2024).

2.2.5 Construction of active ingredient-MAFLD target-pathway network diagram of *Zanthoxylum bungeanum* maxim

CytoScape3.8.2 was used to construct the active ingredient-MAFLD target-pathway network diagram of *Z. bungeanum* Maxim. CytoScape3.8.2 built-in tools were used to analyze the topological characteristics of the network, and the core targets and important active components that exert drug efficacy were analyzed according to the network topology parameters (Liu et al., 2024).

2.2.6 Molecular docking

The molecular docking between the active metabolites and the screened core targets was performed to verify the accuracy of the screened active metabolites of *Z. bungeanum* Maxim. On the potential core targets of MAFLD. The 2D structure of the active components of *Z. bungeanum* Maxim. Was searched by Pubchem website, and then the 3D structure of the active components was optimized by Chem3D software and saved as Mol2 format. Receptor proteins were downloaded from the RSCB PBD database (http://www.rcsb.org/pdb/home/home.do). PyMOL software was used to dehydrate and remove residues, and Autodock 1.5.6 software was used to hydrogenate the protein. The receptor protein and ligand small molecules were transformed into Pdbqt format, and the active components and core targets were molecularly docked using AutoDock Vina 1.1.2 software. According to the binding energy of the receptor and the ligand, the affinity was judged. The smaller the binding energy, the lower the affinity, and then the PyMOL software was used to visualize the active metabolites and target genes with higher molecular docking scores (Deng et al., 2024).

2.3 *In vivo* study

2.3.1 Animal model establishment and grouping

Healthy SPF ICR mice, 4 weeks old, male, weighing 20 ± 2 g, purchased from SPF (Beijing) Biotechnology Co., Ltd., production license number: SCXK (Beijing) 2019–0,010. Feeding in a standard laboratory environment (temperature 20°C–26°C, humidity 40%–70%), light and dark cycles of 12 h, given standard animal feed, free feeding and drinking water. Adaptive feeding for 3 days before the experiment. All animal experiments were ethically approved by the Ethics Committee for Laboratory Animal Welfare of Chengdu University of Traditional Chinese Medicine (Project Ethics No. 2022–76) on 5 May 2022.

After adaptive feeding, all mice were randomly divided into 6 groups, with 8 mice in each group. They are control group (Control), model group (Model), quercetin low dose group (35 mg/kg), quercetin medium dose group (70 mg/kg), quercetin high dose group (140 mg/kg), positive control group (Metformin). The control group was fed a normal diet, and the other groups were fed a high-fat diet. At the beginning of the experiment, the control group was intraperitoneally injected with normal saline, and the other groups were intraperitoneally injected with tetracycline (150 mg/kg) to establish the MAFLD model. After 5 consecutive

days, the quercetin treatment group was given different doses of quercetin (35, 70 and 140 mg/kg), the positive control group was given 200 mg/kg metformin, and the control group and the model group were given an equal volume of 2% Tween-80 once a day for 28 days.

2.3.2 Histopathological examination

The liver tissue was fixed in 4% paraformaldehyde, dehydrated by alcohol and transparentized by xylene. Paraffin embedded and sectioned (thickness 3 μm). The sections were stained with hematoxylin and eosin (H&E) and observed under a microscope after dehydration, and the pathological changes were described (Ali et al., 2024).

2.3.3 Oil red o staining

Oil red O staining was used to observe the accumulation of lipid droplets in liver tissue and hepatocytes. The liver tissue was made into frozen sections, and the cells were made into smears. The cells were fixed with 4% paraformaldehyde and stained in oil red O staining solution. Observed and photographed under an optical microscope (Lu and Wang, 2024).

2.3.4 Biochemical index analysis

The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and triglyceride (TG) in serum or cells were detected by microcoder (Zhang J. et al., 2024). ALT and AST are indicators of liver parenchymatous injury, and AST and ALT are usually measured at the same time, and the ratio of the two can reflect different degrees of liver injury. LDL-C, HDL-C, TC and TG are the basic items of clinical blood lipid detection. Affected by many factors, the elevation of LDL-C is the main risk factor for the occurrence and development of atherosclerosis (Jacobson et al., 2015), A large number of epidemiological data show that serum HDL-C level is negatively correlated with the risk of atherosclerotic cardiovascular disease (ASCVD) (Gotto and Brinton, 2004), TC refers to the sum of cholesterol contained in various lipoproteins in the blood. The increase of TG may have a direct effect on atherosclerosis, and it is often used in combination with LDL-C and HDL-C.

2.3.5 Lipid metabolism analysis

30 mg of liver tissue was weighed, add 200 μL of water and 20 μL of internal lipid standard, vortex at MP, add 800 μL of MTBE, vortex to mix, add 240 μL of pre-cooled methanol, vortex to mix, ultrasonic for 20 min in a low-temperature bath, leave at room temperature for 30 min, centrifugation at 14,000 g for 15 min at 10°C, take the upper layer of the organic phase, nitrogen gas blowing, and add 200 μL of 90% isopropanol/acetonitrile solution to dissolve, vortex thoroughly, take 90 μL of complex solution, centrifugation at 14,000 g for 15 min at 10°C. For mass spectrometry analysis, add 200 μL of 90% isopropanol/acetonitrile solution, vortex thoroughly, take 90 μL of the compound solution, centrifuge at 14,000 g 10°C for 15 min, take the supernatant into the sample for analysis. The separation was performed on a UHPLC Nexera LC-30A ultra performance liquid chromatography system. The chromatographic column was CSH C18. The chromatographic conditions were as follows: mobile phase A (acetonitrile/water = 6:4, v/v) + 0.1% formic acid +0.1 mM ammonium formate, and mobile phase B (acetonitrile/isopropanol = 1:9, v/v)) + 0.1% formic acid +0.1 ammonium formate, at a flow rate of 300 μL/min, and the temperature of the column chamber was 45°C. The samples were analyzed by random injection sequence method to avoid the effect of signal fluctuation. The samples were separated by UHPLC and analyzed by mass spectrometry using a Q Exactive series mass spectrometer (Thermo Scientific). Electrospray ionization (ESI) was used to detect positive and negative ions. The ESI source conditions were as follows: heater temperature 300°C, sheath gas flow rate of 45 arb, auxiliary gas flow rate of 15 arb, scanning gas flow rate of 1 arb, spray voltage of 3.0 KV, capillary temperature of 350°C, S-lens RF level of 50%, MS1 scanning range: 200–1800. Mass-to-charge ratios of lipid molecules and lipid fragments were collected as follows: 10 fragmentation profiles were collected after each full scan (MS2 scan, HCD). 70,000 resolution at M/Z 200 for MS1 and 17,500 resolution at M/Z 200 for MS2 were used for the identification of peaks, peak extraction, and lipid identification (secondary identification) of the lipid molecules and internal standards using LipidSearch. LipidSearch was used to identify the peaks of lipid molecules and internal standards, extract the peaks, and identify the lipids (secondary identification). The main parameters were: precursor tolerance: 5 ppm, product tolerance: 5 ppm, product ion threshold: 5% (Yang et al., 2025).

2.4 *In vitro* experiment

2.4.1 Cell model establishment and grouping

Human hepatoma HepG2 cells (Shanghai Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were used to establish oleic acid-induced MAFLD model. HepG2 cells were cultured in ATCC-modified low-limit Eagle medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin in a 5% carbon dioxide incubator at 37 C.

The concentration of oleic acid modeling and quercetin administration was determined by detecting the cytotoxicity of oleic acid and quercetin. Cell viability was detected by Cell Counting Kit-8 (CCK-8). Cells were seeded into 96-well culture plates at a density of 3×10^4 cells/well, 100 μL per well. The concentration gradient of oleic acid was 0.05, 0.1, 0.15, 0.2, 0.25, 0.5, 0.75, 1.0 mM, and the treatment time was 24 h. Quercetin concentration gradient was 5, 10, 20, 30, 40, 80 μM, treated for 48 h. 10 μL CCK-8 was added to each well and incubated for 1 h. SpectraMax iD3 microplate reader was used to determine the absorbance at 450 nm.

The cells were divided into 5 groups: control group (Control), model group (Model, containing 0.5 mM oleic acid), low-dose quercetin group (5 μM, containing 0.5 mM oleic acid and 5 μM quercetin), medium-dose quercetin group (10 μM, containing 0.5 mM oleic acid and 10 μM quercetin), high-dose quercetin group (20 μM, containing 0.5 mM oleic acid and 20 μM quercetin).

2.4.2 Hepatocyte transcriptome sequencing

Total RNA was isolated from HepG2 cells using TriZol. The quantity and purity of total RNA were controlled by NanoDrop ND-1000 (NanoDrop, Wilmington, DE, United States), and the integrity

TABLE 1 qRT - PCR primer sequence table.

Gene	Primer	Sequences
Epha2	Forward primer	TAAGAGGGCAGACTGTGAA
	Reverse primer	CCAGGAAAGCAAGGGTTT
Dusp1	Forward primer	GCGTCAAGACATTTGCTGAA
	Reverse primer	GTCGTCGGGAATAATACTGGTA
Csf1	Forward primer	CCGTGACTTTCCCTTCCT
	Reverse primer	GTTCACTGCCCTTCCCTA
Golga4	Forward primer	ACCACCGTACTGAAGTTC
	Reverse primer	GTCACCCAATGTCACTCT
Best1	Forward primer	CTAACCTAGAAGTCAGCAAGC
	Reverse primer	TTCATCATCTGGCAGTGTTC
Tmsb4x	Forward primer	AGACCAGACTTCGCTCGTA
	Reverse primer	CCTGCTTGCTTCTCCTGTT
Gapdh	Forward primer	GAAGGTGAAGGTCGGAGTC
	Reverse primer	GAAGATGGTGATGGGATTTC

of RNA was detected by Bioanalyzer 2,100 (Agilent, CA, United States). RNA in cell samples was sequenced using the Illumina Novaseq 6,000 sequencing platform (LC Bio Technology CO., Ltd. Hangzhou, China). The obtained FASTQ format file was further processed into reads, and the reads of all samples were compared with the cell reference genome using HISAT2 (https://daehwankimlab.github.io/hisat2/). StringTie (https://ccb.jhu.edu/software/stringtie/) software was used to estimate the expression level of all transcripts, and the Fragments Per Kilobase Million (FPKM) value was calculated as the expression level of each gene, and the difference between groups was analyzed. DESeq2 software was used to analyze the differential expression of genes in two different groups, and the enrichment analysis of GO function and KEGG pathway was performed on the differentially expressed genes (Yang et al., 2024).

2.4.3 qRT-PCR

Total RNA was isolated from HepG2 using TriZol, and *Gapdh* was used as an internal reference. The relative expression levels of genes in each sample and group were calculated using $2^{-\Delta\Delta CT}$ (Kong et al., 2025). The qRT-PCR primer information is shown in Table 1.

2.4.4 Western blotting test

Total protein was extracted from liver tissue using RIPA lysis buffer at low temperature. The protein concentration was quantitatively determined by BCA protein detection kit. Equal amounts of protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. After blocking in 5% skimmed milk powder for 2 h, the cells were incubated with primary antibody at 4 °C overnight. After washing three times with TBST, the cells were incubated with HRP conjugated Goat Anti-Rabbit IgG for 1 h. TBST was washed three times again and observed with ECL chemiluminescence detection kit. The results were quantified using ImageJ. GAPDH was used as an internal control (Deng et al., 2025).

2.5 Data processing and statistical analysis

The results were expressed as mean ± standard deviation. Data processing and statistical analysis were performed using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, N.Y., United States). One-way analysis of variance (One-way ANOVA) was used when the homogeneity of variance was satisfied. The least significant difference (LSD) test was used for pairwise comparison between groups, and the non-parametric test (Kruskal Wallis test) was used otherwise. $P < 0.05$ was considered statistically significant. The histogram was drawn using GraphPad Prism 8.0 software (GraphPad, Boston, United States).

3 Results

3.1 Results of network analysis

3.1.1 Acquisition of potent metabolites and potential targets and molecular docking validation results for the treatment of MAFLD by *Zanthoxylum bungeanum* maxim

A total of 8 active metabolite and 439 potential targets in *Z. bungeanum* Maxim. Were screened out by TCMSP database and target prediction. A total of 721 disease targets related to MAFLD were obtained from OMIM, Dis genet, TTD and Drug bank disease gene databases. There are 44 common targets between the active components of *Z. bungeanum* Maxim. And MAFLD (Figure 1A). The PPI network diagram was drawn in Cytoscape 3.8.2, and the topological characteristics were analyzed (Figure 1B). The nodes in the network graph represent proteins, and the degree value is represented by the number of edges connected to the same node. After optimizing the network, the larger the degree value, the larger the node area, the darker the color, and the thickness of the line represents the comprehensive score of the node in the PPI network. Eight core targets were identified, including interleukin-6 (IL-6), tumor necrosis factor (TNF), epidermal growth factor receptor (EGFR), peroxisome proliferator-activated receptor gamma (PPARG), Toll-like receptor 4 (TLR4), C-reactive protein (CRP), interleukin-1β (IL-1β) and interleukin-10 (IL-10) (Figure 1C).

The component-target-pathway network diagram was constructed using CytoScape3.8.2. (Figure 1D). The active components with larger degree values were quercetin and hydroxy-α-sanshool, and the targets with larger degree values were IL-1β, TNF, IL-6 and TGF-βI. It is indicated that these components and targets may be the key components and proteins of *Z. bungeanum* Maxim. In the treatment of MAFLD. Molecular docking was carried out between the active components and the core targets of screening, the docking results are shown in Table 2. The results showed that the binding energy of 91% components to their targets was ≤ −4.25 kcal/mol, especially the binding energy of quercetin and diosmetin to IL-6, PPARG and CRP was ≤ −7.0 kcal/mol, suggesting that these components had strong binding activity to the target. Finally, we used PyMOL software to

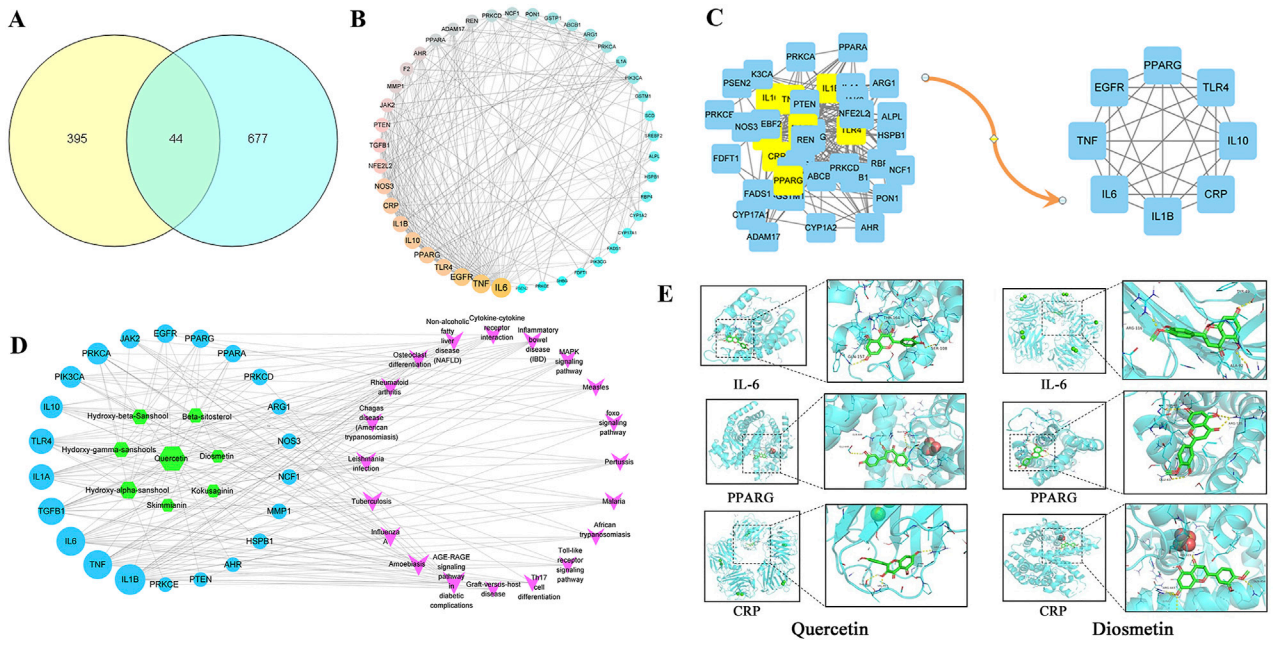

FIGURE 1
Target screening map. **(A)** Venn diagram of cross target of *Zanthoxylum bungeanum* Maxim. And MAFLD, **(B)** PPI network, **(C)** Core target screening map, **(D)** Active metabolites-MAFLD target-pathway network diagram, **(E)** Visualization of molecular docking.

TABLE 2 Binding energies of metabolites to core targets.

Metabolites	Score (Kcal/mol)							
	IL6	TNF	EGFR	PPARG	TLR4	CRP	IL1B	IL10
Kokusaginin	−6.1	−4.6	−4.7	−7.5	−4.3	−6.6	−5.5	−5.2
Skimmianin	−5.9	−4.3	−4	−6.3	−4.9	−6.8	−4.8	−5.3
Diosmetin	−7.1	−5.3	−5.3	−8.3	−5.6	−8.2	−6.9	−6.7
Beta-sitosterol	−6.6	−5	−5.1	−6.7	−6.4	−6.7	−7.2	−6.8
Quercetin	−7.8	−5.2	−5.3	−8.6	−5.2	−8.8	−6.7	−6.7
Hydorxy-gamma-sanshools	−4	−5	−2.5	−5.8	−4.1	−4.8	−3.8	−4.6
Hydroxy-beta-Sanshool	−5.5	−4.5	−4.1	−4.8	−4.6	−4.7	−3.7	−4.7
Hydroxy-alpha-sanshool	−4.5	−4.1	−3.5	−4.5	−4.1	−5.1	−4.8	−4.6

visualize the molecular docking results of quercetin and diosmetin with IL-6, PPARG and CRP (Figure 1E). Therefore, based on the above predictions, we can make a preliminary assessment of the efficacy of the active constituents of *Z. bungeanum* Maxim. Against MAFLD.

3.1.2 GO functional enrichment and KEGG pathway enrichment analysis

After preliminary evaluation, we hypothesized that quercetin may be a key active substance in the therapeutic effects of *Z. bungeanum* Maxim. In MAFLD, but its possible mechanism of action is not clear. Therefore, We used Meta scape platform to conduct enrichment analysis of MF(molecular function), BP(biological process) and CC(cellular component) of 44 potential action targets obtained. it was found that MF mainly involved lipid binding and insulin receptor substrate binding; BP mainly includes regulation of lipid metabolic process and lipid biosynthetic process. CC is mainly involved in membrane rafts and cytoplasmic perinuclear regions. We selected the top 20 pathways based on P-values to further investigate these findings and visualized them using the Bioinformatics online tool. The results of KEGG enrichment analysis mainly revealed AGE-RAGE signaling pathway, MAFLD, NAFLD, MAPK signaling pathway, *etc.* (Figure 2). These findings strongly suggest that quercetin, the main ingredient in *Z. bungeanum* Maxim., may exert its effect on MAFLD by participating in signaling pathways related to lipid metabolism. However, the mechanism needs to be further explored in *ex vivo* and *in vivo* experiments.

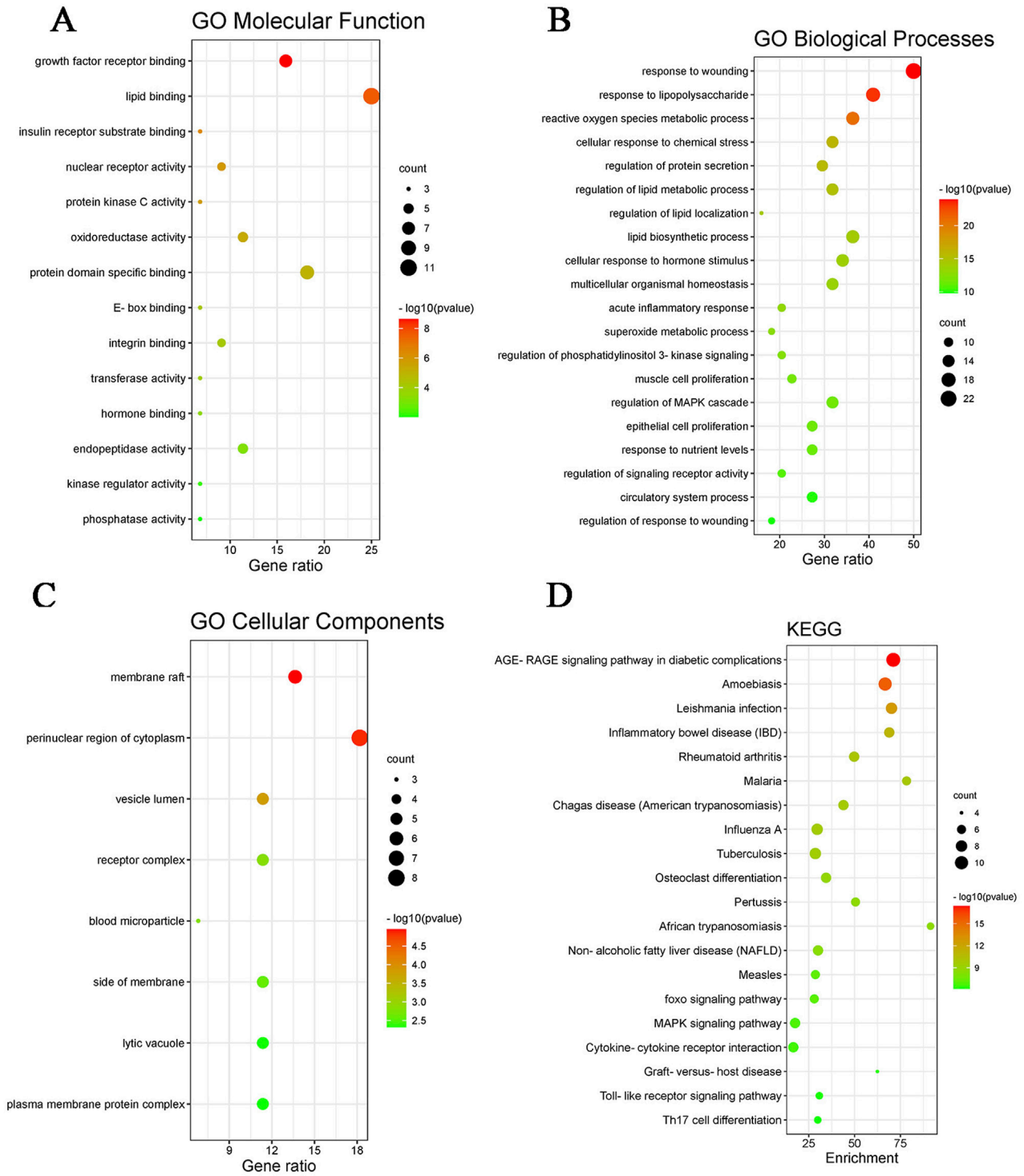

FIGURE 2
GO functional enrichment and KEGG pathway enrichment map. **(A)** GO molecular function map, **(B)** GO biological processes map, **(C)** GO cellular component map, **(D)** KEGG pathway enrichment map.

3.2 Results of *in vivo* experiments

3.2.1 Effects of quercetin on liver histopathology and lipid accumulation in MAFLD mice

The liver tissue morphology of mice in each group was observed by HE staining. It was found that the liver tissue morphology of mice in the control group was basically normal, and the liver of mice in the model group showed a large number of hepatocyte ballooning, hepatocyte atrophy, necrosis and interstitial inflammation. The pathological damage of the liver in the quercetin treatment group was improved to varying degrees, the number of necrotic cells was reduced, and the inflammatory response was alleviated. The number

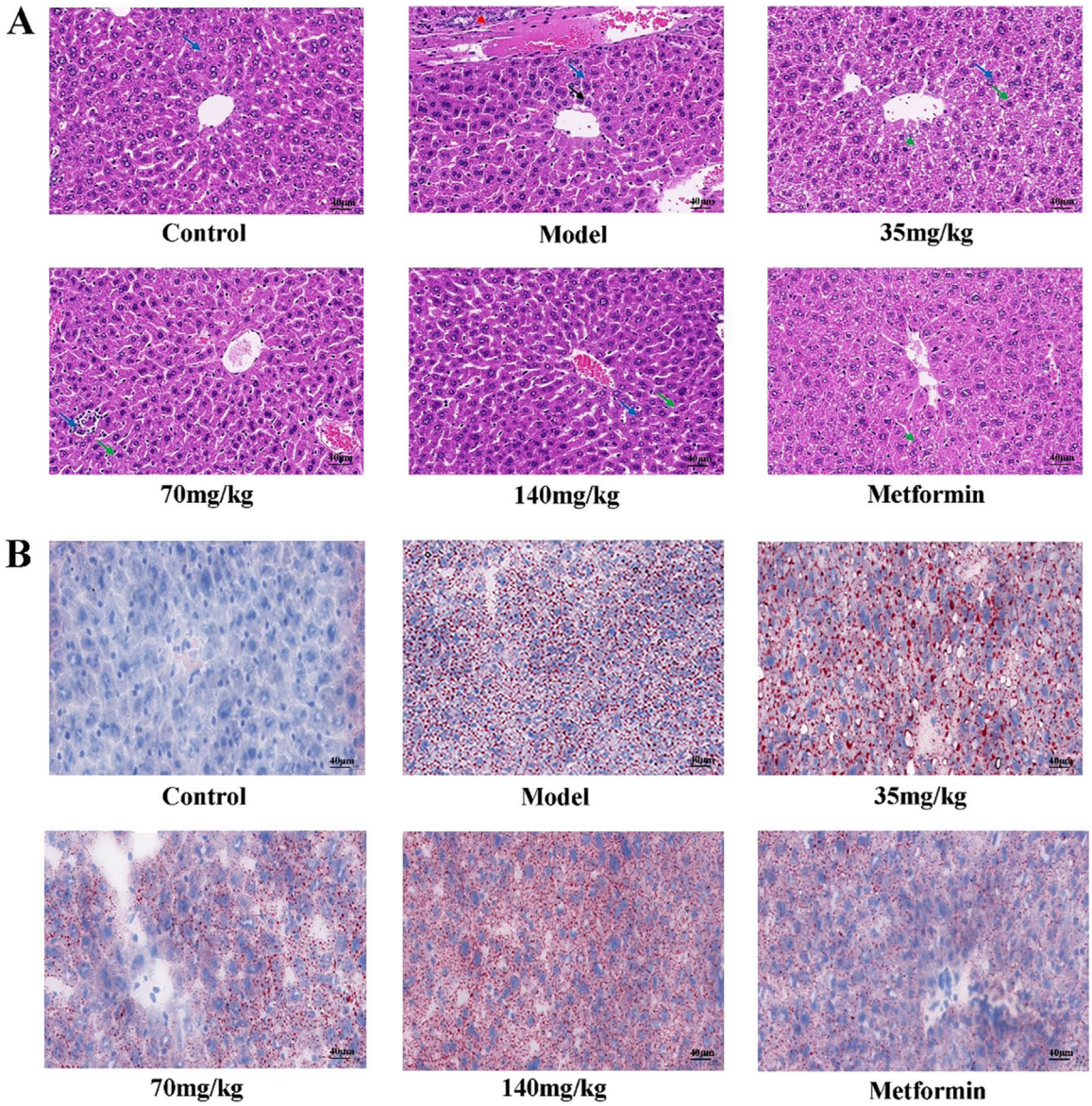

FIGURE 3
(A) Pathological morphology of mouse liver (×400 magnification) (n = 3). Solid blue arrows (hepatocyte necrosis), red dotted arrows (bile duct epithelial cell degeneration or necrosis), solid black arrows (hepatocyte atrophy), and solid green arrows (hepatocyte vesicular steatosis) **(B)** Mouse liver was stained with oil red O (×400 magnification) (n = 3).

of hepatocyte necrosis in the liver of the positive group was significantly reduced, and the interstitial inflammatory response disappeared (Figure 3A). A large number of red lipid droplets were observed in the liver cells of the model group by oil red O staining, indicating that there was a large amount of lipid accumulation. The accumulation of lipid droplets in the quercetin treatment group and the positive group was improved (Figure 3B).

3.2.2 Effects of quercetin on body weight and serum biochemical indexes in MAFLD mice

Compared with the control group, the body weight of the model group was significantly increased ($p < 0.01$). Compared with the model group, the body weight of mice in the quercetin treatment group and the positive group was significantly lower ($p < 0.05$), suggesting that quercetin may improve the weight gain of MAFLD mice (Figure 4A). Compared with the control group, the contents of ALT, AST, LDL-C and TC in the serum of MAFLD model mice were significantly increased, and the content of HDL-C was decreased ($p < 0.05$). After quercetin treatment, the contents of ALT, AST, LDL-C and TC in the serum were reversed to varying degrees. It is suggested that quercetin may improve steatosis and liver injury in MAFLD mice (Figures 4B–F).

3.2.3 Effect of quercetin on lipid metabolism in MAFLD mice

Lipid subclasses and lipid molecules in mouse liver were detected by lipidomics. A total of 45 lipids were identified, including 4,529 lipid molecules (Figure 5A). We used univariate

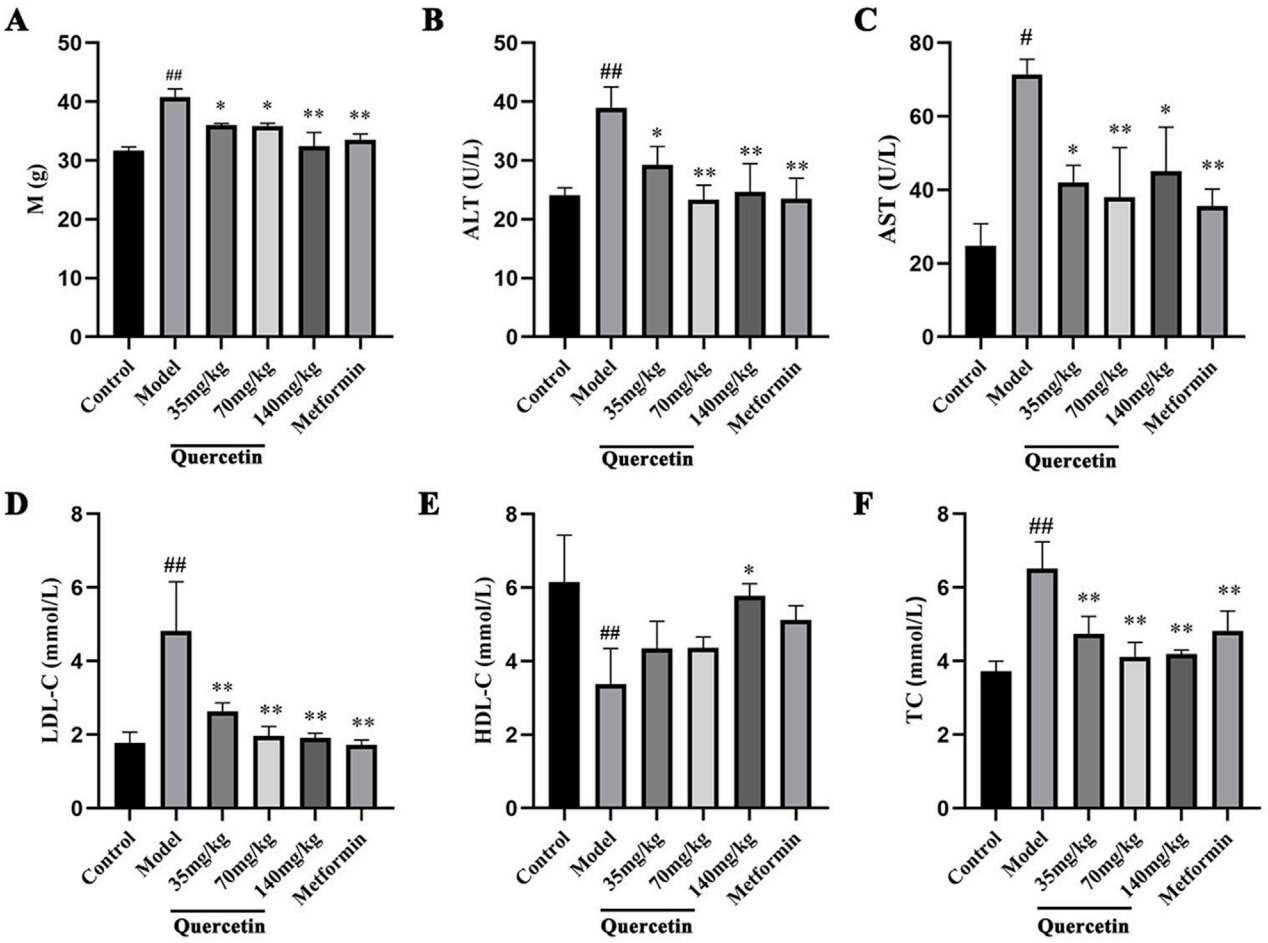

FIGURE 4
Effect of quercetin on MAFLD mice. **(A)** The body weight of ICR mice, **(B–F)** The serum levels of ALT, AST, LDL-C, HDL-C and TC in ICR mice. Values were represented the mean ± SD (n ≥ 3). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Control, *P < 0.05, **P < 0.01 vs. Model.

statistical analysis to analyze the differences of these lipid molecules in the form of volcano maps (Figures 5B, C), in which the fold change (FC) > 1.5, p-value <0.05 was upregulated lipid molecules, FC < 0.67, p-value <0.05 was downregulated lipid molecules. Compared with the control group, there were 693 upregulated lipid molecules and 480 downregulated lipid molecules in the liver of MAFLD model mice. Compared with the model group, there were 41 upregulated lipid molecules and 44 downregulated lipid molecules in the liver of the quercetin treatment group. Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed on the detected lipid molecules (Figure 5D–M). Among the PCA model parameters obtained by 7-fold cross-validation, the R^2X between the model group and the control group was 0.506, and the R^2X between the quercetin group and the model group was 0.597. PLS-DA and OPLS-DA models were tested by permutation test, and no over-fitting was observed.

Significantly different lipid molecules that meet OPLS-DA VIP >1 and p-Value <0.05 are considered as potential biomarkers. A total of 179 significantly different lipid metabolites were screened between the model group and the control group, including 18 categories of substances, including 55 diacylglycerols (DG), 37 phosphatidylethanolamines (PE), 18 phosphatidylcholines (PC), 13 ceramides (Cer), 11 cardiolipin (CL), 10 TG, 7 sphingomyelins (SM), 5 phosphatidylinositols (PI), 5 hexosylceramides (Hex1Cer), 4 Lys phosphatidylethanolamines (LPE), 4 cholesterol esters (ChE), 1 glycerol monoester (MG), two phosphatidylglycerols (PG), 1 pregnenolone ester (Co.), 1 fatty acid (FA), 1 phosphatidylserine (PS), 1 phosphatidylserine (PA) and 1 zymosterol (ZyE). We listed the differential lipid metabolites with OPLS-DA VIP >3 and p-Value <0.05, as shown in Table 3. A total of 29 significantly different lipid metabolites were screened between the quercetin administration group and the model group, including 7 types of substances, including 16 PE types, 4 C L types, 2 Lys phosphatidylcholine (LPC), 2 LPE, 2 DG, 2 Hex1Cer types, and 1 SM type. The specific differential lipid metabolites are shown in Table 4.

Pathway analysis of significant differential metabolites was performed by MetaboAnalysis 5.0 website (https://www.metaboanalyst.ca/). The results showed that quercetin treatment of MAFLD may be involved in seven metabolic pathways, namely, Glycerophospholipid metabolism, Linoleic acid metabolism, alpha-Linolenic acid metabolism, Glycosylphosphatidylinositol (GPI)-anchor biosynthesis, Glycerolipid metabolism, Sphingolipid metabolism, Arachidonic acid metabolism (Figure 6). Among

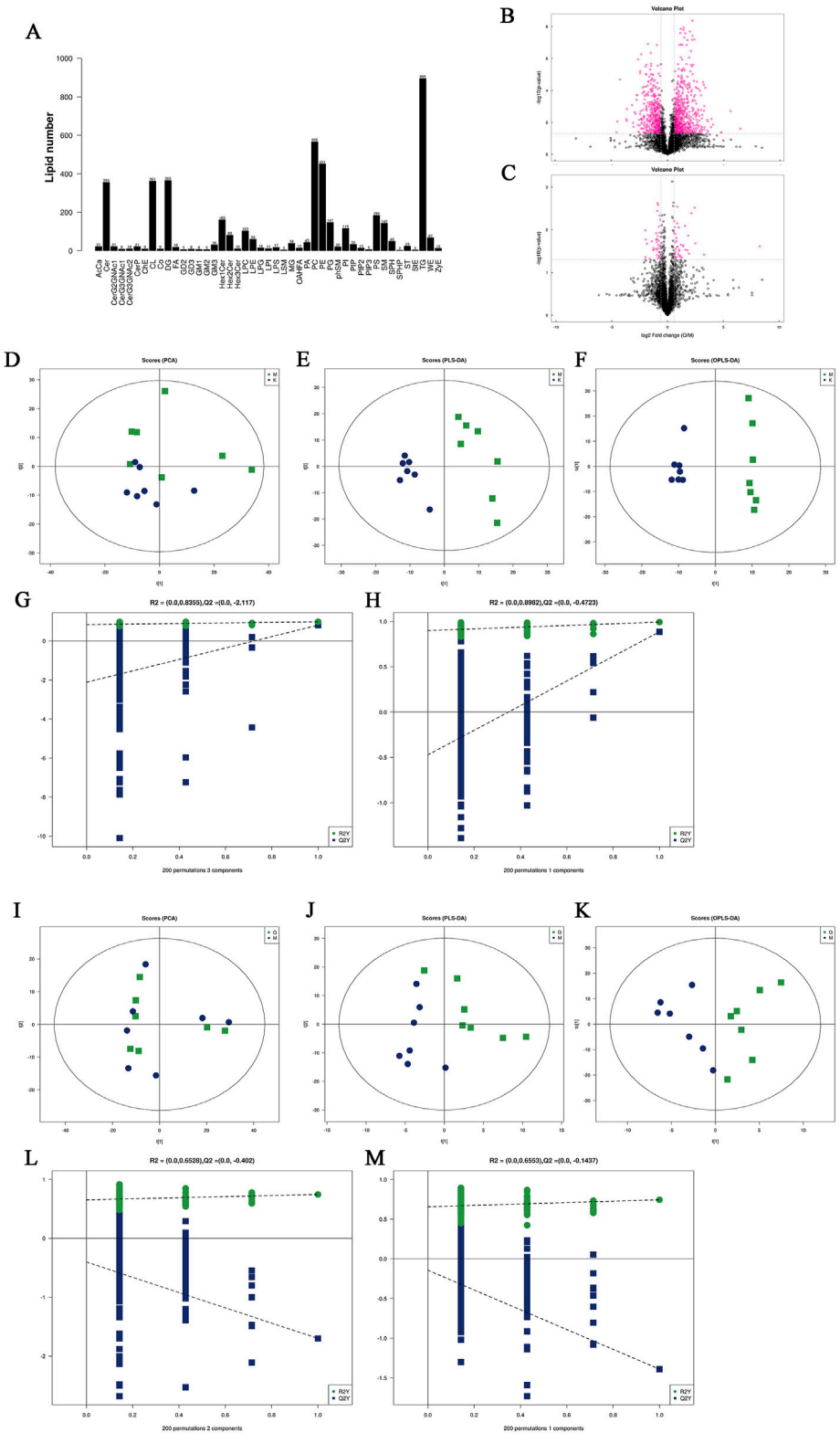

FIGURE 5
(A) Statistics of lipid subclasses and lipid molecules **(B, C)** Volcanic plot **(D)** PCA score plots of differential lipid molecules between model group and control group. **(E, F)** PLS-DA score plots and OPLS-DA score plots differential lipid molecules of differential lipid molecules between model group and control group. **(I)** PCA score plots of differential lipid molecules between quercetin group and model group. **(J, K)** PLS-DA score plots and OPLS-DA score plots differential lipid molecules of differential lipid molecules between quercetin group and model group **(G, H)**, **(L, M)** Cross-validation plot of the PLS-DA and OPLS-DA models with 200 times permutation tests. K: control group, M: model group, Q: quercetin group.

TABLE 3 Differential lipid metabolites in the liver between model group and control group.

No.	Lipidlon	Ion	Class	Molecular formula	CalMz	Rt (min)	VIP	Fold change	p-Value
1	ChE (18:1)	M + H	ChE	$C_{45}H_{82}O_2N_1$	668.634	16.671	15.139	8.115	0.048
2	DG (18:2/18:2)	M + H	DG	$C_{39}H_{72}O_5N_1$	634.541	9.029	13.993	0.413	0.008
3	DG (18:2/22:6)	M + H	DG	$C_{43}H_{72}O_5N_1$	682.541	8.352	13.002	0.422	0.005
4	PC (16:0/18:2)	M + H	PC	$C_{43}H_{81}O_{10}N_1P_1$	802.560	7.687	8.275	0.652	0.010
5	DG (18:0/22:6)	M + H	DG	$C_{43}H_{76}O_5N_1$	686.572	10.044	7.728	9.335	0.000
6	PC (14:0/22:6)	M-H	PC	$C_{43}H_{73}O_8N_1P_1$	762.508	7.546	7.018	0.596	0.018
7	DG (34:1e)	M + H	DG	$C_{37}H_{72}O_4Na_1$	603.532	16.507	6.576	2.116	0.011
8	DG (18:3/18:2)	M + H	DG	$C_{39}H_{70}O_5N_1$	632.525	8.202	6.251	0.238	0.004
9	SM (d42:2)	M + H	SM	$C_{48}H_{94}O_8N_2P_1$	857.675	10.767	5.840	0.647	0.002
10	SM (d40:1)	M + H	SM	$C_{46}H_{92}O_8N_2P_1$	831.660	10.851	5.371	1.539	0.014
11	Hex1Cer(d14:0/22:6)	M + H	Hex1Cer	$C_{43}H_{72}O_{10}N_1$	762.516	7.546	5.110	0.720	0.005
12	Cer(d18:1/24:1)	M + H	Cer	$C_{43}H_{82}O_5N_1$	692.620	12.129	5.081	0.801	0.046
13	PE (16:0/22:6)	M-H	PE	$C_{43}H_{73}O_8N_1P_1$	762.508	7.510	5.050	0.740	0.002
14	DG (20:3/18:2)	M + H	DG	$C_{41}H_{74}O_5N_1$	660.556	9.601	4.860	1.641	0.041
15	ChE (2:0)	M + H	ChE	$C_{29}H_{49}O_2$	429.373	6.009	4.746	9.450	0.002
16	DG (36:4e)	M + H	DG	$C_{39}H_{71}O_4$	603.535	16.413	4.646	2.098	0.012
17	DG (34:1e)	M + H	DG	$C_{37}H_{72}O_4Na_1$	603.532	16.326	4.640	2.107	0.012
18	DG (18:0/18:2)	M + H	DG	$C_{39}H_{76}O_5N_1$	638.572	10.896	4.613	2.122	0.046
19	DG (22:3/18:2)	M + H	DG	$C_{43}H_{78}O_5N_1$	688.587	10.465	4.595	2.833	0.001
20	DG (34:3e)	M + H	DG	$C_{37}H_{68}O_4Na_1$	599.501	14.125	4.588	1.213	0.004
21	DG (36:6e)	M + H	DG	$C_{39}H_{67}O_4$	599.503	14.114	4.560	1.212	0.003
22	Cer (d18:1/18:0)	M + H	Cer	$C_{37}H_{72}O_5N_1$	610.542	9.935	4.108	2.444	0.000
23	Cer (m17:1/19:0 + O)	M + H	Cer	$C_{37}H_{72}O_5N_1$	610.542	9.940	4.063	2.444	0.000
24	TG (18:1/18:1/18:1)	M + H	TG	$C_{57}H_{108}O_6N_1$	902.817	16.442	4.032	3.139	0.012
25	TG (16:0/18:1/18:1)	M + H	TG	$C_{55}H_{106}O_6N_1$	876.801	16.398	3.941	2.057	0.038
26	DG (38:6e)	M + H	DG	$C_{41}H_{71}O_4$	627.535	7.465	3.938	1.503	0.005
27	DG (12:1e/24:2)	M + H	DG	$C_{39}H_{72}O_4Na_1$	627.532	7.459	3.938	1.503	0.005
28	CL (82:11)	M-H	CL	$C_{91}H_{154}O_{17}P_2$	790.534	8.576	3.753	0.787	0.010
29	PE (16:0/19:0)	M + H	PE	$C_{40}H_{81}O_8N_1P_1$	734.569	8.535	3.753	0.789	0.021
30	TG (18:1/18:1/18:2)	M + H	TG	$C_{57}H_{106}O_6N_1$	900.801	16.250	3.724	2.525	0.043
31	PE (18:1/18:2)	M + H	PE	$C_{41}H_{76}O_8N_1P_1Na_1$	764.520	7.529	3.691	0.874	0.022
32	Cer (m40:1 + O)	M + H	Cer	$C_{41}H_{80}O_5N_1$	666.604	12.212	3.679	1.606	0.016
33	TG (18:0/18:1/18:1)	M + H	TG	$C_{57}H_{110}O_6N_1$	904.833	16.805	3.591	4.902	0.006
34	PE (18:0/20:4)	M-H	PE	$C_{43}H_{77}O_8N_1P_1$	766.539	8.985	3.566	1.431	0.000
35	Cer (d18:1/22:0)	M + H	Cer	$C_{41}H_{80}O_5N_1$	666.604	12.212	3.554	1.572	0.017
36	PE (18:1/20:3)	M + H	PE	$C_{43}H_{78}O_8N_1P_1Na_1$	790.536	7.607	3.533	0.855	0.016
37	Cer (d40:1)	M + H	Cer	$C_{41}H_{80}O_5N_1$	666.604	16.309	3.525	1.612	0.039
38	DG (34:4e)	M + H	DG	$C_{37}H_{67}O_4$	575.503	6.527	3.508	0.535	0.000

(Continued on following page)

TABLE 3 (*Continued*) Differential lipid metabolites in the liver between model group and control group.

No.	Lipidlon	Ion	Class	Molecular formula	CalMz	Rt (min)	VIP	Fold change	p-Value
39	DG (34:0e)	M + H	DG	$C_{37}H_{74}O_4Na_1$	605.548	16.746	3.332	2.636	0.004
40	PC (8:1e/10:1)	M + H	PC	$C_{26}H_{51}O_7N_1P_1$	520.340	1.509	3.329	0.607	0.027
41	PE (18:1/20:2)	M + H	PE	$C_{43}H_{80}O_8N_1P_1Na_1$	792.551	8.656	3.306	0.827	0.019
42	Cer (m36:1 + O)	M + H	Cer	$C_{37}H_{72}O_5N_1$	610.542	10.342	3.304	2.143	0.002
43	PE (16:1/18:1)	M-H	PE	$C_{39}H_{73}O_8N_1P_1$	714.508	8.052	3.246	0.756	0.006
44	Hex1Cer (d32:2)	M + H	Hex1Cer	$C_{39}H_{72}O_{10}N_1$	714.516	8.033	3.246	0.756	0.006
45	CL (78:10)	M-H	CL	$C_{87}H_{148}O_17P_2$	763.510	7.504	3.245	0.766	0.001
46	PE (18:0/18:1)	M + H	PE	$C_{41}H_{80}O_8N_1P_1Na_1$	768.551	9.001	3.228	1.329	0.000
47	PE (16:1e/22:4)	M-H	PE	$C_{43}H_{77}O_7N_1P_1$	750.544	9.388	3.144	2.718	0.000
48	DG (16:1/22:6)	M + H	DG	$C_{41}H_{70}O_5N_1$	656.525	7.927	3.143	0.349	0.003
49	DG (18:0/18:1)	M + H	DG	$C_{39}H_{78}O_5N_1$	640.587	11.925	3.040	3.352	0.000

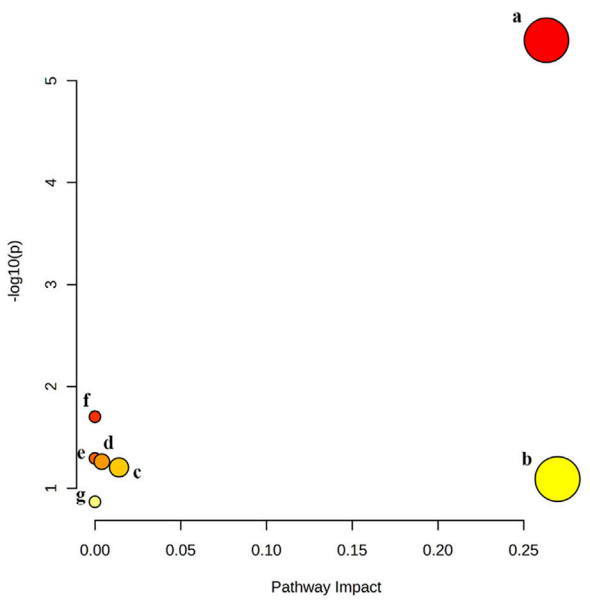

FIGURE 6
Six metabolic pathways related to changed biomarkers. **(a)** glycerophospholipid metabolism; **(b)** linoleic acid metabolism; **(c)** alpha-Linolenic acid metabolism; **(d)** glycosylphosphatidylinositol (GPI)-anchor biosynthesis; **(e)** glycerolipid metabolism; **(f)** sphingolipid metabolism; **(g)** arachidonic acid metabolism.

them, Glycerophospholipid metabolism is the most likely metabolic pathway involved in quercetin treatment of MAFLD.

3.3 Results of *in vitro* experiment

3.3.1 Oleic acid-induced lipid accumulation in HepG2 cells

The effect of different concentrations of oleic acid on the viability of HepG2 cells was detected by CCK 8. Compared with the control group, oleic acid had no effect on cell viability when it was lower than 0.2 mM ($p > 0.05$), and had a significant effect on cell viability when it was higher than 0.25 mM ($p < 0.05$) (Figure 7A). Oil red O staining was used to detect the lipid accumulation of HepG2 cells induced by oleic acid. Compared with the control group, the accumulation of lipid droplets was obvious after induction with 0.5 mM and 0.75 mM oleic acid, suggesting a large amount of lipid accumulation. However, 0.75 mM oleic acid induced significant changes in cell morphology. Therefore, we chose 0.5 mM oleic acid to establish the MAFLD model of HepG2 cells (Figure 8).

3.3.2 Quercetin improved the number of lipid droplets in HepG2 cells induced by oleic acid

Firstly, CCK 8 was used to detect the effect of quercetin on the viability of HepG2 cells. Compared with the control group, the cell viability of the model group was significantly decreased ($p < 0.01$). Compared with the model group, 5–20 μM quercetin significantly increased cell viability in a dose-dependent manner (Figure 7B). Secondly, oil red O staining showed that compared with the model group, 10 μM and 20 μM quercetin could significantly reduce the area of lipid droplets induced by oleic acid, and the reduction effect of 20 μM dose was more obvious. Quercetin above 30 μM could reduce the number of lipid droplets, but the morphology of lipid droplets increased, indicating that higher doses of quercetin may affect cells (Figure 9). In Figures 7C–E, compared with the control group, the TC, TG and LDL-C of the model group were significantly increased ($p < 0.01$); compared with the model group, quercetin treatment significantly reduced the levels of these three lipid markers ($p < 0.05$).

3.3.3 Quercetin improves transcriptomic sequencing of MAFLD cell model

In order to explore the potential molecular mechanism of quercetin in improving MAFLD in cell models, we performed RNA sequencing on cells in the control group, model group, and 20 μM quercetin group. The sequencing quality factor Q30 of all samples reached more than 97.3%, indicating that the quality of

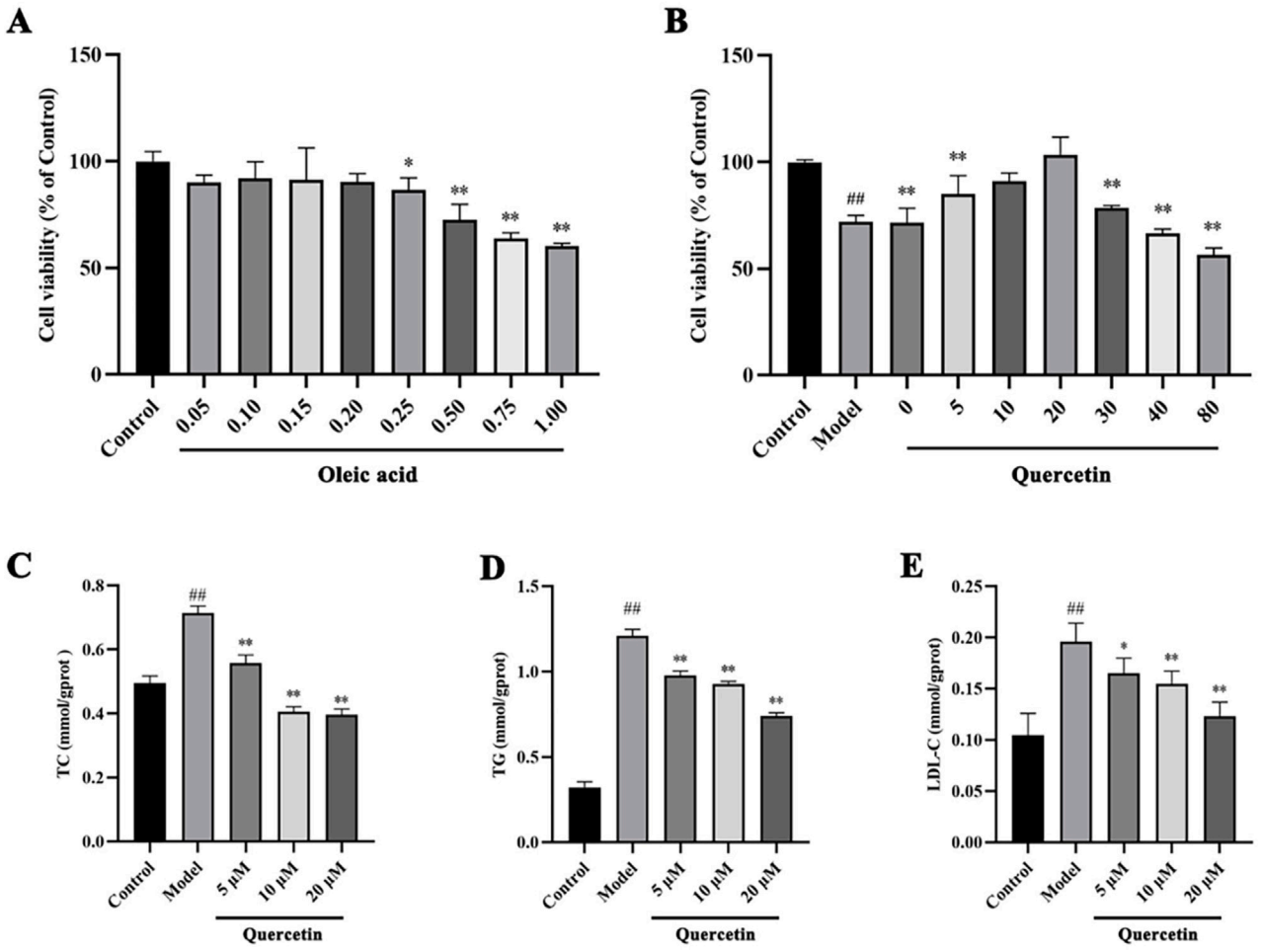

FIGURE 7
The effects of oleic acid and quercetin on HepG2 cells **(A)** Effect of oleic acid on the viability of HepG2 cells **(B)** Effect of quercetin on the viability of HepG2 cells induced by oleic acid **(C–E)** Effect of quercetin on the level of lipid markers in HepG2 cells induced by oleic acid. Values were represented the mean ± SD ($n \geq 3$). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Control, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Model.

sequencing data was good. Quantitative analysis of differential gene expression based on FPKM, as shown in Figure 10A. Compared with the control group, there were 133 differentially expressed genes (DEGs) in the model group, of which 79 genes were upregulated and 54 genes were downregulated. Compared with the model group, there were 141 differential genes in the quercetin group, of which 47 genes were upregulated and 94 genes were downregulated (Figures 10B, C).

GO enrichment analysis of differential genes showed that the main functions of differential genes in the model group and the control group were transcriptional regulation, positive regulation of transcription by RNA polymerase II, cytokine-mediated signal transduction pathway and immune response during biological processes. The differentially expressed genes mainly enriched in cell components include cell membrane and its organic composition, nucleus, cytoplasm, etc. In the molecular functional classification, including protein binding, metal ion binding, DNA binding, nucleic acid and nucleotide binding (Figures 10D, E). The main enriched functions of differential genes in quercetin group and model group were signal transduction, nervous system development, ion transport, multicellular organism development and immune response in biological processes. The differentially expressed genes mainly enriched in cell components included cell membrane and its organic composition, cytoplasm and cytoplasmic membrane, nucleus, etc. In the molecular functional classification, including protein binding, metal ion binding (Figures 10F, G).

3.3.4 Quercetin decreased the expression levels of *Epha2, Dusp1, Csf1, Golga4, Best1* and *Tmsb4x* genes in HepG2 cells induced by oleic acid

The relative expression levels of *Epha2, Dusp1, Csf1, Golga4, Best1* and *Tmsb4x* genes were consistent with the change trend of the corresponding abundance value (FPKM) in RNA-Seq analysis. Compared with the control group, the expression levels of *Epha2, Dusp1, Csf1, Golga4, Best1* and *Tmsb4x* genes in the model group were significantly upregulated ($p < 0.01$). After quercetin treatment, the expression levels of *Epha2, Dusp1, Csf1, Golga4, Best1* and *Tmsb4x* genes were significantly downregulated ($p < 0.01$) (Table 5).

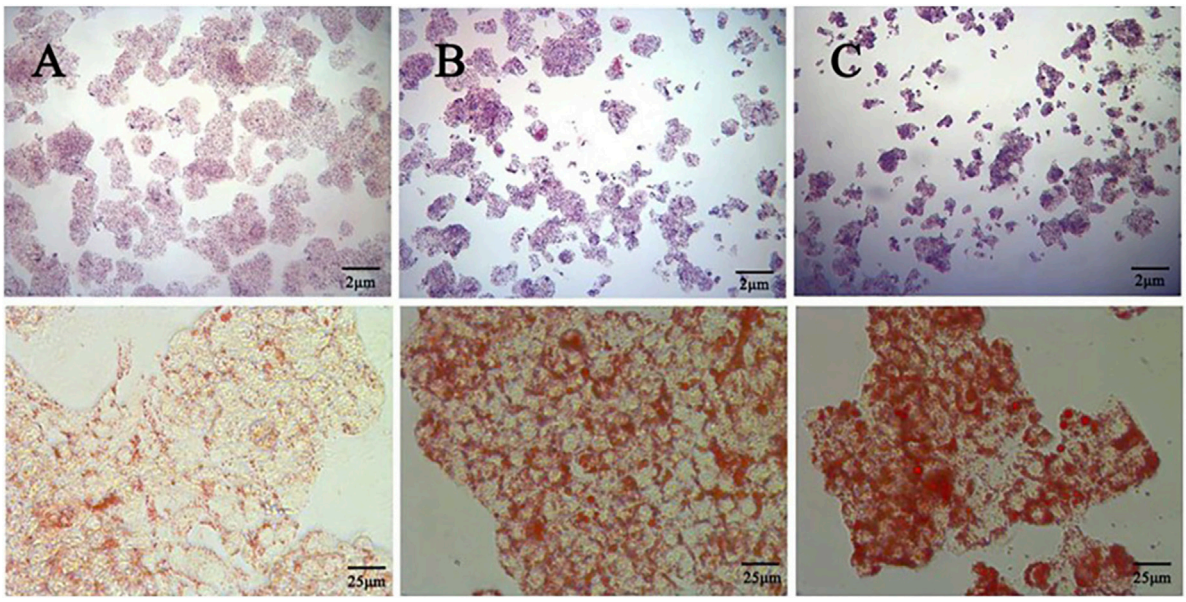

FIGURE 8
Comparison of oil red O staining between the control group and the OA induced group (400x) **(A)** Control group **(B)** 0.5 mM oleic acid group **(C)** 0.75 mM oleic acid group.

3.3.5 Quercetin can improve oleic acid-induced MAFLD by up-regulating p-p38 and pERK1/2 and down-regulating GPX4, SCL3A2 and xCT

MAPK signaling pathway plays an important role in the metabolism of MAFLD. Western blot analysis showed that compared with the control group, the expression of phosphorylated p38 MAPK and ERK1/2 protein in HepG2 cells treated with oleic acid was significantly decreased ($p < 0.01$), while quercetin treatment reversed this situation and upregulated the expression of phosphorylated p38 MAPK and ERK1/2 protein. Compared with the control group, the expression of GPX4, SCL3A2 and xCT protein in HepG2 cells induced by oleic acid was significantly decreased ($p < 0.01$), and the expression of GPX4, SCL3A2 and xCT protein was increased after quercetin treatment ($p < 0.05$) (Figure 11). It is suggested that quercetin may inhibit ferroptosis through the p38 MAPK/ERK signaling pathway, thereby alleviating the progression of MAFLD.

4 Discussion

MAFLD has become a leading cause of chronic liver disease worldwide. Disturbed lipid metabolism is the main predisposing factor for MAFLD, which has been reported to affect up to 70% of overweight people and more than 90% of morbidly obese people (Bourganou et al., 2025). However, there is still no satisfactory strategy to treat MAFLD induced by obesity. Ideal drugs for the treatment of MAFLD not only inhibit hepatic steatosis, but also ameliorate metabolic diseases associated with obesity.

Zanthoxylum plants have been shown to have a wide range of biological activities, including *Z. bungeanum* Maxim (Huang Y. et al., 2023).、*Zanthoxylum rhetsa* (Imphat et al., 2021; Rahman et al., 2002)、*Zanthoxylum khasianum*, etc. Santhanam et al. found that ethyl acetate extract of *Z. rhetsa* significantly inhibited the increase of pro-inflammatory cytokines in HDF cells induced by ultraviolet radiation b (Santhanam et al., 2018). Barman et al. 's study suggested that *Z. rhetsa* ethanol extract had significant free radical scavenging activity, and oral glucose tolerance test found that the extract could effectively reduce the blood glucose level of diabetic mice. Recent studies have shown that *Z. bungeanum* Maxim. Can attenuate high-fat diet (HFD)-induced MAFLD by improving fat accumulation (Peng et al., 2024).

In this study, we predicted the important active components that play a role in the treatment of MAFLD by *Z. bungeanum* Maxim. Including quercetin, geranylgeranyl, hydroxy-α-sanshool and hydroxy-β-sanshool based on network analysis, among which quercetin corresponded to the most potential targets, network analysis hypothesizes that quercetin may be the main active ingredient in the therapeutic effects of *Z. bungeanum* Maxim. On MAFLD. Quercetin is a secondary metabolite of plants with a variety of health benefits. Numerous studies have found quercetin to have some anti-inflammatory effects in MAFLD mice (Jiang et al., 2025; Lu et al., 2024; Markowska et al., 2024). The combination of quercetin and metformin has also been found to reduce cirrhosis by stimulating autophagy and reducing inflammatory cytokines via the cAMP/AMPK/SIRT1 signaling pathway (Afshari et al., 2023). A study by Cao P. et al. Found that quercetin prevents MAFLD via AMPK-mediated mitochondrial autophagy (Cao et al., 2023), and it has also been suggested that quercetin, by down-regulating the mTOR/YY1 signaling pathway converts cholesterol to bile acids, which leads to an increase in CYP7A1 activity, restores cholesterol homeostasis, and exerts hepatoprotective effects against T2DM-associated MAFLD (Yang et al., 2023).

In recent years, the "histology" approach has been increasingly applied to the study of drug mechanism of action, especially the combination of multi-omics techniques has provided new ideas for

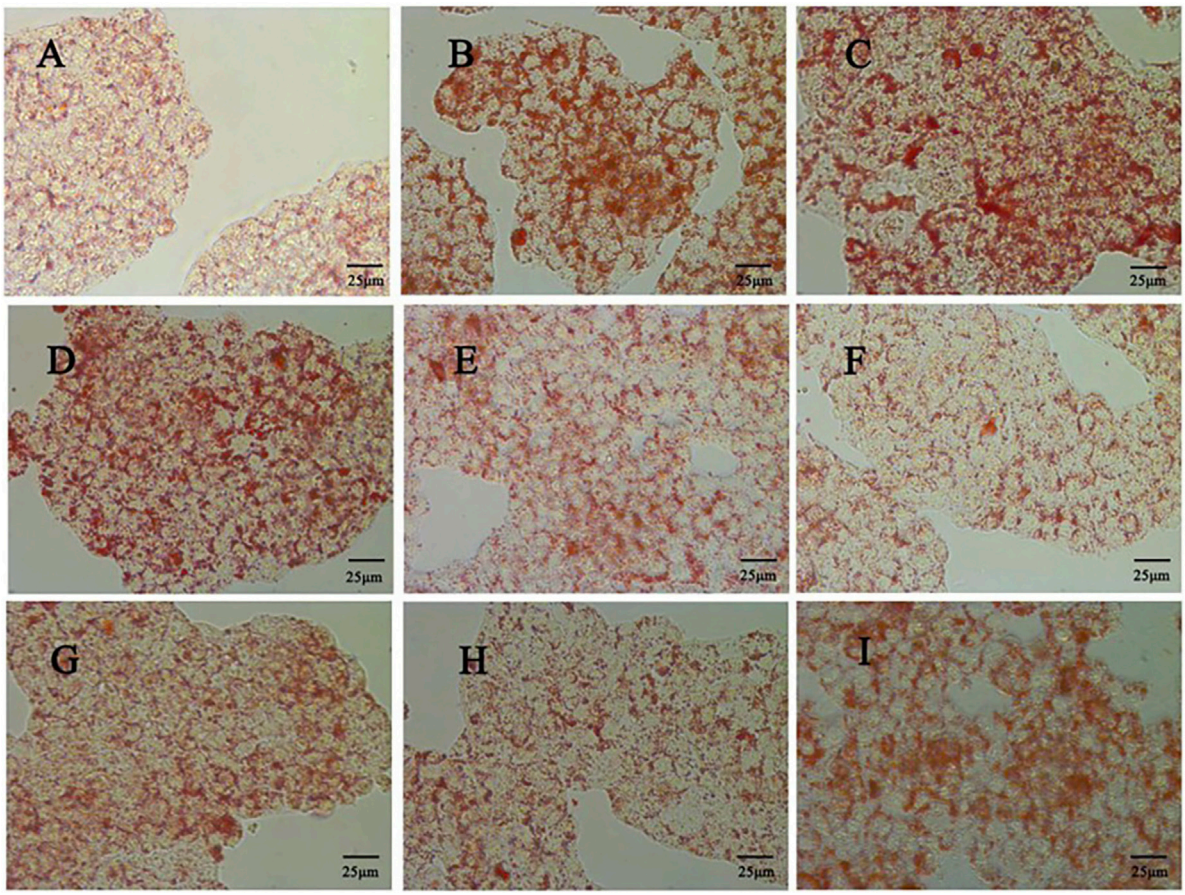

FIGURE 9
Effect of different doses of quercetin on lipid droplet accumulation in HepG2 cells (400x) **(A)** Control group **(B)** Model group; **(C)** 0 μM quercetin group **(D)** 5 μM quercetin group; **(E)** 10 μM quercetin group **(F)** 20 μM quercetin group; **(G)** 30 μM quercetin group **(H)** 40 μM quercetin group **(I)** 80 μM quercetin group.

the elucidation of drug mechanism of action and drug discovery. In the present study, network analysis and molecular docking results predicted that quercetin may be the main active ingredient in *Z. bungeanum* Maxim. Exerting therapeutic effects in MAFLD, in addition, by establishing a mouse model and a cell model of MAFLD, and by detecting serum biochemical indexes, pathological HE staining and oil red O staining, it was found that quercetin could regress the MAFLD mice serum ALT, AST, LDL-C, HDL -C and TC levels, hepatic pathological changes were alleviated, and hepatic lipid accumulation was improved. Further analysis of hepatic lipid metabolism in MAFLD mice using lipidomics technology revealed that quercetin treatment of MAFLD may improve hepatic lipid metabolism disorders in mice through the Glycerophospholipid metabolic pathway. In addition, we evaluated the effect of quercetin on oleic acid-induced HepG2 cells by detecting TC, TG, LDL-C content and lipid accumulation in the cells. Differential genes and signaling pathways were screened by transcriptome sequencing and further validated by Western blot, and we obtained that quercetin may improve MAFLD through p38 MAPK/ERK signaling pathway.

The predicted results of network analysis in this study indicated that the core targets of *Z. bungeanum* Maxim. With therapeutic effects on MAFLD were IL-1β, TNF, IL-6 and TGF-βI. The molecular docking results further predicted that quercetin might be the main active substance in the therapeutic effects of Zanthoxylum bungeanum Maxim. On MAFLD. Quercetin is a flavonoid widely found in the plant kingdom, and studies have shown that quercetin has hepatoprotective effects against MAFLD, especially against hepatic steatosis and hepatitis (Chen et al., 2021b). A study found that quercetin reversed MAFLD symptoms by reducing oleic acid-induced secretion of inflammatory factors IL-8 and TNF-α in HepG2 cells. This is consistent with our network analysis predicting that the core targets of quercetin for MAFLD are IL-1β, TNF, IL-6 and TGF-βI, among others (Chen et al., 2021a). Subsequently, we used tetracycline combined with high-fat diet to establish a mouse MAFLD model and oleic acid to establish a HepG2 cell model. The liver is an important organ for lipid metabolism, and when hepatic fatty acid synthesis or uptake exceeds the liver's capacity for oxidation or its output, lipid droplets accumulate in the liver parenchyma and triglyceride levels increase, causing MAFLD when lipids account for more than 5% of the liver's wet weight (Gutiérrez-Cuevas et al., 2021). Tetracycline, an antibiotic that induces steatosis (Zhong et al., 2020), has been found to induce the transport of lipids to the liver, which in

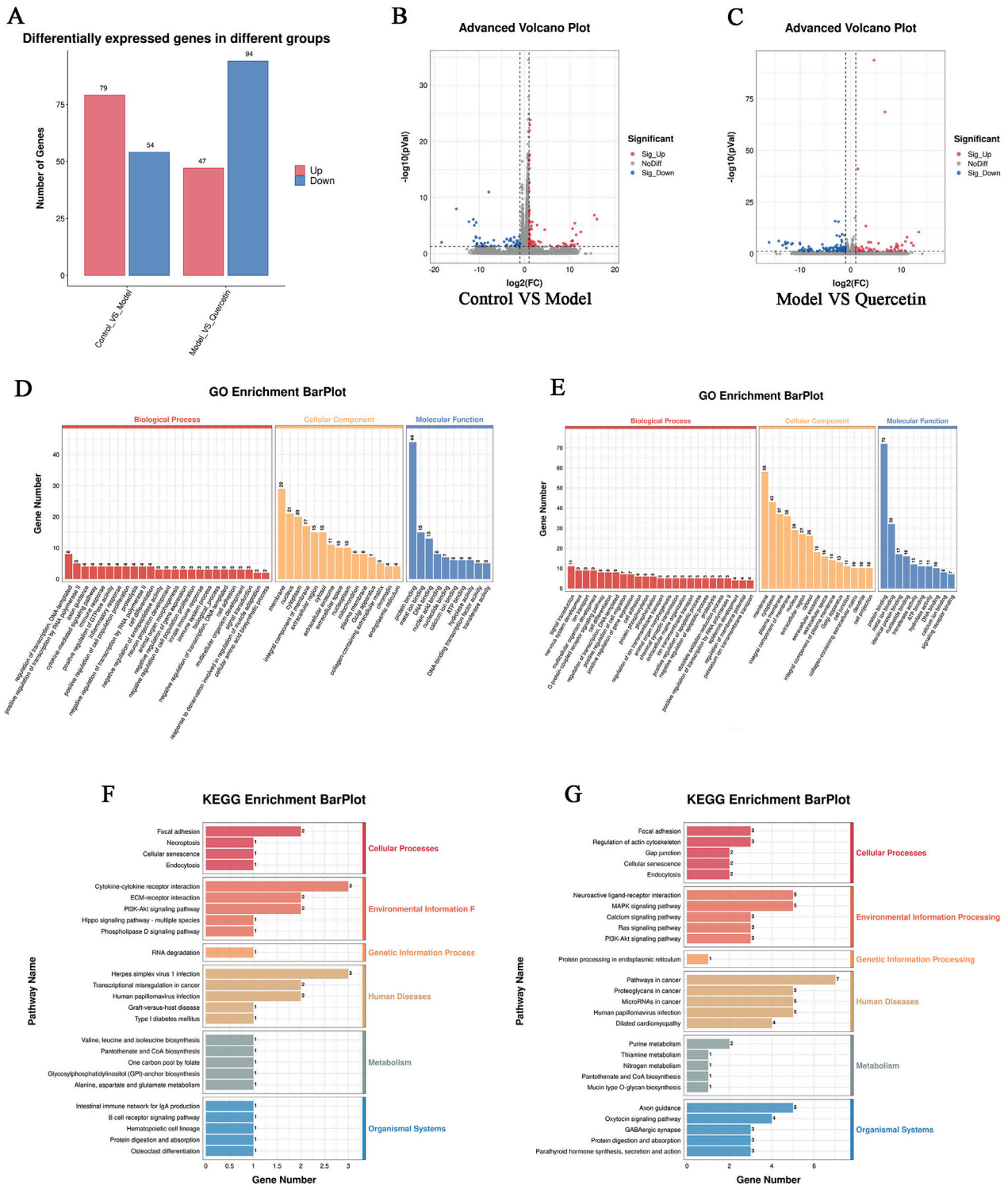

FIGURE 10
(A) Statistical map of up and down frequency modulation of differentially expressed genes, **(B)** Differential expression volcano map of control group and model group **(C)** Differential expression volcano map of model group and quercetin group. In the differential expression volcano map, the upregulated expression genes were represented by red dots, and the downregulated expression genes were represented by blue dots, **(D, F)** GO and KEGG enrichment analysis of differential genes between model group and control group **(E, G)** GO and KEGG enrichment analysis of differential genes between quercetin group and model group.

TABLE 4 Differential lipid metabolites in the liver between quercetin group and model group.

No.	Lipidlon	Ion	Class	Molecular formula	CalMz	Rt (min)	VIP	Fold change	p-Value
1	PE (38:5e)	M-H	PE	$C_{43}H_{77}O_7N_1P_1$	750.544	9.388	3.068	0.834	0.011
2	LPC (18:2)	M + H	LPC	$C_{27}H_{51}O_9N_1P_1$	564.331	3.175	2.992	309.998	0.024
3	LPE (18:0)	M-H	LPE	$C_{23}H_{47}O_7N_1P_1$	480.310	2.453	2.506	1.594	0.020
4	Hex1Cer (d33:0 + O)	M + H	Hex1Cer	$C_{39}H_{78}O_9N_1$	704.567	7.364	2.300	0.876	0.043
5	PE (18:0e)	M + H	PE	$C_{23}H_{49}O_7N_1P_1$	482.324	2.452	2.190	1.338	0.005
6	CL (72:7)	M-H	CL	$C_{81}H_{143}O_{17}P_2$	1,449.981	14.667	2.139	0.825	0.045
7	PE (36:2e)	M + H	PE	$C_{41}H_{80}O_7N_1P_1Na_1$	752.556	9.514	1.971	0.847	0.011
8	PE (38:4p)	M + H	PE	$C_{43}H_{79}O_7N_1P_1$	752.559	9.844	1.971	0.847	0.011
9	LPC (18:2)	M + H	LPC	$C_{26}H_{51}O_7N_1P_1$	520.340	2.349	1.849	9.726	0.038
10	LPE (16:0)	M-H	LPE	$C_{21}H_{43}O_7N_1P_1$	452.278	1.849	1.825	1.598	0.037
11	PE (16:0e)	M + H	PE	$C_{21}H_{45}O_7N_1P_1$	454.293	1.854	1.771	1.379	0.005
12	PE (36:4p)	M + H	PE	$C_{41}H_{75}O_7N_1P_1$	724.528	8.373	1.755	0.830	0.004
13	PE (37:1e)	M + H	PE	$C_{42}H_{85}O_7N_1P_1$	746.606	9.305	1.724	0.792	0.028
14	DG (38:8e)	M + H	DG	$C_{41}H_{66}O_4Na_1$	645.485	13.396	1.720	0.763	0.040
15	PE (36:5e)	M-H	PE	$C_{41}H_{73}O_7N_1P_1$	722.513	8.353	1.644	0.850	0.027
16	PE (38:1)	M + H	PE	$C_{43}H_{84}O_8N_1P_1Na_1$	796.583	9.749	1.515	0.818	0.016
17	PE (40:4)	M-H	PE	$C_{45}H_{81}O_8N_1P_1$	794.571	9.733	1.483	0.817	0.010
18	CL (82:7)	M-H	CL	$C_{91}H_{162}O_{17}P_2$	794.565	9.727	1.483	0.817	0.010
19	PE (38:4p)	M + H	PE	$C_{43}H_{79}O_7N_1P_1$	752.559	9.354	1.292	0.746	0.043
20	PE (37:1e)	M + H	PE	$C_{42}H_{84}O_7N_1P_1Na_1$	768.588	8.215	1.215	0.814	0.030
21	PE (39:4e)	M + H	PE	$C_{44}H_{83}O_7N_1P_1$	768.590	8.205	1.202	0.819	0.044
22	PE (40:4p)	M + H	PE	$C_{45}H_{83}O_7N_1P_1$	780.590	10.330	1.195	0.775	0.010
23	PE (40:5e)	M-H	PE	$C_{45}H_{81}O_7N_1P_1$	778.576	10.281	1.173	0.800	0.019
24	CL (79:2)	M-H	CL	$C_{88}H_{166}O_{17}P_2$	778.581	10.269	1.173	0.800	0.019
25	CL (78:3)	M-H	CL	$C_{87}H_{162}O_{17}P_2$	770.565	10.171	1.161	0.607	0.012
26	PE (40:8)	M-H	PE	$C_{45}H_{73}O_8N_1P_1$	786.508	6.642	1.104	0.821	0.031
27	Hex1Cer (d38:8)	M + H	Hex1Cer	$C_{45}H_{72}O_{10}N_1$	786.516	6.642	1.104	0.821	0.031
28	SM (d44:5)	M + H	SM	$C_{49}H_{92}O_6N_2P_1$	835.669	11.370	1.081	5.429	0.003
29	DG (38:3e)	M + H	DG	$C_{41}H_{76}O_4Na_1$	655.564	9.751	1.006	0.738	0.012

TABLE 5 Validation of qRT-PCR for candidate differentially expressed genes ($\bar{x} \pm s$, $n \geq 3$).

Group	Dose (μM)	*Epha2*	*Dusp1*	*Csf1*	*Golga4*	*Best1*	*Tmsb4x*
Control	-	0.28 ± 0.05	0.25 ± 0.05	0.41 ± 0.05	0.45 ± 0.04	0.45 ± 0.04	0.27 ± 0.05
Model	-	1.07 ± 0.01$^{\#\#}$	1.20 ± 0.09$^{\#\#}$	0.74 ± 0.29	1.28 ± 0.24$^{\#}$	1.28 ± 0.24$^{\#}$	0.59 ± 0.16
Quercetin	20	0.46 ± 0.01**	0.36 ± 0.11**	0.45 ± 0.00	0.26 ± 0.05**	0.26 ± 0.05**	0.56 ± 0.25

Values were represented the mean ± SD (n = 3). $^{\#}P < 0.05$. $^{\#\#}P < 0.01$ vs. Control. $^{*}P < 0.05$. $^{**}P < 0.01$ vs. Model.

turn triggers the infiltration of hepatic fat and the formation of vesicular fatty liver disease. As fatty acids in the liver continue to rise, the level of oxidative stress also continues to rise, which results in a series of attacks on functional proteins, impeding the normal metabolism of fatty acids, and leading to the abnormal accumulation of triglycerides, which in turn exacerbates the

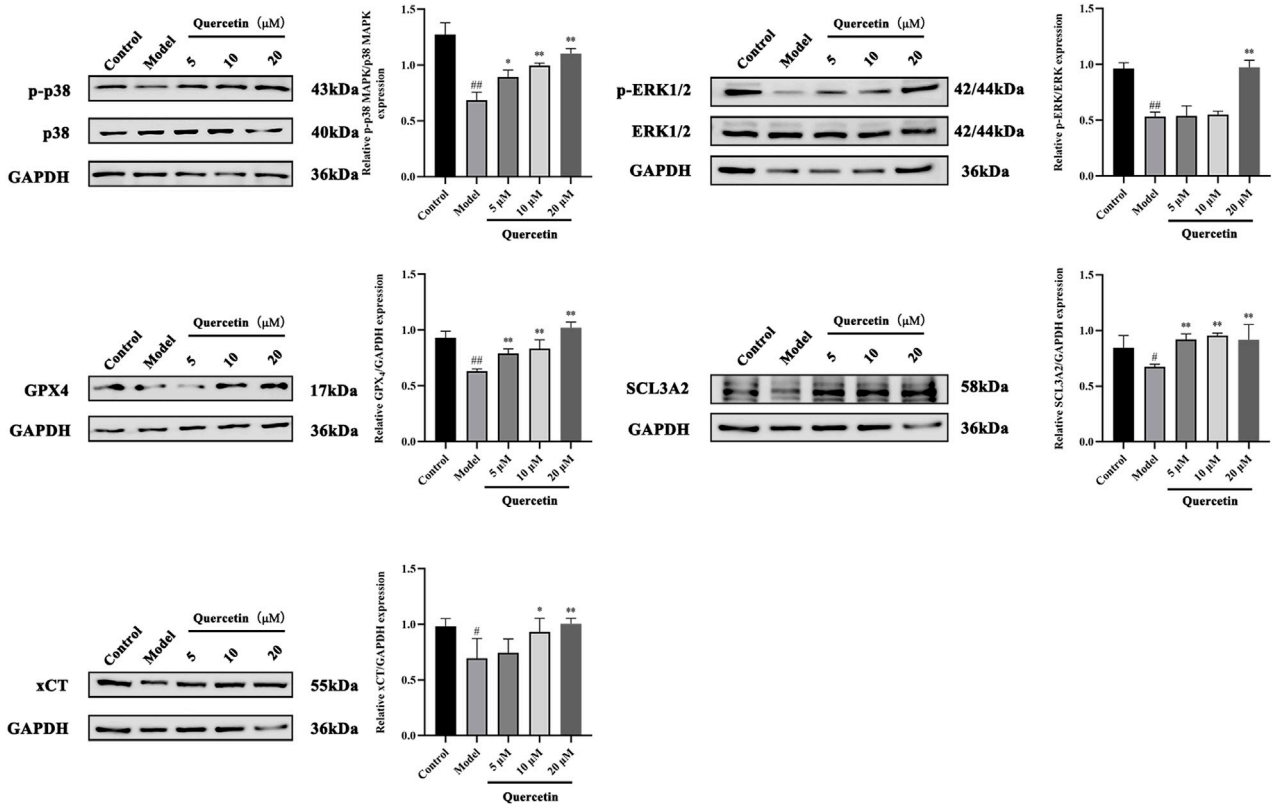

FIGURE 11
Effect of quercetin on expression of related proteins in mouse liver tissue. Values were represented the mean ± SD (n = 3). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Control, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Model.

formation of fatty liver disease. As fatty acid levels rise in the liver, oxidative stress levels also rise, causing a series of functional proteins to be attacked, preventing the normal metabolism of fatty acids and leading to an abnormal accumulation of triglycerides, which in turn exacerbates fatty liver formation (Steinberg et al., 2025). Therefore, we used tetracycline combined with a high-fat diet to model MAFLD. Similarly, *in vitro* models of steatosis are often used to explore the role of a drug in the treatment of fatty liver disease. Oleic acid is a monounsaturated fatty acid found in animals and plants, and the synthesis of palmitic acid by acyl coenzyme A is extended to stearic acid, which is desaturated to give oleic acid, which occupies about 40%–50% of the total free fatty acids, and is an important constituent of triglycerides stored in the cytoplasm. In some studies, 0.1 mM oleic acid was used to establish MAFLD model of HepG2 cells for *in vitro* experiments (Kaixuan et al., 2022). However, by combining the results of CCK-8 and oil red O staining, it was observed that the viability of HepG2 cells was significantly inhibited when the concentration of oleic acid was 0.5mM, and the accumulation of cell lipid drops was significantly increased, and the cell morphology did not change significantly. Therefore, in this study, the steatosis of HepG2 cells induced by 0.5 mM oleic acid was used to establish an *in vitro* model of MAFLD.

The p38 mitogen-activated protein kinase (p38 MAPK) is an important inflammatory factor and the basis of oxidative stress, and is involved in the regulation of nuclear factor E2-related factor 2 (Nrf2) and NF-κB in liver and metabolic disorders (Wang et al., 2023). We enriched the gene targets obtained by transcriptome sequencing to the MAPK signaling pathway may be involved in the treatment of MAFLD by quercetin, and further validated the related proteins on the MAPK signaling pathway by Western blot, and found that quercetin inhibits iron death through the p38 MAPK/ERK signaling pathway, thus alleviating the extension of MAFLD disease. It has been shown that quercetin significantly ameliorates hepatic dysfunction and host metabolic disorders in MAFLD mice (Shi et al., 2022) and inhibits MAFLD through AMPK-mediated mitochondrial phagocytosis (Cao et al., 2023). Quercetin also exhibits hepatoprotective activity in early-stage MAFLD rats by modulating fatty acids, inflammation, oxidative stress, and related metabolites (Xu et al., 2019). Flavonoids have been shown to improve hepatic steatosis by regulating glycerophospholipid metabolism in the treatment of MAFLD. Du Siyu et al. found that total flavonoids in Garcinia cambogia tea could reduce the *de novo* synthesis of fatty acids and regulate glycerophospholipid metabolism by targeting the PPAR signaling pathway, thereby improving hepatic steatosis (Du et al., 2024). Co-loaded liposomes prepared from Antarctic krill oil and quercetin have also been shown to be protective against oleic acid-induced lipoatrophy and oxidative stress in HepG2 cells (Li et al., 2024).

In addition, quercetin-rich buckwheat tartare extracts can prevent alcoholic liver disease by regulating hepatic glycerophospholipid metabolism (Cao et al., 2022). Similarly, our study found that quercetin is metabolized *via* the Glycerophospholipid metabolic pathway regulates hepatic lipid metabolites in MAFLD mice, thereby ameliorating liver injury.

In the present study, quercetin was hypothesized to be the main active metabolite of *Z. bungeanum* Maxim. For the treatment of MAFLD, and its potential mechanism of action was further elucidated by *in vitro* experiments based on *in vivo* experiments. However, some limitations need to be recognized. First, the results of the network analysis predicted that quercetin might be the primary active substance for the pharmacological effects of *Z. bungeanum* Maxim., but quercetin itself belongs to the class of pan-assay interfering compound (PAINS) substances, which are substances with nonspecific, pharmacologically irrelevant *in vitro* and computer-simulated data. Therefore, it may non-specifically interfere with multiple assay systems in vitro experiments, and more rigorous control systems are needed to validate the specificity of its action (e.g., designing *in vitro* experiments using other non-PAINS active substances in peppercorns for multi-faceted validation). In addition, the mechanism of action of quercetin needs to be verified *in vivo* experiments because of such properties of quercetin. In this study, the mechanism of action of quercetin was only predicted *in vivo* by lipidomics techniques, but the mechanism was not further verified *in vivo* experiments. To address this issue, future studies should compare the efficacy of quercetin with other non-PAINS bioactive compounds derived from *Z. bungeanum* Maxim. And focus primarily on *in vivo* experimental data to further confirm its therapeutic specificity and to better interpret and validate its pharmacological findings. Second, although lipidomic analysis revealed seven key pathways for quercetin enrichment, we focused only on the glycerophospholipid metabolism pathway for subsequent validation. Other interesting pathways, such as linoleic acid metabolism and α-linolenic acid metabolism, deserve further exploration. Finally, in addition to lipidomics, the metabolomics dataset generated in this study still has untapped potential to provide insights into the mechanisms of MAFLD progression and interventions.

5 Conclusion

In conclusion, our study showed that quercetin, as a major active ingredient in *Z. bungeanum* Maxim., may regulate hepatic lipid metabolites via glycerophospholipid metabolism pathway in MAFLD mice, thereby ameliorating hepatic lipid accumulation and liver injury. Meanwhile, our results also indicated that quercetin was able to ameliorate MAFLD by reducing oleic acid-induced lipid accumulation in HepG2 cells and slow down the progression of MAFLD disease by inhibiting iron death through the p38 MAPK/ERK signaling pathway. Our study reveals the potential mechanism by which quercetin isolated from *Z. bungeanum* Maxim. Ameliorates MAFLD, and these results provide a theoretical basis for the mechanism of *Z. bungeanum* Maxim. Treatment for MAFLD.

Data availability statement

The transcriptomics data presented in this study have been submitted to the (NCBI) BioProject repository, with the accession number: PRJNA1241200. The original data of this article can be found in the article/Supplementary Material.

Ethics statement

The animal study was approved by the Ethics Committee for Laboratory Animal Welfare of Chengdu University of Traditional Chinese Medicine. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

YC: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Writing–original draft, Writing–review and editing. FR: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Writing–original draft, Writing–review and editing. NY: Formal Analysis, Investigation, Methodology, Writing–original draft. QX: Formal Analysis, Investigation, Methodology, Writing–original draft. SG: Formal Analysis, Investigation, Methodology, Writing–original draft. WP: Formal Analysis, Investigation, Methodology, Writing–original draft. ZY: Formal Analysis, Investigation, Methodology, Writing–original draft. QL: Funding acquisition, Resources, Supervision, Validation, Writing–review and editing. SL: Funding acquisition, Resources, Supervision, Validation, Writing–review and editing. CR: Investigation, Resources, Supervision, Validation, Writing–review and editing.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1517291/full#supplementary-material

References

Afshari, H., Noori, S., and Zarghi, A. (2023). Hepatic steatosis alleviated by a novel metformin and quercetin combination activating autophagy through the cAMP/AMPK/SIRT1 pathway. *Iran. J. Pharm. Res.* 22 (1), e136952. doi:10.5812/ijpr-136952

Ali, G., Zeb, A., Usman, M., and Al-Babili, S. (2024). Walnut extract protects against hepatic inflammation and toxicity induced by a high-fat diet. *Food Sci. Nutr.* 12 (10), 8340–8352. doi:10.1002/fsn3.4405

Bourganou, M. V., Chondrogianni, M. E., Kyrou, I., Flessa, C. M., Chatzigeorgiou, A., Oikonomou, E., et al. (2025). Unraveling metabolic dysfunction-associated steatotic liver disease through the use of omics technologies. *Int. J. Mol. Sci.* 26 (4), 1589. doi:10.3390/ijms26041589

Cai, Y., Fang, L., Chen, F., Zhong, P., Zheng, X., Xing, H., et al. (2025). Targeting AMPK related signaling pathways: a feasible approach for natural herbal medicines to intervene non-alcoholic fatty liver disease. *J. Pharm. Anal.* 15 (1), 101052. doi:10.1016/j.jpha.2024.101052

Cao, P., Wang, Y., Zhang, C., Sullivan, M. A., Chen, W., Jing, X., et al. (2023). Quercetin ameliorates nonalcoholic fatty liver disease (NAFLD) via the promotion of AMPK-mediated hepatic mitophagy. *J. Nutr. Biochem.* 120, 109414. doi:10.1016/j.jnutbio.2023.109414

Cao, P., Wu, Y., Li, Y., Xiang, L., Cheng, B., Hu, Y., et al. (2022). The important role of glycerophospholipid metabolism in the protective effects of polyphenol-enriched Tartary buckwheat extract against alcoholic liver disease. *Food Funct.* 13 (20), 10415–10425. doi:10.1039/d2fo01518h

Chandran, U., Mehendale, N., Patil, S., Chaguturu, R., and Patwardhan, B. (2017). "Chapter 5 - network pharmacology," in *Innovative approaches in drug discovery*. Editors B. Patwardhan and R. Chaguturu (Boston: Academic Press), 127–164.

Chen, H., Yan, S., Xiang, Q., Liang, J., Deng, X., He, W., et al. (2024). Network analysis and experimental verification of Salvia miltiorrhiza Bunge-Reynoutria japonica Houtt. drug pair in the treatment of non-alcoholic fatty liver disease. *BMC Complement. Med. Ther.* 24 (1), 305. doi:10.1186/s12906-024-04600-4

Chen, L., Liu, J., Mei, G., Chen, H., Peng, S., Zhao, Y., et al. (2021a). Quercetin and non-alcoholic fatty liver disease: a review based on experimental data and bioinformatic analysis. *Food Chem. Toxicol.* 154, 112314. doi:10.1016/j.fct.2021.112314

Chen, L., Liu, J., Mei, G., Chen, H., Peng, S., Zhao, Y., et al. (2021b). Quercetin and non-alcoholic fatty liver disease: a review based on experimental data and bioinformatic analysis. *Food Chem. Toxicol.* 154, 112314. doi:10.1016/j.fct.2021.112314

Daina, A., Michielin, O., and Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.* 7, 42717. doi:10.1038/srep42717

Daina, A., Michielin, O., and Zoete, V. (2019). SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res.* 47 (W1), W357-W364–w364. doi:10.1093/nar/gkz382

Deng, Y., Chen, Y., Zheng, H., Li, B., Liang, L., Su, W., et al. (2025). Xuetongsu ameliorates synovial inflammatory hyperplasia in rheumatoid arthritis by inhibiting JAK2/STAT3 and NF-κB signaling pathways. *J. Ethnopharmacol.* 337 (Pt 1), 118786. doi:10.1016/j.jep.2024.118786

Deng, Z., Ou, M., Shi, Y., Li, G., and Lv, L. (2024). Ginsenoside Rg3 attenuates the stemness of breast cancer stem cells by activating the hippo signaling pathway. *Toxicol. Appl. Pharmacol.* 494, 117158. doi:10.1016/j.taap.2024.117158

Dong, Y., Zhao, Q., and Wang, Y. (2021). Network pharmacology-based investigation of potential targets of astragalus membranaceous-angelica sinensis compound acting on diabetic nephropathy. *Sci. Rep.* 11 (1), 19496. doi:10.1038/s41598-021-98925-6

Du, S., Chen, X., Ren, R., Li, L., Zhang, B., Wang, Q., et al. (2024). Integration of network pharmacology, lipidomics, and transcriptomics analysis to reveal the mechanisms underlying the amelioration of AKT-induced nonalcoholic fatty liver disease by total flavonoids in vine tea. *Food Funct.* 15 (9), 5158–5174. doi:10.1039/d4fo00586d

Gotto, A. M., and Brinton, E. A. (2004). Assessing low levels of high-density lipoprotein cholesterol as a risk factor in coronary heart disease: a working group report and update. *J. Am. Coll. Cardiol.* 43 (5), 717–724. doi:10.1016/j.jacc.2003.08.061

Gutiérrez-Cuevas, J., Santos, A., and Armendariz-Borunda, J. (2021). Pathophysiological molecular mechanisms of obesity: a link between MAFLD and nash with cardiovascular diseases. *Int. J. Mol. Sci.* 22 (21), 11629. doi:10.3390/ijms222111629

Huang, X., Yuan, Z., Liu, X., Wang, Z., Lu, J., Wu, L., et al. (2023a). Integrative multi-omics unravels the amelioration effects of Zanthoxylum bungeanum Maxim. on non-alcoholic fatty liver disease. *Phytomedicine* 109, 154576. doi:10.1016/j.phymed.2022.154576

Huang, Y., Gong, Z., Yan, C., Zheng, K., Zhang, L., Li, J., et al. (2023b). Investigation on the mechanisms of Zanthoxylum bungeanum for treating diabetes mellitus based on network pharmacology, molecular docking, and experiment verification. *Biomed. Res. Int.* 2023, 9298728. doi:10.1155/2023/9298728

Imphat, C., Thongdeeying, P., Itharat, A., Panthong, S., Makchuchit, S., Ooraikul, B., et al. (2021). Anti-inflammatory investigations of extracts of Zanthoxylum rhetsa. *Evidence-Based Complementary Altern. Med.* 2021 (1), 5512961. doi:10.1155/2021/5512961

Jacobson, T. A., Ito, M. K., Maki, K. C., Orringer, C. E., Bays, H. E., Jones, P. H., et al. (2015). National lipid association recommendations for patient-centered management of dyslipidemia: Part 1—full report. *J. Clin. Lipidol.* 9 (2), 129–169. doi:10.1016/j.jacl.2015.02.003

Jiang, L., Yi, R., Chen, H., and Wu, S. (2025). Quercetin alleviates metabolic-associated fatty liver disease by tuning hepatic lipid metabolism, oxidative stress and inflammation. *Anim. Biotechnol.* 36 (1), 2442351. doi:10.1080/10495398.2024.2442351

Kaixuan, Z., Dong, Z., Huiwei, B., and Lijing, L. I. (2022). Network pharmacology and molecular docking study on the effect of Kaempferol in treatment of metabolic associated fatty liver disease. *J. Tradit. Chin. Med.* 42 (5), 788–794. doi:10.19852/j.cnki.jtcm.20220815.003

Katsaros, I., Sotiropoulou, M., Vailas, M., Kapetanakis, E. I., Valsami, G., Tsaroucha, A., et al. (2024). Quercetin's potential in MASLD: investigating the role of autophagy and key molecular pathways in liver steatosis and inflammation. *Nutrients* 16 (22), 3789. doi:10.3390/nu16223789

Kong, Q., Liang, Q., Tan, Y., Luo, X., Ling, Y., Li, X., et al. (2025). Induction of ferroptosis by SIRT1 knockdown alleviates cytarabine resistance in acute myeloid leukemia by activating the HMGB1/ACSL4 pathway. *Int. J. Oncol.* 66 (1), 2. doi:10.3892/ijo.2024.5708

Li, Y. J., Chi, H., and Liu, G. Q. (2024). Preparation and characterization of Antarctic krill oil/quercetin co-loaded liposomes and their protective effect on oleic acid-induced steatosis and oxidative stress *in vitro*. *Food Funct.* 15 (4), 2103–2114. doi:10.1039/d3fo04291j

Li, Z., Xu, R., Li, C., Chi, C., Yang, X., Yang, S., et al. (2025). The protective effect and potential mechanism of Zanthoxylum bungeanum Maxim. on atherosclerosis. *Fitoterapia* 181, 106394. doi:10.1016/j.fitote.2025.106394

Liu, G., Yang, L., Tang, Y., Lin, J., Wang, F., Shen, J., et al. (2024). Study on the action mechanism of the Polygonum perfoliatum L. on non-alcoholic fatty liver disease, based on network pharmacology and experimental validation. *J. Ethnopharmacol.* 319 (Pt 3), 117330. doi:10.1016/j.jep.2023.117330

Lu, J., and Wang, C. (2024). Ferulic acid from Angelica sinensis (Oliv.) Diels ameliorates lipid metabolism in alcoholic liver disease via AMPK/ACC and PI3K/AKT pathways. *J. Ethnopharmacol.* 338, 119118. doi:10.1016/j.jep.2024.119118

Lu, Q., La, M., Wang, Z., Huang, J., Zhu, J., and Zhang, D. (2024). Investigation of active components of meconopsis integrifolia (maxim.) franch in mitigating non-alcoholic fatty liver disease. *Int. J. Mol. Sci.* 26 (1), 50. doi:10.3390/ijms26010050

Luo, Z., Huang, C., Chen, J., Chen, Y., Yang, H., Wu, Q., et al. (2024). Potential diagnostic markers and therapeutic targets for non-alcoholic fatty liver disease and

ulcerative colitis based on bioinformatics analysis and machine learning. *Front. Med. (Lausanne)* 11, 1323859. doi:10.3389/fmed.2024.1323859

Ma, J., Deng, Y., Yang, T., Li, M., and Shang, J. (2023). Esculetin alleviates nonalcoholic fatty liver disease on high-cholesterol-diet-induced larval zebrafish and FFA-induced BRL-3A hepatocyte. *Int. J. Mol. Sci.* 24 (2), 1593. doi:10.3390/ijms24021593

Mansour, R. M., Abdel Mageed, S. S., Abulsoud, A. I., Sayed, G. A., Lutfy, R. H., Awad, F. A., et al. (2025). From fatty liver to fibrosis: the impact of miRNAs on NAFLD and NASH. *Funct. Integr. Genomics* 25 (1), 30. doi:10.1007/s10142-025-01544-x

Markowska, J., Kasprzak-Drozd, K., Niziński, P., Dragan, M., Kondracka, A., Gondek, E., et al. (2024). Quercetin: a promising candidate for the management of metabolic dysfunction-associated steatotic liver disease (masld). *Molecules* 29 (22), 5245. doi:10.3390/molecules29225245

Peng, W., He, C. X., Li, R. L., Qian, D., Wang, L. Y., Chen, W. W., et al. (2024). Zanthoxylum bungeanum amides ameliorates nonalcoholic fatty liver via regulating gut microbiota and activating AMPK/Nrf2 signaling. *J. Ethnopharmacol.* 318 (Pt A), 116848. doi:10.1016/j.jep.2023.116848

Rahman, M. T., Alimuzzaman, M., Ahmad, S., and Chowdhury, A. A. (2002). Antinociceptive and antidiarrhoeal activity of Zanthoxylum rhetsa. *Fitoterapia* 73 (4), 340–342. doi:10.1016/s0367-326x(02)00083-7

Santhanam, R. K., Fakurazi, S., Ahmad, S., Abas, F., Ismail, I. S., Rukayadi, Y., et al. (2018). Inhibition of UVB-induced pro-inflammatory cytokines and MMP expression by Zanthoxylum rhetsa bark extract and its active constituent hesperidin. *Phytotherapy Res.* 32 (8), 1608–1616. doi:10.1002/ptr.6092

Shi, R., Chai, K., Wang, H., Zhou, J., Yang, S., Li, J., et al. (2025). Clinical assessment of common medications for nonalcoholic fatty liver disease: a systematic review and bayesian network meta-analysis. *J. Evid. Based Med.* 18 (1), e70002. doi:10.1111/jebm.70002

Shi, Z., Zhang, C., Lei, H., Chen, C., Cao, Z., Song, Y., et al. (2022). Structural insights into amelioration effects of quercetin and its glycoside derivatives on NAFLD in mice by modulating the gut microbiota and host metabolism. *J. Agric. Food Chem.* 70 (46), 14732–14743. doi:10.1021/acs.jafc.2c06212

Steinberg, G. R., Valvano, C. M., De Nardo, W., and Watt, M. J. (2025). Integrative metabolism in MASLD and MASH: pathophysiology and emerging mechanisms. *J. Hepatol.* doi:10.1016/j.jhep.2025.02.033

UniProt Consortium (2021). UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res.* 49 (D1), D480–d489. doi:10.1093/nar/gkaa1100

Wan, X., Ma, J., Bai, H., Hu, X., Ma, Y., Zhao, M., et al. (2025). Drug advances in NAFLD: individual and combination treatment strategies of natural products and small-synthetic-molecule drugs. *Biomolecules* 15 (1), 140. doi:10.3390/biom15010140

Wang, C., Zhao, M., Yue, Y., Hu, C., Zhou, C., Zhang, Z., et al. (2024). Protective effect of modified suanmei-tang on metabolic-associated fatty liver disease: an integrated strategy of network pharmacology, metabolomics, and transcriptomics. *Drug Des. Devel Ther.* 18, 5161–5182. doi:10.2147/dddt.S478072

Wang, M., Zhang, F., Zhou, J., Gong, K., Chen, S., Zhu, X., et al. (2023). Glabridin ameliorates alcohol-caused liver damage by reducing oxidative stress and inflammation via p38 MAPK/Nrf2/NF-κB pathway. *Nutrients* 15 (9), 2157. doi:10.3390/nu15092157

Xu, Y., Han, J., Dong, J., Fan, X., Cai, Y., Li, J., et al. (2019). Metabolomics characterizes the effects and mechanisms of quercetin in nonalcoholic fatty liver disease development. *Int. J. Mol. Sci.* 20 (5), 1220. doi:10.3390/ijms20051220

Yang, H., Zhang, C., Kim, W., Shi, M., Kilicliogl u., M., Bayram, C., et al. (2025). Multi-tissue network analysis reveals the effect of JNK inhibition on dietary sucrose-induced metabolic dysfunction in rats. *Elife* 13. doi:10.7554/eLife.98427

Yang, L., Liu, X., Zhen, L., Liu, Y., Wu, L., Xu, W., et al. (2024). ANXA4 restricts HBV replication by inhibiting autophagic degradation of MCM2 in chronic hepatitis B. *BMC Med.* 22 (1), 521. doi:10.1186/s12916-024-03724-1

Yang, T., Wang, Y., Cao, X., Peng, Y., Huang, J., Chen, L., et al. (2023). Targeting mTOR/YY1 signaling pathway by quercetin through CYP7A1-mediated cholesterol-to-bile acids conversion alleviated type 2 diabetes mellitus induced hepatic lipid accumulation. *Phytomedicine* 113, 154703. doi:10.1016/j.phymed.2023.154703

Ye, Q. N., Shi, X. F., and Yang, J. L. (2023). Research advance on structure and function of amides in Zanthoxylum plants. *Zhongguo Zhong Yao Za Zhi* 48 (9), 2406–2418. doi:10.19540/j.cnki.cjcmm.20230120.201

Yuan, H. M., Qiu, L., Song, Y., Zou, L., Yang, L. F., and Fu, Q. (2021). Phenylpropanoids from Zanthoxylum species and their pharmacological activities: a review. *Zhongguo Zhong Yao Za Zhi* 46 (22), 5760–5772. doi:10.19540/j.cnki.cjcmm.20210531.601

Zhang, J., Yuan, Y., Gao, X., Li, H., Yuan, F., Wu, D., et al. (2024a). Scopoletin ameliorates hyperlipidemia and hepatic steatosis via AMPK, Nrf2/HO-1 and NF-κB signaling pathways. *Biochem. Pharmacol.* 231, 116639. doi:10.1016/j.bcp.2024.116639

Zhang, T., Zheng, J., Chen, M., Li, D., Sun, Y., Liu, R., et al. (2024b). A mini review of polysaccharides from Zanthoxylum bungeanum maxim: their extraction, purification, structural characteristics, bioactivity and potential applications. *Int. J. Biol. Macromol.* 282 (Pt 3), 137007. doi:10.1016/j.ijbiomac.2024.137007

Zhong, F., Zhou, X., Xu, J., and Gao, L. (2020). Rodent models of nonalcoholic fatty liver disease. *Digestion* 101 (5), 522–535. doi:10.1159/000501851

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Ginseng and its functional components in non-alcoholic fatty liver disease: therapeutic effects and multi-target pharmacological mechanisms

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Background: Non-alcoholic fatty liver disease (NAFLD) is a common type of chronic liver disease and its incidence is increasing. Its disease progression is closely related to non-alcoholic steatohepatitis and liver fibrosis. Effective treatment is currently lacking. The traditional Chinese medicine ginseng (*Panax ginseng*) shows unique advantages in NAFLD intervention, but its complex compositional system and molecular mechanism network still need to be systematically analyzed.

Objective: This paper systematically integrates evidence from nearly 20 years of research to elucidate the multi-target pharmacological mechanism of ginseng for the treatment of NAFLD.

Methods: Relevant information was sourced from Pubmed, Web of science, Embase and CNKI databases. Using BioRender and visio to draw biomedical illustrations.

Results: The active ingredients of ginseng contain 2 classes of saponins (tetracyclic triterpene saponins, pentacyclic triterpene saponins and other modified types) and non-saponins. Different cultivation methods, processing techniques and extraction sites have expanded the variety of ginseng constituents and demonstrated different pharmacological activities. Studies have shown that ginseng and its functional components have the ability to regulate lipid metabolism disorders, inflammation, oxidative stress, endoplasmic reticulum stress, insulin resistance, disruption of intestinal flora structure, cell death and senescence. Demonstrates the potential of ginseng for the treatment of NAFLD.

Conclusion: This study reveals for the first time the integrative mechanism of ginseng in the treatment of NAFLD through the tertiary mode of action of "multi-component multi-target multi-pathway". The multilevel modulatory ability of ginseng provides a new direction for the development of comprehensive therapeutic strategies for NAFLD.

KEYWORDS

ginseng (*Panax ginseng*), non-alcoholic fatty liver disease (NAFLD), lipid metabolism, lipotoxic injury, multi-target pharmacological mechanism

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by hepatocellular steatosis and fat accumulation without secondary causes of hepatic fat accumulation, such as heavy alcohol consumption, long-term use of lipotropic drugs, or monogenic genetic disorders (Wei et al., 2024). In recent years, NAFLD has become the most common chronic liver disease worldwide. Epidemiologic data show that the global prevalence of NAFLD is as high as 30%, and the prevalence of NAFLD in overweight and obese groups is even as high as 70% (Lou et al., 2024; Quek et al., 2023). Markov modeling predicts that the disease burden of NAFLD-associated cirrhosis and hepatocellular carcinoma will increase by 115%–130% by 2040, leading to serious challenges for healthcare systems worldwide (Devarbhavi et al., 2023). The pathogenesis of NAFLD begins with nonalcoholic fatty liver, which progresses to non-alcoholic steatohepatitis (NASH) in about 20% of patients, driven by oxidative stress, inflammation, and other pathologic factors. Among patients with NASH, approximately 15%–20% progress to cirrhosis within 30–40 years (Li et al., 2024c; Loomba et al., 2021).

Current clinical management strategies for NAFLD are slightly limited. The common treatment of NAFLD is aimed primarily at alleviating the associated metabolic comorbidities and cardiovascular disease. For example, reducing hyperlipidemia, insulin resistance (IR) and hyperglycemia associated with NAFLD, rather than directly treating the disease itself (Xu et al., 2022). NAFLD patients who do not develop NASH or liver fibrosis have a good prognosis, and their disease can even be reversed (Loomba et al., 2021). First-line therapies are based on lifestyle interventions, but patient compliance is poor, resulting in a high risk of disease progression (Younossi et al., 2023). Pharmacologic therapy is primarily directed at patients who have progressed to NASH and liver fibrosis, but approved drugs (vitamin E and pioglitazone) have limited efficacy. Clinical studies have shown that pioglitazone therapy for NASH has a 47% remission rate, but does not improve fibrosis and may cause weight gain and fluid retention (Paternostro and Trauner, 2022). Although novel agents "glucagon-like peptide-1 receptor agonists and farnesoid X receptor (FXR) agonists" have entered clinical trials, their long-term safety, cost-effectiveness, and efficacy in advanced fibrosis remain controversial (Newsome et al., 2021; Younossi et al., 2022). End-stage patients are dependent on liver transplantation, but donor shortages and postoperative immunosuppressive complications result in 5-year survival rates of only 65% (Terrault et al., 2023). Therefore, the development of novel therapeutic strategies with multi-targeted effects, high safety profile and the ability to block early disease progression is urgent.

Compared with synthetic drugs, traditional Chinese medicine (TCM), as a complementary therapy for liver diseases, has become a new direction for NAFLD drug development due to its wide range of efficacy and multi-component-multi-target properties (Li et al., 2024b; Liu et al., 2022; Zhang and Feng, 2022). Complementary herbal therapies are mostly used clinically for the comprehensive regulation of NAFLD and NASH, among which the hepatoprotective effects of ginseng and its compounds have attracted much attention (Chen et al., 2021). Clinical studies have confirmed that ginseng and its functional components improve metabolic disorders through the following mechanisms: (1) Improve blood glucose and lipid levels by regulating the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) signaling pathway. (2) Inhibiting inflammation-related signaling pathways "nuclear factor kappa-B (NF-κB), mitogen-activated protein kinases, janus kinase 2/signal transducer and activator of transcription (STAT) 5" to reduce inflammation. (3) Scavenges free radicals and reduces cellular damage from oxidative stress (Zhou et al., 2023). Notably, ginseng has shown more comprehensive efficacy than a single synthetic drug in animal models of NAFLD, providing simultaneous improvement in steatosis, IR, and hepatic fibrosis, while demonstrating low toxicity and conferring many benefits (Wang et al., 2021; Yang K. et al., 2023). In this paper, for the first time, we systematically elucidate the multidimensional mechanism of ginseng intervention in NAFLD by integrating nearly 20 years of experimental research evidence, focusing on resolving its key target networks in lipid metabolism regulation, lipotoxic damage repair, cell death and senescence reversal, IR and gut microbiota remodeling (Figure 1). By revealing the component-target-pathway interaction patterns, this study aims to promote the progress of traditional medicine modernization research and provide a theoretical basis for the development of innovative strategies based on the multi-targets of TCM for the treatment of NAFLD.

2 Pathogenesis of NAFLD

The pathogenesis of NAFLD is complex. The early "two-hit" hypothesis was considered the central model to explain the progression of NAFLD (Buzzetti et al., 2016). This hypothesis suggests that the first hit is triggered by excessive hepatic lipid deposition, while the second hit is driven by secondary damage, such as inflammation and endoplasmic reticulum stress (ERS), which ultimately leads to NASH and fibrosis. However, with the deepening of the research, the limitations of the "two-hit" model have gradually appeared, and its assumption of linear phasing is difficult to explain the heterogeneity of NAFLD, multi-organ interactions, and the systemic effects of metabolic syndrome. In this context, scholars have proposed the "multiple-hit" hypothesis, which emphasizes that NAFLD is the result of synergistic or superimposed effects of multiple pathological factors in space and time (Buzzetti et al., 2016; Wang et al., 2023). Unlike the "two-hit" model, the "multiple-hit" hypothesis is no longer limited to localized events in the liver, but incorporates extra-hepatic factors (e.g., adipose tissue dysfunction, intestinal flora disruption) into the core mechanistic framework. Excessive lipid accumulation also promotes the production of lipotoxic substances, which in turn activate oxidative stress (OS), inflammation and ERS processes, leading to secondary liver damage and further inducing cell death or senescence (Mahlapuu et al., 2022; Xu et al., 2020). If pathological factors persist, the liver will undergo repeated injury and repair processes and activate hepatic stellate cells, leading to fibroplasia and even cirrhosis (Taru et al., 2024). Inflammatory cytokines, adipokines, dietary, genetic and environmental factors have important effects on IR and gut microbiota homeostasis, and conversely, IR and gut microbiota disorders exacerbate imbalance of lipid metabolism (Chen and Vitetta, 2020; Fujii et al., 2020; Khan

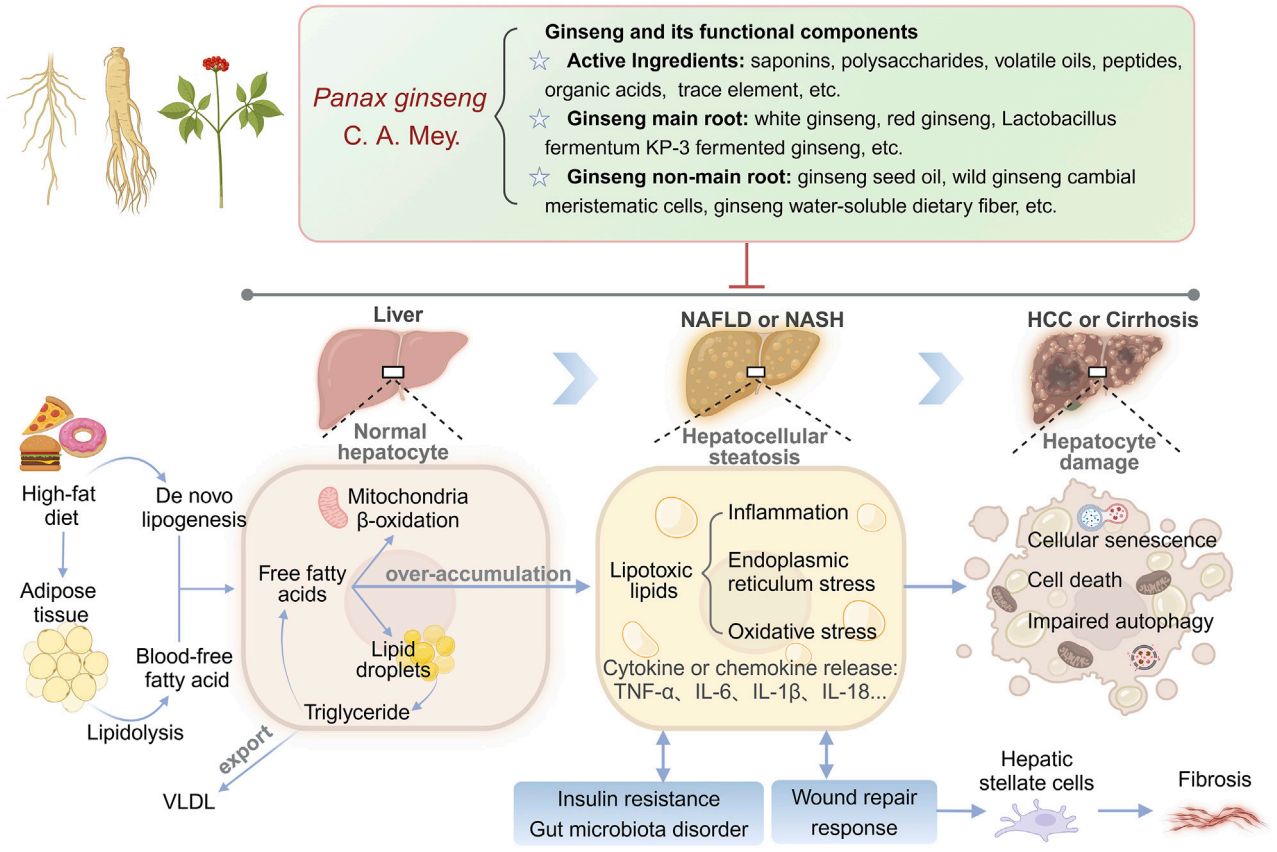

FIGURE 1
Multi-target mechanisms of ginseng in NAFLD/NASH: from lipid metabolism to hepatocyte damage.

et al., 2019; Malesza et al., 2021). Complex pathological mechanisms mutually drive the development of aberrant lipid metabolism in the liver, including excessive nutrient intake, lipotoxicity, activation of the hepatic immune system, OS, ERS, cell death and senescence, IR, disturbances of the gut microbiota, and genetic and epigenetic factors, which are the key risk factors for the development of NAFLD (Buzzetti et al., 2016; Friedman et al., 2018; Huby and Gautier, 2022). Some studies have been reported to demonstrate that if hepatocellular steatosis can be reversed by early intervention through metabolic pathways, the risk causative factors and metabolism-related complications of NAFLD can be mitigated (Loomba et al., 2021). Therefore, we should develop more potential therapeutic approaches to prevent and treat NAFLD.

3 Traditional and modern cognition of ginseng

Ginseng (Latin name: *Panax ginseng* C.A. Mey.) is a perennial herb in the family araliaceae and is widely used worldwide (Ratan et al., 2021). It is widely known as "the king of herbs, the head of all medicines" in East Asian traditional medicine such as China, Korea and Japan (Potenza et al., 2023). In the famous book *Chinese Pharmacopoeia (2020 Edition)*, there is an exhaustive account of ginseng. It has been used in China for more than 2000 years (Li G. et al., 2024). In modern applications of TCM, ginseng is often used to treat lack of power, shortness of breath, palpitations and insomnia, haemorrhage and life-threatening conditions (Li X. et al., 2022; Ma et al., 2023). It exhibits unique energy-boosting properties that fit the clinical manifestations of fatigue, weakness and abdominal distension and discomfort that are common in NAFLD patients (Lu et al., 2021).

Ginseng has a complex composition including saponins, polysaccharides, volatile oils, peptides, organic acids and trace elements, among others (Li X. et al., 2022; Piao et al., 2020). The pharmacological effects of these components have significant target specificity, and the active ingredients commonly used in current experiments can be broadly categorized into the following 2 groups. (1) Ginsenosides are the core active ingredients of ginseng. According to the structure of the glycosides, they can be categorized into tetracyclic triterpene saponins, pentacyclic triterpene saponins and other modified types. The main saponin currently used in the treatment of NAFLD is a tetracyclic triterpene saponin. It includes protopanaxadiol (PPD), protopanaxatriol (PPT) and PPD/PPT-type saponin derivatives (Table 1). (2) Non-saponin components, such as the polyacetylene alcohol compounds: panaxydol. The above multicomponents target multiple pathological aspects such as lipid metabolism, inflammation, OS and fibrosis through synergistic effects, providing a material basis for the multi-targeting properties of ginseng in the treatment of NAFLD.

TABLE 1 Classification and representative components of tetracyclic triterpenoid saponins used in NAFLD.

Aglycone structures	Representative components
Protopanaxadiol (PPD)	Rb_1, Rb_2, Rc, Rd, Rg_3, Rh_2, etc.
Protopanaxatriol (PPT)	Rg_1, Rg_2, Re, Rf, Rh_1, 20(S)-PPT, etc.
PPD/PPT-type saponin derivatives	C17-side-chain variant (Rg_3, Rk_3, etc.), deglycosyl-derived saponins (CK, Mc_1, F_2, etc.)

TABLE 2 Effects of processing parts and processing methods on the composition and mechanism of action of ginseng.

Category	Name	Processing technology	Representative characteristic ingredients	Representative mechanisms of action	Ref.
Ginseng main root	Sun-dried ginseng	Washed and dried	Primary saponins predominant (Rg_1, Re, etc.)	Improve lipid metabolism disorders; strong antioxidant capacity	Chung et al. (2016)
	RG	Steam-dried	Higher content of rare saponins (Rg_3, Rh_2, CK, etc.)	Promotes reverse cholesterol transport; anti-inflammatory	Kwak et al. (2024)
	LFG	*Lactobacillus* fermentum KP-3 fermentation	Rg_2, Rg_3, Rh_1, Rh_2, F_2, Ro, small molecule peptide, etc.	Improve lipid metabolism disorders; anti-inflammatory	Nan et al. (2018)
	MFG	Monascus ruber fermentation	Rg_1, Re, Rc, Rd, polysaccharides, etc.	Increases bile acid excretion; regulates intestinal flora	Zhao et al. (2021)
	FRG	Cordyceps militaris fermentation	Rg_3, Rd, etc.	Modulation of immune functions	Li et al. (2020)
	FG	*Saccharomyces* servazzii GB-07 strain and pectinase fermentation	GBCK25	Reduction of OS; reduction of hepatocyte steatosis apoptosis	Choi et al. (2019a)
Ginseng non-main root	GSO	Cold-pressed ginseng seed oil	Oleic acid, linoleic acid, palmitic acid, phytosterols, etc.	Inhibits *de novo* lipogenesis; improves IR	Kim et al. (2018)
	WGCM	*In vitro* culture of wild ginseng meristematic tissue cells	Rg_3, Rh_2, Rk_1, Rg_5, etc.	Improves mitochondrial function; reduces OS	Lee et al. (2016)
	GWDF	Soluble dietary fiber extracted from lateral root of ginseng	Glucose, galactose, mannose, etc.	Regulates glucose-fat metabolism; promotes colon health; regulates appetite and energy balance	Hua et al. (2021)

The functional components of ginseng are closely related to the cultivation method, processing technology and extraction site. Those sown in the forest and grown naturally are called forest ginseng, and those harvested after 5-6 years of artificial cultivation are commonly called garden ginseng (Li G. et al., 2024). The long time of ginseng cultivation and the small number of wild plants often lead to expensive prices and limited resources (Li G. et al., 2024). Therefore, researchers have expanded its medicinal value through processing optimization, microbial fermentation technology and multi-site development (Wang et al., 2025). According to the sources and preparation methods, they can be categorized into the following 2 groups. (1) The active ingredient profile is altered by physical or biological transformation using the main root of ginseng as the main body. For example, fresh ginseng washed and dried in the sun is known as sun-dried ginseng, which will retain native saponin components to a greater extent and has the effect of improving lipid metabolism disorders in NAFLD (Li et al., 2022c). Fresh ginseng that has been steamed and dried is called red ginseng (RG), which promotes the conversion of native saponins (Rb_1) into rare saponins (Rg_3, Rh_2 and CK). It has been shown to promote reverse cholesterol transport and anti-inflammatory effects (Li et al., 2022c). Microbial fermentation (*Lactobacillus* fermentum KP-3 and Monascus ruber) increased the content of secondary ginsenosides, small-molecule peptides, and polysaccharides (Nan et al., 2018; Zhao et al., 2021). Cordyceps militaris fermentation enhances the content of rare ginsenoside (Rg_3) (Li et al., 2020). In addition, some studies have used modern techniques to efficiently enrich active ingredients from ginseng roots. For example, targeted enzymatic cleavage of ginsenoside glycosyl groups to generate highly bioavailable glycosides (GBCK25) (Choi N. et al., 2019). The active ingredients generated have the ability to reduce fatty apoptosis and cholesterol synthesis in hepatocytes. (2) Exploiting the pharmacological potential of non-primary root parts of the ginseng plant, including cold-pressed extraction of ginseng seed oil (GSO), *in vitro* cultivation of wild ginseng meristematic tissue cells, and extraction of water-soluble dietary fibers from ginseng whiskers (Hua et al., 2021; Kim et al., 2018; Lee et al., 2016). These processed ginsengs have a wider range of pharmacological effects, and we summarize the above ginsengs, concoctions and their characteristic extracts into a table (Table 2).

Natural products as supplementation agents are beneficial in promoting glycolipid metabolism and improving hepatocellular steatosis to halt the progression of NAFLD (Cao et al., 2023; Leng et al., 2021). Compared with other traditional medicinal

TABLE 3 Summary of the mechanisms by which ginseng improves lipid metabolism.

Ginseng	Models	Lipid metabolism	Ref.
20(S)-PPT	Mouse PHs, HepG2 cells	TG↓, LXRα↓, SREBP-1c↓, FAS↓, SCD-1↓	Oh et al. (2015)
Aged ginseng	C57BL/6N male mice	TG↓, TC↓, HDL-C↑, FAS↓, adiponectin↑, leptin resistance↓, appetite↓	Chung et al. (2016)
CK	HuH7 cells	TG↓, AMPK↑, ACC↓, LDs↓, PPAR-α↑, ACOX-1↑	Kim et al. (2013)
FG	male ICR mice	TG↓, LDL-C↓, microsomal triglyceride transfer protein↓, apolipoprotein A4↑	Park et al. (2018)
FRG	C57BL/6N male mice, mouse PHs	LDs↓, ACC↓, FAS↓, SREBP-1c↓, PPAR-α↑, CPT-1↑, ACOX-1↑, FA translocase↓, acyl-CoA synthetase long-chain↓	Choi et al. (2019b)
GBCK25	C57BL/6 male mice, alpha mouse liver 12 cell line, mouse KCs, murine monocyte/macrophage cell line RAW264.7	TG↓, TC↓, FAS↓, ACC-1↓	Choi et al. (2019a)
GDS	C57BL/6 male mice, HepG2 cells	TG↓, TC↓, LDL↓, HDL-C↑, fat-specific protein 27↓, AMPK↑, SREBP-1c↓, ACC↓, FAS↓, CPT-1↑, CPT-2↑, PPAR-α↑	Mi et al. (2024)
GF_2	C57BL/6J male and LXRα deficient mice, mouse BMDMs, mouse PHs	TG↓, LXRα↓, FAS↓, SREBP-1↓	Kim et al. (2018)
Ginsenosides	C57BL/6J male mice	TG↓, TC↓, LDL-C↓, LDL-C/HDL-C↓, SREBP-1c↓, FAS↓, ACC-1↓, CPT-1α↑, leptin resistance↓	Liang et al. (2021)
GSO	C57BL/6J mice, HepG2 cells, rat PHs	TG↓, TC↓, LDL-C↓, HDL-C↑, SIRT1↑, PPAR-α↑, PGC-1α↑, CPT-1α↑, SREBP-1↓, ChREBP↓	Kim et al. (2018)
GWDF	male SD rats	TG↓, ghrelin↑, glucagon-like peptide 1↑, peptide YY↑, cholecystokinin↑	Hua et al. (2021)
LFG	C57BL/6N male mice	TG↓, TC↓, LDL↓	Nan et al. (2018)
Mc_1	C57BL/6 male mice, HepG2 cells	TG↓, SREBP-1c↓, FAS↓, ACC↓	Roh et al. (2020)
MFG	SD male rats	TC↓, LDL-C↓, SREBP-2↓, HMGCR↓, FXRα↓, CYP7A1↑, fecal total bile acids↑	Zhao et al. (2021)
Mixture Rh_1 and Rg_2	C57BL/6 male mice, mouse KCs, mouse PHs, mouse hepatic stellate cells	TC↓, LDs↓, FAS↓, SREBP-1c↓, ChREBP↓, PPAR-α↑, CPT-1α↑	Wang et al. (2021)
Non-fermented ginseng	male ICR mice	TG↓, FFAs↓, microsomal triglyceride transfer protein↓, SREBP-1↓, PPAR-α↑, SCD-1↓	Park et al. (2018)
Rb_1	Zebrafish, male long-evans rats, rat PHs, C57BL/6J male mice, mouse 3T3-L1 fibroblast cells, HepG2 cells	TG↓, TC↓, LDs↓, SREBP-2↓, LDL receptor↓, HMGCR↑, CYP7A1↑, AMPK↑, adiponectin↑, ACC↓, SREBP-1c↓, FAS↓, SCD-1↓, PGC-1α↑, PPAR-α↑, CPT-1↑, ACOX-1↑	Li et al. (2020), Li et al. (2022d), Meng et al. (2023), Shen et al. (2013)
Rb_2	C57BL/KsJ-lepdb (db/db) mice, HepG2 cells	TG↓, TC↓	Huang et al. (2017)
Rc	C57BL/6J mice, mouse PHs	TG↓, TC↓, SREBP-1c↓, FAS↓, CPT-1α↑, CPT-1β↑, SIRT6↑, PPAR-α↑	Yang et al. (2023b)
Rd	C57BL/6J mice, mouse PHs	TG↓, TC↓, SREBP-1c↓, FAS↓, ACC↓, CPT-1α↑, CPT-1β↑, CPT-2↑, SIRT6↑, PPAR-α↑	Cui et al. (2023)
Rf	HepG2 cells	DNMT3L gene, ANXA2 genne	Chen et al. (2022)
RG	thoroughbred riding horses, otsuka long-evans tokushima fatty rats, C57BL/6 mice, mouse PHs and KCs	TG↓, FFAs↓, LXRα↓, LXRβ↓, FAS↓, ACC-1↓, HDL-C↑	Hong et al. (2013), Jeong et al. (2018), Kwak et al. (2024)
Rg_1	HepG2 cells, SD male rats, C57BL/6J mice	TG↓, TC↓, FFAs↓, LDL↓, LDL-C↓, HDL-C↑, acyl-CoA synthetase↑, CPT-1↑, ACOX-1↑, AMPK↑, ACC-1↑, SREBP-1c↓, FAS↓, PPAR-α↑	Hou et al. (2022), Xiao et al. (2019), Xu et al. (2018), Xuan et al. (2015)
Rg_3	C57BL/6 male mice, mouse 3T3-L1pre-adipocyte cells, HepG2 cells	TG↓, TC↓, FFAs↓, AMPK↑, ACC-1↓, SREBP-2↓, HMGCR↓, STAT5↓, PPAR-γ↓, FA binding protein 4↓, SCD-1↓	Lee et al. (2017), Lee et al. (2012)
Rk_3	C57BL/6J male mice, HepG2 cells, LX2 cell lines	PI3K/AKT↑, TG↓, TC↓, LDL↓	Guo et al. (2023)

(Continued on following page)

TABLE 3 (*Continued*) Summary of the mechanisms by which ginseng improves lipid metabolism.

Ginseng	Models	Lipid metabolism	Ref.
WGCM	C57BL/6 male mice	TG↓, TC↓, SREBP-1↓, ACC↓, FAS↓, ChREBP↓, PPAR-α↑, CPT-1α↑	Lee et al. (2016)

Note: BMDMs, bone marrow-derived macrophages; KCs, kupffer cells; PHs, primary hepatocytes; SD, sprague dawley. ↓: inactivate or decrease; ↑: activate or increase.

herbs, ginseng exhibits a variety of irreplaceable multidimensional advantages that make it unique in NAFLD intervention. For example, multiple components of ginseng can simultaneously target multiple pathways such as AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor (PPAR) and sterol-regulatory element binding protein (SREBP) (Mi et al., 2024). The lipid metabolism pathology network of NAFLD can be improved through the "one drug, multiple effects" model. Mc_1 can improve apoptosis and insulin sensitivity, realizing the comprehensive improvement of NAFLD across pathological links, which has more clinical potentials than the herbs with a single mechanism of action (Roh et al., 2020). In addition, ginseng has been used for thousands of years without any serious liver injury, and modern toxicology has confirmed that it has no significant side effects at therapeutic doses (Park et al., 2022). Therefore, ginseng is not only a traditional tonic medicine, but also a multi-target liver metabolism regulator verified by modern science.

4 Pharmacological effects and molecular mechanisms of ginseng against NAFLD

Laboratory studies on ginseng anti-NAFLD have shown that ginseng and its functional components can block multiple underlying pathological mechanisms, including regulating disorders of lipid metabolism in the body, reducing hepatocellular inflammation, ERS, OS, IR, intestinal flora disorders, cellular senescence and death. During the onset of NAFLD, ginseng regulates a variety of hepatic functions such as synthesis, storage, catabolism, transport and secretion, thus exerting a protective effect on the normal structure and function of the liver. A detailed discussion is provided below to facilitate the reader's access to relevant experimental studies, and we use BioRender and visio to draw biomedical illustrations.

4.1 Lipid metabolism

Excessive accumulation of lipids in hepatocytes is the earliest and most common response to NAFLD (Ebert et al., 2023). After years of research, ginseng and its functional components have been found to directly regulate multiple pathways of lipid metabolism, including the regulation of lipid uptake and transport, increase of lipolysis and decrease of lipid synthesis, etc. The following is a systematic and comprehensive review of the role of ginseng and its functional components in targeting the above lipid metabolism pathways (Table 3).

4.1.1 Regulation of lipid uptake and transportation

As the hub of systemic metabolism, the liver is prone to lipid metabolism disorders in hyperlipidemic and obese states, which directly promote the progression of NAFLD (Mato et al., 2019). Studies have shown that ginseng improves lipid homeostasis through the following mechanisms (Figure 2). Significantly reduces triglyceride (TG), total cholesterol (TC), free fatty acids (FFAs), lipid droplets (LDs), low density lipoprotein (LDL), low density lipoprotein cholesterol (LDL-C), LDL-C to high density lipoprotein cholesterol (HDL-C) ratio (LDL-C/HDL-C) in the liver and serum (Guo et al., 2023; Liang et al., 2021; Meng et al., 2023; Xu et al., 2018). In addition evidence of pathologic improvement showed that model animals raised with ginseng had lighter liver weights and body weights, more aligned hepatocytes, fewer vacuoles, and less steatosis (Meng et al., 2023). Analysis of FFAs using molecular networking showed that RG fed thoroughbred riding horses reduced pro-inflammatory FFAs (C12:0, dodecanoic acid; C14:0, myristric acid; C18:1, oleic acid; C18:2, linoleic acid) (Kwak et al., 2024). The above studies demonstrated some intuitive manifestations of the hypolipidemic properties of ginseng and its functional components.

Aged ginseng reduced appetite and decreased the body's lipid intake at the source (Chung et al., 2016). Ginseng water-soluble dietary fiber (GWDF) upregulated glucagon-like peptide 1, peptide YY, cholecystokinin and serum gastric hunger hormone levels. It increased satiety, promoted digestion, delayed gastric emptying, decreased food intake and affected glucolipid metabolism in male SD rats (Hua et al., 2021). Aged ginseng and ginsenosides acted as modulators and significantly reversed abnormally high leptin levels. Restores leptin's appetite-suppressing function by reducing leptin resistance (Chung et al., 2016; Liang et al., 2021).

HDL-C and apolipoprotein A4 (apoA4) have a role in transporting cholesterol from peripheral tissues to the liver for removal. Ginseng and its functional components promotes reverse cholesterol transport into the liver and accelerates cholesterol breakdown and excretion by up-regulating HDL-C and apoA4 (Kim et al., 2018; Park et al., 2018; Xuan et al., 2015). Microsomal triglyceride transfer protein is a lipid transfer protein necessary for the synthesis of very low density lipoprotein in hepatocytes, and its synthesis is upregulated in response to increased TG. Fermented ginseng (FG) and non-fermented ginseng reduces very low density lipoprotein secretion and alleviates hepatic TG accumulation by inhibiting MTP expression (Park et al., 2018). Monascus ruber fermented ginseng (MFG) inhibits FXR receptor, increases cholesterol 7α-hydroxylase (CYP7A1) expression, and promotes bile acid synthesis. Rb_1 directly promotes CYP7A1 activity, accelerating the conversion of cholesterol to bile acids and promoting cholesterol excretion (Li et al., 2020; Zhao et al., 2021).

4.1.2 Regulation of lipid synthesis and decomposition

On the one hand, hepatic nascent fatty acid (FA) are esterified to TG and will be stored as LDs in hepatocytes or secreted in other

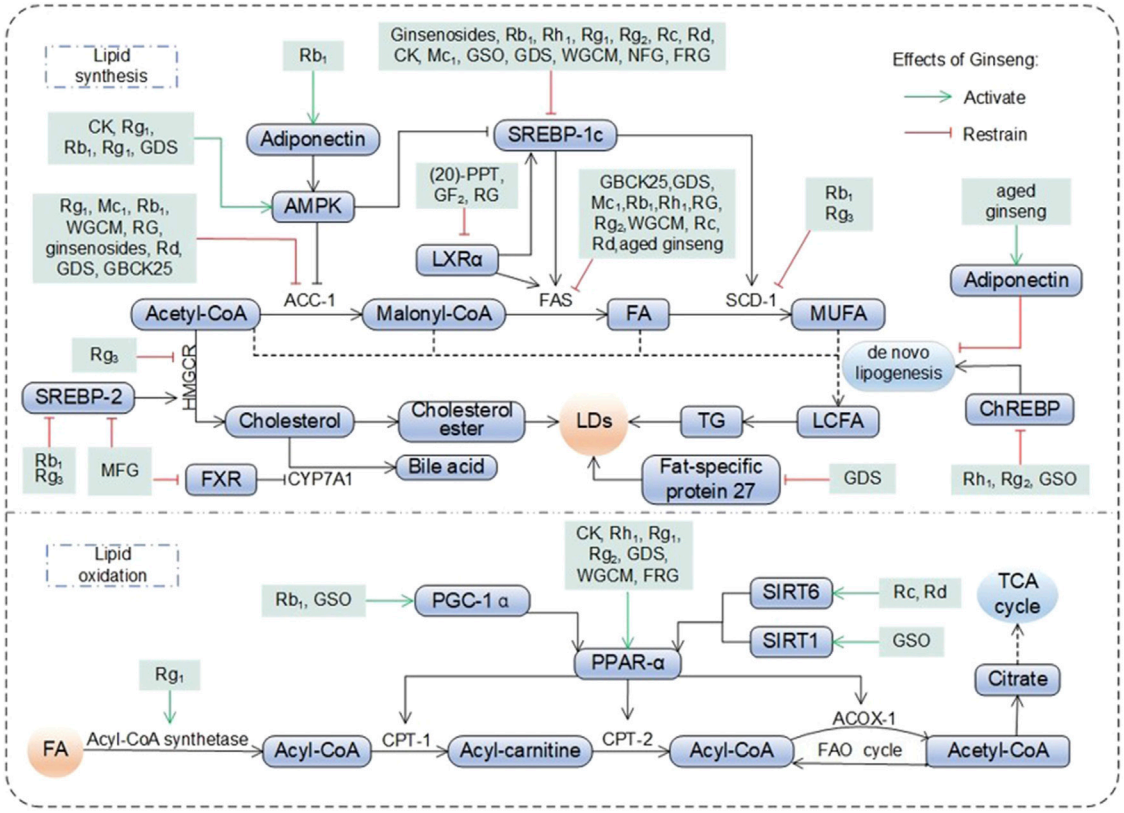

FIGURE 2
Pharmacological effects and molecular mechanisms of ginseng in regulating lipid metabolism. Fatty acids (FA) entering hepatocytes are broken down into acetyl-CoA by acetyl-CoA synthetase, which enters the mitochondria and participates in the fatty acid oxidation cycle (FAO cycle) or the tricarboxylic acid cycle (TCA cycle) under the promotion of various active substances. Among them, acetyl-CoA also participates in lipid synthesis in the organism, generating long chain fatty acid (LCFA, the process is also known as *de novo* lipogenesis) under the promotion of AMP-activated protein kinase (AMPK), acetyl-coenzyme A carboxylase 1 (ACC-1), liver X receptor a (LXRa), sterol-regulatory element binding protein (SREBP) 1c, fatty acid synthase (FAS) and stearoyl-coenzyme A desaturase 1 (SCD-1), on the one hand, lipid droplets (LDs) was generated thereafter. On the other hand, cholesterol was generated under the promotion of SREBP-2 and 3-hydroxy-3-methylglutaryl- coenzyme A reductase (HMGCR), which in turn synthesizes LDs, or is broken down by farnesoid X Receptor (FXR) and CYP7A1 into bile acid and excreted. ACOX-1: acyl-coenzyme A oxidase 1; ChREBP: carbohydrate responsive element-binding protein; CK: compound K; CPT: carnitine palmityl transferase; GDS: ginseng diol saponin; GSO: ginseng seed oil; MFG: monascus ruber fermented ginseng: MUFA: monounsaturated fatty acid; NFG: nonfermented ginseng: PGC-1a: peroxisome proliferators-activated receptor y co-activator a; PPAR-a: peroxisome proliferator-activated receptor a; PPT: protopanaxatriol; SIRT: sirtuins; TG: triglyceride; WGCM: wild ginseng cambial meristematic cells.

forms into the bloodstream, and on the other hand, it is directly metabolised by the β-oxidation pathway (Badmus et al., 2022). Therefore, an increase in nutrients delivered to the liver and FA synthesis, a decrease in FA oxidation and lipid output will result in an excessive accumulation of fat in the liver (Badmus et al., 2022). Ginseng and its functional components are a potent modulator of lipid metabolism (Figure 2).

Ginseng and its functional components plays a key role in reducing FA synthesis by directly inhibiting the expression of lipid synthesis genes such as SREBP-1 gene (isoforms SREBP-1a and SREBP-1c), fatty acid synthase (FAS) gene, stearoyl-coenzyme A desaturase-1 (SCD-1) gene and acetyl-coenzyme A carboxylase (ACC, ACC-1 and ACC-2 subtypes) 1 (Choi N. et al., 2019; Park et al., 2018; Wang et al., 2021). AMPK is a key sensor of cellular energy status. Rg_1 significantly increased AMPK phosphorylation and restored hepatic lipid homeostasis (Xiao et al., 2019). CK and Rg_3 inhibited downstream ACC-1 expression and reduced hepatic lipogenesis by activating AMPK phosphorylation (Kim et al., 2013; Lee et al., 2012). In addition, Rb_1 and ginseng diol saponin (GDS) significantly inhibited SREBP-1c activity upon activation of AMPK, reducing the activation of downstream targets FAS and SCD-1 (Mi et al., 2024; Shen et al., 2013). GSO, Rh_1 and Rg_2 block *de novo* lipogenesis by directly blocking carbohydrate responsive element-binding protein (ChREBP) nuclear translocation and the synthesis of ACC, SCD-1, and FAS. In addition, wild ginseng cambial meristematic cells (WGCM) inhibits DNL by directly reducing the synthesis of the key enzymes ACC, SCD-1, and FAS (Kim et al., 2018; Lee et al., 2016; Wang et al., 2021). Lipocalin is an insulin-sensitive adipocyte-specific cytokine that inhibits *de novo* lipogenesis. Aged ginseng inhibited *de novo* lipogenesis and delayed NAFLD progression by upregulating lipocalin expression (Chung et al., 2016).

RG reduced the expression of hepatic lipid metabolism-related factors liver X receptors (LXR) α and LXRβ. This process regulates intracellular cholesterol and lipid homeostasis and reduces pro-inflammatory factor production. (Jeong et al., 2018). 20(S)-PPT and GF_2 regulated the LXRα by inhibiting the transcription of the adipogenic genes SREBP-1c and FAS to reduce adipogenesis

(Kim K. et al., 2024; Oh et al., 2015). Rb_1, MFG and Rg_3 blocked the SREBP-2/3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) pathway, while Rg_3 directly inhibited the expression of the downstream gene HMGCR (Lee et al., 2012; Li et al., 2020; Zhao et al., 2021). The above processes contribute to the inactivation of key pathways of cholesterol synthesis and reduce cholesterol biosynthesis. GDS targets fat-specific protein, a key protein in lipid droplet synthesis, to reduce its expression and significantly decrease the area of hepatocyte LDs, thereby reducing fat storage (Mi et al., 2024).

During FA oxidation, ginseng and its functional components maintains hepatic lipid homeostasis by stimulating the transcription of PPAR α and PPAR-γ response genes (Liang et al., 2021; Xuan et al., 2015). In addition ginseng and its functional components also synergizes with carnitine palmitoyl transferase (CPT) to accomplish FA oxidation. Examples include the transport and oxidation of acyl-CoA from the cytoplasm to the mitochondrial matrix, and FA oxidation processes involving acyl-CoA synthetase and ester oxygenase (Choi S. Y. et al., 2019; Kim et al., 2013; Mi et al., 2024). One study further found that GSO, Rc and Rd promote PPAR-α-mediated FA oxidation by increasing the expression of sirtuins (SIRT) 1 or SIRT6 proteins (Cui et al., 2023; Kim et al., 2018; Yang Z. et al., 2023). GSO and Rb_1 synergistically regulated PPAR-α-mediated FAO by activating peroxisome proliferators-activated receptor γ co-activator α (PGC-1α) (Kim et al., 2018; Shen et al., 2013). STAT5 promotes the binding of PPAR-γ to PPAR response elements and regulates downstream genes related to adipogenesis, lipid metabolism and glucose homeostasis. However, Rg_3 blocked STAT5 and PPAR-γ associated target adipogenesis and inhibited the adipogenic process in 3T3-L1 cells (Lee et al., 2017). Fermentation of red ginseng with C. militaris (FRG) reduced adipogenesis and lipid uptake by decreasing the expression of acyl-CoA synthetase long-chain and FA translocase (Choi S. Y. et al., 2019). Bioinformatic experiments and cellular experiments validated that Rf downregulated the methylation level of the DNMT3L gene and reversed the aberrant expression of the adipogenesis-related gene ANXA2 (Chen et al., 2022).

4.2 Lipotoxic injury

The liver converts excess FA to TG for storage in an early adaptive protective response. If FAs are continuously supplied in excess or their processing is impaired, they become substrates for the production of lipotoxic lipids and activate a range of pathological response mechanisms, such as inflammation, ERS and OS, causing hepatocellular damage and accelerating the progression of NAFLD (Geng et al., 2021). Not only do various pathophysiological mechanisms play an important role in the development of NAFLD, but there are complex interactions between the mechanisms themselves. Lipotoxic substances induce hepatocyte stress, injury and even death, leading to chronic inflammatory response and generation of large amounts of reactive oxygen species (ROS) causing OS in hepatocytes (Peiseler et al., 2022). OS can further exacerbate inflammation and induce the endoplasmic reticulum to produce a large number of error proteins, activating the ERS and promoting the progression of NAFLD to cirrhosis and hepatocellular carcinoma (Peiseler et al., 2022). Preventing the production of lipotoxic substances will be the key to treatment. Modern medical research has provided a range of evidence at both the cellular and animal levels to support the idea that inhibition of the process of lipotoxic damage by ginseng and its functional components may be a key target for amelioration of NAFLD, suggesting the potential of ginseng and its functional components as novel therapeutic agents for the prevention and treatment of NAFLD (Table 4; Figure 3).

4.2.1 Inflammation

The core pathologic features of NASH and NAFLD encompass lipotoxicity-mediated hepatocellular injury and a chronic inflammatory cascade response. Controlling inflammation is one of the fundamental strategies for treatment (Wiering and Tacke, 2023). In this study, we systematically revealed the molecular mechanisms by which ginseng and its functional components modulate inflammatory signaling networks through multiple targets (Figure 3).

Lipotoxic substances induce hepatocyte damage, leading to abnormal release of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), and lactate dehydrogenase as a diagnostic indicator of the early stages of the disease. Ginseng and its functional components reduces serum levels of damage markers and this effect is directly related to hepatocyte protection (Kim M. Y. et al., 2024; Xuan et al., 2015). In addition, ginseng and its functional components inhibited the production of various inflammatory factors, such as tumor necrosis factor α (TNF-α), interleukin (IL) 6, IL-1β, IL-18, IL-10, IgA, arginase 1, and C-C motif chemokine ligand (CCL) 2. It also reduced the recruitment and activation of immune cells and improved the inflammatory microenvironment (Kim M. Y. et al., 2024; Wang et al., 2021; Zhao et al., 2021).

Lipid overaccumulation leads to aberrant activation of mammalian target of rapamycin C1, causing macrophage 1 polarisation and elevating levels of CCL2, CCL5, IL-1β, IL-6, inducible nitric oxide sythase and TNF-α. FRG reduced the levels of CCL2, CCL5, IL-1β, IL-6, inducible nitric oxide sythase and TNF-α by inhibiting aberrant activation of mTORC1, which reverses macrophage 1 type to macrophage 2 type polarization. It also increases the levels of the macrophage 2 markers CD163 and IL-10, achieving an anti-inflammatory-promoting repair dynamic balance (Choi S. Y. et al., 2019). RG significantly increased natural killer cells counts in otsuka long-evans tokushima fatty rats, improving their immunity (Hong et al., 2013). In addition, RG reduced the phosphorylation of p38 mitogen-activated protein kinase and decreased the secretion of the inflammatory cytokine IL-1β (Kim et al., 2019). RG attenuates downstream hepatic injury and inflammation by inhibiting the FA binding protein4/c-Jun N-terminal kinase (JNK) pathway and reduces the production of ALT, AST, TNF-α, IL-1β, IL-12a, IL-12b, and IL-17a (Jeong et al., 2018).

In the LXRs regulatory module, GF_2 enhances the binding capacity of co-inhibitory factors to LXRα. Reduction of LXRα transcriptional activity improves intracellular lipid homeostasis and correspondingly reduces the number of immune cells and pro-inflammatory factors infiltrating the liver (Kim K. et al., 2024). The body assembles and activates nod-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome after sensing lipotoxic danger signals, which in

TABLE 4 Summary of the mechanisms by which ginseng ameliorates lipotoxic injury.

Ginseng	Models	Lipotoxic injury	Ref.
20(S)-PPT	C57BL/6 male mice, mouse BMDMs, mouse PHs and KCs, human peripheral blood mononuclear cells	ALT↓, AST↓, NLRP3↓, IL-1β↓, TNF-α↓	Lu et al. (2023)
Aged ginseng	C57BL/6N male mice	ALT↓, AST↓, TNF-α↓	Chung et al. (2016)
FRG	C57BL/6N male mice, mouse PHs	ALT↓, AST↓, IL-1β↓, IL-6↓, TNF-α↓, CCL2↓, CCL5↓, inducible nitric oxide sythase↓, IL-10↑, CD163↑	Choi et al. (2019b)
GBCK25	C57BL/6 male mice, alpha mouse liver 12 cell line, mouse KCs, murine monocyte/macrophage cell line RAW264.7	JNK↓, cytochrome P450 2E1↓, MDA↓, ALT↓, TNF-α↓, IL-6↓, IL-1β↓	Choi et al. (2019a)
GDS	C57BL/6 male mice, HepG2 cells	PPAR-γ↑, NF-κB↓, NLRP3↓, AST↓, ALT↓, AMPK↑, nuclear factor-erythroid 2 related factor 2/heme oxygenase 1↑, MDA↓, SOD↑	Mi et al. (2024)
GF_2	C57BL/6J male and LXRα deficient mice, mouse BMDMs, mouse PHs	LXRα↓, IL-1β↓, TNF-α↓, IL-6↓	Kim et al. (2024a)
Ginsenosides	C57BL/6J male mice	NF-κB↓, ALT↓, AST↓, TNF-α↓, IL-1β↓, IL-6↓	Liang et al. (2021)
LFG	C57BL/6N male mice	ALT↓, AST↓, TNF-α↓	Nan et al. (2018)
Mc_1	C57BL/6 male mice, HepG2 cells	GRP78↓, CHOP↓	Roh et al. (2020)
MFG	SD male rats	IgA↓	Zhao et al. (2021)
Panaxydol	C57BL/6 male mice, mouse BMDMs and KCs	TNF-α↓, lactate dehydrogenase↓, NLRP3↓, Caspase-1↓, IL-1β↓, IL-18↓	Kim et al. (2024b)
Rb_1	C57BL/6J male mice, mouse 3T3-L1 fibroblast cells, HepG2 cells	ALT↓, AST↓	Li et al. (2022d)
Rc	C57BL/6J mice, mouse PHs	AST/ALT↓, SIRT6↑, TNF-α↓, IL-6↓, ROS↓	Yang et al. (2023b)
Rd	C57BL/6J mice, mouse PHs	SIRT6↑, NF-κB↓, TNF-α↓, IL-6↓, IL-1β↓, ROS↓, ALT↓, AST↓	Cui et al. (2023)
Rf	HepG2 cells	MMP9 gene	Chen et al. (2022)
RG	Thoroughbred riding horses, otsuka long-evans tokushima fatty rats, C57BL/6 mice, mouse PHs and KCs	ALT↓, AST↓, TNF-α↓, IL-1β↓, IL-12a↓, IL-12b↓, IL-17a↓, FA binding protein 4↓, JNK↓, natural killer cells activity↑, p38 mitogen-activated protein kinase↓	Hong et al. (2013), Jeong et al. (2018), Kim et al. (2019)
Rg_1	SD male rats, HepG2 cells, C57BL/6J mice	ALT↓, AST↓, AKP↓, NF-κB↓, IL-6↓, IL-1β↓, IL-18↓, TNF-α↓, MCP-1↓, forkhead box O1 protein↑, SOD↑, catalase↑, glutathione↑, MDA↓, NLRP3↓, GPR78↓, CHOP↓, Caspase-12↓,ACOX-2↑, ATF3↓	Gu et al. (2021), Hou et al. (2022), Qi et al. (2020), Xiao et al. (2019), Xu et al. (2018), Xuan et al. (2015)
Rg_3	C57BL/6 male mice, mouse 3T3-L1 pre-adipocyte cells	TNF-α↓, IL-1β↓, IL-6↓, IL-10↓	Lee et al. (2017)
Mixture Rh_1 and Rg_2	C57BL/6 male mice, mouse KCs and PHs, mouse hepatic stellate cells	ALT↓, TNF-α↓, CCL2↓, arginase 1↓, IL-10↓, IL-1β↓, ROS↓, NLRP3↓	Wang et al. (2021)
Rk_3	C57BL/6J male mice, HepG2 cells, LX2 cell lines	ALT↓, AST↓, AKP↓, NLRP3↓, IL-6↓, IL-1β↓, TNF-α↓	Guo et al. (2023)
WGCM	C57BL/6 male mice	PGC-1α↑, nuclear respiratory factor 1↑, mitochondrial transcription factor A↑, ALT↓, MDA↓, glutamate dehydrogenase↑, glutathione↑	Lee et al. (2016)

Note: BMDMs, bone marrow-derived macrophages; KCs, kupffer cells; PHs, primary hepatocytes; SD, sprague dawley. ↓: inactivate or decrease; ↑: activate or increase.

turn induces the maturation of cysteine-containing aspartate-specific protease (Caspase) 1 and catalyses the production of pro-inflammatory cytokines IL-18 and IL-1β (Mridha et al., 2017; Yu et al., 2022). Ginseng and its functional components modulated NLRP3 inflammasome-mediated inflammation by blocking the activation of NLRP3 inflammasome and Caspase-1 (Kim M. Y. et al., 2024; Xu et al., 2018). Ginsenosides, Rg_1 and Rd reduced the expression of pro-inflammatory factors by directly inhibiting the activation of NF-κB, which is an important nuclear transcriptional regulator of pro-inflammatory genes (Cui et al., 2023; Liang et al.,

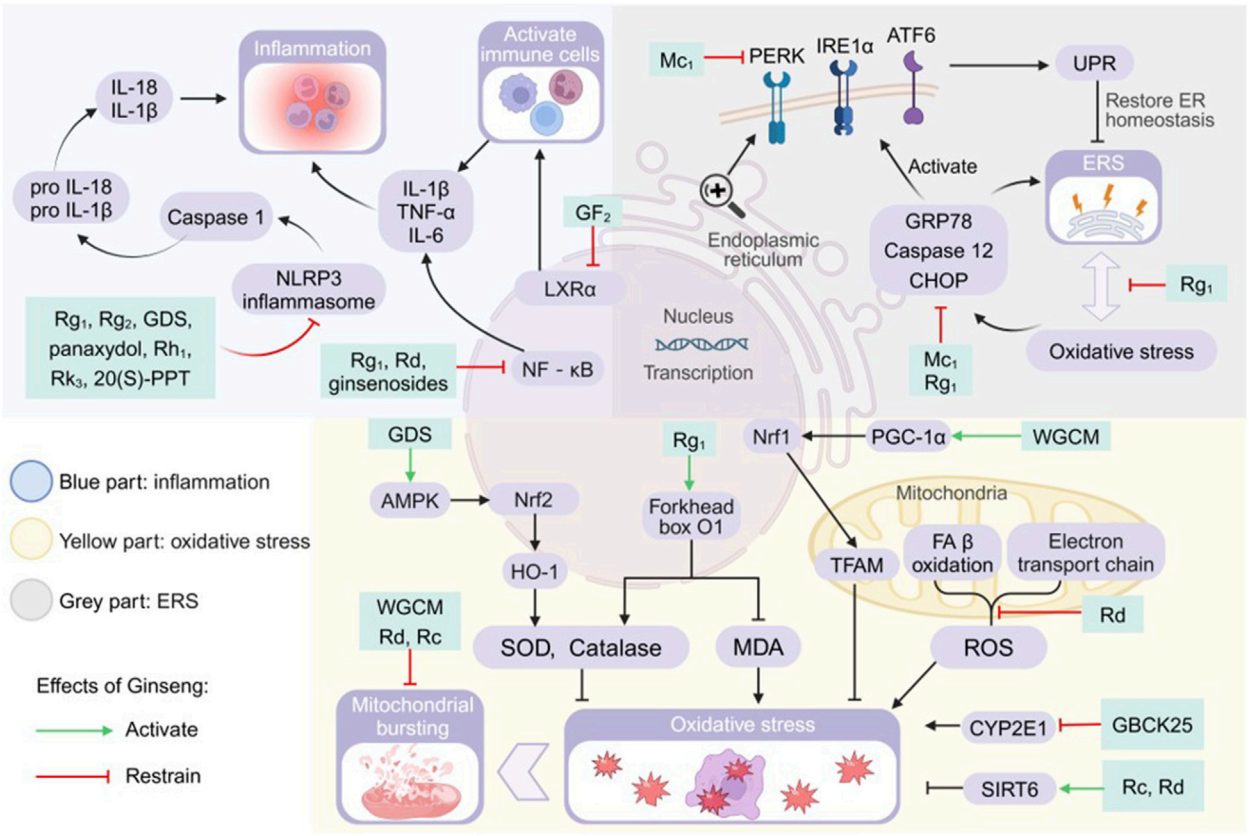

FIGURE 3
Pharmacological effects and molecular mechanisms of ginseng in ameliorating lipotoxic injury. Blue part: activation of nod-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome, nuclear factor kappa-B (NF-kB) pathway, and liver X receptor a (LXRa) all promote the release of inflammatory factors or the activation of immune cells, but ginseng and its characteristic extracts have the effect of inhibiting the activation of the above substances or pathways. Gray part: increased glucose-regulated protein78 (GRP78), cysteine-containing aspartate-specific proteases (Caspase) 12 and CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) promote endoplasmic reticulum stress (ERS), and they also promote unfolded protein response (UPR) by activating the signaling cascade promoters PRKR-like ER kinase (PERK), inositol-requiring enzyme 1a (IRE1a) and activating transcription factor 6 (ATF6). Ginseng has a mitigating effect on ERS and UPR. Yellow part: the AMP-activated protein kinase/nuclear factor-erythroid 2 related factor 2/heme oxygenase 1 (AMPK/Nrf2/HO-1) pathway, forkhead box 01 genes, mitochondrial transcription factor A (TFAM) and sirtuins (SIRT) 6 promote the production of antioxidants or directly inhibit oxidative stress, and ginseng strengthens their efficacy. Mitochondrial ETC and fatty acid (FA) ß oxidation generated reactive oxygen species (ROS) and cytochrome P450 2E1 (CYP2E1) both promoted oxidative stress, and ginseng inhibited their activities. GDS: ginseng diol saponin; IL: interleukin; MDA: malondialdehyde; Nrf1: nuclear respiratory factor 1; PGC-1a: peroxisome proliferators-activated receptor y co-activator a; 20(S)-PPT: 20(S)-protopanaxatriol; SOD: superoxide dismutase; TNF-a: tumor necrosis factor a; WGCM:wild ginseng cambial meristematic cells.

2021; Xiao et al., 2019; Xu et al., 2018). GDS inhibits nuclear translocation of NF-κB and downregulates transcription of key inflammatory factor genes through activation of AMPK/PPAR-γ (Mi et al., 2024). Another study found that Rc and Rd regulate the production of inflammatory factors TNF-α, IL-6 and IL-1β by increasing SIRT6 protein expression (Cui et al., 2023; Yang Z. et al., 2023). Rg_1 specifically upregulates activating transcription factor (ATF) 3/acyl-coenzyme A oxidase (ACOX) 2 to alleviate NAFLD, and how Rg_1 regulates NAFLD through these two genes is unknown (Gu et al., 2021). Leukocyte expression of active the MMP9 gene enhanced its ability to infiltrate during the inflammatory process, and Rf regulation of NAFLD inflammation may be related to the MMP9 gene (Chen et al., 2022).

4.2.2 Oxidative stress

The occurrence of OS in the body indicates that the antioxidant system scavenges ROS at a slower rate than the rate of ROS production, which is an important factor in liver injury and NAFLD progression (Kumar et al., 2021). The tricarboxylic acid cycle transfers the generated NADH and FADH2 to oxygen *via* the electron transport chain, which generates large amounts of ATP and promotes ROS production (Chen et al., 2020). In addition non-electron transport chain sources of ROS, especially the compensatory acceleration of β-oxidation due to hepatic steatosis, appear to generate more ROS and cause OS (Clare et al., 2022). Ginseng improves OS by intervening in the following targets (Figure 3).

Rg_1, WGCM, Rh_1 and Rg_2 reduced abnormally elevated levels of ROS and malondialdehyde (MDA) and increased the vigour of antioxidants such as superoxide dismutase (SOD), catalase, glutamate dehydrogenase and glutathione (Hou et al., 2022; Lee et al., 2016; Wang et al., 2021). Further studies revealed that GDS supplementation activated AMPK and further activated the nuclear factor-erythroid 2 related factor 2/heme oxygenase 1 signalling

pathway, elevating the level of SOD and enhancing the antioxidant capacity of hepatocytes (Mi et al., 2024). Rg_1 elevated nuclear FOXO1 protein levels and subsequently targeted to increase SOD and CAT expression and decrease MDA levels (Qi et al., 2020).

Rd repairs abnormal mitochondrial morphology, ameliorates REDOX disorder, and reduces ROS generation due to electron leakage from ETC (Cui et al., 2023). WGCM elevated the levels of mitochondrial biogenesis related factors, such as PGC-1α, nuclear respiratory factor 1 and mitochondrial transcription factor A, which inhibited mitochondrial OS and promoted mitochondrial biogenesis (Lee et al., 2016). SIRT6 protein attenuated the mitochondrial stress and ameliorated redox deficits in the organism, and ginseng Rc and Rd improved OS status in normal mice better than in SIRT6-deficient mice (Cui et al., 2023; Yang Z. et al., 2023). Rg_1 attenuated OS injury-induced cellular senescence and mitigated hepatic OS by improving biological processes such as cellular matrix composition, membrane receptors, and cellular responses to the outside (Hou et al., 2022). GBCK25 inhibited cytochrome P450 2E1 expression and blocked the lipid peroxidation chain reaction, while down-regulating JNK phosphorylation and attenuating OS-associated cell injury (Choi N. et al., 2019).

4.2.3 Endoplasmic reticulum stress

The accumulation of large amounts of FFAs in fatty liver promotes the production of lipotoxic substances causing ER structural disruption and decrease in number, and ERS occurs in hepatocytes in order to restore homeostasis of the internal environment (Lebeaupin et al., 2018; Lei et al., 2023). During this process, the ER will trigger the unfolded protein response (UPR) to restore the protein homeostasis of the ER, and if the ERS remains at a high level for a long period of time, the terminal UPR programme will trigger cell death (Senft and Ronai, 2015; Zhang et al., 2022). Lipotoxicity induced ERS-OS interaction network constitutes the core pathology axis. We outline the evidence that ginseng intervention in NAFLD is associated with ERS and discuss possible points of intervention (Figure 3).

ERS and OS are both adaptive responses to NAFLD in the early stages of the body, and become damaging factors when they exceed a certain limit, and they form a two-way positive feedback between them. Rg_1 was found to achieve synergistic hepatoprotection through dual regulation of ERS-OS cross-talk (Xu et al., 2018). Furthermore, Rg_1 significantly reduced OS-induced ERS marker levels, suah as CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP), glucose-regulated protein78 (GRP78) and Caspase-12. It also attenuated NLRP3 inflammasome-mediated associated inflammation. Binding of the ER chaperone protein GRP78 to unfolded or misfolded proteins will activate ERS sensors, including inositol-requiring enzyme 1α, PRKR-like ER kinase, and ATF6, which are the initiators of the major signalling cascade of the UPR and the survival mechanism of the ERS (Xu et al., 2018). UPR promotes restoration of ER homeostasis, but failure of restoration induces increased synthesis of pro-apoptotic proteins and triggers cell death. Mc_1 reduced the expression levels of GRP78 and CHOP proteins, which inhibited ERS and UPR activation, and Mc_1 has not been found to ameliorate ERS through activation of the AMPK signalling pathway (Roh et al., 2020).

4.3 Other ways

Recent reports have suggested that the pathogenesis of NAFLD is 'multiple-hit' and that the causative factors are unlikely to be the same in all patients, so that co-morbidities of multiple factors are necessary for the development of NAFLD (Wang et al., 2023). Many molecular pathways that promote the development of NAFLD, such as early IR, gut microbiota and metabolites, are involved in hepatocyte lipotoxic stress and injury, and further contribute to hepatocyte senescence and death, stellate cell activation and fibrosis (Wang et al., 2023). These pathways and mechanisms ultimately lead to the transition of NAFLD to NASH and possibly to end-stage liver disease (Loomba et al., 2021). The mechanisms by which ginseng affects these deficiencies are unclear and are corresponding summarised in this paper (Table 5).

4.3.1 Cellular senescence and death

Hepatocyte death (apoptosis, pyroptosis, impaired autophagy) and fibrosis are key components of NAFLD progression to NASH (Shojaie et al., 2020). Hepatic injury factors such as fat deposition, inflammation, OS and ERS are involved in the pathological changes and accelerate the process of hepatocyte death and fibrosis. Ginseng significantly ameliorates a variety of cell death and aging-related pathological processes through the following multi-targeted mechanisms (Figure 4).

Upon stimulation of the organism from internal or external factors, pro-Caspase-3, the common downstream effector part of multiple apoptotic pathways, will be cleaved to the activator Caspase-3 and activate the cascade reaction of apoptosis (McComb et al., 2019). FRG, Rg_1 and Mc_1 significantly downregulated the expression levels of pro-apoptotic proteins Bcl-2-associated X (Bax) and Caspase-3, while up-regulating the expression levels of anti-apoptotic proteins B-cell lymphoma-2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xL) to reduce hepatocyte apoptosis (Choi S. Y. et al., 2019; Li G. et al., 2022; Roh et al., 2020). Supplementation of Mc_1 lowered reduced CHOP and Bax activity. This process avoids prolonged high levels of ERS, which activates apoptosis induced by the terminal UPR program (Roh et al., 2020). Rg_1 reduces sphingosine-1-phosphate cleavage by inhibiting sphingosine-1-phosphate lyase 1. Enhanced sphingosine-1-phosphate/p-AKT survival signaling in the liver and promoted hepatocyte survival (Li G. et al., 2022). GBCK25 reduced FFAs-induced steatotic apoptosis in hepatocytes by inhibiting the activation of the JNK pathway (Choi N. et al., 2019). Activation of the NLRP3 inflammasome promotes pro-Caspase-1 cleavage and activation. Activated Caspase-1 promotes both the maturation and release of IL-1β and IL-18, as well as being involved in the process of cellular pyroptosis. Panaxydol inhibited NLRP3 inflammatory vesicle activation (ASC oligomerization) and synthesis of lactate dehydrogenase (LDH), a marker of cellular cell death. The reduction of Caspase-1 activity and LDH content reversed the Caspase-1-dependent pathway of cellular cell death (Kim M. Y. et al., 2024). Lipophagy is the interaction of LC3 protein on the autophagosome membrane with envelope proteins on LDs to encapsulate LDs in autophagosomes for degradation. Abnormal lipophagy or degradation of autophagosomes (generation of p62 protein) will cause cell death. Rb_1 and Rb_2 increased autophagic vesicle survival by increasing LC3 protein and decreasing p62 protein content. Rb_2

TABLE 5 Summary of cellular senescence, cell death, IR and gut microbiota disorder by which ginseng ameliorates NAFLD.

Ginseng	Models	Cellular senescence, cell death, IR or gut microbiota disorder	Ref.
Aged ginseng	C57BL/6N male mice	Glucose-6-phosphatase↓, phosphoenolpyruvate carboxykinase↓, glucokinase↑, malic enzyme↓, glucose-6-phosphate dehydrogenase↓, FBG↓, fasting insulin↓	Chung et al. (2016)
FRG	C57BL/6N male mice, mouse PHs	Bcl-2↑, mitophagy↑	Choi et al. (2019b)
GDS	C57BL/6 male mice, HepG2 cells	FBG↓, glucose homeostasis↑	Mi et al. (2024)
GF_2	C57BL/6J male and LXRα deficient mice, mouse BMDMs, mouse PHs	HOMA-IR↓	Kim et al. (2024a)
Ginsenosides	C57BL/6J male mice	ZO-1↑, occludin↑, gut microbiota disorder↓, F/B↓	Liang et al. (2021)
GSO	C57BL/6J mice, HepG2 cells, rat PHs	HOMA-IR↓, FBG↓, fasting insulin↓, CCL2↓, COL1↓	Kim et al. (2018)
GWDF	male SD rats	HOME-IR↓, gut microbiota disorder↓, F/B↓	Hua et al. (2021)
Mc_1	C57BL/6 male mice, HepG2 cells	Caspase 3↓, Bax↓, Bcl-xL↑, JNK↓, TNF-α↓, IL-6↓, HOMA-IR↓	Roh et al. (2020)
MFG	SD male rats	F/B↓, gut microbiota disorder↓	Zhao et al. (2021)
Panaxydol	C57BL/6 male mice, mouse BMDMs and KCs	Lactate dehydrogenase↓, COL3↓, TIMP1↓, α-smooth muscle actin↓, transforming growth factor β↓	Kim et al. (2024b)
Rb_1	C57BL/6J male mice, Mouse 3T3-L1 fibroblast cells, HepG2 cells	LC3 protein↑, p62 protein↓, miR-128↓, transcription factor EB↑, adiponectin↑, HOME-IR↓, FBG↓	Li et al. (2022d), Meng et al. (2023)
Rb_2	C57BL/KsJ-Lepdb (db/db) mice, HepG2 cells	SIRT1↑, AMPK↑, p62 protein↓, LC3 protein↑, FBG↓	Huang et al. (2017)
Rc	C57BL/6J mice, mouse PHs	HOMA-IR↓, phosphoenolpyruvate carboxykinase↓, glucose-6-phosphatase↓, FBG↓, fasting insulin↓	Yang et al. (2023b)
Rd	C57BL/6J mice, mouse PHs	SIRT6↑, HOMA-IR↓, glucose intolerance↓	Cui et al. (2023)
Rf	HepG2 cells	BAZ1A gene	Chen et al. (2022)
RG	C57BL/6 male mice, mouse PHs and KCs	TIMP1↓, transforming growth factor β↓	Jeong et al. (2018)
Rg_1	SD male rats, C57BL/6J mice, HHl-5 hepatocytes	Bax↓, Bcl-2↑, sphingosine-1-phosphate lyase 1↓, phospho-extracellular regulated protein kinases 1/2↑, p-AKT↑, tumor protein P53↓, CDKN2A↓, senescence-associated β-galactosidase↓, phospho-histone H2A.X↓, CDKN1A↓, STAT1↑, epidermal growth factor receptor↑	Hou et al. (2022), Li et al. (2022a), Qi et al. (2020), Xuan et al. (2015)
Mixture Rh_1 and Rg_2	C57BL/6 male mice, mouse KCs, mouse PHs, mouse hepatic stellate cells	TIMP1↓, COL1↓, COL3↓, lysyl oxidas↓, CCL2↓	Wang et al. (2021)
Rk_3	C57BL/6J male mice, HepG2 cells, LX2 cell lines	PI3K/AKT↑, COL1↓, hypoxia-inducible factor 1α↓, TIMP1↓, F/B↓, gut microbiota disorder↓	Guo et al. (2023)

Note: BMDMs, bone marrow-derived macrophages; KCs, kupffer cells; PHs, primary hepatocytes; SD, sprague dawley. ↓: inactivate or decrease; ↑: activate or increase.

accelerated LD degradation by activating AMPK/SIRT1, upregulating LC3 protein and decreasing p62 protein (Huang et al., 2017; Meng et al., 2023). Rb_1 enhances the transcription of transcription factor EB nuclear translocation and its downstream lysosome-associated genes by inhibiting miR-128. This resulted in increased lysosomal degradation of autophagic lipids and alleviated palmitic acid-induced autophagic flux block (Meng et al., 2023).

Additionally it has been proposed that although ginseng lowered Bax levels, it also elevated Bcl-2 during modeling of the disease. Because the Bax/Bcl-2 changes during treatment were slight, it is possible that the improvement in the course of Rg_1 treatment for NAFLD was not cell death (Qi et al., 2020). In addition, impaired autophagy and free radical attack on mitochondria both contribute to cellular senescence. Rg_1-treated mitochondria showed reduced swelling and vacuolization, more intact cytoplasmic matrix, and restoration of cellular autophagy. The expression of the key signals tumor protein P53, senescence-associated β-galactosidase, the nuclear damage marker phospho-histone H2A.X, and cyclin-dependent kinase inhibitor (CDKN) 1A/CDKN2A was decreased in the early stage of senescence (Hou et al., 2022; Qi et al., 2020). Validated by bioinformatic experiments and cellular experiments, Rf was found to inhibit aging-related phenotypes by affecting the BAZ1A gene (Chen et al., 2022). Hepatocyte injury or necrosis activates the body to repair damaged cells, and it causes hepatic fibroproliferation when repair persists. Panaxydol, Rg_1, Rh_1 and Rg_2 was found to be effective in reducing the size of fibrotic areas, the number of inflammatory foci in the periportal and pericentric areas. Decreased the levels of liver fibrosis markers collagen (COL) 1, COL3, lysyl oxidas, tissue inhibitor of metalloproteinase 1 (TIMP1), α-smooth muscle actin, transforming growth factor β and hypoxia-inducible factor 1α (Kim M. Y. et al., 2024; Wang et al., 2021; Xu et al., 2018). In addition, Rk_3 reduced the expression of hypoxia-

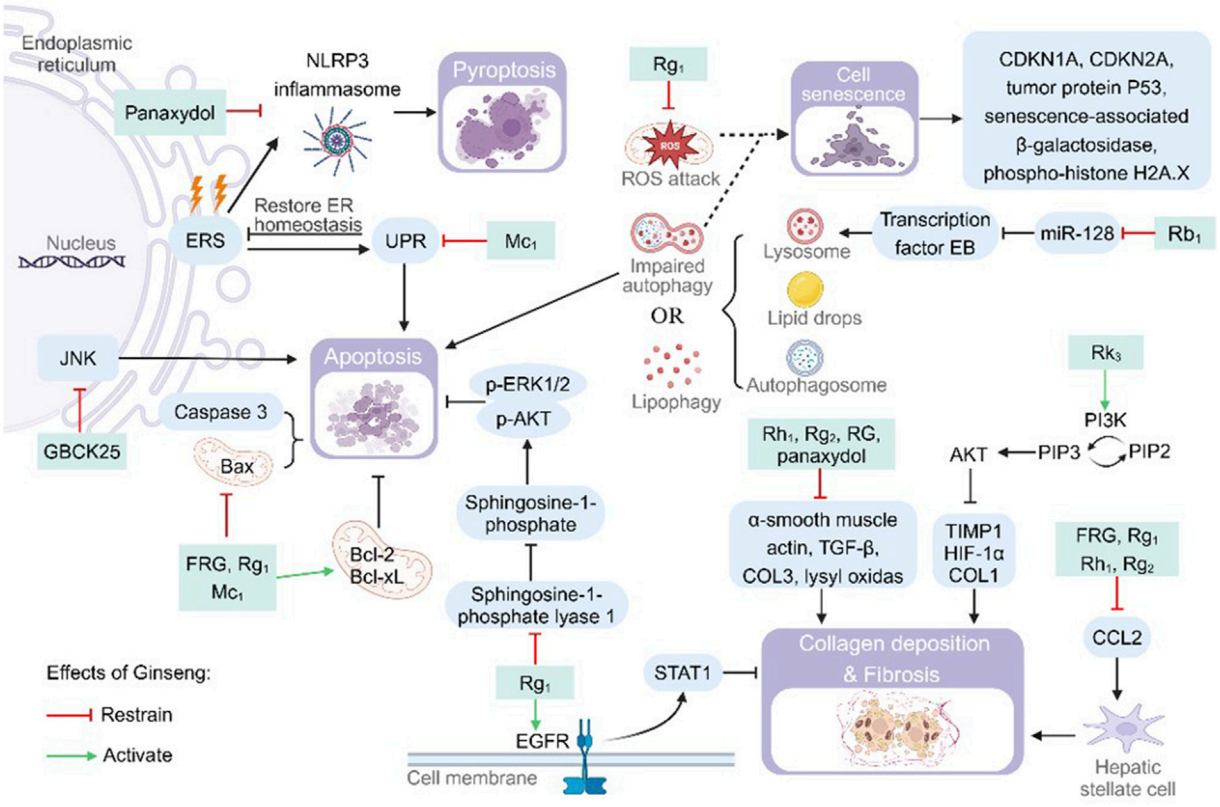

FIGURE 4
Pharmacological effects and molecular mechanisms of ginseng in reducing cellular senescence and death. Pathways or substances such as c-Jun N-terminal kinase (JNK), Bcl2-associated X (Bax), cysteine-containing aspartate-specific proteases (Caspase) 3 and sphingosine-1-phosphate lyase 1 have apoptosis-promoting effects, but ginseng and its characteristic metabolites inhibit the above processes. Endoplasmic reticulum stress (ERS) promotes cellular pyroptosis by upregulating nod-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome synthesis or apoptosis by promoting the unfolded protein response (UPR), but ginseng inhibits this process. Lysosomes, autophagosomes, and lipid droplets interact to cause lipophagy, and impairment of this process promotes cellular senescence. Ginseng attenuates cellular senescence by inhibiting miR-128 to increase the number of lysosomes or by inhibiting mitochondrial damage. Ginseng inhibits the expression of substances associated with collagen (COL) deposition and fibrosis, such as a-smooth muscle actin, transforming growth factor B (TGF-B), COL3 and lysyl oxidas, or inhibits liver fibrosis by activating the epidermal growth factor receptor (EGFR) and protein kinase B (PI3K/AKT) pathways. Bcl-2: B-cell lymphoma-2; Bcl-XL: B-cell lymphoma-extra large; CDKN: cyclin-dependent kinase inhibitor; ER: endoplasmic reticulum; HIF-1a: hypoxia-inducible factor 1a; p-AKT: phospho-protein kinase B; p-ERK: phospho-extracellular regulated protein kinases; PI3K: phosphatidylinositol-3-kinase; PIP2: phosphatidylinositol bisphosphate; PIP3: phosphatidylinositol trisphosphate; ROS: reactive oxygen species; STAT: signal transducer and activator of transcription; TIMP1: tissue inhibitor of metalloproteinase 1.

inducible factor 1α, COL1 and hepatic TIMP1 through activation of the PI3K/AKT signaling pathway, and attenuated COL deposition and fibrosis in hepatocytes (Guo et al., 2023). FRG, Rg_1, Rh_1 and Rg_2 inhibit high CCL2 expression. Reduced hepatic fibrosis and even cirrhosis formation by blocking CCL2 hepatic stellate cell activation (Choi S. Y. et al., 2019; Qi et al., 2020; Wang et al., 2021). Rg_1 promotes the expression of downstream negative regulators of hepatic fibrosis and increases extracellular matrix degradation by regulating the epidermal growth factor receptor/STAT1 axis (Hou et al., 2022).

4.3.2 Insulin resistance

As a hub of systemic metabolic regulation, hepatic lipotoxicity injury (inflammation/OS/ERS) triggers systemic IR through multiple cascade reactions, which is manifested by (1) decreased glucose disposal in non-hepatic tissues (including adipose tissues and muscles). (2) dysregulation of lipolysis leading to aberrant lipid release. (3) impaired hepatic glycogen storage, which causes metabolic abnormalities and exacerbates systemic IR (Sakurai et al., 2021). In this study, we systematically elucidated the action network of ginseng in ameliorating disorders of glucolipid metabolism through multi-target synergism (Figure 5).

Ginseng and its functional components reduced fasting blood glucose (FBG) levels, fasting insulin and homeostatic model assessment for insulin resistance index (HOMA-IR) in animals of NAFLD disease model and improved glucose homeostasis, glucose tolerance and insulin tolerance in the organism (Cui et al., 2023; Kim K. et al., 2024; Li et al., 2022d). JNK elevates levels of TNF-α and IL-6 during obesity-driven macrophage activation, subsequently triggering IR. Mc_1 inhibited the above process. In addition Mc_1 restored insulin receptor substrate 1 tyrosine phosphorylation levels and increased insulin sensitivity in the process of driving down JNK phosphorylation levels (Roh et al., 2020). In regulating glucose metabolic activities, aged ginseng activates glucose uptake and

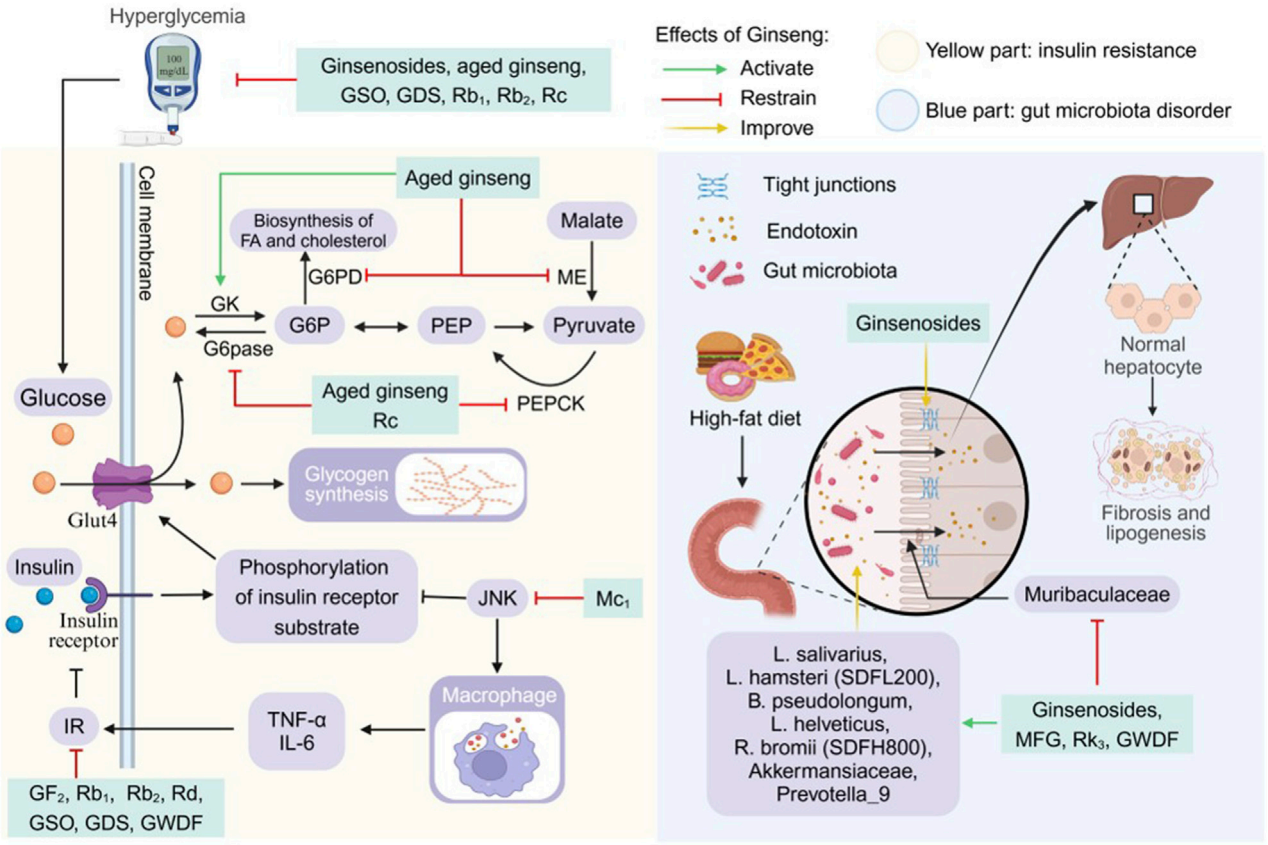

FIGURE 5
Pharmacological effects and molecular mechanisms of ginseng in ameliorating insulin resistance and gut microbiota disorders. Blue part: ginseng improved gut microbiota disorders and impaired intestinal vascular barrier induced by high-fat diet, and attenuated hepatic lipid deposition and hepatic fibrosis. Gray part: JNK reduces insulin sensitivity by inhibiting phosphorylation of insulin receptor substrateand promoting the production of inflammatory cytokines, but ginseng inhibits c-Jun N-terminal kinase (JNK) activation and insulin resistance (IR). Ginseng promotes the expression of glucokinase (GK), which activates glucose uptake and utilization, and inhibits glucose and lipid synthesis by inhibiting the activity of glucose-6-phosphatase (G6pase), phosphoenolpyruvate carboxykinase (PEPCK), malic enzyme (ME), and glucose-6-phosphate dehydrogenase (G6PD). FA: fatty acid; G6P: glucose-6-phosphate; GDS: ginseng diol saponin; Glu4: glucose transporter type 4; GSO: ginseng seed oil; GWDF: ginseng water-soluble dietary fiber; IL-6; interleukin 6; MFG: monascus ruber fermented ginseng; PEP: phosphoenolpyruvate; TNF-a: tumor necrosis factor a.

utilization by decreasing insulin, FBG levels and increasing glucokinase expression. It also inhibits the synthesis of pyruvate, FAs and cholesterol by inhibiting malic enzyme and glucose-6-phosphate dehydrogenase enzyme activities (Chung et al., 2016). In addition both Rc and aged ginseng reduce the expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. This process reduces gluconeogenesis, decreases the body's blood glucose level and improves glucose intolerance (Chung et al., 2016; Yang Z. et al., 2023). Rb_1 improvement of IR and glucose tolerance is partially dependent on lipocalin. Specifically, it improves systemic glucose metabolism, insulin homeostasis, FA oxidation, and hepatic insulin sensitivity after activation of AMPK using lipocalin (Li et al., 2022d).

4.3.3 Gut microbiota disorder

The microbial community in the human gastrointestinal tract has been shown to be involved in a variety of physiopathological processes in the gut, and a unique gut microbiome signature exists for NAFLD (Aron-Wisnewsky et al., 2020; Vallianou et al., 2021). The intestinal microecological disorder of NAFLD is characterized by the imbalance of "flora-intestinal-hepatic axis", and the present study systematically reveals that ginseng can improve the metabolic abnormalities by regulating the homeostasis of intestinal flora in a multi-dimensional way (Figure 5) (Hsu and Schnabl, 2023).

High-fat diets increases intestinal permeability and exposes the liver to endotoxins, and a disturbed gut microbiota also disrupts the intestinal vascular barrier, enhancing intestinal permeability and bacterial lipopolysaccharide leakage into the circulation (Kobayashi et al., 2022; Mouries et al., 2019). Ginsenosides treatment increased the tight junction proteins ZO-1 and occludin levels in a dose-dependent manner, attenuating microecological dysregulation-mediated intestinal leakage and metabolic endotoxaemia (Liang et al., 2021). Qualitative and quantitative abnormalities of the gut microbiota are involved in the pathogenesis of NAFLD and NASH and promote hepatic lipogenesis and fibrosis (Hu et al., 2020; Kolodziejczyk et al., 2019). The study show that GWDF significantly increased the abundances of biomarkers such as L. salivarius, L. hamsteri (SDFL200), B. pseudolongum, *L. helveticus*, and R. bromii (SDFH800), and show a significant prebiotic effect. MFG and Rk_3 selectively promoted colonisation by the probiotic

bacteria Akkermansiaceae and Prevotella_9 and reduce the relative abundance of Muribaculaceae (Guo et al., 2023; Hua et al., 2021; Zhao et al., 2021). GWDF, ginsenosides, MFG and Rk_3 had the effect of decreasing the Firmicutes and Bacteroidetes ratio, increasing the concentration of fecal short-chain FA, the abundance and diversity of intestinal flora species, and ameliorating the imbalance of gut microbiota induced by high-fat diets (Guo et al., 2023; Hua et al., 2021; Liang et al., 2021; Zhao et al., 2021). The above results demonstrated that ginseng has a three-in-one action model of "flora reconstruction, barrier repair and metabolic regulation".

5 Conclusion and outlook

The burden caused by NAFLD is rising and treating NAFLD and its comorbidities is an important clinical challenge. Targeting lipid metabolism is gaining attention as a potential therapeutic target, and herbal medicine has been widely used as a complementary therapy for a long time. Ginseng and its functional components have hepatoprotective effects on patients and experimental animals, but ginseng itself has numerous components and complex targets and pathways, so finding safe and effective components and sorting out the mechanism of action deserve in-depth exploration. In this paper, we present a systematic review of the main features of ginseng in improving lipid metabolism in NAFLD through multiple components, pathways and targets.

In summary, ginseng and its functional components was found to help slow the progression of non-alcoholic fatty liver to NASH. Increased lipid uptake and synthesis, decreased catabolism and excretion, inflammation, ERS, OS, IR, cellular senescence, cell death and gut microbiota disorder are common pathogenic pathways. Modern studies have shown that ginseng and its functional components works by targeting AMPK, PPAR/PGC-1α, adipocytokine, FXR, LXR, SIRT, PI3K/AKT, JNK/insulin receptor substrate, sphingosine-1-phosphate/AKT/extracellular regulated protein kinases, nuclear factor-erythroid 2 related factor 2/heme oxygenase 1, PGC-1α/nuclear respiratory factor 1/mitochondrial transcription factor A, epidermal growth factor receptor, NLRP3 and NF-κB mechanisms to exert lipid-lowering and alleviate hepatic lipotoxicity. In addition, it can also affect the dynamics of lipid synthesis, oxidation and excretion by biometabolic pathways such as lipid *de novo* lipogenesis, β-oxidation, tricarboxylic acid cycle, gluconeogenesis, UPR, and mitochondrial biogenesis. This "network targeting-pharmacological" action characterizes the nature of NAFLD, which is characterized by multiple organ interactions and multiple pathologies.

At the level of mechanism research, we note that there is a cognitive bias in the current academic community that emphasizes on component mechanisms but not on systematic evaluation. Although ginseng has been shown to reduce hepatic cholesterol accumulation by activating FXR, it is puzzling that ginseng has been shown to promote cholesterol synthesis by up-regulating HMGCR expression (Li et al., 2020). It is worth noting that, compared with the compensatory metabolic escape phenomenon that often occurs with single-target chemical drugs. Ginsenosides may be more conducive to maintaining the long-term stability of glucose-lipid metabolism homeostasis by improving IR through the bi-directional regulation of insulin receptor substrate PI3K/AKT and JNK signaling (Roh et al., 2020). This paradoxical phenomenon suggests that there may be antagonistic effects among the complex components of Chinese medicines, and that relying solely on single component studies may result in misjudgment of the overall efficacy. Therefore, we strongly suggest that future studies should establish a three-dimensional evaluation system of "chemical component-biological effect-clinical phenotype," and pay special attention to the key regulatory effects of non-saponin components such as ginseng polysaccharides and volatile oils on the intestinal flora-liver axis.

To date, ginseng is considered an edible and medicinal plant with a long history of medicinal use and a wide range of pharmacological effects. Ginseng and its functional components blocks the above pathogenesis of NAFLD through multi-components, multi-pathways, multi-targets and multi-levels, and has hepatoprotective effects on experimental animals. To summarise the relevant studies, the most relevant components of ginseng are ginsenosides, whose pharmacological mechanisms focus on the regulation of lipid metabolism and the subsequent reduction of lipotoxicity injury, and therefore we consider them to be the typical mechanisms of ginseng in the treatment of NAFLD. A study using network-based approaches to investigate the therapeutic effects and key mechanisms of ginseng revealed similar results (Kim Y. W. et al., 2024). Although the methods of these two studies are different, we adopt the attitude of seeking common ground while reserving differences, and believe that the results of the two studies can confirm and complement each other, verifying the rationality of ginseng in treating liver diseases. However, there are some slight differences between the two studies, such as network pharmacology found that regulation of protein function may be a key core target of ginseng in the treatment of liver-related diseases, but the experimental data on this aspect are slightly insufficient. From the perspective of clinical practice, we believe that ginseng has important application value because of its "both symptoms and root causes." It can rapidly reduce hepatic TG deposition through acute activation of AMPK, and can also regulate the secretion of adipokines to realize the long-term benefit of metabolic memory. This dual-phase regulation advantage is extremely rare among existing chemical drugs, and we look forward to exploring it in more relevant studies in the future.

Taken together, these findings demonstrate the effectiveness of ginseng and its functional components in terms of key mechanisms against NAFLD. However, there are still some unknowns to be confirmed in this study. Firstly there are a few studies that showed discrepancies in the results, for example, ginseng reduced cholesterol transport by decreasing the activity of LDL receptor and increased the expression of HMGCR thereby increasing cholesterol synthesis (Li et al., 2020). We need more high-quality experiments to strengthen the level of evidence from controversial experiments. Secondly, in view of the shortcomings of the current quality control system, the current evaluation standard of saponin content as a single quality control index has seriously lagged behind the progress of basic research. We believe that a multidimensional quality control model should be constructed based on the correlation feature of "component-pathway-function," which includes the assessment of bioavailability of metabolome and the detection of metabolic transformation ability of intestinal flora. For example, emphasizing ginseng's absorption, disposition, metabolism,

excretion and biosynthesis will remedy the shortcomings of the current quality control methods in order to better achieve the effect of comprehensive evaluation of the quality of TCM (Li X. et al., 2022). This innovative quality control concept may lead to a paradigm shift in TCM modernization research. Finally, there are still many important unexplored signalling molecules or pathways to be added in modern research. At the translational medicine level, for example, breakthroughs in the cutting-edge area of macrophage polarization regulation may lead to clinical benefits (Loomba et al., 2021). In genetic medicine, individualized intervention studies of ginseng for people with mutations in patatin-like phospholipase domain containing protein 3 may provide new ideas to address the therapeutic dilemma of genetically susceptible NAFLD (Park et al., 2023). It is also of interest that the regulation of the mitochondrial quality control network by the SIRT family has not yet been fully elucidated in ginseng studies, which may be an important bridge connecting lipotoxic injury to cellular aging mechanisms (Xu et al., 2024). However, the role of ginseng in this regard has been slightly underreported and more work needs to be done in these area.

Overall, the practice of ginseng functional components in the treatment of NAFLD is derived from the TCM theory, and its efficacy has been further verified by modern studies. We summarise the relevant studies and confirm that ginseng and functional components achieve therapeutic effects on NAFLD through multi-components, multi-targets, and multi-pathways. We believe that ginseng and its functional components play an ameliorative role in NAFLD, which has a complex pathogenesis, through multi-targets, and have significant pharmacological effects on NAFLD, reducing hepatic lipid accumulation by regulating lipid uptake and transport, increasing lipid catabolism, and decreasing lipid synthesis and other related mechanisms. In addition, ginseng ameliorates hepatic steatosis and hepatocyte injury by inhibiting lipotoxicity-related factors and pathways to attenuate hepatocyte inflammation, ERS, OS, cell death, cellular senescence and gut microbiota disorders. All these can promote the understanding and application of ginseng to compensate for the lack of NAFLD drugs and the development of green and natural medicines, so that more NAFLD patients can be helped.

Author contributions

PX: Investigation, Visualization, Writing – original draft, Writing – review and editing. ZY: Formal Analysis, Investigation, Writing – review and editing. XL: Formal Analysis, Investigation, Writing – review and editing. QF: Supervision, Writing – review and editing. YS: Conceptualization, Funding acquisition, Writing – review and editing.

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Conflict of interest

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

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

References

Aron-Wisnewsky, J., Vigliotti, C., Witjes, J., Le, P., Holleboom, A. G., Verheij, J., et al. (2020). Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat. Rev. Gastroenterol. Hepatol.* 17 (5), 279–297. doi:10.1038/s41575-020-0269-9

Badmus, O. O., Hillhouse, S. A., Anderson, C. D., Hinds, T. D., and Stec, D. E. (2022). Molecular mechanisms of metabolic associated fatty liver disease (MAFLD): functional analysis of lipid metabolism pathways. *Clin. Sci. (Lond)* 136 (18), 1347–1366. doi:10.1042/cs20220572

Buzzetti, E., Pinzani, M., and Tsochatzis, E. A. (2016). The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 65 (8), 1038–1048. doi:10.1016/j.metabol.2015.12.012

Cao, Y., Fang, X., Sun, M., Zhang, Y., Shan, M., Lan, X., et al. (2023). Preventive and therapeutic effects of natural products and herbal extracts on nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Phytother. Res.* 37 (9), 3867–3897. doi:10.1002/ptr.7932

Chen, J., and Vitetta, L. (2020). Gut microbiota metabolites in NAFLD pathogenesis and therapeutic implications. *Int. J. Mol. Sci.* 21 (15), 5214. doi:10.3390/ijms21155214

Chen, L., Wang, L., Ao, W., Chen, Y., Li, S., Huang, Z., et al. (2022). Bioinformatics study of the potential therapeutic effects of ginsenoside Rf in reversing nonalcoholic fatty liver disease. *Biomed. Pharmacother.* 149, 112879. doi:10.1016/j.biopha.2022.112879

Chen, M., Xie, Y., Gong, S., Wang, Y., Yu, H., Zhou, T., et al. (2021). Traditional Chinese medicine in the treatment of nonalcoholic steatohepatitis. *Pharmacol. Res.* 172, 105849. doi:10.1016/j.phrs.2021.105849

Chen, Z., Tian, R., She, Z., Cai, J., and Li, H. (2020). Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. *Free Radic. Biol. Med.* 152, 116–141. doi:10.1016/j.freeradbiomed.2020.02.025

Choi, N., Kim, J. W., Jeong, H., Shin, D. G., Seo, J. H., Kim, J. H., et al. (2019a). Fermented ginseng, GBCK25, ameliorates steatosis and inflammation in nonalcoholic steatohepatitis model. *J. Ginseng Res.* 43 (2), 196–208. doi:10.1016/j.jgr.2017.10.002

Choi, S. Y., Park, J. S., Shon, C. H., Lee, C. Y., Ryu, J. M., Son, D. J., et al. (2019b). Fermented Korean red ginseng extract enriched in Rd and Rg3 protects against non-alcoholic fatty liver disease through regulation of mTORC1. *Nutrients* 11 (12), 2963. doi:10.3390/nu11122963

Chung, S. I., Nam, S. J., Xu, M., Kang, M. Y., and Lee, S. C. (2016). Aged ginseng (Panax ginseng Meyer) reduces blood glucose levels and improves lipid metabolism in high fat diet-fed mice. *Food Sci. Biotechnol.* 25 (1), 267–273. doi:10.1007/s10068-016-0039-1

Clare, K., Dillon, J. F., and Brennan, P. N. (2022). Reactive oxygen species and oxidative stress in the pathogenesis of MAFLD. *J. Clin. Transl. Hepatol.* 10 (5), 939–946. doi:10.14218/jcth.2022.00067

Cui, T., Xiao, X., Pan, Z., Tang, K., Zhong, Y., Chen, Y., et al. (2023). Harnessing the therapeutic potential of ginsenoside Rd for activating SIRT6 in treating a mouse model of nonalcoholic fatty liver disease. *ACS Omega* 8 (32), 29735–29745. doi:10.1021/acsomega.3c04122

Devarbhavi, H., Asrani, S. K., Arab, J. P., Nartey, Y. A., Pose, E., and Kamath, P. S. (2023). Global burden of liver disease: 2023 update. *J. Hepatol.* 79 (2), 516–537. doi:10.1016/j.jhep.2023.03.017

Ebert, T., Widman, L., Stenvinkel, P., and Hagström, H. (2023). Increased risk for microvascular outcomes in NAFLD-A nationwide, population-based cohort study. *J. Intern Med.* 294 (2), 216–227. doi:10.1111/joim.13673

Friedman, S. L., Neuschwander-Tetri, B. A., Rinella, M., and Sanyal, A. J. (2018). Mechanisms of NAFLD development and therapeutic strategies. *Nat. Med.* 24 (7), 908–922. doi:10.1038/s41591-018-0104-9

Fujii, H., Kawada, N., and Japan Study Group Of Nafld, J.-N. (2020). The role of insulin resistance and diabetes in nonalcoholic fatty liver disease. *Int. J. Mol. Sci.* 21 (11), 3863. doi:10.3390/ijms21113863

Geng, Y., Faber, K. N., de Meijer, V. E., Blokzijl, H., and Moshage, H. (2021). How does hepatic lipid accumulation lead to lipotoxicity in non-alcoholic fatty liver disease? *Hepatol. Int.* 15 (1), 21–35. doi:10.1007/s12072-020-10121-2

Gu, D., Yi, H., Jiang, K., Fakhar, S. H., Shi, J., He, Y., et al. (2021). Transcriptome analysis reveals the efficacy of ginsenoside-Rg1 in the treatment of nonalcoholic fatty liver disease. *Life Sci.* 267, 118986. doi:10.1016/j.lfs.2020.118986

Guo, M., Zhu, C., Fu, R., Ma, X., Duan, Z., and Fan, D. (2023). Ginsenoside Rk3 regulates nonalcoholic steatohepatitis by modulation of intestinal flora and the PI3K/AKT signaling pathway in C57bl/6 mice. *J. Agric. Food Chem.* 71 (24), 9370–9380. doi:10.1021/acs.jafc.3c00789

Hong, S. H., Suk, K. T., Choi, S. H., Lee, J. W., Sung, H. T., Kim, C. H., et al. (2013). Anti-oxidant and natural killer cell activity of Korean red ginseng (Panax ginseng) and urushiol (Rhus vernicifera Stokes) on non-alcoholic fatty liver disease of rat. *Food Chem. Toxicol.* 55, 586–591. doi:10.1016/j.fct.2013.01.022

Hou, J., Ma, R., Zhu, S., and Wang, Y. (2022). Revealing the therapeutic targets and mechanism of ginsenoside Rg1 for liver damage related to anti-oxidative stress using proteomic analysis. *Int. J. Mol. Sci.* 23 (17), 10045. doi:10.3390/ijms231710045

Hsu, C. L., and Schnabl, B. (2023). The gut-liver axis and gut microbiota in health and liver disease. *Nat. Rev. Microbiol.* 21 (11), 719–733. doi:10.1038/s41579-023-00904-3

Hu, H., Lin, A., Kong, M., Yao, X., Yin, M., Xia, H., et al. (2020). Intestinal microbiome and NAFLD: molecular insights and therapeutic perspectives. *J. Gastroenterol.* 55 (2), 142–158. doi:10.1007/s00535-019-01649-8

Hua, M., Fan, M. L., Li, Z. M., Sha, J. Y., Li, S. S., and Sun, Y. S. (2021). Ginseng soluble dietary fiber can regulate the intestinal flora structure, promote colon health, affect appetite and glucolipid metabolism in rats. *J. Funct. Foods* 83, 104534. doi:10.1016/j.jff.2021.104534

Huang, Q., Wang, T., Yang, L., and Wang, H. Y. (2017). Ginsenoside Rb2 alleviates hepatic lipid accumulation by restoring autophagy via induction of Sirt1 and activation of AMPK. *Int. J. Mol. Sci.* 18 (5), 1063. doi:10.3390/ijms18051063

Huby, T., and Gautier, E. L. (2022). Immune cell-mediated features of non-alcoholic steatohepatitis. *Nat. Rev. Immunol.* 22 (7), 429–443. doi:10.1038/s41577-021-00639-3

Jeong, H., Kim, J. W., Yang, M. S., Park, C., Kim, J. H., Lim, C. W., et al. (2018). Beneficial effects of Korean red ginseng in the progression of non-alcoholic steatohepatitis via FABP4 modulation. *Am. J. Chin. Med.* 46, 1581–1607. doi:10.1142/s0192415x18500817

Khan, R. S., Bril, F., Cusi, K., and Newsome, P. N. (2019). Modulation of insulin resistance in nonalcoholic fatty liver disease. *Hepatology* 70 (2), 711–724. doi:10.1002/hep.30429

Kim, G. W., Jo, H. K., and Chung, S. H. (2018). Ginseng seed oil ameliorates hepatic lipid accumulation *in vitro* and *in vivo*. *J. Ginseng Res.* 42 (4), 419–428. doi:10.1016/j.jgr.2017.04.010

Kim, J. C., Jeon, J. Y., Yang, W. S., Kim, C. H., and Eom, D. W. (2019). Combined amelioration of ginsenoside (Rg1, Rb1, and rg3)-enriched Korean red ginseng and probiotic lactobacillus on non-alcoholic fatty liver disease. *Curr. Pharm. Biotechnol.* 20 (3), 222–231. doi:10.2174/1389201020666190311143554

Kim, K., Kim, M. H., Kang, J. I., Baek, J. I., Jeon, B. M., Kim, H. M., et al. (2024a). Ginsenoside F2 restrains hepatic steatosis and inflammation by altering the binding affinity of liver X receptor coregulators. *J. Ginseng Res.* 48 (1), 89–97. doi:10.1016/j.jgr.2023.10.001

Kim, M. S., Lee, K. T., Iseli, T. J., Hoy, A. J., George, J., Grewal, T., et al. (2013). Compound K modulates fatty acid-induced lipid droplet formation and expression of proteins involved in lipid metabolism in hepatocytes. *Liver Int.* 33 (10), 1583–1593. doi:10.1111/liv.12287

Kim, M. Y., Jeong, B., Lee, G. S., Jeon, H., Yang, Y. M., Yang, H., et al. (2024b). Panaxydol extracted from Panax ginseng inhibits NLRP3 inflammasome activation to ameliorate NASH-induced liver injury. *Int. Immunopharmacol.* 128, 111565. doi:10.1016/j.intimp.2024.111565

Kim, Y. W., Bak, S. B., Song, Y. R., Kim, C. E., and Lee, W. Y. (2024c). Systematic exploration of therapeutic effects and key mechanisms of Panax ginseng using network-based approaches. *J. Ginseng Res.* 48 (4), 373–383. doi:10.1016/j.jgr.2024.01.005

Kobayashi, T., Iwaki, M., Nakajima, A., Nogami, A., and Yoneda, M. (2022). Current research on the pathogenesis of NAFLD/NASH and the gut-liver Axis: gut microbiota, dysbiosis, and leaky-gut syndrome. *Int. J. Mol. Sci.* 23 (19), 11689. doi:10.3390/ijms231911689

Kolodziejczyk, A. A., Zheng, D., Shibolet, O., and Elinav, E. (2019). The role of the microbiome in NAFLD and NASH. *EMBO Mol. Med.* 11 (2), e9302. doi:10.15252/emmm.201809302

Kumar, S., Duan, Q., Wu, R., Harris, E. N., and Su, Q. (2021). Pathophysiological communication between hepatocytes and non-parenchymal cells in liver injury from NAFLD to liver fibrosis. *Adv. Drug Deliv. Rev.* 176, 113869. doi:10.1016/j.addr.2021.113869

Kwak, Y. B., Yoo, H. H., and Yoon, J. (2024). The impact of the administration of red ginseng (Panax ginseng) on lipid metabolism and free fatty acid profiles in healthy horses using a molecular networking approach. *Front. Vet. Sci.* 11, 1285000. doi:10.3389/fvets.2024.1285000

Lebeaupin, C., Vallée, D., Hazari, Y., Hetz, C., Chevet, E., and Bailly-Maitre, B. (2018). Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. *J. Hepatol.* 69 (4), 927–947. doi:10.1016/j.jhep.2018.06.008

Lee, J. B., Yoon, S. J., Lee, S. H., Lee, M. S., Jung, H., Kim, T. D., et al. (2017). Ginsenoside Rg3 ameliorated HFD-induced hepatic steatosis through downregulation of STAT5-PPARγ. *J. Endocrinol.* 235 (3), 223–235. doi:10.1530/joe-17-0233

Lee, S., Lee, M. S., Kim, C. T., Kim, I. H., and Kim, Y. (2012). Ginsenoside Rg3 reduces lipid accumulation with AMP-Activated Protein Kinase (AMPK) activation in HepG2 cells. *Int. J. Mol. Sci.* 13 (5), 5729–5739. doi:10.3390/ijms13055729

Lee, S. B., Cho, H. I., Jin, Y. W., Lee, E. K., Ahn, J. Y., and Lee, S. M. (2016). Wild ginseng cambial meristematic cells ameliorate hepatic steatosis and mitochondrial dysfunction in high-fat diet-fed mice. *J. Pharm. Pharmacol.* 68 (1), 119–127. doi:10.1111/jphp.12487

Lei, N., Song, H., Zeng, L., Ji, S., Meng, X., Zhu, X., et al. (2023). Persistent lipid accumulation leads to persistent exacerbation of endoplasmic reticulum stress and inflammation in progressive NASH via the ire1α/TRAF2 complex. *Molecules* 28 (7), 3185. doi:10.3390/molecules28073185

Leng, Y. R., Zhang, M. H., Luo, J. G., and Zhang, H. (2021). Pathogenesis of NASH and promising natural products. *Chin. J. Nat. Med.* 19 (1), 12–27. doi:10.1016/s1875-5364(21)60002-x

Li, F., Wu, Z., and Sui, X. (2020). Biotransformation of ginsenoside Rb1 with wild Cordyceps sinensis and Ascomycota sp. and its antihyperlipidemic effects on the diet-induced cholesterol of zebrafish. *J. Food Biochem.* 44 (6), e13192. doi:10.1111/jfbc.13192

Li, G., Wang, H., Dong, Z. Q., Wang, Y. P., Wang, T., Yao, X., et al. (2024a). Development situation and trends of Ginseng Radix et Rhizoma industry in China. *Zhongguo Zhong Yao Za Zhi* 49 (17), 4818–4828. doi:10.19540/j.cnki.cjcmm.20240615.102

Li, G., Xie, H., Cao, X., Ma, C., Li, Y., and Chen, L. (2022a). Ginsenoside Rg1 exerts anti-apoptotic effects on non-alcoholic fatty liver cells by downregulating the expression of SGPL1. *Mol. Med. Rep.* 25 (5), 178. doi:10.3892/mmr.2022.12694

Li, X., Liu, J., Zuo, T. T., Hu, Y., Li, Z., Wang, H. D., et al. (2022b). Advances and challenges in ginseng research from 2011 to 2020: the phytochemistry, quality control, metabolism, and biosynthesis. *Nat. Prod. Rep.* 39 (4), 875–909. doi:10.1039/d1np00071c

Li, X., Xu, X., Tao, S., Su, Y., Wen, L., Wang, D., et al. (2024b). Gut microbes combined with metabolomics reveal the protective effects of Qijia Rougan decoction against CCl(4)-induced hepatic fibrosis. *Front. Pharmacol.* 15, 1347120. doi:10.3389/fphar.2024.1347120

Li, X., Zhou, L., Xu, X., Liu, X., Wu, W., Feng, Q., et al. (2024c). Metabolic reprogramming in hepatocellular carcinoma: a bibliometric and visualized study from 2011 to 2023. *Front. Pharmacol.* 15, 1392241. doi:10.3389/fphar.2024.1392241

Li, Y., Yang, B., Guo, W., Zhang, P., Zhang, J., Zhao, J., et al. (2022c). Classification of three types of ginseng samples based on ginsenoside profiles: appropriate data normalization improves the efficiency of multivariate analysis. *Heliyon* 8 (12), e12044. doi:10.1016/j.heliyon.2022.e12044

Li, Y., Zhang, S., Zhu, Z., Zhou, R., Xu, P., Zhou, L., et al. (2022d). Upregulation of adiponectin by Ginsenoside Rb1 contributes to amelioration of hepatic steatosis induced by high fat diet. *J. Ginseng Res.* 46 (4), 561–571. doi:10.1016/j.jgr.2021.10.005

Liang, W., Zhou, K., Jian, P., Chang, Z., Zhang, Q., Liu, Y., et al. (2021). Ginsenosides improve nonalcoholic fatty liver disease via integrated regulation of gut microbiota, inflammation and energy homeostasis. *Front. Pharmacol.* 12, 622841. doi:10.3389/fphar.2021.622841

Liu, C., Huang, R., Yu, H., Gong, Y., Wu, P., Feng, Q., et al. (2022). Fuzheng Xiaozheng prescription exerts anti-hepatocellular carcinoma effects by improving lipid and glucose metabolisms via regulating circRNA-miRNA-mRNA networks. *Phytomedicine* 103, 154226. doi:10.1016/j.phymed.2022.154226

Loomba, R., Friedman, S. L., and Shulman, G. I. (2021). Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* 184 (10), 2537–2564. doi:10.1016/j.cell.2021.04.015

Lou, T. W., Yang, R. X., and Fan, J. G. (2024). The global burden of fatty liver disease: the major impact of China. *Hepatobiliary Surg. Nutr.* 13 (1), 119–123. doi:10.21037/hbsn-23-556

Lu, B., Wang, D., Xie, D., Wu, C., and Sun, M. (2023). 20(S)-Protopanaxatriol ameliorates MAFLD by inhibiting NLRP3 inflammasome. *Eur. J. Pharmacol.* 940, 175468. doi:10.1016/j.ejphar.2022.175468

Lu, G., Liu, Z., Wang, X., and Wang, C. (2021). Recent advances in Panax ginseng C.A. Meyer as a herb for anti-fatigue: an effects and mechanisms review. *Foods* 10 (5), 1030. doi:10.3390/foods10051030

Ma, R., Yang, P., Jing, C., Fu, B., Teng, X., Zhao, D., et al. (2023). Comparison of the metabolomic and proteomic profiles associated with triterpene and phytosterol accumulation between wild and cultivated ginseng. *Plant Physiol. Biochem.* 195, 288–299. doi:10.1016/j.plaphy.2023.01.020

Mahlapuu, M., Caputo, M., Xia, Y., and Cansby, E. (2022). GCKIII kinases in lipotoxicity: roles in NAFLD and beyond. *Hepatol. Commun.* 6 (10), 2613–2622. doi:10.1002/hep4.2013

Malesza, I. J., Malesza, M., Walkowiak, J., Mussin, N., Walkowiak, D., Aringazina, R., et al. (2021). High-fat, western-style diet, systemic inflammation, and gut microbiota: a narrative review. *Cells* 10 (11), 3164. doi:10.3390/cells10113164

Mato, J. M., Alonso, C., Noureddin, M., and Lu, S. C. (2019). Biomarkers and subtypes of deranged lipid metabolism in non-alcoholic fatty liver disease. *World J. Gastroenterol.* 25 (24), 3009–3020. doi:10.3748/wjg.v25.i24.3009

McComb, S., Chan, P. K., Guinot, A., Hartmannsdottir, H., Jenni, S., Dobay, M. P., et al. (2019). Efficient apoptosis requires feedback amplification of upstream apoptotic signals by effector caspase-3 or -7. *Sci. Adv.* 5 (7), eaau9433. doi:10.1126/sciadv.aau9433

Meng, Z., Lu, J., Ge, G., Wang, G., Zhang, R., Li, Y., et al. (2023). Ginsenoside Rb1 induces autophagic lipid degradation via miR-128 targeting TFEB. *Food Funct.* 14 (1), 240–249. doi:10.1039/d2fo02719d

Mi, A., Hu, Q., Liu, Y., Zhao, Y., Shen, F., Lan, J., et al. (2024). Hepatoprotective efficacy and interventional mechanism of the panaxadiol saponin component in high-fat diet-induced NAFLD mice. *Food Funct.* 15 (2), 794–808. doi:10.1039/d3fo03572g

Mouries, J., Brescia, P., Silvestri, A., Spadoni, I., Sorribas, M., Wiest, R., et al. (2019). Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. *J. Hepatol.* 71 (6), 1216–1228. doi:10.1016/j.jhep.2019.08.005

Mridha, A. R., Wree, A., Robertson, A. A. B., Yeh, M. M., Johnson, C. D., Van Rooyen, D. M., et al. (2017). NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. *J. Hepatol.* 66 (5), 1037–1046. doi:10.1016/j.jhep.2017.01.022

Nan, B., Liu, Y. L., You, Y., Li, W. C., Fan, J. J., Wang, Y. S., et al. (2018). Protective effects of enhanced minor ginsenosides in Lactobacillus fermentum KP-3-fermented ginseng in mice fed a high fat diet. *Food Funct.* 9 (11), 6020–6028. doi:10.1039/c8fo01056k

Newsome, P. N., Buchholtz, K., Cusi, K., Linder, M., Okanoue, T., Ratziu, V., et al. (2021). A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis. *N. Engl. J. Med.* 384 (12), 1113–1124. doi:10.1056/NEJMoa2028395

Oh, G. S., Yoon, J., Lee, G. G., Oh, W. K., and Kim, S. W. (2015). 20(S)-protopanaxatriol inhibits liver X receptor α-mediated expression of lipogenic genes in hepatocytes. *J. Pharmacol. Sci.* 128 (2), 71–77. doi:10.1016/j.jphs.2015.05.007

Park, C. H., Kim, M., Woo, M., Lee, K. H., An, B. K., Yokozawa, T., et al. (2018). Comparison of the effects of nonfermented and fermented Panax ginseng root against hypertriglycemia in high-fat diet-fed mice. *J. Med. Food* 21 (4), 317–321. doi:10.1089/jmf.2017.4035

Park, J., Zhao, Y., Zhang, F., Zhang, S., Kwong, A. C., Zhang, Y., et al. (2023). IL-6/STAT3 axis dictates the PNPLA3-mediated susceptibility to non-alcoholic fatty liver disease. *J. Hepatol.* 78 (1), 45–56. doi:10.1016/j.jhep.2022.08.022

Park, S. K., Kim, S. W., Seo, H. W., Hyun, S. H., Kyung, J. S., Youn, S. H., et al. (2022). Long-term evaluation of safety and biological effects of Korean Red Ginseng (Panax Ginseng): a long-term *in vivo* study. *BMC Complement. Med. Ther.* 22 (1), 284. doi:10.1186/s12906-022-03736-5

Paternostro, R., and Trauner, M. (2022). Current treatment of non-alcoholic fatty liver disease. *J. Intern Med.* 292 (2), 190–204. doi:10.1111/joim.13531

Peiseler, M., Schwabe, R., Hampe, J., Kubes, P., Heikenwälder, M., and Tacke, F. (2022). Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease - novel insights into cellular communication circuits. *J. Hepatol.* 77 (4), 1136–1160. doi:10.1016/j.jhep.2022.06.012

Piao, X., Zhang, H., Kang, J. P., Yang, D. U., Li, Y., Pang, S., et al. (2020). Advances in saponin diversity of Panax ginseng. *Molecules* 25 (15), 3452. doi:10.3390/molecules25153452

Potenza, M. A., Montagnani, M., Santacroce, L., Charitos, I. A., and Bottalico, L. (2023). Ancient herbal therapy: a brief history of Panax ginseng. *J. Ginseng Res.* 47 (3), 359–365. doi:10.1016/j.jgr.2022.03.004

Qi, R., Jiang, R., Xiao, H., Wang, Z., He, S., Wang, L., et al. (2020). Ginsenoside Rg1 protects against d-galactose induced fatty liver disease in a mouse model via FOXO1 transcriptional factor. *Life Sci.* 254, 117776. doi:10.1016/j.lfs.2020.117776

Quek, J., Chan, K. E., Wong, Z. Y., Tan, C., Tan, B., Lim, W. H., et al. (2023). Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis. *Lancet Gastroenterol. Hepatol.* 8 (1), 20–30. doi:10.1016/s2468-1253(22)00317-x

Ratan, Z. A., Haidere, M. F., Hong, Y. H., Park, S. H., Lee, J. O., Lee, J., et al. (2021). Pharmacological potential of ginseng and its major component ginsenosides. *J. Ginseng Res.* 45 (2), 199–210. doi:10.1016/j.jgr.2020.02.004

Roh, E., Hwang, H. J., Kim, J. W., Hong, S. H., Kim, J. A., Lee, Y. B., et al. (2020). Ginsenoside Mc1 improves liver steatosis and insulin resistance by attenuating ER stress. *J. Ethnopharmacol.* 259, 112927. doi:10.1016/j.jep.2020.112927

Sakurai, Y., Kubota, N., Yamauchi, T., and Kadowaki, T. (2021). Role of insulin resistance in MAFLD. *Int. J. Mol. Sci.* 22 (8), 4156. doi:10.3390/ijms22084156

Senft, D., and Ronai, Z. A. (2015). UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. *Trends Biochem. Sci.* 40 (3), 141–148. doi:10.1016/j.tibs.2015.01.002

Shen, L., Xiong, Y., Wang, D. Q., Howles, P., Basford, J. E., Wang, J., et al. (2013). Ginsenoside Rb1 reduces fatty liver by activating AMP-activated protein kinase in obese rats. *J. Lipid Res.* 54 (5), 1430–1438. doi:10.1194/jlr.M035907

Shojaie, L., Iorga, A., and Dara, L. (2020). Cell death in liver diseases: a review. *Int. J. Mol. Sci.* 21 (24), 9682. doi:10.3390/ijms21249682

Taru, V., Szabo, G., Mehal, W., and Reiberger, T. (2024). Inflammasomes in chronic liver disease: hepatic injury, fibrosis progression and systemic inflammation. *J. Hepatol.* 81 (5), 895–910. doi:10.1016/j.jhep.2024.06.016

Terrault, N. A., Francoz, C., Berenguer, M., Charlton, M., and Heimbach, J. (2023). Liver transplantation 2023: status report, current and future challenges. *Clin. Gastroenterol. Hepatol.* 21 (8), 2150–2166. doi:10.1016/j.cgh.2023.04.005

Vallianou, N., Christodoulatos, G. S., Karampela, I., Tsilingiris, D., Magkos, F., Stratigou, T., et al. (2021). Understanding the role of the gut microbiome and microbial metabolites in non-alcoholic fatty liver disease: current evidence and perspectives. *Biomolecules* 12 (1), 56. doi:10.3390/biom12010056

Wang, C., Tang, X., Yan, F., Wang, Y., Wang, X., Zhao, D., et al. (2025). Different processing methods affect the chemical composition and *in vitro* anti-tumor activity of ginseng. *J. Food Sci.* 90 (1), e17639. doi:10.1111/1750-3841.17639

Wang, F., Park, J. S., Ma, Y., Ma, H., Lee, Y. J., Lee, G. R., et al. (2021). Ginseng saponin enriched in Rh1 and Rg2 ameliorates nonalcoholic fatty liver disease by inhibiting inflammasome activation. *Nutrients* 13 (3), 856. doi:10.3390/nu13030856

Wang, Y. D., Wu, L. L., Qi, X. Y., Wang, Y. Y., Liao, Z. Z., Liu, J. H., et al. (2023). New insight of obesity-associated NAFLD: dysregulated "crosstalk" between multi-organ and the liver? *Genes Dis.* 10 (3), 799–812. doi:10.1016/j.gendis.2021.12.013

Wei, S., Wang, L., Evans, P. C., and Xu, S. (2024). NAFLD and NASH: etiology, targets and emerging therapies. *Drug Discov. Today* 29 (3), 103910. doi:10.1016/j.drudis.2024.103910

Wiering, L., and Tacke, F. (2023). Treating inflammation to combat non-alcoholic fatty liver disease. *J. Endocrinol.* 256 (1), e220194. doi:10.1530/joe-22-0194

Xiao, Q., Zhang, S., Yang, C., Du, R., Zhao, J., Li, J., et al. (2019). Ginsenoside Rg1 ameliorates palmitic acid-induced hepatic steatosis and inflammation in HepG2 cells via the AMPK/NF-κB pathway. *Int. J. Endocrinol.* 2019, 7514802. doi:10.1155/2019/7514802

Xu, G., Quan, S., Schell, J., Gao, Y., Varmazyad, M., Sreenivas, P., et al. (2024). Mitochondrial ACSS1-K635 acetylation knock-in mice exhibit altered metabolism, cell senescence, and nonalcoholic fatty liver disease. *Sci. Adv.* 10 (20), eadj5942. doi:10.1126/sciadv.adj5942

Xu, H., Zhao, Q., Song, N., Yan, Z., Lin, R., Wu, S., et al. (2020). AdipoR1/AdipoR2 dual agonist recovers nonalcoholic steatohepatitis and related fibrosis via endoplasmic reticulum-mitochondria axis. *Nat. Commun.* 11 (1), 5807. doi:10.1038/s41467-020-19668-y

Xu, X., Poulsen, K. L., Wu, L., Liu, S., Miyata, T., Song, Q., et al. (2022). Targeted therapeutics and novel signaling pathways in non-alcohol-associated fatty liver/steatohepatitis (NAFL/NASH). *Signal Transduct. Target Ther.* 7 (1), 287. doi:10.1038/s41392-022-01119-3

Xu, Y., Yang, C., Zhang, S., Li, J., Xiao, Q., and Huang, W. (2018). Ginsenoside Rg1 protects against non-alcoholic fatty liver disease by ameliorating lipid peroxidation, endoplasmic reticulum stress, and inflammasome activation. *Biol. Pharm. Bull.* 41 (11), 1638–1644. doi:10.1248/bpb.b18-00132

Xuan, P., De-Bin, H., Mi-Ya, Y., and Shi-Jun, P. (2015). Ginsenoside Rg1 improves liver function by regulating fat metabolism in rats with non-alcoholic fatty liver disease. *Chin. J. Pathophysiol.* 31 (5), 864–870. doi:10.3892/mmr.2022.12694

Yang, K., Kim, H. H., Shim, Y. R., and Song, M. J. (2023a). The efficacy of Panax ginseng for the treatment of nonalcoholic fatty liver disease: a systematic review and meta-analysis of preclinical studies. *Nutrients* 15 (3), 721. doi:10.3390/nu15030721

Yang, Z., Yu, Y., Sun, N., Zhou, L., Zhang, D., Chen, H., et al. (2023b). Ginsenosides Rc, as a novel SIRT6 activator, protects mice against high fat diet induced NAFLD. *J. Ginseng Res.* 47 (3), 376–384. doi:10.1016/j.jgr.2020.07.005

Younossi, Z. M., Stepanova, M., Nader, F., Loomba, R., Anstee, Q. M., Ratziu, V., et al. (2022). Obeticholic acid impact on quality of life in patients with nonalcoholic steatohepatitis: REGENERATE 18-month interim analysis. *Clin. Gastroenterol. Hepatol.* 20 (9), 2050–2058.e12. doi:10.1016/j.cgh.2021.07.020

Younossi, Z. M., Zelber-Sagi, S., Henry, L., and Gerber, L. H. (2023). Lifestyle interventions in nonalcoholic fatty liver disease. *Nat. Rev. Gastroenterol. Hepatol.* 20 (11), 708–722. doi:10.1038/s41575-023-00800-4

Yu, L., Hong, W., Lu, S., Li, Y., Guan, Y., Weng, X., et al. (2022). The NLRP3 inflammasome in non-alcoholic fatty liver disease and steatohepatitis: therapeutic targets and treatment. *Front. Pharmacol.* 13, 780496. doi:10.3389/fphar.2022.780496

Zhang, J., and Feng, Q. (2022). Pharmacological effects and molecular protective mechanisms of Astragalus polysaccharides on nonalcoholic fatty liver disease. *Front. Pharmacol.* 13, 854674. doi:10.3389/fphar.2022.854674

Zhang, J., Guo, J., Yang, N., Huang, Y., Hu, T., and Rao, C. (2022). Endoplasmic reticulum stress-mediated cell death in liver injury. *Cell Death Dis.* 13 (12), 1051. doi:10.1038/s41419-022-05444-x

Zhao, C., Qu, Q., Yang, F., Li, Z., Yang, P., Han, L., et al. (2021). Monascus ruber fermented Panax ginseng ameliorates lipid metabolism disorders and modulate gut microbiota in rats fed a high-fat diet. *J. Ethnopharmacol.* 278, 114300. doi:10.1016/j.jep.2021.114300

Zhou, G., Wang, C. Z., Mohammadi, S., Sawadogo, W. R., Ma, Q., and Yuan, C. S. (2023). Pharmacological effects of ginseng: multiple constituents and multiple actions on humans. *Am. J. Chin. Med.* 51 (5), 1085–1104. doi:10.1142/s0192415x23500507

Glossary

ACC	acetyl-coenzyme A carboxylase
ACOX	acyl-coenzyme A oxidase
AKP	alkaline phosphatase
AKT	protein kinase B
ALT	alanine aminotransferase
AMPK	AMP-activated protein kinase
AST	aspartate aminotransferase
ATF	activating transcription factor
Bax	Bcl-2-associated X
Bcl-2	B-cell lymphoma-2
Bcl-XL	B-cell lymphoma-extra large
Caspase	cysteine-containing aspartate-specific protease
CCL	C-C motif chemokine ligand
CDKN	cyclin-dependent kinase inhibitor
CHOP	CCAAT/enhancer binding protein (C/EBP) homologous protein
ChREBP	carbohydrate responsive element-binding protein
COL	collagen
CPT	carnitine palmitoyl transferase
CYP7A1	cholesterol 7α-hydroxylase
ERS	endoplasmic reticulum stress
FA	fatty acid
FAS	fatty acid synthase
FBG	fasting blood-glucose
FFAs	free fatty acids
FG	fermented ginseng
FRG	fermentation of red ginseng with C. militaris
FXR	farnesoid X receptor
GDS	ginseng diol saponin
GRP78	glucose-regulated protein78
GSO	ginseng seed oil
GWDF	ginseng water-soluble dietary fiber
HDL-C	high density lipoprotein cholesterol
HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase
HOMA-IR	insulin resistance index
IL	interleukin
IR	insulin resistance
JNK	c-Jun N-terminal kinase
LDL	low density lipid protein
LDL-C	low density lipoprotein cholesterol
LDs	lipid droplets
LFG	*lactobacillus* fermentum KP-3 fermented ginseng
LXR	liver X receptor
MDA	malondialdehyde
MFG	monascus ruber fermented ginseng
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NF-κB	nuclear factor kappa-B
NLRP3	nod-like receptor thermal protein domain associated protein 3
Nrf2	nuclear factor-erythroid 2 related factor 2
PGC-1α	peroxisome proliferators-activated receptor γ co-activator α
PI3K	phosphatidylinositol-3-kinase
PPAR	peroxisome proliferator-activated receptor
PPD	protopanaxadiol
PPT	protopanaxatriol
RG	red ginseng
ROS	reactive oxygen species
SCD-1	stearoyl-coenzyme A desaturase-1
SIRT	sirtuins
SOD	superoxide dismutase
SREBP	sterol-regulatory element binding protein
STAT	signal transducer and activator of transcription
TC	total cholesterol
TCM	traditional Chinese medicine
TG	triglyceride
TIMP	tissue inhibitor of metalloproteinase 1
TNF-α	tumor necrosis factor α
UPR	unfolded protein response
WGCM	wild ginseng cambial meristematic cells

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Gut microbiota-mitochondrial crosstalk in obesity: novel mechanistic insights and therapeutic strategies with traditional Chinese medicine

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Obesity rates are rising globally and have become a major public health issue. Recent research emphasizes the bidirectional communication between gut microbiota and mitochondrial function in obesity development. Gut microbiota regulates energy metabolism through metabolites that impact mitochondrial processes, such as oxidative phosphorylation, biogenesis, and autophagy. In turn, alterations in mitochondrial function impact microbiota homeostasis. Traditional Chinese medicine (TCM), which encompasses TCM formulas and the metabolites of botanical drugs, employs a holistic and integrative approach that shows promise in regulating gut microbiota–mitochondrial crosstalk. This review systematically explores the intricate interactions between gut microbiota and mitochondrial function, underscoring their crosstalk as a critical mechanistic axis in obesity pathogenesis. Furthermore, it highlights the potential of TCM in developing innovative, targeted interventions, paving the way for personalized approaches in obesity treatment through the precise modulation of gut microbiota–mitochondrial interactions, offering more effective and individualized therapeutic options.

KEYWORDS

gut microbiota, mitochondria, obesity, metabolism, traditional Chinese medicine

1 Introduction

The global incidence of obesity, a multifaceted metabolic disorder, has surged in recent decades, becoming a pressing public health challenge (Welsh et al., 2024). While excessive calorie intake and inactivity are known causes of obesity, attention is shifting toward gut microbiota and mitochondrial interactions (Belda et al., 2022). In a healthy state, the gut microbiota supports host energy metabolism, lipid metabolism, and glucose homeostasis through diverse metabolic activities (Fan and Pedersen, 2021). This microbiota supports the intestinal barrier functionality through the modulation of the host's cellular processes and immune responses (Leshem et al., 2020). However, a disruption in the gut microbial balance can increase intestinal permeability, thereby allowing bacterial toxins and metabolic products to enter the bloodstream and disrupt the body's overall metabolic equilibrium (Gasmi et al., 2021). Unfortunately, the exact mechanisms within the intestinal microbial community that trigger these metabolic abnormalities remain poorly understood (Michaudel and Sokol, 2020).

Gut microbiota–mitochondria interactions play a crucial role in various health conditions has become increasingly evident (Ballard and Towarnicki, 2020; Li Y. et al., 2023). By metabolizing dietary elements, gut microbiota generates short-chain fatty acids (SCFAs) and secondary bile acids (SBAs), which influence mitochondrial oxidative phosphorylation (OXPHOS), biogenesis, dynamics, and autophagy mechanisms, thereby significantly affecting energy balance and metabolism (Yoo et al., 2021; Fogelson et al., 2023). As the main cellular powerhouses, mitochondria are pivotal in regulating fat metabolism, thermoregulation, and oxidative balance (Brestoff et al., 2021; Xia et al., 2024). Dysfunctional mitochondria can produce excess reactive oxygen species (ROS) and organic acids, subsequently altering the gut microbiota composition and function, leading to intricate two-way interactions (Singh et al., 2022).

The ancient Chinese medical text *Lingshu Jing* of the *Huangdi Neijing* states that obesity is characterized by "people having fat, ointment, and flesh." It describes obesity as a condition primarily caused by spleen and kidney deficiency and liver qi stagnation, with phlegm, dampness, heat, and blood stasis as its key pathological manifestations. These factors interact with one another, affecting both energy metabolism and material metabolism in the body. Based on this understanding, traditional Chinese medicine (TCM) has been widely applied in the clinical treatment of obesity, demonstrating safe, gentle, and long-lasting effects, and has accumulated extensive experience through long-term practice (Li et al., 2020; Chen Y. K. et al., 2023; Zhang Q. et al., 2023). Rooted in TCM, TCM formulas and metabolites of botanical drugs offer a promising approach to managing obesity by modulating gut microbiota and mitochondrial function. By restoring microbial balance and enhancing mitochondrial efficiency, it addresses key dysfunctions within the gut microbiota–mitochondria axis, providing a novel and integrative approach to targeted obesity interventions.

Although early research has revealed a sophisticated relationship between gut microbiota and mitochondria, the precise mechanisms underlying this interaction in obesity development remain to be fully understood. This review systematically examines the intricate crosstalk between gut microbiota and mitochondria, emphasizing their role as a mechanistic axis in obesity. Furthermore, it explores the potential of TCM-based interventions in modulating this axis, highlighting their prospective applications in precision medicine approaches for obesity treatment.

2 Review methodology

This review conducted a comprehensive literature search across PubMed, Web of Science, ScienceDirect, Google Scholar, and CNKI using a combination of controlled vocabulary and free-text keywords, including "gut microbiota," "mitochondria," "obesity," "metabolic disorders," "plant metabolites," "botanical drugs," and "Traditional Chinese Medicine." Boolean operators (AND, OR) and database-specific filters were applied to refine results and exclude irrelevant publications. Inclusion criteria encompassed studies investigating gut microbiota-mitochondria interactions in obesity and metabolic disorders using rigorously designed *in vivo* or *in vitro* models. Exclusion criteria comprised studies with unclear experimental design, insufficient mechanistic evaluation, inadequate sample sizes, unsupported conclusions, or redundant/duplicate publications.

3 Mitochondrial dysfunction in obesity

Mitochondria generate adenosine triphosphate (ATP) through OXPHOS, providing the necessary energy for cellular activities. Beyond energy production, mitochondria are integral to numerous vital cellular functions, including apoptosis control, calcium homeostasis, lipid metabolism, and thermogenesis. Maintaining mitochondrial homeostasis involves balancing and regulating various processes, including OXPHOS, dynamics, biogenesis, and autophagy, all of which are critical for sustaining energy metabolism. Disruptions in these processes can lead to obesity (Figure 1).

3.1 Mitochondrial oxidative phosphorylation

Mitochondrial OXPHOS, a key component of cellular respiration, converts chemical energy from nutrients into ATP, the primary energy source for cellular functions (Harrington et al., 2023; Talari et al., 2023). Under normal conditions, electrons move through the electron transport chain sequentially from complexes I to IV, where they ultimately combine with oxygen to form water. As the electrons travel, their movement drives the transfer of protons from the mitochondrial matrix to the intermembrane space. This process creates an electrochemical gradient across the inner mitochondrial membrane. (Pedersen et al., 2022). ATP synthase utilizes this gradient to synthesize ATP from ADP and inorganic phosphate, fueling cellular functions. However, dysfunctional mitochondria impair this process by reducing ATP production and increasing oxidative stress (CA et al., 2021). Obese individuals exhibit significantly reduced activity in complexes I, III, and IV, impairing the effectiveness of the electron transport chain and disrupting ATP production. This dysfunction also increases electron leakage from the chain, leading to the excessive production of ROS (MacLean et al., 2023; Zotta et al., 2024). The accumulation of ROS exacerbates damage to mitochondria and other cell structures, perpetuating a detrimental cycle. In addition, impaired OXPHOS leads to the accumulation of metabolic intermediates, such as lactate, which interferes with normal metabolic processes (Sergio et al., 2021; Abu Shelbayeh et al., 2023).

3.2 Mitochondrial biogenesis

Mitochondrial biogenesis refers to the generation and proliferation of new mitochondria within cells, a process essential for maintaining cellular energy supply and metabolic equilibrium. This process includes the replication of mitochondrial DNA, the formation of mitochondrial membranes, and the synthesis and assembly of mitochondrial proteins (Chen L. et al., 2023; Lapatto et al., 2023; Malik et al., 2023). Overconsumption of nutrients results in elevated free fatty acid levels enhanced mitochondrial ROS

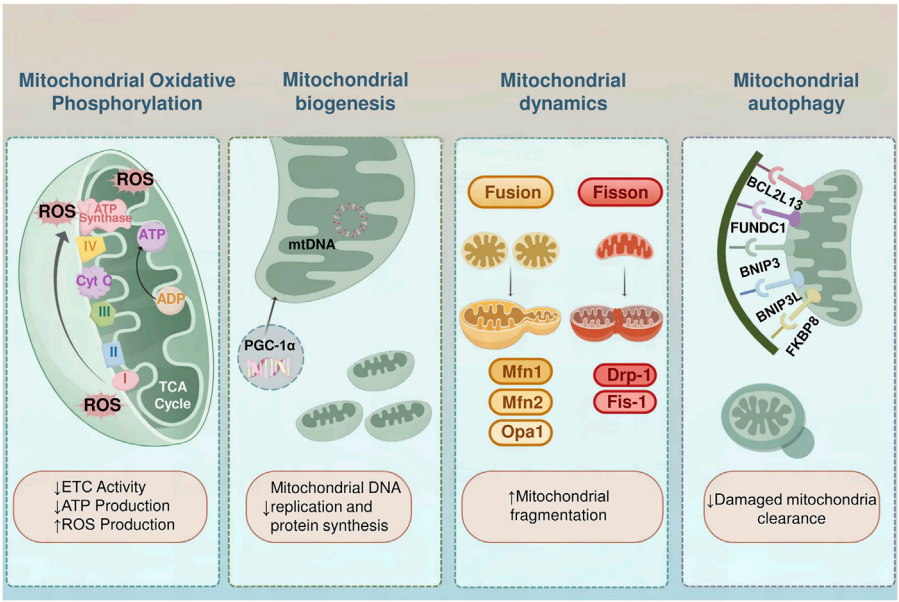

FIGURE 1
Mitochondrial Dysfunction in Obesity. In individuals with obesity, diminished electron transport chain function results in lower ATP generation and elevated ROS levels, inducing oxidative stress and harm to cells. The process of mitochondrial biogenesis is controlled by PGC-1α, with reduced biogenesis resulting in decreased mitochondrial DNA duplication and protein production. The balance of mitochondrial dynamics is compromised, characterized by enhanced fission facilitated by Drp1 and Fis1, coupled with reduced fusion facilitated by Mfn1/2 and Opa1, leading to a deterioration of network cohesiveness. Mitochondrial autophagy, the process of clearing damaged mitochondria, is impaired, leading to their accumulation. Key mitophagy receptors interacting with the LC3 are essential for the management of mitochondrial quality. BCL2L13 and FUNDC1 bind LC3 to facilitate autophagic clearance, especially under hypoxic conditions. The BNIP3 and BNIP3L possess LC3-interacting region motifs essential for mitochondrial recycling under stressful conditions. FKBP8 also interacts with LC3 to facilitate the clearance of dysfunctional mitochondria.

generation, compromised mitochondrial performance in fat cells, decreased mitochondrial formation and DNA quantity, and lowered β-oxidation rates. Subsequently, fat cell metabolic processes undergo changes, as evidenced by modifications in fat synthesis, breakdown, fatty acid bonding, and the production of lipocalin by adipocytes. These metabolic shifts lead to reduced insulin responsiveness (Bénédicte A. et al., 2021). Obesity leads to downregulation of the gene expression of mitochondrial respiratory complex components, with the protein peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) being a key regulator of mitochondrial biogenesis. PGC-1α stimulates mitochondrial creation by interacting with various nuclear receptors and gene transcription regulators, including peroxisome proliferator-activated receptor γ (PPARγ). However, the expression and function of PGC-1α are significantly impaired in individuals with obesity (Rius-Perez et al., 2020).

3.3 Mitochondrial dynamics

Mitochondrial dynamics are governed by a series of GTPase proteins. Dynamin-related protein 1 (Drp1) is primarily responsible for the division of mitochondrial membranes, while mitochondrial fission protein 1 (Fis1) also plays a crucial role in regulating mitochondrial fragmentation (Duan et al., 2021; Yu et al., 2021; Huan et al., 2023). On the other hand, GTPase optic atrophy 1 (Opa1) is responsible for the fusion of the inner mitochondrial membrane, and mitochondrial fusion proteins 1 (Mfn1) and 2 (Mfn2) control the fusion of the outer mitochondrial membrane (von der Malsburg et al., 2023). In the obese state, mitochondrial division tends to be overactive, leading to mitochondrial fragmentation and dysfunction. Excessive mitochondrial division not only decreases the number of mitochondria but also increases ROS production, triggering a cellular stress response and metabolic dysregulation (Al Ojaimi et al., 2022). Moreover, reduced mitochondrial fusion limits mitochondrial repair and functional optimization, exacerbating obesity-related metabolic issues (Garcia et al., 2022). Changes in the proteins that regulate mitochondrial structure and function are closely linked to obesity-related mitochondrial dysfunction. Specifically, several studies have shown that decreased expression of OPA1 and Mfn2 is linked to impaired mitochondrial functionality in obesity (Mancini et al., 2019; Zheng et al., 2023). Pereira et al. indicated that deletion of the mitochondrial protease OMA1 disrupts OPA1 processing, affecting metabolic balance and leading to increased fat mass and reduced energy expenditure (Pereira et al., 2021). Similarly, Da et al. reported that Mfn2 deficiency can lead to elevated hydrogen peroxide and ROS levels, contributing to mitochondrial dysfunction in liver and muscle tissues (Da Dalt et al., 2024). Further investigations in HFD-fed mice revealed that changes in mitochondrial dynamics—from fusion to increased fission—are associated with respiratory challenges and reduced ATP production in skeletal muscle, strengthening the connection between impaired mitochondrial dynamics and metabolic disturbances in obesity (Yang H.-M. et al., 2023; Kim et al., 2024).

TABLE 1 Mitochondrial dysfunction in different stages of obesity.

Stage of obesity	Main characteristics	Specific manifestations of mitochondrial dysfunction	References
Early Stage	Weight gain, adipocyte hypertrophy	Decreased mitochondrial biogenesis, and impaired oxidative phosphorylation	Baldini et al. (2021)
Intermediate Stage	Chronic low-grade inflammation, onset of insulin resistance	Elevated ROS levels, and dysregulated lipid oxidation, exacerbated insulin resistance	Wang et al. (2021)
Advanced Stage	High risk of metabolic complications	Mitochondrial DNA damage, defective mitophagy, increased apoptosis and necrosis	Montgomery (2019)
Metabolic Disease Stage	Metabolic syndrome, type 2 diabetes, cardiovascular diseases	Tissue-specific mitochondrial dysfunction, chronic inflammation, fibrosis, disrupted metabolic homeostasis	Arruda et al. (2014), Cavaliere et al. (2023)

3.4 Mitochondrial autophagy

Mitochondrial autophagy, a crucial process for maintaining mitochondrial quality and function, selectively targets and removes damaged mitochondria. Research has shown that impaired regulation of mitochondrial autophagy in the skeletal muscle of obese individuals leads to a decrease in both the quantity and functionality of mitochondria (Pileggi et al., 2021). Interestingly, while a short-term high-fat diet can trigger mitochondrial autophagy in skeletal muscle, it does not affect mitochondrial respiratory capacity. This phenomenon might be associated with lipid-induced oxidative stress, indicating that mitochondrial autophagy might serve a protective role during the early stages of obesity (Ehrlicher et al., 2021). Additionally, impaired mitochondrial autophagy in adipose tissue may exacerbate the progression of insulin resistance and obesity by influencing the release of adipokines and cytokines (Turchi et al., 2020). Enhanced lipid autophagy and mitochondrial autophagy in brown and beige adipocytes, especially in cold environments, promote adaptive thermogenesis, helping the body cope with cold stimuli (Sakers et al., 2022). Recent studies have also shown that triiodothyronine (T3) modulates mitochondrial homeostasis by inducing lipophagy and mitochondrial autophagy in the liver and skeletal muscle and stimulates thermogenesis (Yau et al., 2021; Hatziagelaki et al., 2022). This mechanism triggers the expression of mitochondrial uncoupling protein 1 (UCP1), enhances autophagy-dependent fatty acid oxidation, and regulates mitochondrial autophagy, functionality, and renewal in brown adipose tissue (BAT) and aging skeletal muscle (Wang et al., 2023). Furthermore, as shown in Table 1, mitochondrial dysfunction presents differently at various stages of obesity.

To address these challenges, antioxidants have been investigated for their potential to reduce oxidative stress and protect mitochondrial function. Among them is mitoquidone (MitoQ), a chemically modified form of coenzyme Q10 that selectively enters mitochondria and accumulates in their inner membrane, thereby safeguarding them from oxidative stress (Chen et al., 2022). Additionally, MitoQ has been shown to normalize metabolic profiles, reduce lipid peroxidation, and restore UCP-2 protein levels to normal values in obese rats (Xu et al., 2021). Research also indicate that MitoQ improves NF-κB activation and mitigates endoplasmic reticulum stress by regulating mitochondrial function (Cojocaru et al., 2023). Water-soluble Szeto-Schiller (SS) peptides, another group of mitochondria-targeted antioxidants, are small molecules that are quickly and selectively taken up into the inner mitochondrial membrane (IMM) across various cell types (Zong et al., 2024). Studies have demonstrated that SS-31 reduces mitochondrial ROS production, suppresses changes in mitochondrial membrane permeability, and safeguards cells from oxidative stress-induced death (Peng et al., 2021; Yang et al., 2021).

4 Gut microbiota and its role in obesity

4.1 Gut microbiota and its metabolites

The gut microbiota consists of many microorganisms, including archaea, protozoa, fungi, viruses, and bacteria, with bacterial species being the most abundant and extensively studied group (Zhang et al., 2022). The major intestinal bacterial phyla are *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria*, and *Verrucromicrobia*, with *Firmicutes* and *Bacteroidetes* being 90% of the total bacterial population. Notably, although certain bacteria are less abundant, this does not necessarily indicate a secondary functional role (Fassarella et al., 2021). Different microbial concentration gradients are observed throughout the gut, and understanding this distribution is crucial for investigating the impact of the gut microbiota on the host's health. The lower microbial density in the upper gastrointestinal tract helps prevent pathogen colonization. In contrast, the more abundant microbiota in the lower intestine facilitates the breakdown of complex carbohydrates and the production of SCFAs, which are metabolic byproducts essential for the host's energy metabolism and intestinal health (Wen et al., 2022).

The intestinal microbial community begins to form at birth and potentially even earlier in the womb. It is influenced by numerous factors, such as the delivery method, infant nutrition, lifestyle choices, genetic predisposition, medication use, and dietary habits (Valles-Colomer et al., 2023). Typically, the intestinal microbiota attains an adult-like configuration within the first 5 years of life, though it continues to evolve dynamically throughout an individual's lifetime (Roswall et al., 2021). The symbiotic relationships between humans and their gut microbiota are essential for maintaining health. The intestinal microbiota plays a vital role in various physiological processes, including preserving the integrity of the intestinal barrier, providing against pathogens, and modulating immune responses and diverse metabolic functions (Lee et al., 2022; Loh et al., 2024; Zhu et al., 2024).

Along with the importance of the gut microbiota, the byproducts of microbial metabolism also play crucial roles. The host absorbs and utilizes the gut microbiota metabolites SCFAs, providing additional energy and regulating host metabolic processes through various mechanisms (Xiong et al., 2022). Previous studies have extensively documented key bacteria involved in SCFA production's metagenomic characteristics and associated pathways (Tsukuda et al., 2021; Frolova et al., 2022).

Currently, *Akkermansia muciniphila*, *Bacteroides* spp., *Bacteroides vulgatus*, *Bifidobacterium* spp., *Lactobacillus* spp., and *Prevotella* spp. are the primary producers of acetate and propionate, while *Coprococcus* spp., *Eubacterium* spp., *Faecalibacterium prausnitzii, and Roseburia* spp. are the main butyrate-producing bacteria (Kim et al., 2020; Lordan et al., 2020). In addition to SCFAs, the gut microbiota can influence host metabolic processes through metabolites such as SBAs, tryptophan metabolites, and hydrogen sulfide (H_2S). By metabolizing primary bile acids and producing tryptophan metabolites via the tryptophan metabolic pathway, *Bacteroides* and *Clostridium* are converted to SBAs (Matthew F. et al., 2020; Xiaomin S. et al., 2022; Xiaomin T. et al., 2022). *Desulfovibrio* and *Bilophila* species produce H_2S through cysteine degradation, while *Fusobacterium nucleatum* generates H_2S during digestion (DJ et al., 2021; Matthew W. et al., 2023).

4.2 Gut microbiota and its metabolites in obesity

HFD consumption, lifestyle choices, antibiotics use, and psychological or physical stress are key factors that alter the composition of intestinal microbial and its metabolic products, potentially disrupting gut homeostasis, leading to dysbiosis (IJ et al., 2021; Francois et al., 2022). Extensive research using both animal models and human participants has demonstrated that imbalances in gut microbiota interfere with energy metabolism and lead to obesity (Lee et al., 2020; Wachsmuth et al., 2022; Takeuchi et al., 2023). Certain bacterial species have been identified as either harmful or beneficial in the progression of these conditions. Compared to individuals with normal weight, obese individuals exhibit reduced microbial diversity and richness in their intestines, along with structural changes in the microbiome and a disproportionate *Firmicutes*-to-*Bacteroidetes* ratio, typically characterized by an increase in *Firmicutes* and a decrease in *Bacteroidetes* (Manor et al., 2020a). Similar findings have been observed in animal studies, where the expression of *Lactobacillus* and *Bifidobacterium*, among others, was reduced in the gut of obese mice (Huo et al., 2020; Zhihao L. et al., 2022). Conversely, in cases of obesity induced by excessive fat consumption, elevated levels of bacterial lipopolysaccharide (LPS) have been reported (Ramos-Romero et al., 2020). This condition disrupts intestinal structure, increases intestinal permeability, and allows significant amounts of LPS to enter the bloodstream, triggering chronic inflammatory and accelerating the progression of obesity (Camilleri, 2023; Di Vincenzo et al., 2024).

Given the gut microbiota's central role in obesity and its associated metabolic dysregulation, targeted modulation of the intestinal ecosystem emerges as a promising therapeutic avenue. The microbiota's significant adaptability in its makeup and function makes it a promising target for treating and preventing various health conditions. Strategies such as probiotics, prebiotics, or symbiotics are commonly explored. In the field of functional foods and nutritional supplements, *Bifidobacterium* and *Lactobacillus* are among the most frequently used probiotics (Sharma et al., 2021). Notably, the South Korean Food and Drug Administration has approved *Lactobacillus gasseri* BNR17 as a functional component for reducing adipose tissue (Kim et al., 2018). New-generation probiotics, such as *F. prausnitzii* strains, are also gaining attention, as they are commonly found in the microbiota of healthy individuals. Studies have linked a decline in these strains to a higher likelihood of an increased risk of developing obesity (Kallassy et al., 2023; Yang M. et al., 2023). *Faecalibacterium prausnitzii* exhibits anti-inflammatory properties, as its supernatant suppresses the NF-κB pathway *in vitro* and *in vivo* (Auger et al., 2022). It also synthesizes butyrate and other SCFAs, contributing to its beneficial effects (NM et al., 2017). Prebiotics, defined as nutritional components that undergo selective fermentation, can potentially modify the intestinal microbiota's composition or functionality, leading to beneficial outcomes for the host's health (Miyamoto et al., 2023). Most evidence supporting their efficacy stems from studies on dietary constituents, primarily categorized into two chemical groups: inulin-type fructans and galacto-oligosaccharides (GOSs) (Piotr P. et al., 2022). Fibers rich in prebiotics stimulate enteroendocrine L-cell development and elevate the anorexigenic peptides PYY and GLP1 concentrations in both the intestinal lumen and bloodstream, consequently diminishing caloric consumption (Akhlaghi, 2024). Human-based research has demonstrated that consuming diets abundant in prebiotics correlates with decreased food consumption, lowered adipose tissue, and minimized weight increase, particularly among individuals with obesity (Huwiler et al., 2022; Jagielski et al., 2023). Synbiotics, which combine prebiotics and probiotics, offer a promising strategy for addressing imbalances in the intestinal microbiota (Saadati et al., 2024). Dietary symbiotics, incorporating carefully selected bacterial strains—such as *L. gasseri* variants known for their weight-reducing and anti-inflammatory properties, paired with galactomannan or inulin fibers may provide enhanced benefits for tackling obesity by promoting SCFA production and restructuring the microbiome (Li X. et al., 2023). Studies have shown a significant decrease in obesity among mice on high-fat diets when administered a symbiotic blend of D-allulose and the probiotic strains *Lactobacillus sakei* and *Leuconostoc kimchii* (Choi et al., 2018). Moreover, introducing symbiotics to mice early in life modulates their gut microbiota, protecting against diet-related obesity as they age (Kang et al., 2023).

The precise function of gut microbiota in the onset of metabolic disorders remains unclear. Recent research has emphasized the connections between microbiota diversity, composition, and mitochondrial function. Importantly, gut microbiota and their metabolic byproducts significantly regulate different factors, transcriptional coactivators, and enzymatic processes that affect mitochondrial functionality (Qi et al., 2022). In addition, abnormal mitochondrial function may cause excessive ROS release, dysregulation of glucolipid metabolism, and abnormal adipose tissue function, which affect gut microbiota homeostasis and result in bodily imbalance, triggering obesity (Zhu et al., 2023). The following sections will delve into the complex interplay between

gut microbiota and mitochondrial operations, exploring potential pathways through which these interactions could be leveraged to develop innovative approaches for obesity management.

5 Gut microbiota–mitochondrial crosstalk in obesity

5.1 Role of mitochondrial energy metabolism in the gut microbiota

Emerging research highlights an interactive relationship between mitochondrial energy metabolism and the gut microbiota. Efficient mitochondrial energy production relies on the integration of fatty acid oxidation, the tricarboxylic acid cycle (TCA cycle), and OXPHOS. OXPHOS utilizes NADH and FADH2, produced via fatty acid oxidation and the TCA cycle, to generate significant amounts of ATP (Guo et al., 2023). Key byproducts of this process are essential in regulating the gut environment and shaping the gut microbiota, thus influencing obesity development (Wan et al., 2020; Bhattacharjee et al., 2023). ROS are produced during mitochondrial OXPHOS. Obese individuals exhibit markedly altered proportions of *Bacteroides* and *Firmicutes* in their gut microbiota, which is closely associated with disrupted mitochondrial OXPHOS and ROS production (Jinhua et al., 2022). Interestingly, ROS serves a dual role in intestinal inflammation, functioning as important signaling molecules produced by mitochondria during OXPHOS. Moderate levels of ROS support intestinal homeostasis by regulating cell proliferation, differentiation, and immune responses. Studies have also demonstrated that ROS can activate the Nrf2 signaling pathway, promoting the expression of antioxidant genes and enhancing cellular antioxidant defenses (He et al., 2022).

However, in obese individuals, excessive ROS production leads to oxidative stress, causing damage to proteins, lipids, and DNA (Abdolmaleky and Zhou, 2024). This disrupts intestinal barrier integrity, triggers an inflammatory response, and alters the gut microenvironment, allowing the colonization of harmful bacteria, such as *Enterobacteriaceae*, while inhibiting the growth of beneficial bacteria like *Bifidobacteria* (Jeong and Kang, 2023). Organic acids, natural byproducts of the TCA cycle, inhibit the growth of *Escherichia coli* and *Salmonella* by lowering intestinal pH, while promoting the proliferation of *Lactobacillus* and *Bifidobacterium*. A HFD raises intestinal pH, disrupting gut microbiota equilibrium and affecting the gut's metabolic state (Guo et al., 2021; Ekkachai et al., 2023). Ketone bodies, including β-hydroxybutyrate (BHB) and acetoacetate, are produced by mitochondria during fatty acid oxidation. These ketones are not only crucial energy metabolites but also important signaling molecules. Metabolism and inflammatory responses in host cells are regulated by BHB through activation of the G protein-coupled receptor GPR109A and suppression of NF-κB and NLRP3 inflammasomes, leading to reduced intestinal inflammation and improved balance of the gut microbiota (Valentina et al., 2022). In an *in vitro* human colon microbiota model, BHB supplementation increased butyric acid production (Kengo et al., 2020). Furthermore, BHB influences cellular gene expression and metabolic pathways by inhibiting histone deacetylase (HDACs) and modifying SCFA production, which subsequently impacts the host's energy balance and insulin sensitivity. This process is essential for maintaining the diversity and functionality of the gut microbiota, particularly in metabolic disorders related to obesity (KB et al., 2021).

Moreover, mitochondrial dysfunction exacerbates obesity-related metabolic dysregulation by influencing the gut microbiota through immune and neural pathways. Regarding immune regulation, mitochondrial dysfunction causes the release of mitochondrial DNA into the cytoplasm, where it acts as a pathogen-associated molecular pattern. This mtDNA then interacts with Toll-like receptor 9 (TLR9), triggering a MyD88-dependent signaling cascade that stimulates the production of inflammatory factors, altering the intestinal microenvironment and impacting the composition of the intestinal microbiota (Ren et al., 2020). Additional research has shown that depletion of the mitochondrial membrane potential leads to intracellular potassium ion outflow, subsequently activating various inflammatory vesicles, such as NLRP3. Potassium ion efflux not only activates these inflammatory vesicles but also compromises the integrity of intestinal epithelial cells, impairing gut barrier function and increasing permeability, which further disrupts microbiota homeostasis (Diwakar et al., 2021; Wei et al., 2021; Matthew Y. et al., 2023). Moreover, the state of mitochondrial metabolism significantly affects dendritic cell function. Mitochondrial stress signals can modulate the antigen-presenting function and cytokine secretion of dendritic cells, thereby affecting the immune response in the gut (Adamik et al., 2022). In obesity, mitochondrial stress promotes increased secretion of proinflammatory cytokines by dendritic cells, resulting in an increase in Th17 cells, which are crucial in intestinal inflammation (van der Zande et al., 2023). In terms of neuromodulation, mitochondrial dysfunction disrupts the synthesis and degradation of serotonin and dopamine, key neurotransmitters that regulate intestinal function, affecting intestinal peristalsis and secretion. This in turn alters the composition of the microbiota (Xie et al., 2020). Imbalances in the autonomic nervous system, particularly an underactive or overactive sympathetic and parasympathetic response, also affect the gut microbiota in the obese state. The vagal nerve regulates gut peristalsis, secretion, and barrier function by releasing acetylcholine. In obese individuals, decreased vagal activity leads to increased colonization of *E. coli* and *Salmonella*, while inhibiting the growth of *Lactobacillus* and *Bifidobacterium* (Bénédicte et al., 2017). Additionally, the enteric nervous system (ENS), often referred to as the "second brain," directly controls intestinal behavior. Mitochondrial dysfunction in obese individuals impairs the energy-dependent functions of ENS neurons leading to changes in intestinal movement and enzymatic secretion. A decrease in digestive enzyme secretion can lead to incomplete food digestion, increased retention of undigested material in the intestine, and provide nutrients for harmful bacteria. Conversely, excessive secretion may dilute intestinal contents, affecting the growth of beneficial bacteria (Bénédicte N. et al., 2021). In obese individuals, ENS dysfunction also compromises epithelial cell function and reduces tight junction protein expression, enhancing intestinal permeability. This impaired barrier function enables the increased entry of pathogenic bacteria and toxins into the bloodstream, triggering a widespread inflammatory response and

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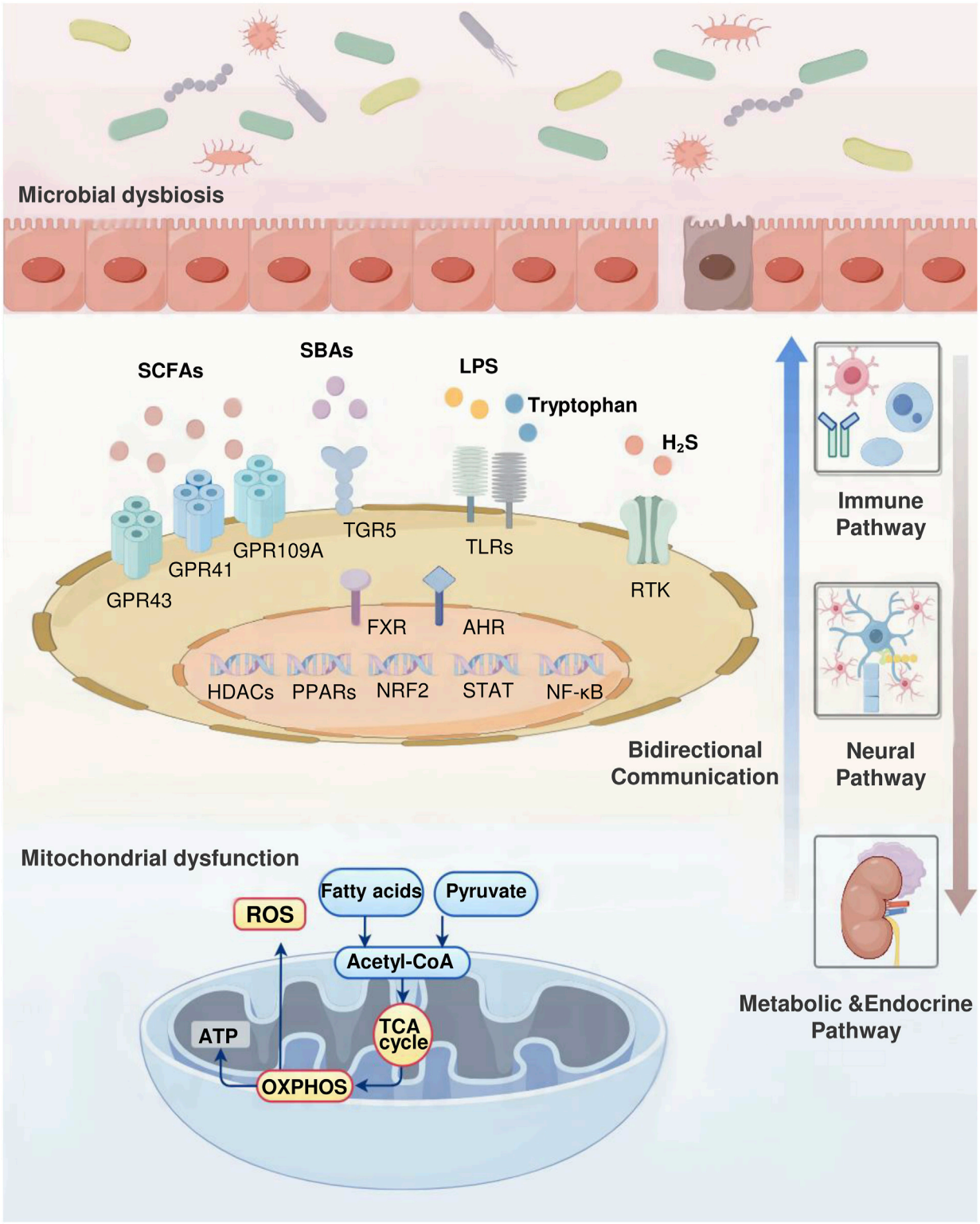

FIGURE 2
Gut microbiota–mitochondrial crosstalk in obesity. The complex interactions between the gut microbiota and mitochondrial function underscore their key role in regulating obesity. This bidirectional communication occurs through immune, neural, and metabolic-endocrine pathways. Gut microbial metabolites such as SCFAs, SBAs, tryptophan, H2S, and LPS maintain body homeostasis and directly influence the development of obesity by regulating mitochondrial activity. Conversely, mitochondrial energy metabolites such as ROS, organic acids, and ketone bodies alter the intestinal microenvironment, affecting microbial composition. This process involves multiple signaling molecules. Receptors including GPR43, GPR41, GPR109A, FXR, TGR5, TLRs, AHR, RTK. Key transcription factors include Nrf2, NF-κB, STAT, and PPARs. Enzymes including HDACs.

further destabilizing the gut microbiota equilibrium (Bénédicte et al., 2020).

In summary, mitochondria regulate the gut microbiota through their metabolic products while maintaining gut microbiota balance by preventing oxidative stress, immune dysregulation, and neuroregulatory imbalances. This underscores the impact of mitochondrial dysfunction on gut microbiota homeostasis and its close association with the development of obesity.

5.2 The role of gut microbiota in mitochondrial energy metabolism

Changes in gut microbiota composition can lead to alterations in environmental metabolites, shedding light on the mechanisms behind obesity development. Extensive studies using germ-free mice that were inoculated with gut microbiota from obese individuals have strongly confirmed the influence of gut microbiota on the host's energy metabolism, glucose tolerance, and insulin resistance (Diwakar et al., 2022). Additionally, metabolites produced by gut microbiota play a vital role in maintaining homeostasis in the body and affect the progression of obesity through their impact on mitochondrial function. The following section explores the key metabolites derived from gut microbiota that have recently been associated with the advancement of obesity (Figure 2).

5.2.1 SCFAs

Among the metabolites produced by gut microbiota, SCFAs primarily include acetic, propionic, and butyric acids. Acetic acid regulates adipocytes' mitochondrial function and fatty acid oxidation by activating the GPR43 receptor. This process increases cAMP production, which, in turn, activates protein kinase A (PKA), influencing fat accumulation and energy metabolism (Manor et al., 2020b). Acetic acid modulates glucose metabolism in obesity-related cell types, including adipocytes and macrophages, via the GPR43 receptor (Li et al., 2024). Propionic acid's activation of GPR41 and GPR43 receptors triggers the ERK and AMPK signaling cascades, stimulating mitochondrial biogenesis and lipolysis. This process enhances insulin sensitivity, mitigates oxidative stress, and reduces mitochondrial damage, thereby combating the metabolic disorders associated with obesity (Hiroki et al., 2019; Xiaomin W. et al., 2022). Studies have revealed that propionate metabolism involves acetyl coenzyme A synthase short-chain family member 3 (ACSS3) in the IMM. ACSS3 plays a crucial role in propionate metabolism, and ACSS3 deficiencies lead to serum propionic acid accumulation, causing reduced BAT, increased white adipose tissue (WAT), and insulin resistance (Zhihao J. et al., 2022). Research has also shown that propionic acid stimulates thermogenic responses and promotes the browning of adipose tissue through various mechanisms, including the upregulation of PGC-1α expression, a key regulator of mitochondrial biosynthesis (CC et al., 2021). Among the SCFAs, butyric acid improves mitochondrial function through GPR109A receptor activation, AMPK signaling pathway modulation, enhanced mitochondrial autophagy, and attenuation of inflammatory responses (Xaomin et al., 2020). Additionally, research has shown that butyrate enhances UCP1-induced BAT thermogenesis and WAT browning by upregulating PGC-1α expression (YM et al., 2020).

5.2.2 SBAs

In addition to SCFAs, other microbial-derived metabolites are associated with metabolic processes and obesity. SBAs, including deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA), influence mitochondrial energy metabolism primarily through the actions of the farnesoid X receptor (FXR) and the G protein-coupled bile acid receptor 5 (TGR5). FXR reduces hepatic lipid accumulation by suppressing SREBP-1c, regulates hepatic gluconeogenesis by enhancing FGF15/19 and PPARα expression, lowers blood glucose levels, and stimulates mitochondrial biogenesis and fatty acid oxidation. These actions collectively reduce lipid accumulation and enhance insulin sensitivity (JYL et al., 2020; Lulu et al., 2021; Matthew et al., 2021). TGR5, through its regulation of the AMPK signaling pathway, promotes BAT thermogenesis, increases energy expenditure, and enhances mitochondrial OXPHOS (SR et al., 2020).

5.2.3 Tryptophan and H_2S

Tryptophan metabolites primarily include indole propionic acid (IPA), indole-3-acetic acid (IAA), kynurenine, and quinolinic acid (QA). IPA promotes fat metabolism and reduces fat accumulation by stimulating the PPARγ-AMPK pathway, which enhances mitochondrial OXPHOS, promotes lipid metabolism, and decreases fat accumulation (Piotr K. et al., 2022). IAA regulates intestinal immune responses, reduces inflammation-related metabolic disorders, and influences mitochondrial biogenesis and autophagy by activating the TLR4-JNK pathway (Ruiz et al., 2020; Chowdhury et al., 2021). The kynurenine pathway produces metabolites that activate the aromatic hydrocarbon receptor, regulate gene expression, influence immune and inflammatory responses, and promote mitochondrial biogenesis (Ren et al., 2022). QA modulates cellular proliferation and metabolic processes by stimulating the mammalian target of rapamycin protein (mTOR) signaling cascade, improving mitochondrial performance, and regulating lipid biosynthesis and energy utilization (Bénédicte H. et al., 2021).

Additionally, hydrogen sulfide (H_2S), a metabolite of the intestinal microbiota, affects mitochondrial function. H_2S is produced by intestinal anaerobic sulfate-reducing bacteria, and high-fat diets lead to an increase in these bacteria in the gut of obese rats, resulting in elevated H_2S production (Francois et al., 2021). H_2S comprehensively modulates cellular metabolism, mitochondrial performance, and inflammatory pathways by stimulating the PI3K/Akt and AMPK signaling pathways, while concurrently suppressing the NF-κB pathway (Diwakar et al., 2020; Bénédicte W. et al., 2021; Alex et al., 2022).

5.2.4 Lipopolysaccharides

Under normal physiological conditions, LPS generated from intestinal microbiota metabolism is nonpathogenic. However, prolonged stimulation induces macrophages to secrete inflammatory mediators, triggering ROS generation in the jejunum and ileum. This cascade results in mitochondrial enlargement, reduced membrane potential, structural abnormalities, and impaired functionality of intestinal epithelial

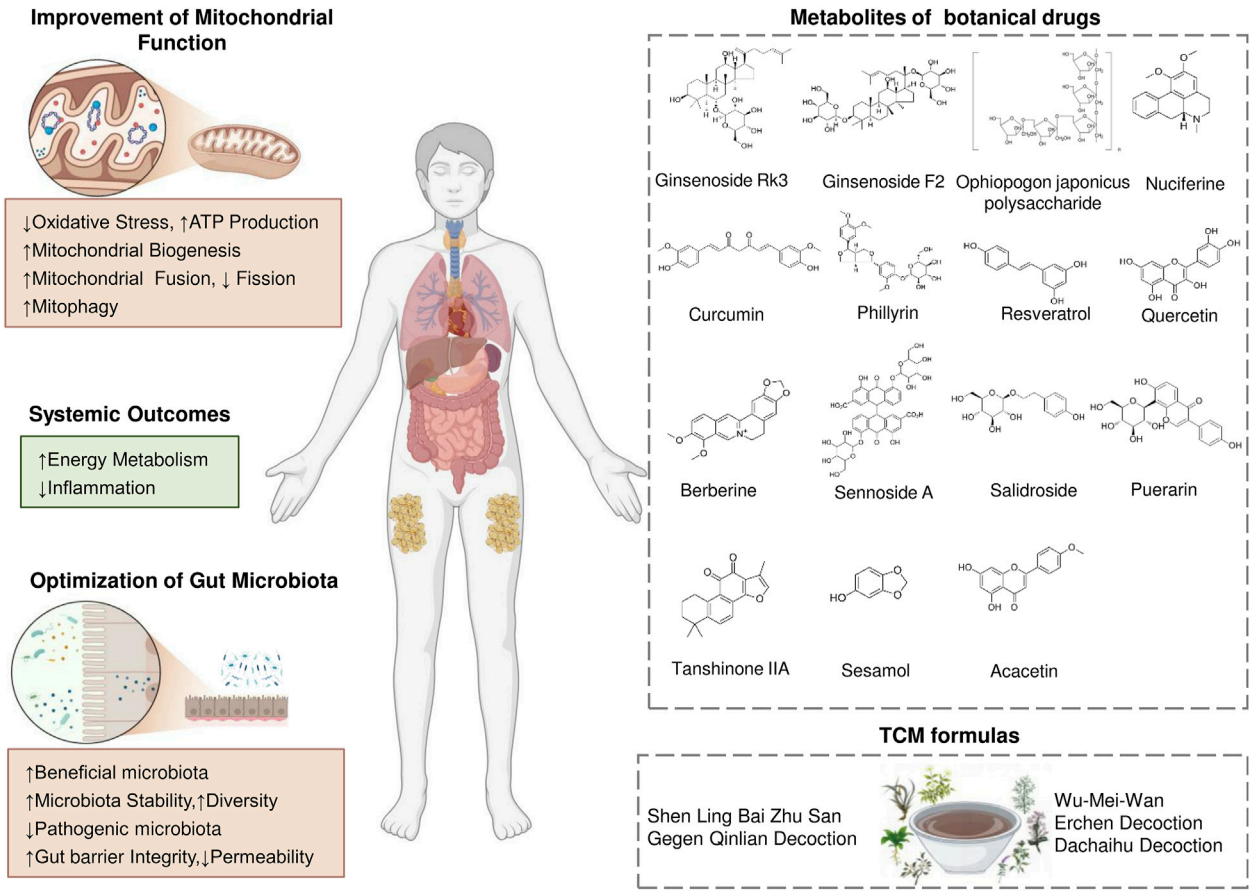

FIGURE 3
Therapeutic Strategies Targeting the Gut Microbiota–Mitochondrial Crosstalk for Obesity. Traditional Chinese medicine works synergistically to enhance mitochondria-gut crosstalk. This mutual reinforcement between improved mitochondrial function and a healthier gut microbiota leads to better energy metabolism, enhanced insulin sensitivity, and effective inflammation control, offering a comprehensive strategy for obesity management.

cells (Matthew and PY, 2020). Additional research has demonstrated that LPS increases intestinal permeability, allowing more LPS molecules to enter the bloodstream and activate a systemic immune response. In the bloodstream, LPS forms complexes with LPS-binding proteins and CD14, engaging Toll-like receptor 4 (TLR4). This interaction triggers various inflammatory signaling pathways, leading to proinflammatory cytokines like IL-6 and TNF-α secretion (He et al., 2019). These mediators subsequently compromise mitochondrial performance and decrease metabolic energy efficiency (Matthew X. et al., 2020; Zhang X. et al., 2021). Additionally, LPS promotes adipose tissue proliferation and inflammation by activating the JAK/STAT pathway (CC et al., 2019).

6 Therapeutic potential of targeting the gut microbiota and mitochondria from traditional Chinese medicine

Accumulating evidence suggests that therapies targeting the interplay between mitochondria and the microbiota may offer a new approach to treating these diseases or reducing their complications, providing a broader range of effects than targeting mitochondria or the microbiota alone. Recent studies have uncovered multiple molecular mechanisms mediating the gut microbiota and mitochondria interaction, potentially paving the way for the development of precisely targeted therapeutic interventions. Conversely, alterations in mitochondrial activity can influence the microbial community, thereby affecting disease progression and highlighting the gut microbiota and mitochondria bidirectional communication. In this context, TCM formulas and metabolites of botanical drugs which are guided by TCM theory, offer unique advantages (Law et al., 2022; Zhang Q. et al., 2023). These therapeutic providing integrative strategies that align with the complexity of this bidirectional interaction (Figure 3).

Contemporary research has increasingly shown that metabolites of botanical drugs can contribute to obesity prevention and management by influencing mitochondrial energy processes and the gut microbiota (Cheng et al., 2023; Luo et al., 2023; Zhi et al., 2024). The following metabolites of botanical drugs that have demonstrated significant effects in relevant studies are shown in Table 2.

While the therapeutic potential of metabolites of botanical drugs offers a holistic approach to obesity treatment, contemporary research has also highlighted TCM formulas, through their synergistic interactions and multi-target effects (Zhang C. H.

TABLE 2 Metabolites of botanical drugs for the treatment of obesity.

Sources information	Plant metabolites	Types of study	Experiment object	Dosage	Intervention mode	Modeling methods	Effects on Gut Microbiota–Mitochondrial Crosstalk	Mechanisms	Metabolic Outcomes	References
Panax ginseng C.A. Mey. [Araliaceae; Ginseng Radix et Rhizoma]	Ginsenoside Rk3	*In vivo*	Male C57BL/6JFandd mice	20,60 mg/kg	Gavage 2 weeks	Antibiotic-induced gut microbiota dysbiosis model (3 g/kg lincomycin administration)	↑ *Bacteroides, Alloprevotella, and Blautia*,↓ *Firmicutes*; ↑Mitochondrial membrane integrity	↑ AMPK/Akt; ↓ TNF-α, IL-1β, IL-6, IL-17; ↓NLRP3	↓Inflammatory response,↑SCFAs production,↓Lipid accumulation	Bai et al. (2021)
Panax ginseng C.A. Mey. [Araliaceae; Ginseng Radix et Rhizoma]	Ginsenoside F2	*In vivo* and *In vitro*	*In vivo*:Male C57BL/6J mice *In vitro*:3T3-L1 Cells	*In vivo*: 50,100 mg/kg; *In vitro*:12.5, 25, 50, 100 μM	Gavage 4 weeks	*In vivo*:High-fat diet *In vitro*:3T3-L1 preadipocyte differentiation model	↑Mitochondrial biogenesis,↑ Mitochondrial function	↑ AMPK/ACC phosphorylation; ↓ PPARγ and C/EBPα	↓Adipocyte differentiation,↑Energy metabolism,↓ oxidative stress	Zhou et al. (2021)
Ophiopogon japonicus (Thunb.) Ker Gawl. [Asparagaceae; Ophiopogonis radix]	*Ophiopogon japonicus* polysaccharide	*In vivo*	Male C57BL/6J mice	300 mg/kg	Gavage, 12 weeks	High-fat diet	↑ *Lactobacillus*;↑Activity of mitochondrial respiratory chain complexes	↑ AMPK and Nrf2; ↓NF-κB	↑Oxygen consumption,↑Energy expenditure,↓Lipid metabolism disorder	Shi et al. (2015)
Curcuma longa L. [Zingiberaceae; Curcumae longa rhizoma]	Curcumin	*In vivo* and *In vitro*	*In vivo*:Male C57BL/6J mice *In vitro*:3T3-L1 Cells	*In vivo*:50 mg/kg; *In vitro*: 10–35 μM	Gavage 8 weeks	*In vivo*:High-fat diet *In vitro*:3T3-L1 preadipocyte differentiation model	↑ Mitochondrial oxygen consumption,↑ Mitochondrial respiration and ATP production	PPARγ Pathway Activation; ↑ Browning of WAT; ↑ UCP1, PGC-1α, PRDM16	↓ Body weight, ↓ Fat accumulation, ↓ Inflammation, ↑ Energy expenditure, ↑ Insulin sensitivity	Zhao et al. (2021a)
		In vitro	Male C57BL/6J mice	0.2% (w/w) in diet	Oral supplementation 10 weeks	High-fat diet	↓ *Firmicutes/Bacteroidetes* ratio, ↓ *Desulfovibrio*,↑ *Bacteroides*, *Parabacteroides*, *Alistipes* and *Alloprevotella*	↑SCFA; ↓LPS	↓ Body weight, ↓ Hepatic steatosis, ↓ Inflammation, ↑ Insulin sensitivity	Li et al. (2021b)
Forsythia suspensa (Thunb.) Vahl [Oleaceae; Forsythiae fructus]	Phillyrin	*In vivo*	Male C57BL/6J mice	25,50 mg/kg	Gavage 8 weeks	High-fat diet	↓Mitochondrial membrane permeability; ↑Fatty acid oxidation	↑ AMPK	↓Lipid accumulation	Fang et al. (2022)
Nelumbo nucifera Gaertn. [Nelumbonaceae; Nelumbinis folium]	Nuciferine	*In vivo*	Male Sprague-Dawley rats	10 mg/kg	Gavage 8 weeks	High-fat diet	↓ *Firmicutes/Bacteroidetes* ratio, ↓ *Desulfovibrio*	↓SREBP-1, PPARγ, FAS; ↑PPARα	↓Obesity,Fat Accumulation; ↑ Insulin sensitivity	Wang et al. (2020b)
Reynoutria japonica Houtt. [Polygonaceae; Polygoni cuspidati rhizoma et radix]	Resveratrol	*In vivo*	Male C57BL/6J mice	300 mg/kg	Gavage 16 weeks	High-fat diet	↑ *Blautia*, ↓ *Desulfovibrio*, ↓ *Lachnospiraceae_NK4A136_group*;↓ Oxidative stress	Influenced metabolic pathways	↓ Body weight, ↓ Fat accumulation, ↓ Inflammation, ↑ Insulin sensitivity	Wang et al. (2020a)
Scutellaria baicalensis Georgi [Lamiaceae; Scutellariae radix]	Quercetin	*In vivo*	Spotted seabass (Lateolabrax maculatus)	0.5 g/kg、1.0 g/kg	Oral supplementation 8 weeks	High-fat diet	↑ Mitochondrial biogenesis, ↑ Mitophagy, ↓ Endoplasmic reticulum stress	↑ PGC-1α,PINK1; ↓ ATF6,IRE1	↓ Liver triglycerides, ↓ Fat accumulation,↑ Antioxidant capacity	Dong et al. (2021)
		In vivo	Male C57BL/6J mice	50 mg/kg	Gavage 20 weeks	High-fat diet	↑ *Akkermansia, Coprococcus_1, Lactococcus* and *Allobaculum*,↓ *Adlercreutzia*	↓ TLR4-MyD88-NF-κB signaling; ↓TNF-α, IL-6, IL-1β	↓ Body weight, ↓ Fat accumulation, ↓ Inflammation, ↓ Insulin resistance	Su et al. (2022)

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TABLE 2 (*Continued*) Metabolites of botanical drugs for the treatment of obesity.

Sources information	Plant metabolites	Types of study	Experiment object	Dosage	Intervention mode	Modeling methods	Effects on Gut Microbiota–Mitochondrial Crosstalk	Mechanisms	Metabolic Outcomes	References
Coptis chinensis Franch. [Ranunculaceae; Coptidis rhizoma]	Berberine	*In vivo*	Male C57BL/6J mice	100 μg/kg	Gavage 15 weeks	High-fat diet	↑ *Akkermansia muciniphila*, ↑ SCFA-producing bacteria (*Butyricimonas*, *Eubacterium*, *Clostridium*),↓ *Firmicutes*/*Bacteroidetes* ratio	↓ TLR4-MyD88-NF-κB signaling; ↓ TNF-α, IL-6, iNOS	↓ Insulin resistance,↓ LPS levels,↓ Body weight	Li et al. (2022)
		In vivo	Sprague-Dawley rats	100 mg/kg	Oral supplementation 4 weeks	High-fat diet	↑Mitochondrial biogenesis,↓ Oxidative stress,↑ATP,↑ Mitochondrial calcium retention	↑SirT3	↑ Insulin sensitivity,↓ Liver triglycerides, ↓ Hepatic steatosis	Teodoro et al. (2013)
Rheum palmatum L. [Polygonaceae; Rhei radix et rhizoma]	Sennoside A	*In vivo*	Male C57BL/6J mice	30 mg/kg	Oral supplementation 8 weeks	High-fat diet	↑ *Akkermansia muciniphila*,↓ *Firmicutes*/*Bacteroidetes* ratio; ↑ Mitochondrial function	↑GLP-1; ↑SCFA production	↑ Insulin sensitivity,↑ Energy metabolism	Le et al. (2019)
Rhodiola crenulata (Hook. f. et Thoms.)H. Ohba [Crassulaceae; Rhodiae crenulatae radix et rhizoma]	Salidroside	*In vivo*	Male C57BL/6J mice	15 mg/kg	Gavage 8 weeks	High-fat diet	↓ *Lachnospiraceae, Alistipes finegoldii, Bacteroides sartorii*;↓ Oxidative stress	↓SREBP-1c, FAS, ACC-1	↓ Insulin resistance,↓Lipid accumulation	Liu et al. (2023)
Pueraria montana var. lobata (Willd.) Maesen and S.M.Almeida ex Sanjappa and Predeep [Fabaceae; Puerariae lobatae radix]	Puerarin	*In vivo*	Male C57BL/6J mice	100 mg/kg	Gavage 4 weeks	High-fat diet	↓*Proteobacteria*, *Bacteroidetes*,↑*Akkermansia muciniphila*, *Clostridium celatum*;↑Mitochondrial function and mitophagy	↓ CYP7A1; ↑FXR, BSEP	↓Metabolic disorders	Yang et al. (2024)
Salvia miltiorrhiza Bunge [Lamiaceae; Salviae miltiorrhizae radix et rhizoma]	Tanshinone IIA	*In vivo*	Male C57BL/6J mice	15 g/kg	Gavage 8 weeks	High-fat diet	↓ *Firmicutes*, ↑ *Bacteroidota*, ↑ *Verrucomicrobiota*	Tanshinones activate TFEB nuclear translocation	↑Energy expenditure,↑Insulin sensitivity	Zheng et al. (2024)
		In vivo and *In vitro*	*In vivo*:Male C57BL/KsJ-Lepr$^{-/-}$ (db/db) mice, Ucp1$^{-/-}$ mice *In vitro*:3T3-L1 Cells	*In vivo*:30 mg/kg; *In vitro*:10, 30, 50 nM	Gavage 8 weeks	High-fat diet	↑ Mitochondrial content in adipocytes,↑ Mitochondrial activity; ↑ UCP1, PGC-1α	AMPK-PGC-1α Pathway Activation		Ma et al. (2022)
Sesamum indicum L. [Pedaliaceae; Sesami semen nigrum]	Sesamol	*In vivo and In vitro*	*In vivo*:Male C57BL/6J mice *In vitro*:3T3-L1 Cells	*In vivo*: 100 mg/kg *In vitro*:12.5, 25, 50 μM	Gavage 8 weeks	*In vivo*:High-fat diet;*In vitro*:3T3-L1 preadipocyte differentiation model	↑ Mitochondrial biogenesis,↑ Mitochondrial number in adipocytes	β3-AR/PKA Pathway Activation; ↑ UCP1, PGC-1α, NRF1, TFAM; ↓Mitophagy	↓ Body fat; ↓ Serum triglycerides (TG), total cholesterol (TC); ↑ Energy expenditure through increased thermogenesis; ↓Lipid accumulation in adipocytes	Lin et al. (2021)
		In vivo	Male C57BL/6J mice	0.2% (w/w) in diet	Gavage 2 weeks	High-fat diet	↑ *Bifidobacterium*, *Akkermansia*, ↓ *Dorea*, *Sutterella*	↑Antioxidant enzyme activities; ↓ MDA; ↑ GSH	↓ Liver lipid accumulation; ↓ Oxidative stress in liver and colon	Wang et al. (2022)
Scutellaria baicalensis Georgi [Lamiaceae; Scutellariae radix]	Acacetin	*In vivo* and *In vitro*	*In vivo*:Male C57BL/6J mice *In vitro*:3T3-L1 Cells	*In vivo*:20 mg/kg *In vitro*:20 μmol/L and 40 μmol/L	*In vivo*: Intraperitoneal injection	*In vivo*:High-fat diet *In vitro*:3T3-L1 preadipocyte differentiation model	↑ Mitochondrial content,↑ Mitochondrial respiratory function; ↓ Lipid accumulation in adipocytes	↑ UCP1, PRDM16, PGC1-α; ↑ cAMP; Activation of AC-cAMP-PKA pathway	↓ Body fat and weight; ↓ Lipid accumulation; Improved glucose and lipid metabolism	Zhang et al. (2023b)

10.3389/fphar.2025.1574887

TABLE 3 TCM formulas for the treatment of obesity.

TCM formulas	Composition of the formula	Extraction	Types of study	Experiment object	Dosage	Intervention mode	Modeling methods	Effects on gut Microbiota–Mitochondrial crosstalk	Mechanisms	Metabolic outcomes	References
Shen Ling Bai Zhu San	Panax ginseng C.A. Mey. [Araliaceae; Ginseng Radix et Rhizoma], Atractylodes macrocephala Koidz. [Asteraceae; Atractylodis macrocephalae rhizoma],Poria cocos (Schw.) Wolf [Polyporaceae; Poria],Dioscorea oppositifolia L. [Dioscoreaceae; Dioscoreae rhizoma], *Nelumbo nucifera* Gaertn. [Nelumbonaceae; Nelumbinis semen],Lablab purpureus subsp. purpureus [Fabaceae; Lablabi semen album], Coix lacryma-jobi var. ma-yuen (Rom.Caill.) Stapf [Poaceae; Coicis semen], Wurfbainia villosa (Lour.) Škorničk. and A.D.Poulsen [Zingiberaceae; Amomi fructus], Platycodon grandiflorus (Jacq.) A.DC. [Campanulaceae; Platycodonis radix], Glycyrrhiza uralensis Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma]	Mix in a ratio of 5:5:5:5:3:4:3:2:2:3 g in sequence	*In vivo*	Male C57BL/6J mice	21.8 g/kg	Gavage 6 weeks	High-fat diet	↑ *Bifidobacterium*, *Parvibacter*, ↓ *Erysipelatoclostridium*, *Lachnoclostridium*	↓TPH1, 5-HT, HTR2A; ↓IL-6, IL-1β, TNF-α, MCP-1, IL-18	↓ Body weight, ↓ Hepatic steatosis, ↓ Inflammation, ↑ Insulin sensitivity	Chen et al. (2024)
Erchen decoction	Pinellia ternata (Thunb.) Makino [Araceae; Pinelliae Rhizoma],Citrus reticulata Blanco [Rutaceae; Citrus reticulatae pericarpium],Poria cocos (Schw.) Wolf [Polyporaceae; Poria],Glycyrrhiza uralensis Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma]	Mixed in a ratio of 15:15:9:4.5 g in sequence. They were decocted twice, and the two decoctions were combined and concentrated to 50 mL	*In vivo*	Male C57BL/7J mice	8.7 g/kg	Gavage 4 weeks	High-fat diet	↑ATP, ↑ Mitochondrial respiration,↑ Mitochondrial membrane potential, ↓ Hepatic lipid accumulation	↓mTORC1, S6K, SREBP1,↑CAV1; ↓TG,TC; ↓IL-6, IL-1β, TNF-α, MCP-1, IL-18	↓ Fasting blood glucose, ↓ Insulin levels, ↓ HOMA-IR	Ding et al. (2024)
		Mixed in a ratio of 15:15:9:4.5 g in sequence. They were soaked in distilled water (1:8, w/v) for 2 h, then boiled at high heat and simmered for 30 min. The extraction was repeated twice, and the filtrates were combined and concentrated to final crude drug concentrations	*In vivo*	Male Zucker Diabetic Fatty rats	2.28, 4.57, 9.14 g/kg	Gavage 4 weeks	High-fat diet	↓ *Prevotella*, *Blautia*, *Ruminococcus*, *Holdemania*, ↑ *Akkermansia*	↑p-IRS1/IRS1 and p-AKT/AKT,↓ p-PKA/PKA and p-HSL/HSL	↓ Body weight, ↓ Fat accumulation, ↓ Inflammation, ↑ Insulin sensitivity, ↓ Circulating free fatty acids	Zhao et al. (2021b)
Dachaihu Decoction	Bupleurum chinense DC. [Apiaceae; Bupleuri Radix],Scutellaria baicalensis Georgi [Lamiaceae; Scutellariae radix],Citrus × aurantium L. [Rutaceae; Aurantii Fructus Immaturus],Paeonia lactiflora Pall.	Mixed in a ratio of 15:9:9:9:9:6:15:12 g in sequence	*In vivo*	Male Sprague-Dawley rats	4.25, 8.5, 17 g/kg	Gavage 8 weeks	High-fat diet	↑ Mitochondrial membrane potential, ↓ Mitochondrial swelling and cristae damage,↑ ATP	CREB/PGC-1α Pathway Activation	↓ Hepatic triglycerides, ↓ Total cholesterol,↓ Fasting blood glucose, ↓ Insulin levels	Li et al. (2021a)

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10.3389/fphar.2025.1574887

TABLE 3 (*Continued*) TCM formulas for the treatment of obesity.

TCM formulas	Composition of the formula	Extraction	Types of study	Experiment object	Dosage	Intervention mode	Modeling methods	Effects on gut Microbiota–Mitochondrial crosstalk	Mechanisms	Metabolic outcomes	References
	[Paeoniaceae; Paeoniae Radix Alba],Pinellia ternata (Thunb.) Makino [Araceae; Pinelliae Rhizoma],Rheum palmatum L. [Polygonaceae; Rhei Radix et Rhizoma],Zingiber officinale Roscoe [Zingiberaceae; Zingiberis Rhizoma Recens],Ziziphus jujuba Mill. [Rhamnaceae; Jujubae Fructus]										
Gegen Qinlian Decoction	Pueraria montana var. lobata (Willd.) Maesen and S.M.Almeida ex Sanjappa and Predeep [Fabaceae; Puerariae lobatae radix],Scutellaria baicalensis Georgi [Lamiaceae; Scutellariae radix],Glycyrrhiza uralensis Fisch. ex DC. [Fabaceae; Glycyrrhizae radix et rhizoma],Coptis chinensis Franch. [Ranunculaceae; Coptidis rhizoma]	Mixed in a ratio of 15:9:6:9 g in sequence. They were soaked (1:4, w/v) for 30 min, then boiled for 1 h, followed by a second extraction (1:3, w/v) for 40 min. The filtrates were combined, and concentrated	*In vivo*	Male C57BL/6J mice	15, 45 g/kg	Gavage 8 weeks	High-fat diet	↑ Oxygen consumption, ↑ Mitochondrial UCP1	↑ UCP1 in adipose tissue, ↓ Lipogenic genes	↓ Body weight, ↓ Adipose tissue mass, ↓ Serum triglycerides and cholesterol, ↓ Hepatic lipid accumulation, ↑ Insulin sensitivity	Wang and Hu (2021)
			In vivo	Male C57BL/6J mice	12.48, 24.96 g/kg	Gavage 4 weeks	High-fat diet	↓ *Firmicutes/Bacteroidetes* ratio, ↑ *Bacteroidetes*, ↓ *Firmicutes*, ↑ *Verrucomicrobia*	↓ Hepatic triglyceride (TG) and total cholesterol (TC) levels; ↓ Pro-inflammatory cytokines (TNF-α, IL-6, IL-1β)	↓ Body weight, ↓ Hepatic triglycerides and cholesterol, ↓ Fasting blood glucose, ↓ Insulin resistance	Zhang and Zhong (2020)
Wu-Mei-Wan	Prunus mume (Siebold) Siebold and Zucc. [Rosaceae; Mume Fructus],Asarum heterotropoides F.Schmidt [Aristolochiaceae; Asari Radix et Rhizoma],Zingiber officinale Roscoe [Zingiberaceae; Zingiberis Rhizoma Recens],Aconitum carmichaelii Debeaux [Ranunculaceae; Aconiti Lateralis Radix Praeparata],Zanthoxylum bungeanum Maxim. [Rutaceae; Zanthoxyli Pericarpium],Neolitsea cassia (L.) Kosterm. [Lauraceae; Cinnamomi Ramulus],Coptis chinensis Franch. [Ranunculaceae; Coptidis rhizoma],Phellodendron chinense C.K.Schneid. [Rutaceae; Phellodendri Chinensis Cortex],Panax ginseng C.A. Mey. [Araliaceae; Ginseng Radix et Rhizoma],Angelica sinensis (Oliv.) Diels [Apiaceae; Angelicae Sinensis Radix]	Mixed in a ratio of 19.2: 7.2:12:7.2:4.8: 7.2:19.2:7.2:7.2:4.8 g in sequence. They were soaked in 500 mL water for 1 h, then boiled for 2 h, with then concentrated	*In vivo*	Male C57BL/6J mice	0.48, 0.96, 1.92 g/mL	Gavage 4 weeks	High-fat diet	↑ Mitochondrial DNA content in BAT, ↑ Thermogenesis-related genes (UCP1, PGC-1α, COX III, CPT1β, CIDEA, ATPsyn, FATP1, MCAD)	↓ TLR3/IL-6/JAK1/STAT3 pathway,↑ BMP7/Smad1/5/9 pathway	↓ Body weight, ↓ White adipose tissue mass, ↑ Brown adipose tissue activity, ↑ Energy expenditure, ↓ Serum triglycerides and total cholestero	Wu et al. (2020)
		Mixed in a ratio of 24: 9:15:9:6:9:24:9:9: 6 g in sequence	*In vivo*	Male C57BL/6J mice	4800 mg/kg	Gavage 4 weeks	High-fat diet	↑ *Bacteroidetes*, *Parabacteroides goldsteinii*, *Akkermansia muciniphila*, ↓ *Firmicutes*, *Blautia, Lactobacillus*	↓ Serum triglyceride and total cholesterol,↓ Pro-inflammatory cytokines (TNF-α, IL-6, IL-1β),↑ Short-chain fatty acids	↓ Body weight, ↓ White adipose tissue mass, ↓ Inflammation, ↓ Serum triglycerides and total cholesterol	Nie et al. (2021a)

10.3389/fphar.2025.1574887

et al., 2021). Table 3 summarizes the potential mechanisms of the TCM formulas in detail. Shenling Baizhu San (SLBZS) has the capacity to modulate the proportion of *Bacteroidetes* to *Firmicutes*, enhance the presence of beneficial *Bifidobacterium*, decrease LPS expression, mitigate systemic chronic inflammation, and enhance metabolic efficiency (Yang et al., 2014; Huang et al., 2022). Erchen decoction (ECD) demonstrates notable efficacy in lowering body mass and blood triglyceride concentrations; enhancing gut microbial diversity; and boosting the proportional presence of *Lactobacillus*, *Bifidobacterium*, and *Butyricicoccus*; while diminishing the abundances of *Bacteroides*, *Parabacteroides*, and *Sediminibacterium* (Matthew et al., 2021; Lulu et al., 2023). Subsequent research indicated that ECD facilitated the restoration of compromised membrane potential, and rectified lipid metabolic abnormalities in mice with obesity (Sergio et al., 2018). Dachaihu decoction (DCHD) can notably improve the reduction in the mitochondrial membrane potential and swelling, activate key factors of mitochondrial synthesis, increase mitochondrial function, and balance the abnormalities of energy metabolism in obese rats (Li L. et al., 2021). Furthermore, DCHD enhanced the intestinal microbiota by restoring *Lactobacillus*, *Akkermansia*, and *Bifidobacterium* levels and elevating the proportion of *Bacteroidetes* to *Firmicutes*, thus contributing to additional weight reduction (Hussain et al., 2016). Gegen Qinlian decoction (GQD) can decrease the production of LPS, and improve abnormalities in lipid metabolism (Xiong, 2020). Moreover, GQD is capable of suppressing IL-6 expression, alleviating obesity-related inflammation, and modulating energy metabolism through stimulating WAT browning and enhancing caloric expenditure (Zhang X. et al., 2021). Wu-Mei-Wan (WMW) can reduce the body weight of obese mice and decrease the ratio of *Firmicutes* to *Bacteroidetes*, thereby adjusting the gut microbiota structure (Nie et al., 2021b). Additionally, WMW treatment resulted in a reduction of white adipocytes, a diminution in lipid droplet quantity, an upregulation of UCP1 expression, an augmentation of mitochondrial count in BAT, and an elevation in energy consumption (Wu et al., 2020).

TCM formulas and metabolites of botanical drugs which are guided by TCM theory targets the bidirectional communication between the gut microbiota and mitochondria, offering a multilevel intervention strategy for obesity treatment. However, further research is needed to elucidate these therapies' long-term effects and potential side effects across different individuals and clinical settings.

7 Conclusion and prospects

The intricate crosstalk between the gut microbiota and mitochondria is crucial in obesity and related metabolic disorders. The gut microbiota and its metabolites substantially affect host adipogenesis, modulating mitochondrial energy production and metabolism with reciprocal impacts. This bidirectional interaction highlights the gut microbiota's capacity to influence the host's metabolic wellbeing, while also indicating that mitochondria, essential cellular structures responsive to internal and external stimuli, reciprocally affect the gut microbiota by modulating mucosal immunity and intestinal barrier. Consequently, this reciprocal relationship between mitochondria and the gut microbiota holds promise for addressing obesity. Given the evolutionary origin of mitochondria as primordial bacterial symbionts, the intricate connection between these organelles and bacteria is understandable. Emerging evidence suggests that TCM formulas and metabolites of botanical drugs could provide novel insights into restoring this gut microbiota–mitochondria axis. This review consolidates findings supporting a connection between gut microbiota imbalances in obesity and mitochondria, offering potential pathways for investigating innovative treatment strategies for obesity.

However, TCM emphasizes individualized treatment, with personalized prescriptions based on the patient's pathological state of obesity and its comorbidities according to the different stages of development. Most current studies rely on simple obesity or simple metabolic syndrome models, which fail to effectively simulate the complex pathology of obese patients, especially when multiple metabolic complications coexist, and the interactions between the gut microbiota and mitochondrial function may show more complex dynamics. In addition, although studies have proposed that the interaction between gut microbiota and mitochondria plays a key role in the combined effects of obesity onset and progression. Limited comparability of data obtained from different experimental approaches further constrains the integrative interpretation of these findings. Therefore, although formulas and metabolites of botanical drugs have shown potential to alleviate obesity by modulating mitochondria and gut microbiota, their specific mechanisms through gut microbiota-mitochondria interactions still need to be further validated.

Future research should prioritize the development of multifactorial obesity models that more accurately reflect human pathological characteristics, particularly in cases where obesity is accompanied by metabolic comorbidities such as type 2 diabetes, metabolic dysfunction-associated fatty liver disease, and cardiovascular diseases. These models will enable a more precise simulation of clinical conditions, enhancing the translational relevance of research findings and facilitating the optimization of TCM formulations and metabolites of botanical drugs for different pathological states. Furthermore, integrating organoid technology and tissue engineering to establish multi-organ co-culture systems will allow for a more physiologically relevant simulation of systemic interactions, providing a robust platform for investigating the gut microbiota–mitochondria axis and its potential therapeutic mechanisms. Additionally, the combination of synthetic biology and intelligent compounding technologies offers opportunities to optimize drug delivery strategies. Approaches such as nanocarrier systems or pre-drug design can enhance targeting efficiency while reducing off-target effects, thereby providing a strong scientific foundation for the precise intervention of TCM formulations and the metabolites of botanical drugs through modulation of the gut microbiota–mitochondrial crosstalk in obesity and metabolic diseases.

Author contributions

LW: Conceptualization, Writing – original draft. KY: Formal Analysis, Writing – review and editing. JW: Validation, Writing – review and editing. HZ: Methodology, Writing – review and editing. WD: Funding acquisition, Project administration, Supervision, Writing – review and editing.

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References

Abdolmaleky, H. M., and Zhou, J. R. (2024). Gut microbiota dysbiosis, oxidative stress, inflammation, and epigenetic alterations in metabolic diseases. *Antioxidants* 13 (8), 985. doi:10.3390/antiox13080985

Abu Shelbayeh, O., Arroum, T., Morris, S., and Busch, K. B. (2023). PGC-1α is a master regulator of mitochondrial lifecycle and ROS stress response. *Antioxidants* 12 (5), 1075. doi:10.3390/antiox12051075

Adamik, J., Munson, P. V., Hartmann, F. J., Combes, A. J., Pierre, P., Krummel, M. F., et al. (2022). Distinct metabolic states guide maturation of inflammatory and tolerogenic dendritic cells. *Nat. Commun.* 13 (1), 5184. doi:10.1038/s41467-022-32849-1

Akhlaghi, M. (2024). The role of dietary fibers in regulating appetite, an overview of mechanisms and weight consequences. *Crit. Rev. Food Sci. Nutr.* 64 (10), 3139–3150. doi:10.1080/10408398.2022.2130160

Alex, C., Sara, F., Samantha, C., and Badiei, A. (2022). Cystathionine γ-lyase and hydrogen sulfide modulates glucose transporter Glut1 expression via NF-κB and PI3k/Akt in macrophages during inflammation. *PLoS One* 17 (12), e0278910. doi:10.1371/journal.pone.0278910

Al Ojaimi, M., Salah, A., and El-Hattab, A. W. (2022). Mitochondrial fission and fusion: molecular mechanisms, biological functions, and related disorders. *Membranes* 12 (9), 893. doi:10.3390/membranes12090893

Arruda, A. P., Pers, B. M., Parlakguel, G., Gueney, E., Inouye, K., and Hotamisligil, G. S. (2014). Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. *Nat. Med.* 20 (12), 1427–1435. doi:10.1038/nm.3735

Auger, S., Kropp, C., Borras-Nogues, E., Chanput, W., Andre-Leroux, G., Gitton-Quent, O., et al. (2022). Intraspecific diversity of microbial anti-inflammatory molecule (MAM) from Faecalibacterium prausnitzii. *Int. J. Mol. Sci.* 23 (3), 1705. doi:10.3390/ijms23031705

Bai, X., Fu, R. Z., Duan, Z. G., Wang, P., Zhu, C. H., and Fan, D. D. (2021). Ginsenoside Rk3 alleviates gut microbiota dysbiosis and colonic inflammation in antibiotic-treated mice. *Food Res. Int.* 146, 110465. doi:10.1016/j.foodres.2021.110465

Baldini, F., Fabbri, R., Eberhagen, C., Voci, A., Portincasa, P., Zischka, H., et al. (2021). Adipocyte hypertrophy parallels alterations of mitochondrial status in a cell model for adipose tissue dysfunction in obesity. *Life Sci.* 265, 118812. doi:10.1016/j.lfs.2020.118812

Ballard, J. W. O., and Towarnicki, S. G. (2020). Mitochondria, the gut microbiome and ROS. *Cell. Signal.* 75, 109737. doi:10.1016/j.cellsig.2020.109737

Belda, E., Voland, L., Tremaroli, V., Falony, G., Adriouch, S., Assmann, K. E., et al. (2022). Impairment of gut microbial biotin metabolism and host biotin status in severe obesity: effect of biotin and prebiotic supplementation on improved metabolism. *Gut* 71 (12), 2463–2480. doi:10.1136/gutjnl-2021-325753

Bénédicte, A.-N., S, C.-P., A, V., and Id, O. (2020). Gut microbiota and intestinal trans-epithelial permeability. *Int. J. Mol. Sci.* 21 (17), 6402. doi:10.3390/ijms21176402

Bénédicte, A., R, S., and Mw, G. (2021a). Adipose tissue and insulin resistance in obese. *Biomed. Pharmacother.* 137 (1950-6007), 111315. doi:10.1016/j.biopha.2021.111315

Bénédicte, B., V, S., and S, P. (2017). The vagus nerve in the neuro-immune Axis: implications in the pathology of the gastrointestinal tract. *Front. Immunol.* 2 (8), 1452. doi:10.3389/fimmu.2017.01452

Bénédicte, H., W, Y., L, L., Y, J., X, X., J, H., et al. (2021b). Mammalian target of rapamycin signaling pathway regulates mitochondrial quality control of Brown adipocytes in mice. *Front. Physiol.* 12, 638352. doi:10.3389/fphys.2021.638352

Bénédicte, N., Id, O., S, K., Id- Orcid, X., Ie, D., Id, O., et al. (2021c). Disorders of the enteric nervous system - a holistic view. *Nat. Rev. Gastroenterol. Hepatol.* 18 (6), 393–410. doi:10.1038/s41575-020-00385-2

Bénédicte, W., Id, O., Z, S., M, G., L, N., Y, S., et al. (2021d). Hydrogen sulfide protects against IL-1β-induced inflammation and mitochondrial dysfunction-related apoptosis in chondrocytes and ameliorates osteoarthritis. *J. Cell Physiol.* 236 (6), 4369–4386. doi:10.1002/jcp.30154

Bhattacharjee, P., Carlson, C., Ryan, C., Fadlaoui, A., and Sunny, N. (2023). Impact of sustained *de novo* lipogenesis on mitochondrial dysfunction and hepatocellular redox during fatty liver disease. *Physiology* 38. doi:10.1152/physiol.2023.38.S1.5730204

Brestoff, J. R., Wilen, C. B., Moley, J. R., Li, Y., Zou, W., Malvin, N. P., et al. (2021). Intercellular mitochondria transfer to macrophages regulates white adipose tissue homeostasis and is impaired in obesity. *Cell Metab.* 33 (2), 270–282.e8. doi:10.1016/j.cmet.2020.11.008

Camilleri, M. (2023). Is intestinal permeability increased in obesity? A review including the effects of dietary, pharmacological and surgical interventions on permeability and the microbiome COMMENT. *Diabetes Obes. and Metabolism* 25 (2), 325–330. doi:10.1111/dom.14899

Ca, P., G, P., Me, H., and Id, O. (2021). The lifecycle of skeletal muscle mitochondria in obesity. *Obes. Rev.* 22, e13164. doi:10.1111/obr.13164

Cavaliere, G., Cimmino, F., Trinchese, G., Catapano, A., Petrella, L., D'Angelo, M., et al. (2023). From obesity-induced low-grade inflammation to lipotoxicity and mitochondrial dysfunction: altered multi-crosstalk between adipose tissue and metabolically active organs. *Antioxidants* 12 (6), 1172. doi:10.3390/antiox12061172

Cc, C., Ch, K., Yl, L., and Sh, W. (2021). Corylin reduces obesity and insulin resistance and promotes adipose tissue browning through SIRT-1 and β3-AR activation. *Pharmacol. Res.* 164, 105291. doi:10.1016/j.phrs.2020.105291

Cc, C., Kc, S., Jf, C., Cm, L., Cm, Y., Ky, H., et al. (2019). Lipopolysaccharide promoted proliferation and adipogenesis of preadipocytes through JAK/STAT and AMPK-regulated cPLA2 expression. *Int. J. Med. Sci.* 16 (1), 167–179. doi:10.7150/ijms.24068

Chen, D. L., Wang, Y. F., Yang, J. M., Ou, W. Y., Lin, G. R., Zeng, Z., et al. (2024). Shenling Baizhu San ameliorates non-alcoholic fatty liver disease in mice by modulating gut microbiota and metabolites. *Front. Pharmacol.* 15, 1343755. doi:10.3389/fphar.2024.1343755

Chen, L., Zhou, M., Li, H., Liu, D., Liao, P., Zong, Y., et al. (2023a). Mitochondrial heterogeneity in diseases. *Signal Transduct. Target. Ther.* 8 (1), 311. doi:10.1038/s41392-023-01546-w

Chen, Y., Yang, F., Chu, Y., Yun, Z., Yan, Y., and Jin, J. (2022). Mitochondrial transplantation: opportunities and challenges in the treatment of obesity, diabetes, and nonalcoholic fatty liver disease. *J. Transl. Med.* 20 (1), 483. doi:10.1186/s12967-022-03693-0

Chen, Y. K., Liu, T. T., Teia, F. K. F., and Xie, M. Z. (2023b). Exploring the underlying mechanisms of obesity and diabetes and the potential of Traditional Chinese Medicine: an overview of the literature. *Front. Endocrinol.* 14, 1218880. doi:10.3389/fendo.2023.1218880

Cheng, H., Zhang, D. D., Wu, J., Liu, J., Zhou, Y. C., Tan, Y. Z., et al. (2023). Interactions between gut microbiota and polyphenols: a mechanistic and metabolomic review. *Phytomedicine* 119, 154979. doi:10.1016/j.phymed.2023.154979

Choi, B.-R., Kwon, E.-Y., Kim, H.-J., and Choi, M.-S. (2018). Role of synbiotics containing D-allulose in the alteration of body fat and hepatic lipids in diet-induced obese mice. *Nutrients* 10 (11), 1797. doi:10.3390/nu10111797

Chowdhury, M. M. I., Tomii, A., Ishii, K., Tahara, M., Hitsuda, Y., Koto, Y., et al. (2021). TLR4 may be a novel indole-3-acetic acid receptor that is implicated in the regulation of CYP1A1 and TNFα expression depending on the culture stage of Caco-2 cells. *Biosci. Biotechnol. Biochem.* 85 (9), 2011–2021. doi:10.1093/bbb/zbab128

Cojocaru, K.-A., Luchian, I., Goriuc, A., Antoci, L.-M., Ciobanu, C.-G., Popescu, R., et al. (2023). Mitochondrial dysfunction, oxidative stress, and therapeutic strategies in diabetes, obesity, and cardiovascular disease. *Antioxidants* 12 (3), 658. doi:10.3390/antiox12030658

Da Dalt, L., Moregola, A., Svecla, M., Pedretti, S., Fantini, F., Ronzio, M., et al. (2024). The inhibition of inner mitochondrial fusion in hepatocytes reduces non-alcoholic fatty liver and improves metabolic profile during obesity by modulating bile acid conjugation. *Cardiovasc. Res.* 119 (18), 2917–2929. doi:10.1093/cvr/cvad169

Ding, S., Zhuang, Y., Liao, Y., Kang, J., Zhang, L., Shen, J., et al. (2024). Effect of Erchen Decoction on liver mitochondrial function by inhibiting mTORC1/SREBP1/CAV1 pathway in mice with high-fat diet. *Zhongguo Zhong Yao Za Zhi* 49, 763–769. doi:10.19540/j.cnki.cjcmm.20231103.401

Di Vincenzo, F., Del Gaudio, A., Petito, V., Lopetuso, L. R., and Scaldaferri, F. (2024). Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. *Intern. Emerg. Med.* 19 (2), 275–293. doi:10.1007/s11739-023-03374-w

Diwakar, G., P, G., Jh, C., N, K., Hw, C., Bs, J., et al. (2020). AMPK is essential for IL-10 expression and for maintaining balance between inflammatory and cytoprotective signaling. *Biochim. Biophys. Acta Gen. Subj.* 1864 (8), 129631. doi:10.1016/j.bbagen.2020.129631

Diwakar, L., S, Y., Y, X., L, P., R, Z., Y, Z., et al. (2021). Novel insights and current evidence for mechanisms of atherosclerosis: mitochondrial dynamics as a potential therapeutic target. *Front. Cell Dev. Biol.* 9, 673839. doi:10.3389/fcell.2021.673839

Diwakar, W., H, W., L, X., and F, H. (2022). Cross-Talk between gut microbiota and adipose tissues in obesity and related metabolic diseases. *Front. Endocrinol. (Lausanne)* 13, 908868. doi:10.3389/fendo.2022.908868

Dj, B., X, J., M, P., and Ab, H. (2021). The capacity to produce hydrogen sulfide (H(2)S) via cysteine degradation is ubiquitous in the human gut microbiome. *Front. Microbiol.* 12, 705583. doi:10.3389/fmicb.2021.705583

Dong, Y. Z., Xia, T., Lin, J. B., Wang, L., Song, K., and Zhang, C. X. (2021). Quercetin attenuates high-fat diet-induced excessive fat deposition of spotted seabass lateolabrax maculatus through the regulatory for mitochondria and endoplasmic reticulum. *Front. Mar. Sci.* 8. doi:10.3389/fmars.2021.746811

Duan, C., Kuang, L., Hong, C., Xiang, X., Liu, J., Li, Q., et al. (2021). Mitochondrial Drp1 recognizes and induces excessive mPTP opening after hypoxia through BAX-PiC and LRRK2-HK2. *Cell Death and Dis.* 12 (11), 1050. doi:10.1038/s41419-021-04343-x

Ehrlicher, S. E., Stierwalt, H. D., Newsom, S. A., and Robinson, M. M. (2021). Short-term high-fat feeding does not alter mitochondrial lipid respiratory capacity but triggers mitophagy response in skeletal muscle of mice. *Front. Endocrinol.* 12, 651211. doi:10.3389/fendo.2021.651211

Ekkachai, K., C, C., Id, O., N, L., Id, O., N, M., et al. (2023). Effects of synbiotic lacticaseibacillus paracasei, Bifidobacterium breve, and prebiotics on the growth stimulation of beneficial gut microbiota. *Foods* 12 (20), 3847. doi:10.3390/foods12203847

Fan, Y., and Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nat. Rev. Microbiol.* 19 (1), 55–71. doi:10.1038/s41579-020-0433-9

Fang, Z. Z., Wei, L., Lv, Y. P., Wang, T. S., Hamezah, H. S., Han, R. C., et al. (2022). Phillyrin restores metabolic disorders in mice fed with high-fat diet through inhibition of interleukin-6-mediated basal lipolysis. *Front. Nutr.* 9, 956218. doi:10.3389/fnut.2022.956218

Fassarella, M., Blaak, E. E., Penders, J., Nauta, A., Smidt, H., and Zoetendal, E. G. (2021). Gut microbiome stability and resilience: elucidating the response to perturbations in order to modulate gut health. *Gut* 70 (3), 595–605. doi:10.1136/gutjnl-2020-321747

Fogelson, K. A., Dorrestein, P. C., Zarrinpar, A., and Knight, R. (2023). The gut microbial bile acid modulation and its relevance to digestive health and diseases. *Gastroenterology* 164 (7), 1069–1085. doi:10.1053/j.gastro.2023.02.022

François, B., M, A., P, L., E, A., A, L., and E, K. (2021). Production of hydrogen sulfide by the intestinal microbiota and epithelial cells and consequences for the colonic and rectal mucosa. *Am. J. Physiol. Gastrointest. Liver Physiol.* 320 (2), 125–135. doi:10.1152/ajpgi.00261.2020

François, Z., D, A., Jy, Y., and T, Z. (2022). The gut mycobiome in health, disease, and clinical applications in association with the gut bacterial microbiome assembly. *Lancet Microbe* 3 (12), 969–983. doi:10.1016/S2666-5247(22)00203-8

Frolova, M. S., Suvorova, I. A., Iablokov, S. N., Petrov, S. N., and Rodionov, D. A. (2022). Genomic reconstruction of short-chain fatty acid production by the human gut microbiota. *Front. Mol. Biosci.* 9, 949563. doi:10.3389/fmolb.2022.949563

Garcia, D. A., Powers, A. F., Bell, T. A., Guo, S., and Aghajan, M. (2022). Antisense oligonucleotide-mediated silencing of mitochondrial fusion and fission factors modulates mitochondrial dynamics and rescues mitochondrial dysfunction. *Nucleic Acid. Ther.* 32 (1), 51–65. doi:10.1089/nat.2021.0029

Gasmi, A., Mujawdiya, P. K., Pivina, L., Dosa, A., Semenova, Y., Benahmed, A. G., et al. (2021). Relationship between gut microbiota, gut hyperpermeability and obesity. *Curr. Med. Chem.* 28 (4), 827–839. doi:10.2174/0929867327666200721160313

Guo, D., He, H., Meng, Y., Luo, S., and Lu, Z. (2023). Determiners of cell fates: the tricarboxylic acid cycle versus the citrate-malate shuttle. *Protein and Cell* 14 (3), 162–164. doi:10.1093/procel/pwac026

Guo, W., Zhu, S., Li, S., Feng, Y., Wu, H., and Zeng, M. (2021). Microalgae polysaccharides ameliorates obesity in association with modulation of lipid metabolism and gut microbiota in high-fat-diet fed C57BL/6 mice. *Int. J. Biol. Macromol.* 182, 1371–1383. doi:10.1016/j.ijbiomac.2021.05.067

Harrington, J. S. S., Ryter, S. W. W., Plataki, M., Price, D. R. R., and Choi, A. M. K. (2023). Mitochondria in health, disease, and aging. *Physiol. Rev.* 103 (4), 2349–2422. doi:10.1152/physrev.00058.2021

Hatziagelaki, E., Paschou, S. A., Schoen, M., Psaltopoulou, T., and Roden, M. (2022). NAFLD and thyroid function: pathophysiological and therapeutic considerations. *Trends Endocrinol. Metabolism* 33 (11), 755–768. doi:10.1016/j.tem.2022.08.001

He, J., Xiong, X., Yang, H., Li, D., Liu, X., Li, S., et al. (2022). Defined tumor antigen-specific T cells potentiate personalized TCR-T cell therapy and prediction of immunotherapy response. *Cell Res.* 32 (6), 530–542. doi:10.1038/s41422-022-00627-9

He, W., Rebello, O., Savino, R., Terracciano, R., Schuster-Klein, C., Guardiola, B., et al. (2019). TLR4 triggered complex inflammation in human pancreatic islets. *Biochimica Biophysica Acta-Molecular Basis Dis.* 1865 (1), 86–97. doi:10.1016/j.bbadis.2018.09.030

Hiroki, Y., M, I., and M, A. (2019). Propionate suppresses hepatic gluconeogenesis via GPR43/AMPK signaling pathway. *Arch. Biochem. Biophys.* 672, 108057. doi:10.1016/j.abb.2019.07.022

Huan, Y., Hao, G., Shi, Z., Liang, Y., Dong, Y., and Quan, H. (2023). The role of dynamin-related protein 1 in cerebral ischemia/hypoxia injury. *Biomed. and Pharmacother.* 165, 115247. doi:10.1016/j.biopha.2023.115247

Huang, Y.-H., Wu, Y.-H., Tang, H.-Y., Chen, S.-T., Wang, C.-C., Ho, W.-J., et al. (2022). Gut microbiota and bile acids mediate the clinical benefits of YH1 in male patients with type 2 diabetes mellitus: a pilot observational study. *Pharmaceutics* 14 (9), 1857. doi:10.3390/pharmaceutics14091857

Huo, Y., Lu, X., Wang, X., Wang, X., Chen, L., Guo, H., et al. (2020). Bifidobacterium animalis subsp. lactis A6 alleviates obesity associated with promoting mitochondrial biogenesis and function of adipose tissue in mice. *Molecules* 25 (7), 1490. doi:10.3390/molecules25071490

Hussain, A., Yadav, M. K., Bose, S., Wang, J. H., Lim, D., Song, Y. K., et al. (2016). Daesiho-tang is an effective herbal formulation in attenuation of obesity in mice through alteration of gene expression and modulation of intestinal microbiota. *Plos One* 11 (11), e0165483. doi:10.1371/journal.pone.0165483

Huwiler, V. V., Schonenberger, K. A., Segesser von Brunegg, A., Reber, E., Muhlebach, S., Stanga, Z., et al. (2022). Prolonged isolated soluble dietary fibre supplementation in overweight and obese patients: a systematic review with meta-analysis of randomised controlled trials. *Nutrients* 14 (13), 2627. doi:10.3390/nu14132627

Ij, M., M, M., Id, O., J, W., Id, O., N, M., et al. (2021). High-fat, western-style diet, systemic inflammation, and gut microbiota: a narrative review. *Cells* 10 (11), 3164. doi:10.3390/cells10113164

Jagielski, P., Boleslawska, I., Wybranska, I., Przyslawski, J., and Luszczki, E. (2023). Effects of a diet containing sources of prebiotics and probiotics and modification of the gut microbiota on the reduction of body fat. *Int. J. Environ. Res. Public Health* 20 (2), 1348. doi:10.3390/ijerph20021348

Jeong, S.-R., and Kang, M. (2023). Exploring tumor-immune interactions in Co-culture models of T cells and tumor organoids derived from patients. *Int. J. Mol. Sci.* 24 (19), 14609. doi:10.3390/ijms241914609

Jinhua, G., Id, O., Y, S., H, Z., M, C., M, G., et al. (2022). Gut microbiota characteristics of people with obesity by meta-analysis of existing datasets. *Nutrients* 14 (14), 2293. doi:10.3390/nu14142993

Jyl, C., Id, O., and Jm, F. (2020). Bile acid receptors FXR and TGR5 signaling in fatty liver diseases and therapy. *Am. J. Physiol. Gastrointest. Liver Physiol.* 318 (3), 554–573. doi:10.1152/ajpgi.00223.2019

Kallassy, J., Gagnon, E., Rosenberg, D., Silbart, L. K., and McManus, S. A. (2023). Strains of Faecalibacterium prausnitzii and its extracts reduce blood glucose levels, percent HbA1c, and improve glucose tolerance without causing hypoglycemic side effects in diabetic and prediabetic mice. *Bmj Open Diabetes Res. and Care* 11 (3), e003101. doi:10.1136/bmjdrc-2022-003101

Kang, Y., Ren, P., Shen, X., Kuang, X., Yang, X., Liu, H., et al. (2023). A newly synbiotic combination alleviates obesity by modulating the gut microbiota-fat Axis and inhibiting the hepatic TLR4/NF-κB signaling pathway. *Mol. Nutr. and Food Res.* 67 (24), e2300141. doi:10.1002/mnfr.202300141

Kb, K., Cm, G., H, H., Jk, K., Jl, F., P, C., et al. (2021). Ketogenesis impact on liver metabolism revealed by proteomics of lysine β-hydroxybutyrylation. *Cell Rep.* 36 (5), 109487. doi:10.1016/j.celrep.2021.109487

Kengo, S., D, S., A, H., J, T., and A, K. (2020). *In vitro* human colonic microbiota utilises D-β-hydroxybutyrate to increase butyrogenesis. *Sci. Rep.* 10 (1), 8516. doi:10.1038/s41598-020-65561-5

Kim, H., Jeong, Y., Kang, S., You, H. J., and Ji, G. E. (2020). Co-culture with Bifidobacterium catenulatum improves the growth, gut colonization, and butyrate production of Faecalibacterium prausnitzii: *in vitro* and *in vivo* studies. *Microorganisms* 8 (5), 788. doi:10.3390/microorganisms8050788

Kim, J., Yun, J. M., Kim, M. K., Kwon, O., and Cho, B. (2018). Lactobacillus gasseri BNR17 supplementation reduces the visceral fat accumulation and waist circumference in obese adults: a randomized, double-blind, placebo-controlled trial. *J. Med. Food* 21 (5), 454–461. doi:10.1089/jmf.2017.3937

Kim, M., Paik, J. H., Lee, H., Kim, M. J., Eum, S. M., Kim, S. Y., et al. (2024). Ancistrocladus tectorius extract inhibits obesity by promoting thermogenesis and mitochondrial dynamics in high-fat diet-fed mice. *Int. J. Mol. Sci.* 25 (7), 3743. doi:10.3390/ijms25073743

Lapatto, H. A. K., Kuusela, M., Heikkinen, A., Muniandy, M., van der Kolk, B. W., Gopalakrishnan, S., et al. (2023). Nicotinamide riboside improves muscle mitochondrial biogenesis, satellite cell differentiation, and gut microbiota in a twin study. *Sci. Adv.* 9 (2), eadd5163. doi:10.1126/sciadv.add5163

Law, S. K., Wang, Y. P., Lu, X. C., Au, D. C. T., Chow, W. Y. L., Leung, A. W. N., et al. (2022). Chinese medicinal herbs as potential prodrugs for obesity. *Front. Pharmacol.* 13, 1016004. doi:10.3389/fphar.2022.1016004

Le, J. M., Zhang, X. Y., Jia, W. P., Zhang, Y., Luo, J. T., Sun, Y. N., et al. (2019). Regulation of microbiota-GLP1 axis by sennoside A in diet-induced obese mice. *Acta Pharm. Sin. B* 9 (4), 758–768. doi:10.1016/j.apsb.2019.01.014

Lee, C. J., Sears, C. L., and Maruthur, N. (2020). Gut microbiome and its role in obesity and insulin resistance. *Ann. N. Y. Acad. Sci.* 1461 (1), 37–52. doi:10.1111/nyas.14107

Lee, J.-Y., Tsolis, R. M., and Baumler, A. J. (2022). The microbiome and gut homeostasis. *Science* 377 (6601), 44. doi:10.1126/science.abp9960

Leshem, A., Liwinski, T., and Elinav, E. (2020). Immune-microbiota interplay and colonization resistance in infection. *Mol. Cell* 78 (4), 597–613. doi:10.1016/j.molcel.2020.03.001

Li, C., Zhang, H. L., and Li, X. H. (2020). The mechanism of traditional Chinese medicine for the treatment of obesity. *Diabetes Metabolic Syndrome Obesity-Targets Ther.* 13, 3371–3381. doi:10.2147/dmso.S274534

Li, J. J., Li, J. L., Ni, J. J., Zhang, C. B., Jia, J. L., Wu, G. Y., et al. (2022). Berberine relieves metabolic syndrome in mice by inhibiting liver inflammation caused by a high-fat diet and potential association with gut microbiota. *Front. Microbiol.* 12, 752512. doi:10.3389/fmicb.2021.752512

Li, L., Li, X., Du, X., and Chai, T. (2021a). Da chaihutang protects liver mitochondrial function of nutritionally obese rats via CREB/PGC-1α pathway. *Chin. J. Exp. Traditional Med. Formulae* 27 (09), 26–31. doi:10.13422/j.cnki.syfjx.20210601

Li, S., You, J. M., Wang, Z. R., Liu, Y., Wang, B., Du, M., et al. (2021b). Curcumin alleviates high-fat diet-induced hepatic steatosis and obesity in association with modulation of gut microbiota in mice. *Food Res. Int.* 143, 110270. doi:10.1016/j.foodres.2021.110270

Li, X., Hu, S., Yin, J., Peng, X., King, L., Li, L., et al. (2023a). Effect of synbiotic supplementation on immune parameters and gut microbiota in healthy adults: a double-blind randomized controlled trial. *Gut Microbes* 15 (2), 2247025. doi:10.1080/19490976.2023.2247025

Li, Y., Yang, S., Jin, X., Li, D., Lu, J., Wang, X., et al. (2023b). Mitochondria as novel mediators linking gut microbiota to atherosclerosis that is ameliorated by herbal medicine: a review. *Front. Pharmacol.* 14, 1082817. doi:10.3389/fphar.2023.1082817

Li, Y. N., Yu, H., Zhen, J. H., Liu, T. G., Gao, F., He, J. Z., et al. (2024). Impacts of fecal microbiota transplantation on the gut microbiota-short-chain fatty acids-GPR43-interleukin-18 pathway in rats subjected to a high-calorie diet. *J. Biol. Regul. Homeost. Agents* 38 (5), 3861–3872. doi:10.23812/j.biol.regul.homeost.agents.20243805.305

Lin, C., Chen, J. H., Hu, M. M., Zheng, W. Y., Song, Z. Y., and Qin, H. (2021). Sesamol promotes browning of white adipocytes to ameliorate obesity by inducing mitochondrial biogenesis and inhibition mitophagy via β3-AR/PKA signaling pathway. *Food and Nutr. Res.* 65. doi:10.29219/fnr.v65.7577

Liu, J. X., Cai, J. P., Zhang, N. S., Tai, J. D., Fan, P., Dong, X., et al. (2023). Salidroside protects mice from high-fat diet-induced obesity by modulating the gut microbiota. *Int. Immunopharmacol.* 120, 110278. doi:10.1016/j.intimp.2023.110278

Loh, J. S., Mak, W. Q., Tan, L. K. S., Ng, C. X., Chan, H. H., Yeow, S. H., et al. (2024). Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduct. Target. Ther.* 9 (1), 37. doi:10.1038/s41392-024-01743-1

Lordan, C., Thapa, D., Ross, R. P., and Cotter, P. D. (2020). Potential for enriching next-generation health-promoting gut bacteria through prebiotics and other dietary components. *Gut Microbes* 11 (1), 1–20. doi:10.1080/19490976.2019.1613124

Lulu, S., Id, O., J, C., Fj, G., and Id, O. (2021). The role of farnesoid X receptor in metabolic diseases, and gastrointestinal and liver cancer. *Nat. Rev. Gastroenterol. Hepatol.* 18 (5), 335–347. doi:10.1038/s41575-020-00404-2

Lulu, Z., N, C., L, Z., T, B., W, Z., L, Z., et al. (2023). Erchen Decoction alleviates obesity-related hepatic steatosis via modulating gut microbiota-drived butyric acid contents and promoting fatty acid β-oxidation. *J. Ethnopharmacol.* 317, 116811. doi:10.1016/j.jep.2023.116811

Luo, M. C., Zheng, Y. H., Tang, S. Y., Gu, L. S., Zhu, Y., Ying, R. T., et al. (2023). Radical oxygen species: an important breakthrough point for botanical drugs to regulate oxidative stress and treat the disorder of glycolipid metabolism. *Front. Pharmacol.* 14, 1166178. doi:10.3389/fphar.2023.1166178

Ma, L., Zhao, Z. W., Guo, X. M., Li, J., Xu, L., Mei, W. J., et al. (2022). Tanshinone IIA and its derivative activate thermogenesis in adipocytes and induce beiging of white adipose tissue. *Mol. Cell. Endocrinol.* 544, 111557. doi:10.1016/j.mce.2022.111557

MacLean, A., Legendre, F., and Appanna, V. D. D. (2023). The tricarboxylic acid (TCA) cycle: a malleable metabolic network to counter cellular stress. *Crit. Rev. Biochem. Mol. Biol.* 58 (1), 81–97. doi:10.1080/10409238.2023.2201945

Malik, N., Ferreira, B. I., Hollstein, P. E., Curtis, S. D., Trefts, E., Novak, S. W., et al. (2023). Induction of lysosomal and mitochondrial biogenesis by AMPK phosphorylation of FNIP1. *Science* 380 (6642), eabj5559. doi:10.1126/science.abj5559

Mancini, G., Pirruccio, K., Yang, X., Blucher, M., Rodeheffer, M., and Horvath, T. L. (2019). Mitofusin 2 in mature adipocytes controls adiposity and body weight. *Cell Rep.* 26 (11), 2849–2858. doi:10.1016/j.celrep.2019.02.039

Manor, O., Dai, C. L., Kornilov, S. A., Smith, B., Price, N. D., Lovejoy, J. C., et al. (2020a). Health and disease markers correlate with gut microbiome composition across thousands of people. *Nat. Commun.* 11 (1), 5206. doi:10.1038/s41467-020-18871-1

Manor, O., Dai, C. L., Kornilov, S. A., Smith, B., Price, N. D., Lovejoy, J. C., et al. (2020b). Health and disease markers correlate with gut microbiome composition across thousands of people. *Nat. Commun.* 11 (1), 5206. doi:10.1038/s41467-020-18871-1

Matthew, B., Id, O., S, M., A, C., Id, O., C, D. G., et al. (2021). Bile acids activated receptors in inflammatory bowel disease. *Cells* 10 (6), 1281. doi:10.3390/cells10061281

Matthew, F., Tl, G., M, W., Y, V., Me, M., Lc, B., et al. (2020a). A metabolic pathway for bile acid dehydroxylation by the gut microbiome. *Nature* 582 (7813), 566–570. doi:10.1038/s41586-020-2396-4

Matthew, S., and Py, v.d.W. (2020). Lipopolysaccharides modulate intestinal epithelial permeability and inflammation in a species-specific manner. *Gut Microbes* 11 (3), 421–432. doi:10.1080/19490976.2019.1629235

Matthew, W., Z, W., Dj, L., M, G., W, C., and Id, O. (2023a). Fusobacterium nucleatum and its metabolite hydrogen sulfide alter gut microbiota composition and autophagy process and promote colorectal cancer progression. *Microbiol. Spectr.* 11 (6), e0229223. doi:10.1128/spectrum.02292-23

Matthew, X., L, Z., L, L., M, F., L, H., and Id, O. (2020b). MiR-339 attenuates LPS-induced intestinal epithelial cells inflammatory responses and apoptosis by targeting TLR4. *Genes Genomics* 42 (9), 1097–1105. doi:10.1007/s13258-020-00977-x

Matthew, Y., Jh, W., Jh, S., D, L., Sn, L., Jg, S., et al. (2023b). Pharmaceutical efficacy of novel human-origin Faecalibacterium prausnitzii strains on high-fat-diet-induced obesity and associated metabolic disorders in mice. *Front. Endocrinol. (Lausanne)* 14, 1220044. doi:10.3389/fendo.2023.1220044

Michaudel, C., and Sokol, H. (2020). The gut microbiota at the service of immunometabolism. *Cell Metab.* 32 (4), 514–523. doi:10.1016/j.cmet.2020.09.004

Miyamoto, J., Shimizu, H., Hisa, K., Matsuzaki, C., Inuki, S., Ando, Y., et al. (2023). Host metabolic benefits of prebiotic exopolysaccharides produced by Leuconostoc mesenteroides. *Gut Microbes* 15 (1), 2161271. doi:10.1080/19490976.2022.2161271

Montgomery, M. K. (2019). Mitochondrial dysfunction and diabetes: is mitochondrial transfer a friend or foe? *Biology-Basel* 8 (2), 33. doi:10.3390/biology8020033

Nie, K., Zhao, Y., Su, H., Xu, L., Zou, X., Wang, K., et al. (2021a). Effect of Wu-Mei-Wan on gut microbiota in obese mice. *Chin. J. Hosp. Pharm.* 41 (08), 796–802. doi:10.13286/j.1001-5213.2021.08.05

Nie, K., Zhao, Y., Su, H., Xu, L., Zou, X., Wang, K., et al. (2021b). Effect of Wu-Mei-Wan on gutmicrobiota in obese mice. *Chin. J. Hosp. Pharm.* 41 (08), 796–802. doi:10.13286/j.1001-5213.2021.08.05

Nm, B., C, M., Cs, d.S., Pb, V. B., F, C., Va, A., et al. (2017). Microbial anti-inflammatory molecule (MAM) from Faecalibacterium prausnitzii shows a protective effect on DNBS and DSS-induced colitis model in mice through inhibition of NF-κB pathway. *Front. Microbiol.* 8, 114. doi:10.3389/fmicb.2017.00114

Pedersen, J. S., Rygg, M. O., Chrois, K., Sustarsic, E. G., Gerhart-Hines, Z., Albrechtsen, N. J. W., et al. (2022). Influence of NAFLD and bariatric surgery on hepatic and adipose tissue mitochondrial biogenesis and respiration. *Nat. Commun.* 13 (1), 2931. doi:10.1038/s41467-022-30629-5

Peng, X., Wang, K., Zhang, C., Bao, J.-P., Vlf, C., Gao, J.-W., et al. (2021). The mitochondrial antioxidant SS-31 attenuated lipopolysaccharide-induced apoptosis and pyroptosis of nucleus pulposus cells via scavenging mitochondrial ROS and maintaining the stability of mitochondrial dynamics. *Free Radic. Res.* 55 (11-12), 1080–1093. doi:10.1080/10715762.2021.2018426

Pereira, R. O., Marti, A., Olvera, A. C., Tadinada, S. M., Bjorkman, S. H., Weatherford, E. T., et al. (2021). OPA1 deletion in brown adipose tissue improves thermoregulation and systemic metabolism via FGF21. *Elife* 10, e66519. doi:10.7554/eLife.66519

Pileggi, C. A., Parmar, G., and Harper, M.-E. (2021). The lifecycle of skeletal muscle mitochondria in obesity. *Obes. Rev.* 22 (5), e13164. doi:10.1111/obr.13164

Piotr, K., Id, O., and I, M. (2022a). Biological effects of indole-3-propionic acid, a gut microbiota-derived metabolite, and its precursor tryptophan in mammals' health and disease. *Int. J. Mol. Sci.* 23 (3), 1222. doi:10.3390/ijms23031222

Piotr, P., F, S., Id, O., C, J., Id, O., K, K., et al. (2022b). Prebiotic oligofructose protects against high-fat diet-induced obesity by changing the gut microbiota, intestinal mucus

production, glycosylation and secretion. *Gut Microbes* 14 (1), 2152307. doi:10.1080/19490976.2022.2152307

Qi, Z., W, X., Q, W., Z, T., Y, W., and W, G. (2022). Gut microbiota-mitochondrial inter-talk in non-alcoholic fatty liver disease. *Front. Nutr.* 9, 934113. doi:10.3389/fnut.2022.934113

Ramos-Romero, S., Santocildes, G., Pinol-Pinol, D., Roses, C., Pages, T., Hereu, M., et al. (2020). Implication of gut microbiota in the physiology of rats intermittently exposed to cold and hypobaric hypoxia. *Plos One* 15 (11), e0240686. doi:10.1371/journal.pone.0240686

Ren, J., Zk, H., F, L., S, H., Ss, Z., Wy, G., et al. (2020). Mitophagy-Mediated mtDNA release aggravates stretching-induced inflammation and lung epithelial cell injury via the TLR9/MyD88/NF-κB pathway. *Front. Cell Dev. Biol.* 8, 819. doi:10.3389/fcell.2020.00819

Ren, R., Id, O., Y, F., P, S., Q, L., C, L., et al. (2022). Kynurenine/aryl hydrocarbon receptor modulates mitochondria-mediated oxidative stress and neuronal apoptosis in experimental intracerebral hemorrhage. *Antioxid. Redox Signal* 37 (16-18), 1111–1129. doi:10.1089/ars.2021.0215

Rius-Perez, S., Torres-Cuevas, I., Millan, I., Ortega, A. L., and Perez, S. (2020). PGC-1α, inflammation, and oxidative stress: an integrative view in metabolism. *Oxidative Med. Cell. Longev.* 2020, 1452696. doi:10.1155/2020/1452696

Roswall, J., Olsson, L. M., Kovatcheva-Datchary, P., Nilsson, S., Tremaroli, V., Simon, M.-C., et al. (2021). Developmental trajectory of the healthy human gut microbiota during the first 5 years of life. *Cell Host and Microbe* 29 (5), 765–776.e3. doi:10.1016/j.chom.2021.02.021

Ruiz, E., Penrose, H. M., Heller, S., Nakhoul, H., Baddoo, M., Flemington, E. F., et al. (2020). Bacterial TLR4 and NOD2 signaling linked to reduced mitochondrial energy function in active inflammatory bowel disease. *Gut Microbes* 11 (3), 350–363. doi:10.1080/19490976.2019.1611152

Saadati, S., Naseri, K., Asbaghi, O., Yousefi, M., Golalipour, E., and de Courten, B. (2024). Beneficial effects of the probiotics and synbiotics supplementation on anthropometric indices and body composition in adults: a systematic review and meta-analysis. *Obes. Rev.* 25 (3), e13667. doi:10.1111/obr.13667

Sakers, A., De Siqueira, M. K., Seale, P., and Villanueva, C. J. (2022). Adipose-tissue plasticity in health and disease. *Cell* 185 (3), 419–446. doi:10.1016/j.cell.2021.12.016

Sergio, D., Id, O., J, K., Id, O., L, T., Id, O., et al. (2018). Erchen decoction ameliorates lipid metabolism by the regulation of the protein CAV-1 and the receptors VLDLR, LDLR, ABCA1, and SRB1 in a high-fat diet rat model. *Evid. Based Complement. Altern. Med.* 2018, 5309490. doi:10.1155/2018/5309490

Sergio, R.-C., Id, O., Cj, L., M, C., G, P., M, M.-U., et al. (2021). Allostatic hypermetabolic response in PGC1α/β heterozygote mouse despite mitochondrial defects. *FASEB J.* 35 (9), e21752. doi:10.1096/fj.202100262RR

Sharma, M., Wasan, A., and Sharma, R. K. (2021). Recent developments in probiotics: an emphasis on Bifidobacterium. *Food Biosci.* 41, 100993. doi:10.1016/j.fbio.2021.100993

Shi, L. L., Li, Y., Wang, Y., and Feng, Y. (2015). MDG-1, an Ophiopogon polysaccharide, regulate gut microbiota in high-fat diet-induced obese C57BL/6 mice. *Int. J. Biol. Macromol.* 81, 576–583. doi:10.1016/j.ijbiomac.2015.08.057

Singh, V., Ahlawat, S., Mohan, H., Gill, S. S., and Sharma, K. K. (2022). Balancing reactive oxygen species generation by rebooting gut microbiota. *J. Appl. Microbiol.* 132 (6), 4112–4129. doi:10.1111/jam.15504

SR, S., Y, H., Lp, N., C, T., M, W., Ls, B., et al. (2020). Dysbiosis-induced secondary bile acid deficiency promotes intestinal inflammation. *Cell Host Microbe* 27 (4), 659–670. doi:10.1016/j.chom.2020.01.021

Su, L. J., Zeng, Y. P., Li, G. K., Chen, J., and Chen, X. Y. (2022). Quercetin improves high-fat diet-induced obesity by modulating gut microbiota and metabolites in C57BL/6J mice. *Phytotherapy Res.* 36 (12), 4558–4572. doi:10.1002/ptr.7575

Takeuchi, T., Kubota, T., Nakanishi, Y., Tsugawa, H., Suda, W., Kwon, A. T. J., et al. (2023). Gut microbial carbohydrate metabolism contributes to insulin resistance. *Nature* 621 (7978), 389–395. doi:10.1038/s41586-023-06466-x

Talari, N. K., Mattam, U., Meher, N. K., Paripati, A. K., Mahadev, K., Krishnamoorthy, T., et al. (2023). Lipid-droplet associated mitochondria promote fatty-acid oxidation through a distinct bioenergetic pattern in male Wistar rats. *Nat. Commun.* 14 (1), 766. doi:10.1038/s41467-023-36432-0

Teodoro, J. S., Duarte, F. V., Gomes, A. P., Varela, A. T., Peixoto, F. M., Rolo, A. P., et al. (2013). Berberine reverts hepatic mitochondrial dysfunction in high-fat fed rats: a possible role for SirT3 activation. *Mitochondrion* 13 (6), 637–646. doi:10.1016/j.mito.2013.09.002

Tsukuda, N., Yahagi, K., Hara, T., Watanabe, Y., Matsumoto, H., Mori, H., et al. (2021). Key bacterial taxa and metabolic pathways affecting gut short-chain fatty acid profiles in early life. *Isme J.* 15 (9), 2574–2590. doi:10.1038/s41396-021-00937-7

Turchi, R., Tortolici, F., Guidobaldi, G., Iacovelli, F., Falconi, M., Rufini, S., et al. (2020). Frataxin deficiency induces lipid accumulation and affects thermogenesis in brown adipose tissue. *Cell Death and Dis.* 11 (1), 51. doi:10.1038/s41419-020-2253-2

Valentina, S., G, C., Nt, I., G, F., G, M., M, M., et al. (2022). Activation of G protein-coupled receptors by ketone bodies: clinical implication of the ketogenic diet in metabolic disorders. *Front. Endocrinol. (Lausanne)* 13, 972890. doi:10.3389/fendo.2022.972890

Valles-Colomer, M., Blanco-Miguez, A., Manghi, P., Asnicar, F., Dubois, L., Golzato, D., et al. (2023). The person-to-person transmission landscape of the gut and oral microbiomes. *Nature* 614 (7946), 125–135. doi:10.1038/s41586-022-05620-1

van der Zande, H. J. P., Brombacher, E. C., Lambooij, J. M., Pelgrom, L. R., Zawistowska-Deniziak, A., Patente, T. A., et al. (2023). Dendritic cell-intrinsic LKB1-AMPK/SIK signaling controls metabolic homeostasis by limiting the hepatic Th17 response during obesity. *Jci Insight* 8 (11), e157948. doi:10.1172/jci.insight.157948

von der Malsburg, A., Sapp, G. M., Zuccaro, K. E., von Appen, A., Moss, F. R., Kalia, R., et al. (2023). Structural mechanism of mitochondrial membrane remodelling by human OPA1. *Nature* 620 (7976), 1101–1108. doi:10.1038/s41586-023-06441-6

Wachsmuth, H. R., Weninger, S. N., and Duca, F. A. (2022). Role of the gut-brain axis in energy and glucose metabolism. *Exp. Mol. Med.* 54 (4), 377–392. doi:10.1038/s12276-021-00677-w

Wan, Y., Yuan, J., Li, J., Li, H., Yin, K., Wang, F., et al. (2020). Overweight and underweight status are linked to specific gut microbiota and intestinal tricarboxylic acid cycle intermediates. *Clin. Nutr.* 39 (10), 3189–3198. doi:10.1016/j.clnu.2020.02.014

Wang, L., Chen, W. X., Tian, Y. J., Duan, X. R., Yuan, Y., Wang, N., et al. (2022). Preventive effects of sesamol on deep-frying oil-induced liver metabolism disorders by altering gut microbiota and protecting gut barrier integrity. *Mol. Nutr. and Food Res.* 66 (9), e2101122. doi:10.1002/mnfr.202101122

Wang, L., Hu, J., and Zhou, H. (2021). Macrophage and adipocyte mitochondrial dysfunction in obesity-induced metabolic diseases. *World J. Mens Health* 39 (4), 606–614. doi:10.5534/wjmh.200163

Wang, L., and Hu, K. (2021). Effect of gegen qinlian decoction on lipid metabolism in obese mice and its mechanism. *World J. Integr. Traditional West. Med.* 16 (07), 1231–1236. doi:10.13935/j.cnki.sjzx.210713

Wang, P., Gao, J. P., Ke, W. X., Wang, J., Li, D. T., Liu, R. L., et al. (2020a). Resveratrol reduces obesity in high-fat diet-fed mice via modulating the composition and metabolic function of the gut microbiota. *Free Radic. Biol. Med.* 156, 83–98. doi:10.1016/j.freeradbiomed.2020.04.013

Wang, Y., Li, J., Zhang, Z., Wang, R., Bo, H., and Zhang, Y. (2023). Exercise improves the coordination of the mitochondrial unfolded protein response and mitophagy in aging skeletal muscle. *Life-Basel* 13 (4), 1006. doi:10.3390/life13041006

Wang, Y., Yao, W. F., Li, B., Qian, S. Y., Wei, B. B., Gong, S. Q., et al. (2020b). Nuciferine modulates the gut microbiota and prevents obesity in high-fat diet-fed rats. *Exp. Mol. Med.* 52 (12), 1959–1975. doi:10.1038/s12276-020-00534-2

Wei, L. Q., Cheong, I. H., Yang, G. H., Li, X. G., Kozlakidis, Z., Ding, L., et al. (2021). The application of high-throughput technologies for the study of microbiome and cancer. *Front. Genet.* 12, 699793. doi:10.3389/fgene.2021.699793

Welsh, A., Hammad, M., Pina, I. L., and Kulinski, J. (2024). Obesity and cardiovascular health. *Eur. J. Prev. Cardiol.* 31 (8), 1026–1035. doi:10.1093/eurjpc/zwae025

Wen, L., Xiong, W., Wei, G., Zhang, L., Liu, Y., Zhang, T., et al. (2022). Differential response of ileal and colonic microbiota in rats with high-fat diet-induced atherosclerosis. *Int. J. Mol. Sci.* 23 (19), 11154. doi:10.3390/ijms231911154

Wu, F., Yang, X. P., Hu, M. L., Shao, Q. Q., Fang, K., Li, J. B., et al. (2020). Wu-Mei-Wan prevents high-fat diet-induced obesity by reducing white adipose tissue and enhancing brown adipose tissue function. *Phytomedicine* 76, 153258. doi:10.1016/j.phymed.2020.153258

Xia, W., Veeragandham, P., Cao, Y., Xu, Y., Rhyne, T. E., Qian, J., et al. (2024). Obesity causes mitochondrial fragmentation and dysfunction in white adipocytes due to RalA activation. *Nat. Metab.* 6 (2), 273–289. doi:10.1038/s42255-024-00978-0

Xiaomin, S., Y, G., R, Y., and Id, O. (2022a). Gut microbiota-derived tryptophan metabolites maintain gut and systemic homeostasis. *Cells* 11 (15), 2296. doi:10.3390/cells11152296

Xiaomin, T., S, M., Y, L., Y, S., K, Z., Q, Z., et al. (2020). Evaluating the activity of sodium butyrate to prevent osteoporosis in rats by promoting osteal GSK-3β/nrf2 signaling and mitochondrial function. *J. Agric. Food Chem.* 68 (24), 6588–6603. doi:10.1021/acs.jafc.0c01820

Xiaomin, T., Y, W., C, D., X, Z., Y, L., J, Z., et al. (2022b). Tryptophan was metabolized into beneficial metabolites against coronary heart disease or prevented from producing harmful metabolites by the *in vitro* drug screening model based on Clostridium sporogenes. *Front. Microbiol.* 13, 1013973. doi:10.3389/fmicb.2022.1013973

Xiaomin, W., M, Z., Jj, L., Q, J., Y, Z., W, L., et al. (2022c). Propionate alleviates fatty acid-induced mitochondrial dysfunction, oxidative stress, and apoptosis by upregulating PPARG coactivator 1 alpha in hepatocytes. *J. Dairy Sci.* 105 (5), 4581–4592. doi:10.3168/jds.2021-21198

Xie, Y., Wang, C., Zhao, D., Wang, C., and Li, C. (2020). Dietary proteins regulate serotonin biosynthesis and catabolism by specific gut microbes. *J. Agric. Food Chem.* 68 (21), 5880–5890. doi:10.1021/acs.jafc.0c00832

Xiong, R.-G., Zhou, D.-D., Wu, S.-X., Huang, S.-Y., Saimaiti, A., Yang, Z.-J., et al. (2022). Health benefits and side effects of short-chain fatty acids. *Foods* 11 (18), 2863. doi:10.3390/foods11182863

Xiong, X. (2020). Gegen Qinlian Decoction formula syndrome and its application in diabetes, hypertension, hyperlipidemia and obesity. *Zhongguo Zhong Yao Za Zhi* 45 (12), 2760–2764. doi:10.19540/j.cnki.cjcmm.20190829.501

Xu, J., Shen, J., Yuan, R., Jia, B., Zhang, Y., Wang, S., et al. (2021). Mitochondrial targeting therapeutics: promising role of natural products in non-alcoholic fatty liver disease. *Front. Pharmacol.* 12, 796207. doi:10.3389/fphar.2021.796207

Yang, C. W., Liu, H. M., Chang, Z. Y., Liu, G. H., Chang, H. H., Huang, P. Y., et al. (2024). Puerarin modulates hepatic farnesoid X receptor and gut microbiota in high-fat diet-induced obese mice. *Int. J. Mol. Sci.* 25 (10), 5274. doi:10.3390/ijms25105274

Yang, D.-Q., Zuo, Q.-N., Wang, T., Xu, D., Lian, L., Gao, L.-J., et al. (2021). Mitochondrial-targeting antioxidant SS-31 suppresses airway inflammation and oxidative stress induced by cigarette smoke. *Oxidative Med. Cell. Longev.* 2021, 6644238. doi:10.1155/2021/6644238

Yang, H.-M., Kim, J., Shin, D., Kim, J.-Y., You, J., Lee, H.-C., et al. (2023a). Resistin impairs mitochondrial homeostasis via cyclase-associated protein 1-mediated fission, leading to obesity-induced metabolic diseases. *Metabolism-Clinical Exp.* 138, 155343. doi:10.1016/j.metabol.2022.155343

Yang, M., Wang, J.-H., Shin, J.-H., Lee, D., Lee, S.-N., Seo, J.-G., et al. (2023b). Pharmaceutical efficacy of novel human-origin Faecalibacterium prausnitzii strains on high-fat-diet-induced obesity and associated metabolic disorders in mice. *Front. Endocrinol.* 14, 1220044. doi:10.3389/fendo.2023.1220044

Yang, Q.-H., Xu, Y.-J., Liu, Y.-Z., Liang, Y.-J., Feng, G.-F., Zhang, Y.-P., et al. (2014). Effects of chaihu-shugan-san and shen-ling-Bai-Zhu-San on p38 MAPK pathway in kupffer cells of nonalcoholic steatohepatitis. *Evidence-Based Complementary Altern. Med.* 2014, 671013. doi:10.1155/2014/671013

Yau, W. W., Wong, K. A., Zhou, J., Thimmukonda, N. K., Wu, Y., Bay, B.-H., et al. (2021). Chronic cold exposure induces autophagy to promote fatty acid oxidation, mitochondrial turnover, and thermogenesis in brown adipose tissue. *Iscience* 24 (5), 102434. doi:10.1016/j.isci.2021.102434

Ym, W., Tl, H., C, M., J, Z., and Ny, F. (2020). SIRT1 deacetylates mitochondrial trifunctional enzyme α subunit to inhibit ubiquitylation and decrease insulin resistance. *Cell Death Dis.* 11 (10), 821. doi:10.1038/s41419-020-03012-9

Yoo, W., Zieba, J. K., Foegeding, N. J., Torres, T. P., Shelton, C. D., Shealy, N. G., et al. (2021). High-fat diet-induced colonocyte dysfunction escalates microbiota-derived trimethylamine N-oxide. *Science* 373 (6556), 813–818. doi:10.1126/science.aba3683

Yu, Y., Peng, X.-D., Qian, X.-J., Zhang, K.-M., Huang, X., Chen, Y.-H., et al. (2021). Fis1 phosphorylation by Met promotes mitochondrial fission and hepatocellular carcinoma metastasis. *Signal Transduct. Target. Ther.* 6 (1), 401. doi:10.1038/s41392-021-00790-2

Zhang, C. H., Sheng, J. Q., Xie, W. H., Luo, X. Q., Xue, Y. N., Xu, G. L., et al. (2021a). Mechanism and basis of traditional Chinese medicine against obesity: prevention and treatment strategies. *Front. Pharmacol.* 12, 615895. doi:10.3389/fphar.2021.615895

Zhang, F., Aschenbrenner, D., Yoo, J. Y., and Zuo, T. (2022). The gut mycobiome in health, disease, and clinical applications in association with the gut bacterial microbiome assembly. *Lancet Microbe* 3 (12), 969–983. doi:10.1016/s2666-5247(22)00203-8

Zhang, Q., Bai, Y. Y., Wang, W. X., Li, J. J., Zhang, L., Tang, Y. P., et al. (2023a). Role of herbal medicine and gut microbiota in the prevention and treatment of obesity. *J. Ethnopharmacol.* 305, 116127. doi:10.1016/j.jep.2022.116127

Zhang, W., and Zhong, X. (2020). Exploring the effects of gegen qinlian decoction on glucose and lipid metabolism in high-fat diet-induced obese mice based on gut microbiota. *Lishizhen Med. Materia Medica Res.* 31 (08), 1855–1858. doi:10.3969/j.issn.1008-0805.2020.08.020

Zhang, X., Xu, W., Xiao, X., Ding, J., Jiang, Y., and Tu, J. (2021b). Molecular mechanism of Gegen Qinlian Decoction in promoting differentiation of brown adipose tissue to improve glucose and lipid metabolism disorders in diabetic rats. *Zhongguo Zhong Yao Za Zhi* 46 (17), 4462–4470. doi:10.19540/j.cnki.cjcmm.20210524.403

Zhang, Y. A., Huang, Q. Q., Xiong, X. W., Yin, T. T., Chen, S., Yuan, W. W., et al. (2023b). Acacetin alleviates energy metabolism disorder through promoting white fat browning mediated by AC-cAMP pathway. *J. Physiology Biochem.* 79 (3), 529–541. doi:10.1007/s13105-023-00947-3

Zhao, D. D., Pan, Y. Y., Yu, N., Bai, Y., Ma, R. F., Mo, F. F., et al. (2021a). Curcumin improves adipocytes browning and mitochondrial function in 3T3-L1 cells and obese rodent model. *R. Soc. Open Sci.* 8 (3), 200974. doi:10.1098/rsos.200974

Zhao, T., Zhan, L. B., Zhou, W., Chen, W. X., Luo, J. T., Zhang, L. J., et al. (2021b). The effects of erchen decoction on gut microbiota and lipid metabolism disorders in zucker diabetic fatty rats. *Front. Pharmacol.* 12, 647529. doi:10.3389/fphar.2021.647529

Zheng, L. L., Li, B. Y., Yuan, A. L., Bi, S. J., Puscher, H., Liu, C. Q., et al. (2024). TFEB activator tanshinone IIA and derivatives derived from Salvia miltiorrhiza Bge. Attenuate hepatic steatosis and insulin resistance. *J. Ethnopharmacol.* 335, 118662. doi:10.1016/j.jep.2024.118662

Zheng, P., Ma, W., Gu, Y., Wu, H., Bian, Z., Liu, N., et al. (2023). High-fat diet causes mitochondrial damage and downregulation of mitofusin-2 and optic atrophy-1 in multiple organs. *J. Clin. Biochem. Nutr.* 73 (1), 61–76. doi:10.3164/jcbn.22-73

Zhi, N. N., Chang, X. W., Wang, X. R., Guo, J., Chen, J., and Gui, S. Y. (2024). Recent advances in the extraction, purification, structural-property correlations, and antiobesity mechanism of traditional Chinese medicine-derived polysaccharides: a review. *Front. Nutr.* 10, 1341583. doi:10.3389/fnut.2023.1341583

Zhihao, J., Id, O., X, C., J, C., L, Z., Sn, O., et al. (2022a). ACSS3 in brown fat drives propionate catabolism and its deficiency leads to autophagy and systemic metabolic dysfunction. *Clin. Transl. Med.* 12 (2), e665. doi:10.1002/ctm2.665

Zhihao, L., X, Z., W, W., L, G., C, H., H, S., et al. (2022b). Lactobacillus paracasei 24 attenuates lipid accumulation in high-fat diet-induced obese mice by regulating the gut microbiota. *J. Agric. Food Chem.* 70 (15), 4631–4643. doi:10.1021/acs.jafc.1c07884

Zhou, J., Zhang, J., Li, J. Y., Guan, Y. Q., Shen, T., Li, F., et al. (2021). Ginsenoside F2 suppresses adipogenesis in 3T3-L1 cells and obesity in mice via the AMPK pathway. *J. Agric. Food Chem.* 69 (32), 9299–9312. doi:10.1021/acs.jafc.1c03420

Zhu, B., Wang, Y., Ku, L.-T., van Dijk, D., Zhang, L., Hafler, D. A., et al. (2023). scNAT: a deep learning method for integrating paired single-cell RNA and T cell receptor sequencing profiles. *Genome Biol.* 24 (1), 292. doi:10.1186/s13059-023-03129-y

Zhu, X., Zhao, L., Lei, L., Zhu, Y., Xu, J., and Liu, L. (2024). Fecal microbiota transplantation ameliorates abdominal obesity through inhibiting microbiota-mediated intestinal barrier damage and inflammation in mice. *Microbiol. Res.* 282, 127654. doi:10.1016/j.micres.2024.127654

Zong, Y., Li, H., Liao, P., Chen, L., Pan, Y., Zheng, Y., et al. (2024). Mitochondrial dysfunction: mechanisms and advances in therapy. *Signal Transduct. Target. Ther.* 9 (1), 124. doi:10.1038/s41392-024-01839-8

Zotta, A., Oneill, L. A. J., and Yin, M. (2024). Unlocking potential: the role of the electron transport chain in immunometabolism. *Trends Immunol.* 45 (4), 259–273. doi:10.1016/j.it.2024.02.002

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Scutellaria baicalensis Georgi in metabolic-associated fatty liver disease treatment: research progress

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Metabolic associated fatty liver disease (MAFLD), previously known as nonalcoholic fatty liver disease (NAFLD), is a common liver condition marked by excessive fat accumulation exceeding 5% in the liver without significant alcohol consumption. It is closely linked to metabolic disorders such as obesity, type 2 diabetes, and dyslipidemia, with a rising global prevalence projected to escalate from 25% to 56% over the next decade. The pathogenesis of MAFLD is multifaceted, involving insulin resistance, inflammation, and oxidative stress, with progressive symptoms that can lead to severe liver conditions including non-alcoholic steatohepatitis (NASH). Current treatment options are limited, as established medications show variable efficacy and safety. *Scutellaria baicalensis Georgi* (*S. baicalensis*) a traditional Chinese herb rich in flavonoids, has garnered attention for its potential therapeutic effects on MAFLD. Its pharmacological activities, including anti-inflammatory, antioxidant, and lipid-regulating properties, position *S. baicalensis* as a promising candidate for MAFLD management. This article reviews the latest research progress of *S. baicalensis* in the treatment of MAFLD, explores its mechanism of action, pharmacokinetics, and the development of related products, aiming to clarify the pathogenesis of MAFLD and promote the development of new treatment and prevention strategies based on traditional Chinese medicine.

KEYWORDS

metabolic associated fatty liver disease, *Scutellaria baicalensis Georgi*, pharmacological activities, pharmacokinetics, production development

1 Introduction

NAFLD is a common liver disease characterized by fat accumulation of more than 5% in the liver without excessive alcohol consumption. This disease is closely associated with metabolic syndromes and is commonly observed in individuals with obesity, type 2 diabetes, dyslipidemia, and hypertension (Palma et al., 2022; Zhao et al., 2018). The pathogenesis of NAFLD is complex and involves multiple factors such as insulin resistance, inflammatory responses, and oxidative stress. Clinically, NAFLD presents a wide range of symptoms, with many patients experiencing no noticeable signs in the early stages. As the disease progresses, symptoms like fatigue and discomfort in the right upper abdomen may occur. NAFLD can further advance to NASH, a more severe inflammatory condition of the liver that can lead to fibrosis, cirrhosis, and even hepatocellular carcinoma (Figure 1) (Ohtani et al., 2023). The

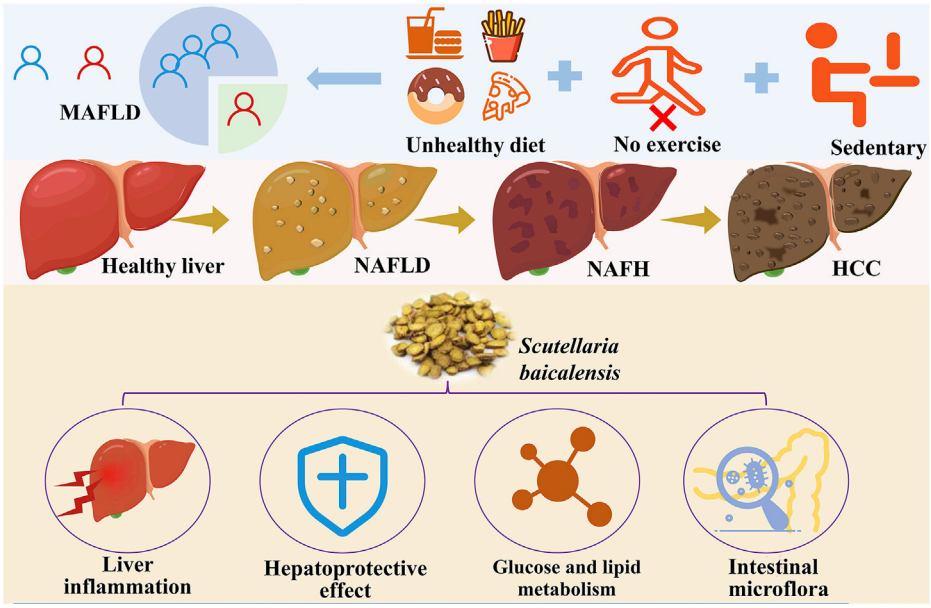

FIGURE 1
Epidemiology, pathogenesis, and potential protective methods of MAFLD (Created by iconfont. cn).

global prevalence of NAFLD is rising annually, with projections indicating that prevalence will increase significantly from the current 25%–56% in China, the United States, and most European countries over the next decade (Huang et al., 2020). Lifestyle factors such as sedentary behavior and poor dietary habits are significant contributors to the development of NAFLD. Therefore, lifestyle interventions—including low-calorie diets, regular exercise, and weight management—are considered effective strategies for treatment and prevention (Elvira-Torales et al., 2019).

As NAFLD gains increasing attention globally, there is a growing recognition of the inherent limitations of the term "nonalcoholic." This characterization places excessive emphasis on the absence of alcohol consumption while neglecting the significance of metabolic risk factors that drive the progression of NAFLD. The term NASH is even more complex, as "steatohepatitis" adds an additional layer of difficulty. In this context, diagnosing NASH not only requires the exclusion of alcohol intake but also histological confirmation of steatohepatitis. Studies have demonstrated significant inter- and intra-observer variability in the histological features, particularly ballooned hepatocytes, which are the main pathological hallmark. Given the lack of clarity regarding the relationship between NAFLD and metabolic risk factors, coupled with the overemphasis on alcohol, an international expert panel suggested in 2020 that the condition be renamed MAFLD. Consequently, this article will consistently use the term MAFLD.

Although several medications, such as metformin, vitamin E, and pioglitazone, are currently used to treat MAFLD (Guo et al., 2022), their efficacy and safety remain debated, and no specific drug for this condition has been established. Metformin has limited efficacy in reducing liver fat in the treatment of MAFLD (Goldberg et al., 2021), and long-term treatment may induce hepatotoxicity (Cone et al., 2010; Hashmi, 2011; Miralles-Linares et al., 2012). Vitamin E supplementation *notably raised* the *risk* of prostate cancer in healthy men (Klein et al., 2011). Pioglitazone is known to increase the risk of heart failure and fractures and might be associated with bladder cancer (Portillo-Sanchez et al., 2019; Azoulay et al., 2012; Violi and Cangemi, 2010). In summary, MAFLD is not only a significant concern for liver health but also poses a challenge to global public health. As its prevalence continues to rise, early detection and intervention will be key to improving patient outcomes and reducing the risk of associated complications. Thus, it is crucial to develop new treatment and prevention strategies, particularly those targeting metabolic abnormalities.

S. baicalensis, commonly known as Huangqin, is a perennial herbaceous plant belonging to the Lamiaceae family, primarily distributed in China and other East Asian regions (Jiang et al., 2017; Zhao Q. et al., 2016). The earliest documentation of *S. baicalensis* can be found in Shennong's Classic of Materia Medica (Shennong Bencao Jing in Chinese), a classic Chinese herbal text written between 200 and 250 AD, which highlights its use for treating conditions related to bitterness, as well as lung and liver issues. The authoritative traditional Chinese medicine text "Bencao Gangmu" first published in 1,593, further reports *S. baicalensis*'s applications in treating diarrhea, dysentery, hypertension, hemorrhage, insomnia, inflammation, and respiratory infections (Chen et al., 2018; Orzechowska et al., 2020; Ji et al., 2015; Zhi et al., 2019; Yoon et al., 2020).

S. baicalensis is rich in flavonoids, including baicalin and baicalein, which contribute to its pharmacological activities (Hai-Juan et al., 2019; Chen et al., 2021). In recent years, there has been growing interest in *S. baicalensis*'s potential role in the treatment of MAFLD, a metabolic disorder characterized by the accumulation of fat in the liver (Chen et al., 2018; Liu et al., 2010; Sun et al., 2019). The pathogenesis of MAFLD is complex, involving factors such as inflammation, oxidative stress, and lipid metabolism dysregulation.

Given *S. baicalensis*'s anti-inflammatory, antioxidant, and lipid-regulating properties, it is emerging as a promising candidate for MAFLD treatment research (Younossi et al., 2016; Semanticscholar, 2019; Yan et al., 2023; Na and sciences, 2019).

This review conducted a comprehensive literature search and screening of studies on *S. baicalensis* and its bioactive metabolites, such as baicalin and wogonin, in the treatment of MAFLD. Databases including PubMed, EMBASE, and Web of Science were systematically searched using combinations of the keywords "*S. baicalensis*" or its bioactive metabolites with "MAFLD" or "NAFLD."The exclusion criteria were as follows: (I) Studies that did not investigate *S. baicalensis* or its bioactive metabolites for the treatment of MAFLD; (II) Studies in which *S. baicalensis* or its derivatives were not the primary intervention; (III) Studies focusing solely on the general pharmacological properties of *S. baicalensis* without specific relevance to MAFLD.

This review summarizes the current studies of *S. baicalensis* used for the treatment of MAFLD of pharmacological activity, mechanisms of action, pharmacokinetics and development of *S. baicalensis*-related products, to provide an important foundation for clarifying the pathogenesis of MAFLD and developing novel approaches for treatment and prevention based on Chinese herbal medicines.

2 The pathogenesis of MAFLD

MAFLD is widely recognized as a complex condition with multifactorial etiology, involving various contributors such as lipid metabolism disorders, endoplasmic reticulum (ER) stress, inflammatory activation, insulin resistance (IR), leptin resistance, oxidative stress, and dysbiosis of the gut microbiota (Rives et al., 2020; Wang et al., 2021; Wei et al., 2016). Among these, lipid metabolism disorders are central to the pathogenesis of MAFLD. Under normal circumstances, the liver regulates lipid synthesis and degradation through multiple mechanisms. However, in MAFLD patients, abnormal lipogenesis leads to significantly elevated levels of free fatty acids while fatty acid oxidation is markedly reduced (Metabolizm, 2012; Veracruz et al., 2021). This metabolic imbalance not only results in the accumulation of triglycerides (TG) in hepatocytes but also promotes the development of insulin resistance, subsequently triggering severe inflammatory responses (Hanley et al., 2004; Leung et al., 2008; Samuel et al., 2004; Singal et al., 2014; Szabo et al., 2015; Vozarova et al., 2002). Additionally, ER stress plays a critical role in the progression of MAFLD. When hepatocytes encounter excess lipids or other stressors, ER dysfunction can lead to the accumulation of unfolded proteins, initiating an ER stress response (Cullinan and Chemistry, 2004; Ajoolabady et al., 2023; Malhotra et al., 2008). This stress state activates multiple signaling pathways, resulting in inflammation and apoptosis of liver cells (Yu et al., 2019). Inflammatory responses are pivotal in the progression from MAFLD to NASH. Activated inflammatory cells in the liver, such as Kupffer cells and macrophages, release various pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which exacerbate hepatocyte damage and promote liver fibrosis (Kazankov et al., 2018).

Furthermore, insulin resistance and leptin resistance are significant metabolic features of MAFLD. Insulin resistance diminishes the liver's response to insulin, disrupting glucose and lipid metabolism, while leptin resistance leads to energy imbalance, further exacerbating hepatic lipid accumulation. These metabolic abnormalities are closely linked to the onset of MAFLD and may contribute to disease progression. Oxidative stress represents another critical pathological mechanism; in MAFLD patients, levels of reactive oxygen species (ROS) are significantly elevated. Excess ROS can induce lipid peroxidation, protein damage, and DNA injury, exacerbating hepatocyte apoptosis and inflammatory responses. According to research by James P. Hardwick (Osborne et al., 2022), CYP4V2 may play a crucial role in regulating the progression of fat liver-related metabolic diseases. As simple steatosis progresses to hepatocellular carcinoma (HCC), ROS levels continue to rise, further underscoring the significance of oxidative stress in the advancement of MAFLD.

In the pathophysiology of MAFLD, the "two-hit" hypothesis is widely accepted. This theory posits that the progression of MAFLD occurs in two phases. The "first hit" refers to the accumulation of triglycerides within hepatocytes (i.e., steatosis) and the development of hepatic insulin resistance, which renders the liver more susceptible to subsequent damage. The "second hit" encompasses the secondary injuries resulting from the first hit, including alterations in the synthesis of lipotoxic factors, increased inflammation, oxidative stress, apoptosis, and liver fibrosis (Borrelli et al., 2018; Byrne and Hepatology, 2015; Haas et al., 2015; Rada et al., 2020). Moreover, ethnic and genetic differences significantly influence the occurrence of MAFLD. Research indicates that certain genetic variations may increase an individual's susceptibility to MAFLD, while environmental factors, such as diet and lifestyle, can interact with genetic predispositions, further impacting the disease's development (Caliceti et al., 2016).

As such, the "two-hit" hypothesis is often viewed by scientists as insufficient to explain all the molecular and metabolic abnormalities associated with MAFLD. In contrast, the "multiple-hit" theory suggests that the onset of MAFLD results from the combined effects of various damaging factors, including insulin resistance, adipokine secretion from adipose tissue, and the interplay between environmental (dietary) and genetic factors, such as epigenetics (Pierantonelli and Transplantation, 2019; Fang et al., 2018). The "multi-organ multiple impact" theory was proposed to explain the progression of MAFLD/NASH, arguing that free fatty acids, derived from lipolysis or *de novo* lipogenesis from dietary fats, exacerbate the burden on the liver. While fatty acids can be metabolized through β-oxidation and triglyceride degradation, saturation of these pathways leads to the formation of lipotoxic lipids, which may cause oxidative damage, ER stress, inflammation, and even cell death. Additionally, non-hepatic organs may directly or indirectly facilitate the development of NASH (Stefan et al., 2019; Kanwal et al., 2021; Long et al., 2022).

Recent studies have highlighted the potential protective effects of *S. baicalensis*, a traditional Chinese medicine, against MAFLD. Active metabolites in *S. baicalensis*, such as baicalin, have been shown to possess antioxidant, anti-inflammatory, and lipid metabolism-improving properties (Liu et al., 2020; Sun et al., 2019; Yang et al., 2020; Shi et al., 2020; Na and sciences, 2019). Research indicates that Scutellaria can reduce fat accumulation in hepatocytes and suppress inflammatory responses by regulating the expression of lipid metabolism-related genes (Shi et al., 2020;

Geethangili et al., 2021). Furthermore, Scutellaria may promote gut health by improving the composition of the gut microbiota, thereby indirectly alleviating the symptoms of MAFLD (Ansari et al., 2020). Therefore, *S. baicalensis* can treat numerous pathophysiological mechanisms associated with NAFLD to ameliorate it.

3 The therapeutic mechanism of *S. baicalensis* for MAFLD

According to a comprehensive analysis of the literature, bioactive substances from *S. baicalensis*, including baicalin and baicalein, have been demonstrated antioxidant and anti-inflammatory qualities (Zhong and Liu, 2018; Xin et al., 2014), thereby alleviating liver damage and improving the pathological state of MAFLD. The therapeutic effects of *S. baicalensis* on MAFLD also involved other pathophysiological processes, including the improvement of insulin resistance, regulation of dyslipidemia, and modulation of the gut microbiota.

It has been suggested that *S. baicalensis* improves insulin sensitivity by modulating signaling pathways such as insulin signaling, thereby ameliorating disorders of glucose and lipid metabolism (Noh et al., 2021). In terms of regulating dyslipidemia, *S. baicalensis* has been found to reduce serum levels of total cholesterol, triglycerides, and other lipids through various mechanisms (Yan et al., 2022). Furthermore, during the combined treatment of MAFLD with other drugs, *S. baicalensis* has been shown to enhance therapeutic efficacy through its multitarget actions. These findings have demonstrate that *S. baicalensis* could treat or improve MAFLD through multiple pathways, and the specific mechanisms will be discussed in the following sections.

3.1 *S. baicalensis* regulates liver inflammation in the treatment of MAFLD

The development of MAFLD is closely associated with chronic inflammation caused by an imbalance between anti-inflammatory and pro-inflammatory markers. Extensive experimental studies have shown that *S. baicalensis* could alleviate hepatic inflammation by blocking the NF-κB signaling pathway, thus treating or delaying the onset of MAFLD. Furthermore, *S. baicalensis* treats MAFLD by controlling hepatic inflammation, altering macrophage polarization (Junior et al., 2021), and exhibiting antioxidant effects through the regulation of Nrf2 transcription factors and other mechanisms (Xin et al., 2014).

In multiple *in vitro* and *in vivo* studies, it has been well established that *S. baicalensis* exhibits potent anti-inflammatory effects and is demonstrated as a potential anti-inflammatory agent that plays an important role in reducing the inflammatory response in MAFLD. Therefore, we will next review the therapeutic effects of *S. baicalensis* on MAFLD inflammation, focusing on three key aspects: the NF-κB signaling pathway, macrophages, and oxidative stress.

3.1.1 The NF-κB signaling pathway

The activation of NLRP3 has plays a critical role in the pathogenesis of MAFLD (Zou et al., 2022). Numerous studies have demonstrated that the NF-κB-mediated signaling pathway increases NLRP3 expression, which subsequently elevates the expression levels of inflammatory factors such as Pro-IL-1β, TNF-α, and IL-6. The NLRP3 inflammasome is composed of three proteins: NLRP3, ASC, and pro-caspase-1 (Shao et al., 2015). Upon activation of NLRP3, pro-caspase-1 undergones autolytic cleavage and cleaves the precursors of inflammatory cytokines, activating their inflammatory activity. It also cleaves GSDMD, releasing its N-terminal fragment, GSDMD-N, which forms pores on the cell membrane, resulting in membrane rupture and pyroptotic cell death (Fu et al., 2024). This process causes fibrosis in normal hepatocytes and accelerates the progression of MAFLD.

While treating MAFLD rats with *S. baicalensis*, researchers have discovered through molecular docking techniques that baicalin exhibited high affinity for TLR4 and NF-κB. This finding suggests that baicalin might alleviate inflammation and treat MAFLD by forming a robust hydrogen bond network with various amino acid residues of TLR4 and NF-κB, thereby blocking the NF-κB signaling pathway (Yan et al., 2023). Another study demonstrated the antifibrotic effects of baicalin in SD rats with thioacetamide-induced cirrhosis, revealing that its mechanism involved inhibition of NLPR3 and NF-κB signaling pathways, thereby reducing the production of inflammatory cytokines (Zaghloul et al., 2022). Gao et al. established a mouse model of MAFLD using a high-fat diet (HFD) and treated the mice with baicalin. They have found that intervention with baicalin inhibited the activity of NF-κB, reduced its nuclear translocation, and downregulated the expression of inflammation-related genes, thereby alleviating hepatic inflammatory responses. Additionally, activation of the Nrf2 signaling pathway and inhibition of SREBP1 together improved overall liver function (Gao et al., 2023). In summary, baicalin has demonstrated significant anti-inflammatory and anti-fibrotic effects by blocking NF-κB activation and inhibiting NLRP3, thereby reducing the production of inflammatory factors and improving hepatic metabolic function, providing new insights for the treatment of MAFLD.

3.1.2 Macrophages

Based on their functional status, macrophages are classified into pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages. M1 macrophages induce robust inflammatory responses by releasing pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-12, and TNF-α, but excessive activation resultes in tissue damage. In contrast, M2 macrophages exhibite anti-inflammatory and reparative functions, primarily through the secretion of Arg-1, IL-10, TGF-β, and matrix metalloproteinases, contributing to tissue repair, wound healing and cellular debris clearance (Arabpour et al., 2021; Gordon and Martinez, 2010). In the treatment of MAFLD, M1 macrophages have been identified as primary effector cells responsible for excessive hepatic inflammatory responses (Junior et al., 2021). A study demonstrated that baicalin could repolarize lipopolysaccharide (LPS)-induced M1 macrophages into an M2 phenotype characterized by the downregulation of IRF5, TNF-α, and IL-23 (Zhu et al., 2016). Furthermore, subsequent research revealed that baicalin could induce the differentiation of macrophages into the

anti-inflammatory M2c subtype and significantly enhance the expression and secretion of anti-inflammatory factors such as IL-10, IL-13 and IL-4 by activating the MERTK signaling pathway and suppressing it at the same time pro-inflammatory cytokines such as IL-12 and IFN-γ. This mechanism not only promoted immune tolerance and tissue repair but also improved the pathological symptoms of diet-induced MAFLD in murine models by enhancing hepatic HDL production and circulation, improving triglyceride transport, and inhibiting fatty acid synthesis (Junior et al., 2021).

Researchers have discovered that baicalin inhibits pan-apoptosis in Kupffer cells in a mouse model of hemophagocytic lymphohistiocytosis, thereby alleviating hepatic inflammation and organ damage (You et al., 2024). Similarly, Liu et al. demonstrated that baicalin treatment in a choline-deficient diet-induced mouse model of MAFLD significantly reduced macrophage infiltration and reversed hepatic lipid accumulation by suppressing the TLR4 signaling cascade. This effect protected the mice from MAFLD progression and inhibited further disease-related fibrosis (Liu et al., 2020). In another study, it was found that baicalin significantly decreased Ly6C^{hi} monocytes, M1 adipose tissue macrophages (ATMs), and M1 Kupffer cells in HFD-induced obese mice. Conversely, it increased anti-inflammatory M2 ATMs, hepatic CD4$^+$ T cells, and the CD4/CD8 ratio (Noh et al., 2021). The study demonstrated that CD4$^+$ T cells suppressed inflammatory responses by regulating the function of ATM, whereas CD8$^+$ T cells promoted the polarization of M1 macrophages. Consequently, increasing the CD4/CD8 ratio enhances immune surveillance, inhibited inflammation, and significantly improved obesity by modulating glucose and lipid metabolism, thereby preventing further hepatic fat accumulation (Conroy et al., 2016; Nishimura et al., 2009). In summary, baicalin has exhibited substantial potential in the treatment of MAFLD by regulating macrophage polarization and inflammatory responses.

3.1.3 Regulating inflammation through oxidative stress

Oxidative stress plays a critical role in the pathological states of the liver (Tauil et al., 2024). Park et al. used a LPS-induced liver injury mouse model and found that heat-treated *S. baicalensis* significantly inhibited reactive ROS level in both serum and liver. The mechanism underlying this effect was likely associated with the suppression of oxidative stress-mediated activation of MAPK (p38, ERK and JNK), NF-κB and AP-1, which effectively ameliorated liver damage in LPS-treated mice (Park et al., 2017). Zhong et al. found that in diet-induced NASH mice, baicalin treatment alleviated oxidative stress damage in liver tissue by reducing MDA levels and significantly enhancing the activity of SOD (Zhong and Liu, 2018). Further studies showed that baicalin or baicalein significantly reduced oxidative stress in MAFLD mice and NASH rats by upregulating the Nrf2/HO-1 signaling pathway in the liver and increasing the activities of SOD and catalase, thereby protecting the liver from ROS -induced damage was protected and preventing further damage (Xin et al., 2014; Liu et al., 2023). In addition, Gao et al. by applying baicalin in a tissue-engineered MAFLD liver model, observed that it significantly alleviated oxidative stress in the model through various mechanisms, including reducing ROS levels, enhancing the expression and activity of antioxidant enzymes, and mitigating cell apoptosis (Gao et al., 2022). Taken together, the therapeutic effects of baicalin and its active metabolites in the treatment of MAFLD may be achieved primarily through antioxidant stress mechanisms (Figure 2).

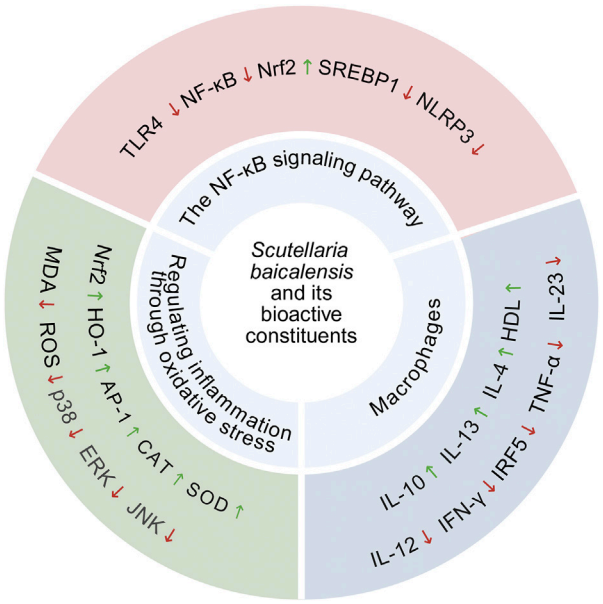

FIGURE 2
S. baicalensis and its bioactive metabolites regulates molecular targets related to oxidative stress and inflammation, including enzymes, proteins, chemical molecules, and inflammatory factors and Anti-inflammatory factor.

3.2 *S. baicalensis* delays MAFLD through its hepatoprotective effect

Multiple studies have shown that *S. baicalensis* exerts significant hepatoprotective effects, with its mechanisms varying depending on the type of liver injury. LPS stimulation induces the release of TNF-α, IL-6, and IL-1β, triggering liver damage and potentially progressing to MAFLD (Laveti et al., 2013). Studies have shown that baicalin significantly reduces the release of inflammatory factors such as TNF-α by downregulating TLR4 expression and inhibiting NF-κB activation, thereby exerting protective effects against LPS-induced liver damage (Cheng et al., 2017).

Researchers have used carbon tetrachloride to induce liver damage to establish a liver fibrosis model. The studies have shown that baicalin can could reduce the serum activities of alanine aminotransferase (ALT), aspartate aminotransferasev (AST) and alkaline phosphatase (ALP), and improve liver damage caused by carbon tetrachloride (CCl_4) (Qiao et al., 2011). Baicalin magnesium, a water-soluble compound isolated from *S. baicalensis* aqueous solution, significantly improves liver injury, lipid deposition, inflammatory response, and oxidative stress in a high-fat diet-induced NASH model, thereby providing protective effects on the liver (Guan et al., 2023).

In drug-induced liver injury, *S. baicalensis* has also shown significant preventive and curative effects. In a mouse model of acetaminophen (APAP)-induced liver injury, baicalin treatment was

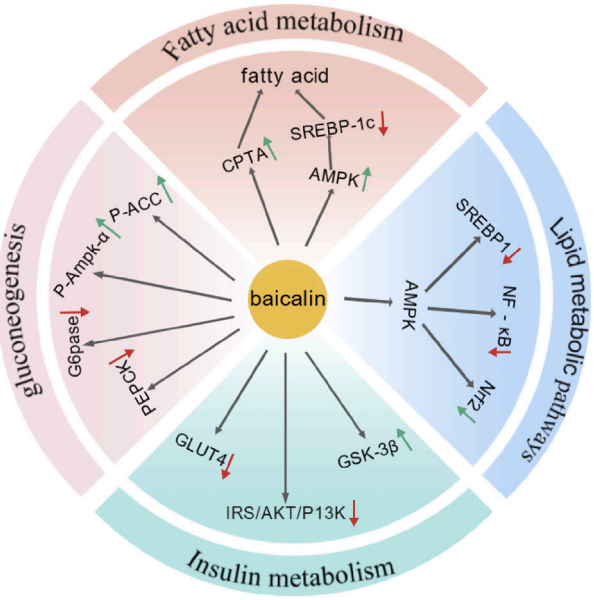

FIGURE 3
S. baicalensis and its active metabolites had exhibited protective effects against various liver injuries by reducing inflammatory factors, activating antioxidant pathways, and regulating related pathways (Created by BioGDP.com).

effective in reducing the levels of TNF-α, IL-6, IL-17, and MPO, thereby achieving a protective effect on the liver (Liao et al., 2016). Another study demonstrated that baicalein and baicalin alleviated APAP-induced liver injury by activating the Nrf2 antioxidant pathway. Molecular docking results indicated that baicalein and baicalin might block the interaction between Nrf2 and Keap1, inducing Nrf2 phosphorylation to activate Nrf2, thereby exerting antioxidant effects through its downstream proteins to reduce APAP-induced hepatotoxicity (Shi et al., 2018). Zhao et al. discovered that exosomes derived from mesenchymal stem cells pretreated with baicalin successfully alleviated hepatocyte ferroptosis following acute liver injury. The main mechanism was that baicalin upregulated the expression of P62, which regulated hepatocyte iron homeostasis through activation of the Keap1-NRF2 pathway (Zhao et al., 2022).

Taken together, *S. baicalensis* exhibites protective effect against various types of liver injury. In LPS-induced inflammation-related liver injury, baicalin inhibited the TLR4-NF-κB pathway to exert its effect. In liver injury models induced by CCl_4 and HFD, metabolites of *S. baicalensis* improved several biomarkers. In case of drug-induced liver injury, *S. baicalensis* alleviated the damage through multiple mechanisms, such as the reduction of inflammatory factors, the activation of antioxidant pathways and the regulation of relevant signaling pathways (Figure 3).

3.3 S. baicalensis is treates MAFLD by improving glucose and lipid metabolism

The key bioactive compounds in *S. baicalensis* significantly improve the symptoms of MAFLD by regulating lipid and glucose metabolism. Multiple mechanisms are involved in improving glucose and lipid metabolism. In terms of lipid metabolism, they regulate fatty acid metabolism, promote fatty acid oxidation, and reduce fatty acid synthesis (Li et al., 2022b). In terms of glucose metabolism, the primary mechanism involved modulation of insulin signaling pathways and inhibition of gluconeogenesis-related pathways, thereby enhancing insulin sensitivity and decreasing glucose production in the liver (Wang et al., 2017).

3.3.1 The lipid metabolism

Baicalin and baicalein have been shown to regulate lipid metabolism by modulating the pathways involved in fatty acid metabolism, lipid synthesis, and fat breakdown, thereby reducing hepatic fat accumulation. The study found that baicalein inhibited SREBP1c/ChREBP, activated AMPK and PPARα signaling pathways, and modulate the processes of fatty acid synthesis, elongation, and oxidation, thereby exerting a preventive effect against liver steatosis caused by fructose consumption in rats (Li et al., 2022b). Dai et al., through chemoproteomics analysis, have identified CPT1A as one of the critical targets within carnitine palmitoyltransferase 1 (CPT1), which serves as a rate-limiting enzyme in mitochondrial fatty acid β-oxidation. Treatment with baicalin resulted in significant reductions in body weight, liver weight and blood lipid levels in mice fed a high-fat diet. Overall, baicalin was found to bind CPT1A, activate its activity, and accelerate fatty acid degradation, thereby significantly alleviating nutritional hepatic steatosis-related symptoms. (Dai et al., 2018).

In addressing alcohol-induced hepatic steatosis, baicalin was able to ameliorate alcohol-induced pathological changes both *in vivo* and *in vitro*. This effect was achieved by activating lipolysis through the regulation of competitive binding between PNPLA3 and ATGL, mediated by SREBP1c (Li et al., 2022a). In non-alcoholic hepatic steatosis, baicalin improves high-fat diet-induced MAFLD by inhibiting SREBP1 and NF-κB signaling pathways via AMPK-mediated mechanisms and activating the Nrf2 signaling pathway (Gao et al., 2023). The activation of AMPK had attenuates the proteolytic processing of SREBP-1c, suppressed lipogenesis, and stimulated fatty acid oxidation, ultimately leading to reduced fat accumulation in the liver (Fang et al., 2018). Moreover, Guo et al. conducted an experiment in which male rats were divided into three groups: normal diet, high-fat diet, and high-fat diet with long-term baicalin administration. Their results demonstrated that prolonged baicalin treatment significantly reduced body weight, liver weight, and blood lipid levels in high-fat diet-induced obese rats (Guo et al., 2009).

3.3.2 The glucose metabolism

Insulin resistance is a key feature of MAFLD, and studies have demonstrated that *S. baicalensis* and its active metabolites improve MAFLD treatment by regulating glucose metabolism. Multiple studies have shown that baicalin activates the insulin signaling pathway and enhances insulin sensitivity. As key regulators of glycogen synthesis, AKT/GSK-3β have been implicated in these processes. In HepG-2 cells with insulin resistance, Wang et al. found that baicalin suppressed gluconeogenesis by activating AMPK or AKT (Wang et al., 2017). Using a similar model, Miao et al. observed in insulin-resistant HepG-2 cells and prediabetic mice that baicalin

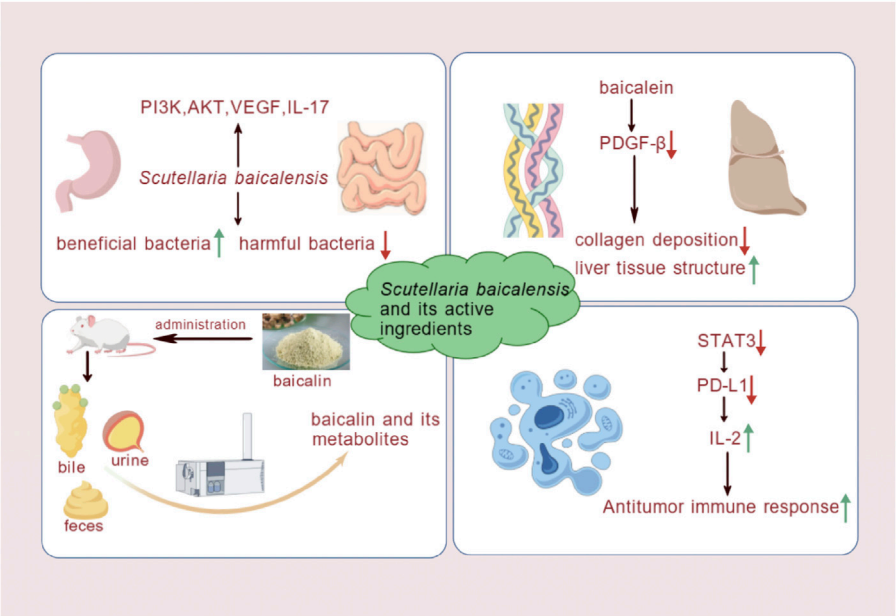

FIGURE 4
Baicalin had treated MAFLD by regulating signaling pathways and mechanisms involved in fatty acid metabolism, lipid synthesis and decomposition, insulin resistance, and gluconeogenesis (Created by BioGDP.com).

prevented downregulation of the IRS/PI3K/AKT signaling pathway, reducing GLUT4 expression, and mitigating enhanced GSK-3β activity, thereby improving dyslipidemia and hyperglycemia in obese mice (Miao et al., 2024). Furthermore, Xi et al. revealed that baicalin activated the AKT signaling pathway and inhibited GSK3β activity, elucidating the mechanism by which baicalin enhanced insulin sensitivity and reduced ectopic fat storage (Xi et al., 2016). Taken together, baicalin alleviates insulin resistance and regulates hepatic glucose metabolism by activating the insulin signaling pathway.

Additionally, baicalin can reduce glucose production by inhibiting the expression of genes related to gluconeogenesis. A HFD induced insulin resistance and ectopic fat storage in skeletal muscle, which is a key feature of metabolic syndrome (Xi et al., 2016). Using high-fat diet-induced obese mice as a model, Fang et al. found that baicalin inhibited the phosphorylation of p38 MAPK, which in turn suppressed the expression and activity of PGC-1α.Acetyl-CoA carboxylase (ACC) is was a key enzyme in fatty acid synthesis, and baicalin alleviated the inhibitory effects of high-fat diet-induced phosphorylation of skeletal muscle ACC and AMP-activated protein kinase (AMPKα) (Xi et al., 2016). Phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) are key enzymes in hepatic gluconeogenesis, and their activity directly impactes blood glucose levels. Baicalin reduced the expression of these key enzymes, thereby decreasing fatty acid synthesis and storage (Fang et al., 2019). Using a high-fat diet-induced obese mouse model, Fang et al. found that baicalin inhibited the phosphorylation of p38 MAPK, which in turn suppressed the expression and activity of PGC-1α. This led to a reduction in the expression and activity of gluconeogenesis-related enzymes, significantly improving hepatic insulin resistance and gluconeogenic activity in high-fat diet-induced obese mice (Fang et al., 2019).

In summary, *S. baicalensis* has significantly improved glucose and lipid metabolism through the regulation of multiple signaling pathways and mechanisms, thereby effectively treating non-alcoholic fatty liver disease (Figure 4).

3.4 *S. baicalensis* treatment for MAFLD by other routes

S. baicalensis and its active metabolites have demonstrated multifaceted effects in the treatment of MAFLD. They not only modulated hepatic inflammation, hepatoprotection, and glucose-lipid metabolism but also exhibited potential mechanisms, including gut microbiota regulation, anti-hepatic fibrosis, and metabolic modulation. These studies have enriched the application of *S. baicalensis* in liver disease treatment and revealed its potential value. A comprehensive analysis of these studies provides a deeper understanding of the multifaceted roles of *S. baicalensis* and its active metabolites in the treatment of liver diseases.

The gut microbiota palys an important role in the pathogenesis of MAFLD. Hu et al. demonstrated that baicalin exerted its effects on the treatment of liver disease by regulating the gut-liver axis, improving gut barrier function, reducing intestinal permeability, and inhibiting inflammatory responses (Hu et al., 2021). Moreover, gut microbiota dysbiosis had been one of the key contributors to MAFLD. Studies have demonstrated that baicalin exerts inhibitory effects on a range of harmful bacteria. At a concentration of 500 μg/mL, baicalin significantly inhibits the biofilm formation of *Streptococcus* mutans by downregulating the expression of genes associated with biofilm formation and reducing the activity of

related enzymes (Elango et al., 2021). Both *in vitro* and *in vivo*, baicalin exhibits notable antibacterial effects against *Pseudomonas aeruginosa*, inhibiting its biofilm formation and motility (Yan et al., 2024). Baicalin had regulates gut microbiota homeostasis by increasing the production of SCFAs, enhancing the abundance of beneficial bacteria such as Bifidobacterium and *Lactobacillus*, and inhibiting the growth of harmful bacteria. This improves gut microbiota balance and alleviated hepatic fat accumulation and inflammation (Ju et al., 2019). Dysbiosis of the gut microbiota may have facilitated the progression of MAFLD to liver fibrosis, hepatocellular carcinoma, and NASH. The mechanisms and pharmacological effects of gut microbiota modulation in MAFLD treatment are still in the early stages of research and further validation through cell and animal studies is required.

In MAFLD, liver fibrosis gradually develops due to the combined effects of metabolic disturbances, oxidative stress, and inflammatory responses. Liver fibrosis, a severe condition characterized by excessive accumulation of extracellular matrix, leads to impaired liver function. Under the stimulation of Wnt signaling caused by PyGO1 mutations, the replication of senescent hepatocytes is halted, activating ductal responses that exacerbate liver fibrosis in MAFLD patients. Consequently, liver fibrosis represents a critical factor in the progression of MAFLD (Fabris et al., 2024). Experimental studies have shown that *S. baicalensis* has shown significant effectiveness in the treatment of heart failure. Sun et al. used a CCl_4-induced rat model of liver fibrosis and administered baicalein for a long period of time. The study found that baicalein inhibited the synthesis of PDGF-β receptor proteins, significantly reducing the degree of CCl_4-induced liver fibrosis, as evidenced by decreased collagen deposition and improved liver tissue architecture (Sun et al., 2010). Taken together, *S. baicalensis* has likely ameliorates liver fibrosis, thereby potentially preventing the progression of MAFLD to more severe liver diseases, providing both theoretical and experimental evidence for the treatment of MAFLD.

HCC is one of the leading cancers worldwide, with over 600,000 deaths occurring annually due to HCC (Song et al., 2012). In the United States, the incidence of HCC has increased significantly due to MAFLD. Analysis of this study indicates that MAFLD was an important cause of HCC (Younossi et al., 2015). *S. baicalensis* and its metabolites have shown antitumor effects, although their mechanisms of interaction with molecular targets are still under investigation. Theoretically, their therapeutic potential in HCC may involve inhibiting cell proliferation, promoting apoptosis and autophagy, suppressing VEGF expression, and exerting anti-inflammatory effects (Ma et al., 2023). Ke et al. further validated through experiments that baicalin and baicalein inhibited STAT3 activity, downregulated IFN-γ-induced PD-L1 expression, restored T cell activity, and increased IL-2 secretion. These effects enhanced T cell-mediated antitumor immune responses, blocked the PD-L1/PD-1 signaling pathway, and strengthened antitumor immunity, thereby synergistically inhibiting tumor growth and improving the immune microenvironment (Ke et al., 2019).

Although flavonoids are generally not well-absorbed after being metabolized in the gut and liver, certain metabolites have shown significant pharmacological activity. As a metabolic disease, MAFLD may be modulated by baicalin and its aglycone through the regulation of metabolic processes related to the pathogenesis of MAFLD, providing new approaches for the treatment of metabolic disorders (Fang et al., 2020). Zhang et al. established a rat liver microsomal-hydrogel system and used the Phase II metabolism (MHCCS-II) cell culture system to predict the metabolic effects of drugs. They found that baicalein (BA) enhanced the antitumor effects on HepG2 and MCF-7 cells through metabolic processes. This study provided a potential framework for *in vitro* investigations into the pharmacological effects of baicalin metabolites, acting as substrates for UDP-glucuronosyltransferases (UGT) during Phase II metabolism (Zhang et al., 2019). Using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), the metabolism and excretion of *S. baicalensis* extract and its metabolites in rats were investigated. The results revealed that five metabolites—baicalin, baicalein, oroxylin A, oroxylin A-7-O-β-D-glucuronide, and scutellarin—were predominantly excreted in the urine, with lower amounts found in feces and bile. Baicalin, baicalein, and their metabolites were primarily eliminated via renal excretion (Zhou et al., 2024; Wang et al., 2012). However, variations in experimental animals, dosage, and detection methods may have produced different results in different studies.

By reviewing the above literature, we have found that the use of *S. baicalensis* in the treatment of MAFLD provides strong scientific evidence. However, further studies are needed to deepen the understanding of its mechanisms of action and promote its clinical application (Figure 5).

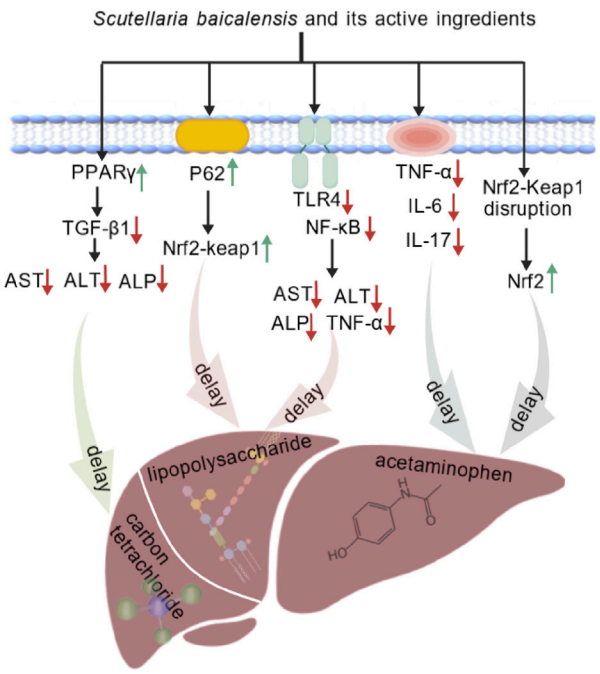

FIGURE 5
S. baicalensis and its active metabolites had provided theoretical and experimental evidence for the treatment of MAFLD by regulating intestinal microbiota, mitigating liver fibrosis, modulating metabolism, and elucidating potential mechanisms of action in liver cancer treatment (Created by BioGDP.com).

4 *S. baicalensis* combined with other drugs for the treatment of MAFLD

The pathogenesis of MAFLD is complex, involving disturbances in lipid metabolism, inflammatory responses, and other factors. In recent years, progress has been made in studies exploring the combined use of *S. baicalensis* and other drugs in the treatment of MAFLD, providing new insights and methods for the comprehensive treatment of this disease.

Zhao et al. established an MAFLD model using HFDs and conducted experiments with different combinations of puerarin, baicalin, and berberine. The results indicated that the combined treatment of puerarin, baicalin, and berberine upregulated the expression levels of liver proliferator-activated receptor (PPAR)-γ and insulin receptor (IR). This contributed to improving insulin resistance as a therapeutic mechanism. Moreover, the combination therapy enhanced overall therapeutic efficacy through multiple targets (Zhao W. et al., 2016). Cui et al. investigated the therapeutic effects of *S. baicalensis* and Coptis chinensis rhizome on type 2 diabetes (T2DM) induced by a HFD combined with low-dose streptozotocin (STZ). The combination therapy primarily improved the pathological features of rats by regulating the expression of pro-inflammatory cytokines, key target proteins in the MAPK signaling pathway, insulin signaling, and the activity of enzymes related to glucose metabolism. The study also indicated that the combination treatment was more effective than monotherapy (Cui et al., 2018).

Liver fibrosis is a key intermediate step in the progression of MAFLD to more severe liver diseases, and its severity has influenced the prognosis and outcome of the disease. *S. baicalensis* has been found to play a therapeutic role in liver fibrosis, blocking the progression of MAFLD to more severe liver conditions. The combination of *S. baicalensis* and Rhei Rhizoma reduced liver fibrosis induced by dimethylnitrosamine (DMN), mainly by regulating redox status, inhibiting oxidative stress, and reducing the expression of α-smooth muscle actin (α-SMA), thereby protecting the liver from fibrotic damage (Pan et al., 2015).

With the increasing integration of Traditional Chinese Medicine (TCM) and Western medicine, not only has the compatibility of *S. baicalensis* with other Chinese herbal medicines attracted attention, but its combined use with modern pharmacological agents has also become a growing area of research interest. A study conducted on Otsuka Long Evans Tokushima Fatty (OLETF) rats demonstrated that co-administration of metformin with *S. baicalensis* resulted in more significant reductions in blood glucose and serum cholesterol levels compared to metformin alone, without adversely affecting the pharmacological actions of metformin (Han et al., 2017). In a 20-week clinical study conducted in 2016, the combination of *S. baicalensis* and metformin for the treatment of type 2 diabetes (T2D) was shown to improve glucose metabolism and significantly enhance glucose tolerance by modulating the composition and function of the gut microbiota, while also effectively reducing inflammatory markers (Shin et al., 2020).

Given the substantial overlap in pathophysiological mechanisms between MAFLD and T2D—particularly the tight association between gut microbiota dysbiosis and metabolic inflammation—this combined therapeutic strategy, which targets the gut microbiota to improve metabolic and inflammatory status, may offer a novel approach and theoretical basis for clinical interventions in MAFLD.

Figure 6 summarizes the potential mechanisms of *S. baicalensis* combined with other drugs in the treatment of MAFLD. The combination of *S. baicalensis*, puerarin, and berberine has been confirmed to upregulate the expression of PPAR-γ and IR in the liver, thereby enhancing insulin receptor sensitivity and reducing the generation of inflammatory cytokines. The combined application of *S. baicalensis* and Coptis chinensis can inhibit the expression of P38, ERK, and JNK proteins in the MAPK signaling pathway, while upregulating the levels of key glycolytic enzymes such as GK, PFK, PK, and GS, thus accelerating glycolytic reactions and fatty acid synthesis, and reducing glucose and lipid levels in the liver. Moreover, the combination of *S. baicalensis* and metformin can modulate the gut microbiota, thereby improving glucose metabolism and reducing inflammation. These effects subsequently impact the development of MAFLD, effectively treating the condition and impeding its progression. Additionally, the combination of *S. baicalensis* and rhubarb can alleviate liver fibrosis by reducing the expression of the important marker α-SMA in activated HSCs. In summary, the combination of *S. baicalensis* with other drugs has shown broad application prospects.

5 *S. baicalensis*-pharmacokinetics, bioavailability, and toxicity

Given the significant pharmacological effects of baicalein in the treatment of MAFLD, its pharmacokinetics had become a key focus of our attention. Numerous studies in the literature have also explored this topic. However, the low oral bioavailability of baicalin (<2.2%) and its poor solubility may limit its clinical efficacy (Lu et al., 2022). The reasons may be as follows: *S. baicalensis* and its major metabolites, such as flavonoid glycosides, are highly polar compounds that cannot pass through the lipid bilayer via passive diffusion, resulting in poor absorption in the intestine (Fang et al., 2017). Baicalin is the predominant metabolite found in blood following oral administration of baicalin and baicalein. Studies indicate that baicalin undergoes a complex metabolic process *in vivo*, which includes gastrointestinal hydrolysis, enterohepatic circulation, and carrier-mediated transport. Baicalin is primarily metabolized via glucuronidation, and it is hydrolyzed in the gastrointestinal tract by microbial enzymes into its active metabolite, baicalein (Xiang et al., 2021). In the liver and intestine, baicalein is predominantly glucuronidated by UDP-glucuronosyltransferases (UGTs), resulting in the formation of baicalin and baicalein-6-O-glucuronide as two major metabolites (Son et al., 2021). Studies investigating the pharmacokinetics of orally administered baicalin, influenced by gut microbiota, and the concurrent role of hepatic enzymes and transport proteins in baicalein metabolism, suggest that baicalin and its metabolites are primarily excreted via bile, with a portion being eliminated in urine (Kang et al., 2014; Zhang et al., 2011).

Although the oral bioavailability of baicalin is relatively low, studies have demonstrated that its tissue distribution is predominantly concentrated in the liver, kidneys, and lungs. After 30 minutes of oral administration of baicalin liposomes, the drug concentrations in the liver, kidneys, and lungs were 5.59, 2.33,

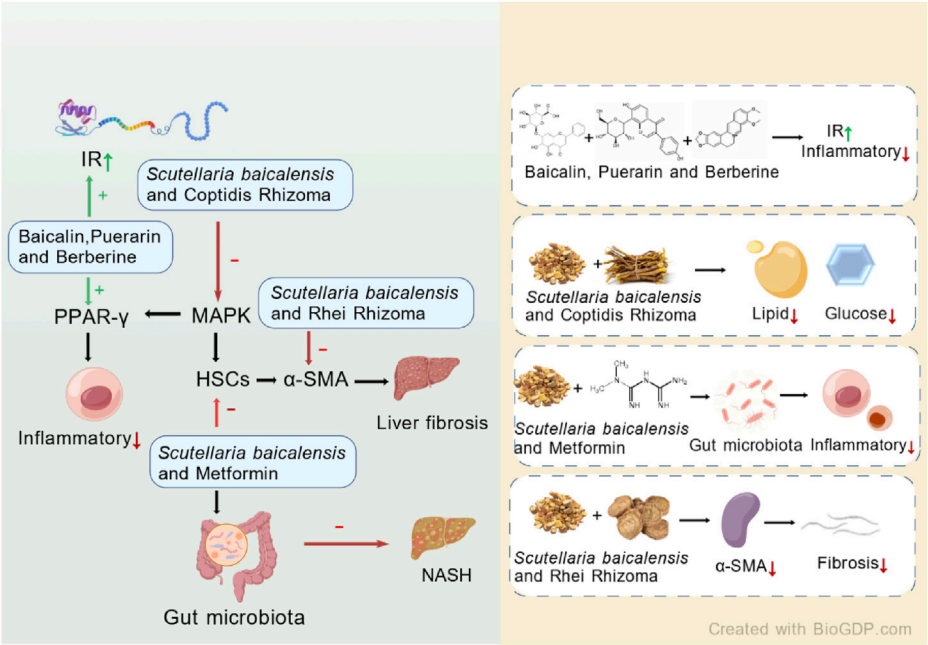

FIGURE 6
Potential mechanisms of *S. baicalensis* combined with other drugs in the treatment of MAFLD (Created by BioGDP.com).

and 1.25 times higher, respectively, compared to the baicalin suspension (Wei et al., 2014). Moreover, studies have shown that the drug concentration in the lungs after intravenous injection was significantly higher than in the liver, kidney, spleen, and other tissues as well as in plasma (Zhao Q. et al., 2016). Moreover, in rats with middle cerebral artery occlusion (MCAO), the baicalin concentration in lung tissue was higher than that in the kidneys or liver after oral administration (Zhu et al., 2013). Other studies have found that the highest concentration of baicalin was reached in the kidneys after oral administration, whereas the compound accumulated predominantly in the lungs after intravenous injection of liposomal baicalin (Zhao Q. et al., 2016).

To improve the efficacy and address the limited bioavailability of single-agent drugs, drug complexes, nanoparticles, nanoliposomes, and other synergistic compound delivery methods have been employed. Numerous studies have shown that liposomal carriers could enhance the oral bioavailability of drugs (Liu et al., 2020; Wei et al., 2014). Targeted nanoparticles promote the *in vivo* and *in vitro* bioavailability of baicalin. In cells treated with baicalin liposomes (BAA1), ApoA1-modified nanoparticles effectively enhanced baicalin uptake in HEPG-2 cells (Xu et al., 2022). An innovative oral baicalein-loaded solid lipid nanoparticle (SLNB) significantly enhanced the bioavailability of baicalein. Compared to free baicalein, the oral relative bioavailability of SLNB increased by approximately 300% (Joshi et al., 2021). Xxx et al. administered baicalin liposomes nasally for the treatment of cerebral ischemia-reperfusion injury in rats. The results demonstrated that baicalin liposomes exhibited favorable pharmacokinetic properties *in vivo*, effectively enhancing the concentration of the drug in brain tissue, while showing good pharmacological efficacy and safety (Xiang et al., 2020). Zhao et al. prepared baicalin-loaded nanoemulsions (BAN-1 and BAN-2) through internal or external drug addition methods and evaluated them both *in vitro* and *in vivo*. Their findings indicated that the baicalin-loaded nanoemulsions, especially BAN-1, were highly effective in improving the oral bioavailability of baicalin (Zhao et al., 2013). Furthermore, the use of novel formulations, such as MSCs-derived exosomes, has the potential to improve bioavailability (Zhao et al., 2022).

The metabolism of *S. baicalensis* and its active metabolites has been shown to influence their activity and toxicity. An oxidative stress study on non-alcoholic fatty liver disease (MAFLD) demonstrated, through CCK-8 assays, that baicalin concentrations ranging from 0.01 nM to 100 μM had no cytotoxic effects on HepG2 cells at 24 and 48 h (Cheng et al., 2017). A dose of 400 $mg \cdot kg^{-1} \cdot d^{-1}$ of baicalin was administered for 14 weeks to high-fat diet mice without toxicity, significantly improving insulin resistance and lipid abnormalities in skeletal muscle. Clinical evaluations of a single oral dose of 100–2,800 mg baicalein in healthy subjects have shown good tolerance with no evidence of liver or kidney toxicity (Li et al., 2014). However, currently, there are no clinical research literatures reporting on the potential risks of long-term use of *S. baicalensis* and its contraindications. While current research has demonstrated the short - term safety and efficacy of *S. baicalensis*, future studies should prioritize long - term clinical trials and translational research to define its potential risks, contraindications, and optimal use in treating MAFLD.

6 Production development

S. baicalensis, a traditional Chinese medicine with a rich history, has garnered significant attention due to its remarkable pharmacological effects. It is extensively utilized in traditional

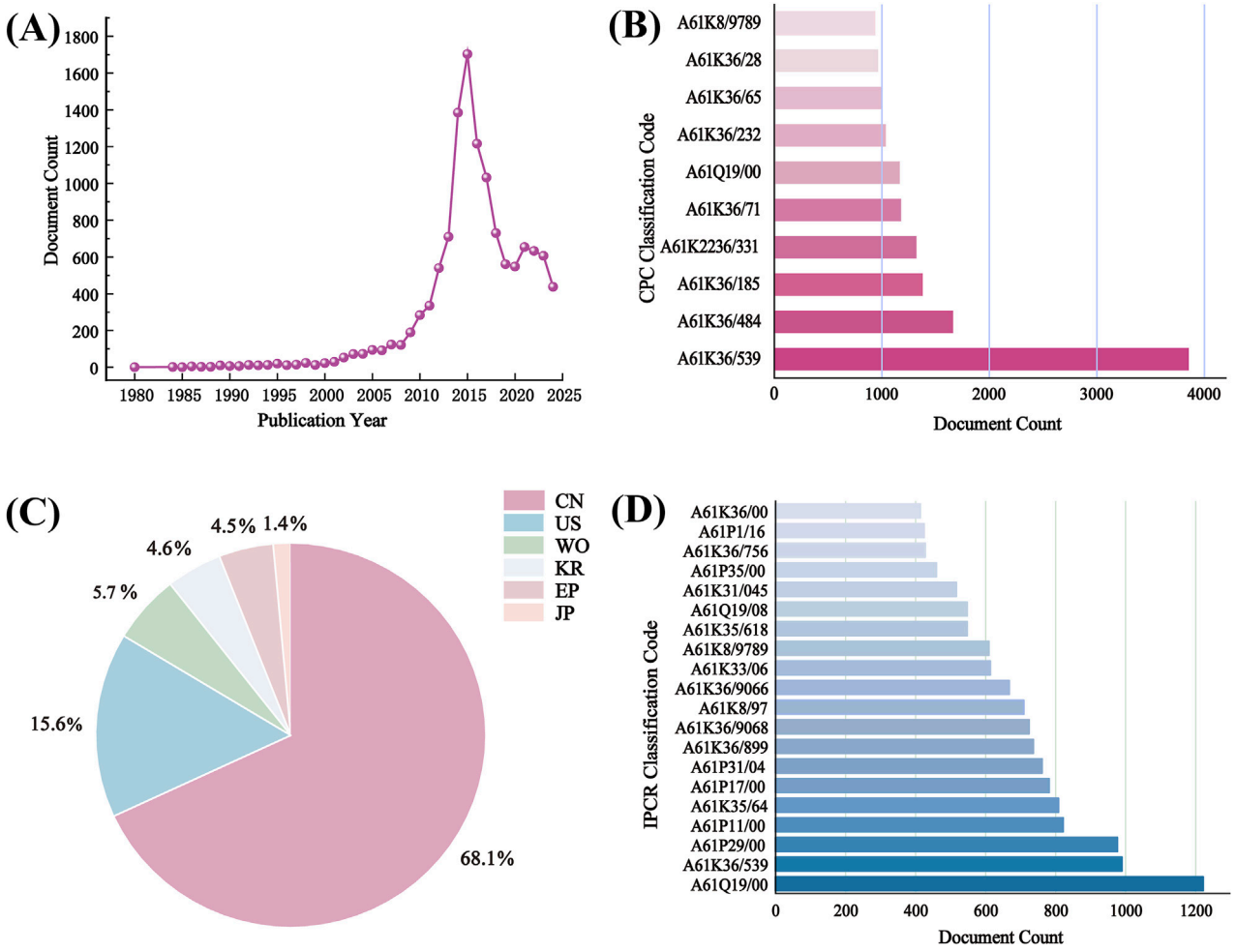

FIGURE 7
Analysis of 12403 patents browsed on LENS.ORG (https://www.lens.org/) using search terms "*S. baicalensis*". **(A)** Patent applications per year; **(B)** CPC classification code; **(C)** Jurisdictions (CN, China; US, United States; WO, World Intellectual property organization; KR, South Korea; EP, European Patent Office; JP, Japan); **(D)** IPCR classification code.

Chinese medicine for its abilities to clear heat and detoxify, reduce inflammation, and protect the liver. Recent modern research has further validated its considerable potential in antibacterial, antiviral, and anti-tumor applications. Currently, the total number of AR patents worldwide has reached 12,403, with China holding the majority, accounting for over three-fifths of the total. Detailed information regarding AR patents is presented in Figure 7. In the 2020 edition of the Chinese Pharmacopoeia, the Traditional Chinese Medicine (TCM) formulas based on *S. baicalensis* include Qinian Pian, Jingtaihong Zhike Granules, Xinqin Pian, Zhaoqin Qingre Heji, Compound Qinlan Oral Liquid, and Gegen Qinlian Wan. Additionally, the 2020 edition of the Chinese Pharmacopoeia includes a total of 248 formulas containing *S. baicalensis*, involving 185 prescriptions. The main dosage forms are as follows: pills (31.05%), tablets (18.95%), capsules (15.32%), granules (14.11%), and oral liquids (10.89%).

The market demand for *S. baicalensis* has been increasing year by year, with widespread applications in the fields of pharmaceuticals, health supplements, and cosmetics. The China Food and Drug Administration (CFDA) (nmpa. gov. cn) has announced 82 *S. baicalensis* related drugs, including Huangqin tablets, Huangqin capsules, Baicalin capsules, Xiongdan Huangqin eye drops, Sanhuang tablets, Yinzhihuang oral solution, etc.

In modern society, the fast-paced lifestyle and increasing work pressures have led many people to gradually overlook the importance of healthy eating and regular exercise. This unhealthy lifestyle has evident impacts on the body, with gastrointestinal issues becoming common among many individuals. To address this, *S. baicalensis* sesame oil soft capsules have emerged as a specialized health product aimed at improving gastrointestinal health.

With the rapid development of the economy, people's awareness of skincare has also been continuously rising. Simultaneously, there is a growing preference for natural and harmless cosmetics, leading to the widespread incorporation of natural plant extracts in products. Plant extracts are commonly used in cosmetics, and *S. baicalensis*, as a traditional Chinese medicinal herb, has found extensive applications in this field. In 2020, the number of recorded extracts from *S. baicalensis* roots exceeded 20,000. Among the recorded metabolites primarily focused on soothing

and anti-aging properties, *S. baicalensis* root extract ranked among the top ten in both quantity and growth rate. Currently, *S. baicalensis* is mainly applied in cosmetics for purposes such as whitening, soothing, and sun protection. To better utilize *S. baicalensis* resources, it is essential to conduct in-depth, multifaceted research on its mechanisms of action, exploring biochemical, cellular, animal, and human perspectives. Additionally, as consumer demand for anti-aging products increases, it is anticipated that *S. baicalensis* will have broad application prospects in this area.

Moreover, with the advancement of modern pharmacological research, the therapeutic potential of *S. baicalensis* in liver injury and liver diseases has garnered increasing attention. *S. baicalensis* and its primary active metabolites are widely incorporated in various TCMs for the treatment of liver damage and liver diseases. For instance, TCM formulations listed in the Chinese Pharmacopoeia, such as Longdan Xiegan Wan and Qinggan Lidan Oral Liquid, contain *S. baicalensis* and are used for clearing heat, detoxification, liver protection, and bile regulation. Additionally, Shugan Ning Injection, composed of *S. baicalensis* extract among other metabolites, is used as an adjunctive therapy for viral hepatitis and liver dysfunction. Furthermore, commercially available Baicalin capsules are commonly employed as supplementary treatments for viral hepatitis and liver dysfunction, exerting hepatoprotective effects through multiple mechanisms, including antioxidant, anti-inflammatory, and antiviral actions. Some compound preparations, such as Chaiqin Qingning Capsules, combine various herbal metabolites and are used to alleviate symptoms of colds and fever while improving liver function. To further expand the therapeutic applications of *S. baicalensis* in liver diseases, it is essential to delve deeper into its pharmacological mechanisms and safety profiles at the cellular, animal model, and clinical levels, facilitating its transition from a traditional herbal remedy to a modern pharmaceutical agent.

Furthermore, *S. baicalensis* has a long history of application in dietary practices, particularly in nourishing diets aimed at enhancing health. This botanical drug can be easily integrated into various culinary creations, seamlessly incorporating health benefits into daily meals. It pairs well with a variety of metabolites, including meats, rice, and vegetables, allowing for the preparation of delicious and nutritious dishes, such as red date and Huangqin chicken stew and Huangqin stewed black beans. The recipe for Huangqin chicken stew, found in the "Medicinal Cuisine Handbook," is a nourishing dish suitable for those suffering from Qi and blood deficiency due to serious illness, prolonged sickness, or excessive postpartum blood loss. *S. baicalensis* is known for its ability to tonify Qi, elevate Yang, strengthen the surface, stop sweating, promote diuresis, nourish blood, expel toxins, and aid in wound healing. The hen used in the dish is beneficial for invigorating Qi and nourishing blood, enhancing overall vitality. Together, they provide a synergistic effect that nourishes Qi, replenishes blood, and strengthens the essence. These traditional recipes not only highlight the versatility of this botanical drug but also emphasize its role in promoting overall health.

Despite the optimistic outlook, challenges remain between foundational research and the industrialization of products centered around *S. baicalensis*. Ongoing research aimed at revealing active metabolites and understanding their mechanisms of action is crucial for overcoming these obstacles. As research progresses, it is expected that effective and safe clinical drugs and health products will soon be developed, further expanding the application prospects of *S. baicalensis*, not only in the realms of food and dietary supplements but also in everyday essentials. Ultimately, this will contribute to the establishment of a healthier society. Through continuous innovation and exploration, the full potential of *S. baicalensis* will be realized, benefiting individuals and communities worldwide.

7 Discussion and outlook

There are currently no internationally approved treatments for MAFLD. The causes are diverse, the cost of screening is high, and the results remain uncertain. No satisfactory preventive or therapeutic medications have been identified (Liu et al., 2023; Younossi et al., 2018; Qu et al., 2021). Interventions based solely on lifestyle modifications have led to limited improvement in MAFLD, and in cases of severe disease, they have failed to prevent further progression. Therefore, research into effective treatments for MAFLD is of great importance.

As shown in Table 1, this review summarizes a total of 57 experimental studies involving various hepatic and metabolic diseases, including fatty liver disease, liver cirrhosis, hepatitis, and diabetes. Among these, seven studies involved *in vitro* experiments, primarily utilizing human hepatocellular carcinoma cell lines such as HepG2 and SMMC-7721, as well as engineered liver models. These studies mainly focused on lipid metabolism and inflammatory pathways, such as AMPK/NF-κB signaling. However, these models lack a complete hepatic microenvironment, including immune interactions and dynamic fibrogenesis, which limits their ability to fully recapitulate the complex pathophysiological features of MAFLD.

In contrast, 50 studies employed *in vivo* models, predominantly using SD rats (29 studies) and C57BL/6 mice (18 studies). The disease models included alcoholic liver disease (ALD); short-term ethanol administration for 4 weeks in SD rats, without the use of classical chronic alcohol consumption models), NAFLD/MAFLD or NASH; induced by high-fat diet or in genetic db/db mice, and liver fibrosis (induced by thioacetamide, TAA). Notably, the ALD models used in these studies feature short intervention durations and simplified pathogenic mechanisms, which differ significantly from the chronic disease course observed in human alcoholism.

It is worth noting that the vast majority of these studies are preclinical, with only one clinical trial reported to date. There remains a lack of robust and consistent clinical data to support the findings. Future research should focus on conducting multicenter, long-term clinical trials (lasting 6 months to 2 years) with large sample sizes encompassing various stages of disease progression. These studies should integrate biochemical markers (e.g., liver enzymes), imaging techniques, and histopathological evaluation to comprehensively assess drug efficacy and safety. Bridging this translational gap between basic research and clinical application remains a critical priority.

S. baicalensis, as a promising therapeutic agent, has been extensively studied for its efficacy in treating MAFLD. A substantial body of research has demonstrated its diverse

TABLE 1 Mechanisms and targets of *S. baicalensis* in treating MAFLD and related diseases: A summary of experimental studies.

Disease	Experimental model	Dose	Duration	Positive control	Targets/ pathways/ mechanisms	Effects	Refs
MAFLD	Male SD rats	Huangqin decoction800 mg/kg/day	8 weeks	Polyene lecithin choline	TLR4/NF-κB/NLRP3	TLR4/NF-κB↓	Yan et al. (2023)
Liver cirrhosis	Adult male SD rats	Baicalin25/75 mg/kg/day	4 weeks	thioacetamide	TGF-β1/NOX4/NF-κB/ NLRP3	NLRP3/Caspase-1↓	Zaghloul et al. (2022)
MAFLD	Male C57BL/6J mice at 8 weeks	Baicalin 100/200/ 400 mg/kg/day	14 weeks	-	SREBP1/Nrf2/NF-κB	TG/TC/LDL↓, HDL↑	Gao et al. (2023)
HLH	C57BL/6J mice at 6–8 weeks	Baicalin 200 mg/kg/day	3 days	-	Kupffer PANoptotic	TNF-α/IFN-γ↓	You et al. (2024)
MAFLD	Male C57BL/6J mice at 6–8 weeks	Baicalin 50 mg/kg/day	4 weeks	-	TLR4	ALT/AST/p-p38/ p-p65↓	Liu et al. (2020)
Obese	Male C57BL/6 mice at 6 weeks	Baicalin 200/ 400 mg/kg/day	8 weeks	-	TNF-α, CCL2, F4/80	Ly6C^{hi}/M1/ATM/ M1/Kupffer↓, M2 ATM/CD4+ T cell↑	Noh et al. (2021)
Liver injury	ICR mice at 6 weeks	Baicalin 100 mg/kg/day	3 days	-	NF-κB	MCP-1/IL-6/ROS/ TNF-α↓	Park et al. (2017)
NASH	Male C57BL/6J mice at 7–8 weeks	Baicalin 0.5% w/w	12 weeks	-	JNK	GSH/SOD/HDL↑, MDA/ALT/ AST/LDL↓	Zhong and Liu (2018)
NASH	Male SD rats at 10 weeks	Baicalein 10 mg/kg/day	8 weeks	-	Nrf2/HO-1	SOD↑,TNF-α/IL-6↓	Xin et al. (2014)
MAFLD	Male db/m mice and db/ db mice	Baicalin 50/100/ 200 mg/kg/day	4 weeks	metformin	p62–Keap1–Nrf2	HO-1/GCLC/SOD/ T-AOC/GSH/ CAT↑, MDA↓	Liu et al. (2023)
MAFLD	Tissue-Engineered liver	Baicalin 100 μM	-	-	ROS	SOD/GSH↑, ROS/MDA↓	Gao et al. (2022)
Acute Liver Injury	C57BL/6 mice at 6–8 weeks	Baicalin 150 μg/mice	-	-	P62 - Keap1 - NRF2	P62/Keap1/NRF2↓	Zhao et al. (2022)
Acute Liver Injury	Male C57BL/6 mice at 8 weeks	Baicalin 30 mg/kg	-	-	IL-17	TNF-α/IL-6/IL-17/MPO↓	Liao et al. (2016)
Acute Liver Injury	Male C57BL/6 mice	Baicalein/Baicalin 40/ 80 mg/kg/day	7 days	-	Keap1-Nrf2	ROS/Keap1/Nrf2↓	Shi et al. (2018)
NASH	Male SD rats at 2–3 weeks	Baicalin magnesium 50/ 150 mg/kg/day	2 weeks	-	NLRP3/Caspase - 1/IL - 1β	NLRP3/Caspase-1/ IL-1β↓	Guan et al. (2023)
Liver Inflammation	1-day-old Beijing white chickens	Baicalin 50/100/ 200 mg/kg	-	-	TLR4 -NF - κB	TLR4/NF-κB↓	Cheng et al. (2017)
Liver injury	Male SD rats	Baicalin 25/50/100 mg/kg	1 week	silymarin (200 mg/kg)	TGFβ1, PPARγ	PPARγ↑,ALT/AST/ ALP/tgf-β1↓	Qiao et al. (2011)
MAFLD	Male SD rats	Baicalein 25,100 mg/kg			SPEBP1C/ChREBP, AMPK/PPARα	SPEBP1C/ChREBP↓ AMPK/PPAα↑	Li et al. (2022a)
hepatic steatosis	DIO mice	Baicalin 400 mg/kg			CPT1A	CPT1A↑	Dai et al. (2018)
ALD	Male 6-week-old SD rats	Baicalin 200 mg/kg			PNPLA3 ATGL	PNPLA3-ATGL↓	Li et al. (2022b)
MAFLD	C57BL/6J mice	Baicalin 100 mg/kg			SREBP1/Nrf2/NF-κB	SREBP1 ↓ NF-κB↓ Nrf2 ↑	Gao et al. (2022)
MAFLD	KK-Ay mice	Baicalin 12.5,25,50 mg/kg			AMPK SREBP	AMPK↑ SREBP-1c↓	Chen et al. (2018)

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TABLE 1 (*Continued*) Mechanisms and targets of *S. baicalensis* in treating MAFLD and related diseases: A summary of experimental studies.

Disease	Experimental model	Dose	Duration	Positive control	Targets/ pathways/ mechanisms	Effects	Refs
MAFLD	Male SD rats. human hepatoma HepG2 cells	Baicalin 80 mg/kg, 5 and 10 μmmol/L			P-AMPK,P-ACC SREBP-1c,AMPK	P-AMPK/P-ACC↑ SREBP-1c↓ AMPKα↑	Guo et al. (2009)
Diabetes	human hepatoma HepG-2 cell	Baicalin 0–50 mM			AMPK,AKT	AMPK↑ AKT↑	Wang et al. (2017)
Diabetes	Insulin-resistant-HepG2 cell Male C57BL/6J mice	Baicalin 20/50 μM 50 and 100 mg/kg			IRS/PI3K/ AKT,GLUT4,GSK-3β	IRS/PI3K/AKT↓ GLUT4↓ GSK-3β↑	Miao et al. (2024)
Insulin resistance	Male C57BL/6J mice	Baicalin 100,200 and 400 mg/kg			AMPK/ACC AKT/ GSK-3β	AMPK/P-ACC↑ AKT↑ GSK-3β↓	XI et al. (2016)
Diabetes	Male C57BL/6Jmice	Baicalein 50 mg/kg			p38MAPK PGC-1α	p38MAPK↓ PGC-1α↓	Fang et al. (2019)
T2D	Male C57BL/6 J mice at 8 weeks old	Baicalin 200 mg/kg			SCFAs	SCFAs↑	Ju et al. (2019)
HF	The SPF grade SD male rats	Baicalin 25 mg/kg			PI3K/AKT、IL-17 VEGF	PI3K/AKT↓ IL-17↓ VEGF↓	Liu et al. (2023)
HF	Male SD rats	Baicalein 20, 40, or 80 mg/kg			PDGF-β	PDGF-β↓	Sun et al. (2010)
HCC	SMMC-7721 cells and HepG2 cell	Baicalein and baicalin 10 μM and 40 μM			STAT3 PD-L1	STAT3↓ PD-L1↓	Ke et al. (2019)
MAFLD	Male SD rats	Baicalein 25, 100 mg/kg	5 weeks		SPEBP1C/ChREBP, AMPK/PPARα	SPEBP1C/ChREBP↓ AMPK/PPAα↑	Li et al. (2022a)
hepatic steatosis	DIO mice	Baicalin 400 mg/kg	12 weeks		CPT1A	CPT1A↑	Dai et al. (2018)
ALD	Male 6-week-old SD rats	Baicalin 200 mg/kg	4 weeks		PNPLA3、ATGL	PNPLA3-ATGL↓	Li et al. (2022b)
MAFLD	C57BL/6J mice	Baicailin 100 mg/kg	24 weeks		SREBP1/Nrf2/NF-κB	SREBP1 ↓ NF-κB↓Nrf2 ↑	Gao et al. (2022)
MAFLD	KK-Ay mice	Baicalin 12.5,25,50 mg/kg	18 days		AMPK/SREBP	AMPK↑ SREBP-1c↓	Chen et al. (2018)
MAFLD	Male SD rats. Human hepatoma HepG2 cells	Baicalin baicalin 80 mg/kg. 5and10 μmmol/L	16 weeks		P-AMPK,P-ACC,SREBP-1c,AMPK	P-AMPK↑P-ACC↑ SREBP-1c↓ AMPKα↑	Guo et al. (2009)
Diabetes	human hepatoma HepG-2 cells	Baicalin 0–50 mM	-		AMPK/AKT	AMPK↑ AKT↑	Wang et al. (2017)
insulin resistance	Male C57BL/6J mice	Baicalin 100, 200, and400 mg/kg	14 weeks		AMPK/ACC Akt/ GSK-3β	AMPK/P-ACC↑ Akt↑GSK-3β↓	XI et al. (2016)
Diabetes	Male C57BL/6Jmice	Baicalein 50 mg/kg	21 days	metformin	p38MAPK/PGC-1α	p38MAPK↓ PGC-1α↓	Fang et al. (2019)
T2D	Male C57BL$^{-/-}$6 J mice (8 weeks old)	Baicalin 200 mg/kg	-	-	SCFAs	SCFAs↑	Ju et al. (2019)
HF	The SPF grade SD male rats	Baicalin 25 mg/kg	8 weeks		PI3K/AkT、IL-17 VEGF	PI3K/AkT↓IL-17↓ VEGF↓	Liu et al. (2023)
HF	male SD rats	Baicalein 20, 40, or 80 mg/kg	10 weeks		PDGF-β	PDGF-β↓	Sun et al. (2010)
HCC	SMMC-7721 cells and HepG2 cell	Baicalein and baicalin 10 μM or40 μM	24 h		STAT3 PD-L1	STAT3↓ PD-L1↓	Ke et al. (2019)

(Continued on following page)

TABLE 1 (*Continued*) Mechanisms and targets of *S. baicalensis* in treating MAFLD and related diseases: A summary of experimental studies.

Disease	Experimental model	Dose	Duration	Positive control	Targets/ pathways/ mechanisms	Effects	Refs
MAFLD	adult male Sprague-Dawley rats	Baicalin and Berberine 100 mg/kg	8 weeks	Rosiglitazone	PPAR-γ, IR	ALT/AST/TC/TG/ LDL↓,HDL/TNF-α/ IL-6↑	Zhao W. et al. (2016)
Liver fibrosis	6-week-old male Wistar rats	SRE 1.25/6.25 mg/kg	3 weeks	-	ROS	ROS/α-SMA↓	Pan et al. (2015)
T2DM	SD rats	crude herbs 6.3 g/kg	1 month	metformin	MAPK	P38/ERK/JNK↓,GK/ PFK/PK/GS↑	Cui et al. (2018)
T2D	OLETF rats	S. baicalensis extract (200 mg/5 mL/kg/day)	12 weeks	metformin	CYP7A1/NR1H4	LDLR↑ HMGCR↓	Han et al. (2017)

therapeutic activities, including anti-inflammatory, antioxidant, metabolic regulation, and hepatoprotective properties, which provide hope for the treatment of MAFLD patients. However, significant challenges remained in translating *S. baicalensis* into clinical therapy for MAFLD.

At present, most clinical studies and animal experiments related to *S. baicalensis* focused primarily on adult animals and adult populations. However, the incidence of MAFLD in children and adolescents is steadily increasing and has become one of the most important pathogenic factors of chronic liver disease in children (Hatton et al., 2018). It is still unclear whether there are differences in the pathophysiological mechanisms of MAFLD between these two age groups (Nobili et al., 2016). Additionally, there are significant differences in drug sensitivity between children and adults (Assunção et al., 2017). Therefore, developing effective treatment strategies for children suffering from this disease holds great value.

(a) During the drug development process, the active metabolites of *S. baicalensis*, due to their complexity and diversity, along with the challenges in standardizing quality, led to instability in therapeutic effects. Therefore, ensuring the quality and stability of *S. baicalensis* materials has become one of the key directions for future research.

In addition, the bioavailability of the active metabolites of *S. baicalensis* was relatively low and the absorption in the gastrointestinal tract was limited after oral administration, which significantly affected the therapeutic efficacy. Pharmacokinetic studies of *S. baicalensis* and its active metabolites revealed that the drug was primarily concentrated in the lungs and kidneys after intravenous injection of baicalin. However, in the context of *treating MAFLD with S. baicalensis*, the goal was to target the liver. Therefore, the development of new formulations of *S. baicalensis* and its active metabolites was necessary to enable targeted delivery to the liver while improving the bioavailability of baicalin. This represented one of the major challenges for future research on *S. baicalensis* and its active metabolites in the treatment of MAFLD.

(b) Although *S. baicalensis* had demonstrated multi-target potential in the treatment of MAFLD, the underlying mechanisms were not yet fully understood. The multi-target mechanisms of *S. baicalensis* were intertwined with the complex pathophysiology of MAFLD, making research more difficult. When designing and evaluating studies on *S. baicalensis* for the treatment of MAFLD, these influencing factors had to be carefully taken into account, and targeted research strategies had to be developed. To date, research on *S. baicalensis* in MAFLD has primarily focused on animal models and small-scale preclinical studies, with a lack of large-scale, multi-center, randomized controlled clinical trials to validate its efficacy and safety in diverse populations.

(c) Existing studies have provided limited insight into the long-term efficacy and potential adverse effects of *S. baicalensis* in the treatment of MAFLD. It remained to be determined whether prolonged use of *S. baicalensis* could affect liver and kidney function or whether significant drug interactions or other side effects had occurred. Further systematic studies were required to ensure safety and feasibility for clinical application.

In conclusion, *S. baicalensis* and its active compounds have demonstrated complex and diverse pharmacological effects that may have had significant implications for the treatment of MAFLD. Mechanisms of action included inhibiting liver inflammation, providing liver protection, and regulating glucose and lipid metabolism. Additionally, *S. baicalensis* alleviated hepatic fat accumulation by modulating gut microbiota homeostasis. Furthermore, *S. baicalensis* alleviated fat accumulation in the liver by modulating the homeostasis of the gut microbiota. In addition, it has shown a synergistic effect in combination with other drugs, offering new possibilities for personalized treatment. These results suggest that *S. baicalensis* has significant potential as a drug candidate for the treatment of MAFLD. However, in order for *S. baicalensis* to be used in the clinical treatment of MAFLD, further research was required to ensure the stability of its quality, investigate the liver-targeting effects of the experimental drug, improve its bioavailability, and explore the underlying mechanism.

Despite existing evidence suggesting that *S. baicalensis* and its major active metabolites, such as baicalin and baicalein, hold therapeutic potential in the management of MAFLD, several limitations remain. Most studies to date have been conducted primarily in animal models, including high-fat diet and alcohol-induced models, with heterogeneous methodologies that undermine the comparability of findings and their clinical translational value. Furthermore, systematic studies on the pharmacokinetic properties,

bioavailability, and liver-targeting distribution characteristics of *S. baicalensis* metabolites are still lacking, particularly with regard to their metabolic transformation mechanisms in relation to gut microbiota. More importantly, clinical investigations focusing on the use of *S. baicalensis* for the treatment of MAFLD are limited, and robust, high-quality clinical evidence supporting its safety and efficacy is still absent.

Future research should focus on a more in-depth understanding of the *in vivo* metabolism of key metabolites of *S. baicalensis*, elucidating their mechanisms of action. Concurrently, the development of novel drug delivery systems, including nanoparticles, liposomes, and prodrugs, is essential to enhance their bioavailability and liver targeting. Further optimization of animal models to better reflect human metabolic characteristics, along with strengthened preclinical toxicology studies, will facilitate the translation of these findings into clinical applications. Additionally, exploring the interactions between *S. baicalensis*, dietary factors, gut microbiota, and host metabolic networks could provide valuable insights for personalized intervention strategies. Overall, continued efforts in both fundamental mechanistic research and formulation optimization, alongside the gradual implementation of systematic clinical trials, are necessary to lay a solid foundation for the application of *S. baicalensis* in the prevention and treatment of MAFLD.

Author contributions

LC: Investigation, Writing – review and editing. EL: Investigation, Writing – original draft, Writing – review and editing. XZ: Resources, Writing – original draft, Writing – review and editing. XL: Writing – review and editing. QD: Writing – review and editing. YL: Writing – review and editing. GH: Writing – original draft, Writing – review and editing. LL: Writing – review and editing. YH: Writing – review and editing.

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Conflict of interest

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

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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References

Ajoolabady, A., Kaplowitz, N., Lebeaupin, C., Kroemer, G., Kaufman, R. J., Malhi, H., et al. (2023). Endoplasmic reticulum stress in liver diseases. *Hepatology* 77: 619–639. doi:10.1002/hep.32562

Ansari, A., Bose, S., Lim, S.-K., Wang, J. H., Choi, Y. H., and Kim, H. (2020). Combination of Scutellaria baicalensis and metformin ameliorates diet-induced metabolic dysregulation in mice via the gut-liver-brain Axis. *Brain Axis* 48, 1409–1433. doi:10.1142/S0192415X2050069X

Arabpour, M., Saghazadeh, A., and Rezaei, N. (2021). Anti-inflammatory and M2 macrophage polarization-promoting effect of mesenchymal stem cell-derived exosomes. *Int. Immunopharmacol.* 97, 107823. doi:10.1016/j.intimp.2021.107823

AssunçãO, S. N. F., Sorte, N. C. B., Alves, C. D., Mendes, P. S. A., and Silva, L. R. (2017). Nonalcoholic fatty liver disease (NAFLD) pathophysiology in obese children and adolescents: update. *Nutr. Hosp.* 34, 727–730. doi:10.20960/nh.723

Azoulay, L., Yin, H., Filion, K. B., Assayag, J., Majdan, A., Pollak, M. N., et al. (2012). The use of pioglitazone and the risk of bladder cancer in people with type 2 diabetes: nested case-control study. *Bmj* 344, e3645. doi:10.1136/bmj.e3645

Borrelli, A., Bonelli, P., Tuccillo, F. M., Goldfine, I. D., Evans, J. L., Buonaguro, F. M., et al. (2018). Role of gut microbiota and oxidative stress in the progression of non-alcoholic fatty liver disease to hepatocarcinoma: current and innovative therapeutic approaches. *Redox Biol.* 15, 467–479. doi:10.1016/j.redox.2018.01.009

Byrne, C. D., and Hepatology, G. T. J. J. O. (2015). NAFLD: a multisystem disease. *J. Hepatol.* 62, S47–S64. doi:10.1016/j.jhep.2014.12.012

Caliceti, C., Franco, P., Spinozzi, S., Roda, A., and Cicero, A. F. G. (2016). Berberine: new insights from pharmacological aspects to clinical evidences in the management of metabolic disorders. *Curr. Med. Chem.* 23, 1460–1476. doi:10.2174/0929867323666160411143314

Cheng, P., Wang, T., Li, W., Muhammad, I., Wang, H., Sun, X., et al. (2017). Baicalin alleviates lipopolysaccharide-induced liver inflammation in chicken by suppressing TLR4-mediated NF-κB pathway. *Front. Pharmacol.* 8, 547. doi:10.3389/fphar.2017.00547

Chen, M., Xiao, H., Chen, B., Bian, Z., and Kwan, H. Y. (2021). The advantages of using Scutellaria baicalensis and its flavonoids for the management of non-viral hepatocellular carcinoma. *J. Funct. Foods* 78, 104389. doi:10.1016/j.jff.2021.104389

Chen, Q., Liu, M., Yu, H., Li, J., Wang, S., Zhang, Y., et al. (2018). Scutellaria baicalensis regulates FFA metabolism to ameliorate NAFLD through the AMPK-mediated SREBP signaling pathway. *J. Nat. Med.* 72, 655–666. doi:10.1007/s11418-018-1199-5

Cone, C. J., Bachyrycz, A. M., and Murata, G. H. (2010). Hepatotoxicity associated with metformin therapy in treatment of type 2 diabetes mellitus with nonalcoholic fatty liver disease. *Ann. Pharmacother.* 44, 1655–1659. doi:10.1345/aph.1P099

Conroy, M. J., Dunne, M. R., Donohoe, C. L., and Reynolds, J. V. (2016). Obesity-associated cancer: an immunological perspective. *Proc. Nutr. Soc.* 75, 125–138. doi:10.1017/S0029665115004176

Cui, X., Qian, D. W., Jiang, S., Shang, E. X., Zhu, Z. H., and Duan, J. A. (2018). Scutellariae radix and Coptidis rhizoma improve glucose and lipid metabolism in T2DM rats via regulation of the metabolic profiling and MAPK/PI3K/akt signaling pathway. *Int. J. Mol. Sci.* 19, 3634. doi:10.3390/ijms19113634

Cullinan, S., and Chemistry, J. D. J. J. O. B. (2004). PERK-Dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J. Biol. Chem.* 279: 20108–20117. doi:10.1074/jbc.M314219200

Dai, J., Liang, K., Zhao, S., Jia, W., Liu, Y., Wu, H., et al. (2018). Chemoproteomics reveals baicalin activates hepatic CPT1 to ameliorate diet-induced obesity and hepatic steatosis. *Proc. Natl. Acad. Sci. U. S. A.* 115, E5896–e5905. doi:10.1073/pnas.1801745115

Elango, A. V., Vasudevan, S., Shanmugam, K., Solomon, A. P., and Neelakantan, P. (2021). Exploring the anti-caries properties of baicalin against Streptococcus mutans: an *in vitro* study. *Biofouling* 37, 267–275. doi:10.1080/08927014.2021.1897789

Elvira-Torales, L. I., Martin-Pozuelo, G., Gonzalez-Barrio, R., Navarro-González, I., Pallarés, F. J., Santaella, M., et al. (2019). Ameliorative effect of spinach on non-alcoholic fatty liver disease induced in rats by a high-fat diet. *Int. J. Mol. Sci.* 20, 1662. doi:10.3390/ijms20071662

Fabris, L., Campello, E., Cadamuro, M., and Simioni, P. (2024). The evil relationship between liver fibrosis and cardiovascular disease in metabolic dysfunction-associated fatty liver disease (MAFLD): looking for the culprit. *Biochim. Biophys. Acta Mol. Basis Dis.* 1870, 166763. doi:10.1016/j.bbadis.2023.166763

Fang, Y.-L., Chen, H., Wang, C.-L., and Liang, L. (2018). Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: from "two hit theory" to "multiple hit model". *"multiple hit model* 24, 2974–2983. doi:10.3748/wjg.v24.i27.2974

Fang, P., Sun, Y., Gu, X., Shi, M., Bo, P., Zhang, Z., et al. (2019). Baicalin ameliorates hepatic insulin resistance and gluconeogenic activity through inhibition of p38 MAPK/PGC-1α pathway. *Phytomedicine* 64, 153074. doi:10.1016/j.phymed.2019.153074

Fang, P., Yu, M., Shi, M., Bo, P., Gu, X., and Zhang, Z. (2020). Baicalin and its aglycone: a novel approach for treatment of metabolic disorders. *Pharmacol. Rep.* 72, 13–23. doi:10.1007/s43440-019-00024-x

Fang, Y., Cao, W., Xia, M., Pan, S., and Xu, X. (2017). Study of structure and permeability relationship of flavonoids in caco-2 cells. *Nutrients* 9, 1301. doi:10.3390/nu9121301

Fu, J., Schroder, K., and Wu, H. (2024). Mechanistic insights from inflammasome structures. *Nat. Rev. Immunol.* 24, 518–535. doi:10.1038/s41577-024-00995-w

Gao, W., Xu, B., Zhang, Y., Liu, S., Duan, Z., Chen, Y., et al. (2022). Baicalin attenuates oxidative stress in a tissue-engineered liver model of NAFLD by scavenging reactive oxygen species. *Nutrients* 14, 541. doi:10.3390/nu14030541

Gao, Y., Liu, J., Hao, Z., Sun, N., Guo, J., Zheng, X., et al. (2023). Baicalin ameliorates high fat diet-induced nonalcoholic fatty liver disease in mice via adenosine monophosphate-activated protein kinase-mediated regulation of SREBP1/Nrf2/NF-κB signaling pathways. *Phytother. Res.* 37, 2405–2418. doi:10.1002/ptr.7762

Geethangili, M., Lin, C.-W., Mersmann, H. J., and Ding, S. T. (2021). Methyl brevifolincarboxylate attenuates free fatty acid-induced lipid metabolism and inflammation in hepatocytes through AMPK/NF-κB signaling pathway. *Int. J. Mol. Sci.* 22, 10062. doi:10.3390/ijms221810062

Goldberg, R. B., Tripputi, M. T., Boyko, E. J., Budoff, M., Chen, Z. Z., Clark, J. M., et al. (2021). Hepatic fat in participants with and without incident diabetes in the diabetes prevention program outcome study. *J. Clin. Endocrinol. Metab.* 106, e4746–e4765. doi:10.1210/clinem/dgab160

Gordon, S., and Martinez, F. O. (2010). Alternative activation of macrophages: mechanism and functions. *Immunity* 32, 593–604. doi:10.1016/j.immuni.2010.05.007

Guan, X., Shen, S., Liu, J., Song, H., Chang, J., Mao, X., et al. (2023). Protective effecs of baicalin magnesium on non-alcoholic steatohepatitis rats are based on inhibiting NLRP3/Caspase-1/IL-1β signaling pathway. *BMC Complement. Med. Ther.* 23, 72. doi:10.1186/s12906-023-03903-2

Guo, H. X., Liu, D. H., Ma, Y., Liu, J. F., Wang, Y., Du, Du , et al. (2009). Long-term baicalin administration ameliorates metabolic disorders and hepatic steatosis in rats given a high-fat diet. *Acta Pharmacol Sin*, 30: 1505–1512.

Guo, X., Yin, X., Liu, Z., and Wang, J. (2022). Non-alcoholic fatty liver disease (NAFLD) pathogenesis and natural products for prevention and treatment. *Int. J. Mol. Sci.* 23, 15489. doi:10.3390/ijms232415489

Haas, J. T., Francque, S., and Physiology, B. S. J. A. R. O. (2015). Pathophysiology and mechanisms of nonalcoholic fatty liver disease. *Annu. Rev. Physiol.* 78: 181–205. doi:10.1146/annurev-physiol-021115-105331

Hai-Juan, Z., Hai-Yan, Z., Yun-Yi, Z., Yan, L., Hong, L., and Dao-Feng, C. (2019). *In vivo* effect of quantified flavonoids-enriched extract of Scutellaria baicalensis root on acute lung injury induced by influenza A virus. *Phytomedicine* 57 105–116. doi:10.1016/j.phymed.2018.12.009

Han, K., Bose, S., Wang, J. H., Lim, S. K., Chin, Y. W., Kim, Y. M., et al. (2017). *In vivo* therapeutic effect of combination treatment with metformin and Scutellaria baicalensis on maintaining bile acid homeostasis. *PLoS One* 12, e0182467. doi:10.1371/journal.pone.0182467

Hanley, A., Williams, K., Festa, A., Wagenknecht, L., D'Agostino, R. B., Jr, Kempf, J., et al. (2004). Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 53, 2623–2632. doi:10.2337/diabetes.53.10.2623

Hashmi, T. (2011). Probable hepatotoxicity associated with the use of metformin in type 2 diabetes. *BMJ Case Rep.* 2011, bcr0420114092. doi:10.1136/bcr.04.2011.4092

Hatton, G., Alterio, T., Nobili, V., and Mann, J. P. (2018). Unmet needs in pediatric NAFLD research: what do we need to prioritize for the future? *Expert Rev. Gastroenterol. Hepatol.* 12, 961–967. doi:10.1080/17474124.2018.1512853

Huang, D. Q., EL-Serag, H. B., and Gastroenterology, R. L. J. N. R. (2020). Global Epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 18: 223–238. doi:10.1038/s41575-020-00381-6

Hu, Q., Zhang, W., Wu, Z., Tian, X., Xiang, J., Li, L., et al. (2021). Baicalin and the liver-gut system: pharmacological bases explaining its therapeutic effects. *Pharmacol. Res.* 165, 105444. doi:10.1016/j.phrs.2021.105444

Jiang, D., Zhao, Z., Zhang, T., Zhong, W., Liu, C., Yuan, Q., et al. (2017). The chloroplast genome sequence of Scutellaria baicalensis provides insight into intraspecific and interspecific chloroplast genome diversity in Scutellaria. *Genes (Basel).* 8, 227. doi:10.3390/genes8090227

Ji, S., Li, R., Wang, Q., Miao, W. j., Li, Z. w., Si, L. l., et al. (2015). Anti-H1N1 virus, cytotoxic and Nrf2 activation activities of chemical constituents from Scutellaria baicalensis. *J. Ethnopharmacol.* 176, 475–484. doi:10.1016/j.jep.2015.11.018

Joshi, H. A., Patwardhan, R. S., Sharma, D., Sandur, S. K., and Devarajan, P. V. (2021). Pre-clinical evaluation of an innovative oral nano-formulation of baicalein for modulation of radiation responses. *Int. J. Pharm.* 595, 120181. doi:10.1016/j.ijpharm.2020.120181

Ju, M., Liu, Y., Li, M., Cheng, M., Zhang, Y., Deng, G., et al. (2019). Baicalin improves intestinal microecology and abnormal metabolism induced by high-fat diet. *Eur. J. Pharmacol.* 857, 172457. doi:10.1016/j.ejphar.2019.172457

Junior, L. A. I. Y. S., Nguyen, H. T., Salmanida, F. P., and Chang, K. T. (2021). MERTK(+/hi) M2c macrophages induced by baicalin alleviate non-alcoholic fatty liver disease. *Int. J. Mol. Sci.* 22, 10604. doi:10.3390/ijms221910604

Kang, M. J., Ko, G. S., Oh, D. G., Kim, J. S., Noh, K., Kang, W., et al. (2014). Role of metabolism by intestinal microbiota in pharmacokinetics of oral baicalin. *Arch. Pharm. Res.* 37, 371–378. doi:10.1007/s12272-013-0179-2

Kanwal, F., Shubrook, J. H., Adams, L. A., Pfotenhauer, K., Wai-Sun Wong, V., Wright, E., et al. (2021). Clinical care pathway for the risk stratification and management of patients with nonalcoholic fatty liver disease. *Gastroenterology* 161, 1657–1669. doi:10.1053/j.gastro.2021.07.049

Kazankov, K., JøRGENSEN, S. M. D., Thomsen, K. L., Møller, H. J., Vilstrup, H., George, H., et al. (2018). The role of macrophages in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Nat. Rev. Gastroenterol. Hepatol.* 16: 145.140–159. doi:10.1038/s41575-018-0082-x

Ke, M., Zhang, Z., Xu, B., Zhao, S., Ding, Y., Wu, X., et al. (2019). Baicalein and baicalin promote antitumor immunity by suppressing PD-L1 expression in hepatocellular carcinoma cells. *Int. Immunopharmacol.* 75, 105824. doi:10.1016/j.intimp.2019.105824

Klein, E. A., Thompson, I. M., JR., Tangen, C. M., Crowley, J. J., Lucia, M. S., Goodman, P. J., et al. (2011). Vitamin E and the risk of prostate cancer: the selenium and vitamin E cancer prevention trial (SELECT). *Jama* 306, 1549–1556. doi:10.1001/jama.2011.1437

Laveti, D., Kumar, M., Hemalatha, R., Sistla, R., Naidu, V. G. M., Talla, V., et al. (2013). Anti-inflammatory treatments for chronic diseases: a review. *Inflamm. Allergy Drug Targets* 12, 349–361. doi:10.2174/18715281113129990053

Leung, D. H., Williams, K., Fraley, J. K., and Klish, W. J. (2008). Age- and ethnic-specific elevation of ALT among obese children at risk for nonalcoholic steatohepatitis (NASH): implications for screening. *Clin. Pediatr.* 48, 50–57. doi:10.1177/0009922808321678

Liao, C. C., Day, Y. J., Lee, H. C., Liou, J. T., Chou, A. H., and Liu, F. C. (2016). Baicalin attenuates IL-17-mediated acetaminophen-induced liver injury in a mouse model. *PLoS One* 11, e0166856. doi:10.1371/journal.pone.0166856

Li, M., Shi, A., Pang, H., Xue, W., Li, Y., Cao, G., et al. (2014). Safety, tolerability, and pharmacokinetics of a single ascending dose of baicalein chewable tablets in healthy subjects. *J. Ethnopharmacol.* 156, 210–215. doi:10.1016/j.jep.2014.08.031

Li, P., Chen, Y., Ke, X., Zhang, R., Zuo, L., Wang, M., et al. (2022a). Baicalin ameliorates alcohol-induced hepatic steatosis by suppressing SREBP1c elicited PNPLA3 competitive binding to ATGL. *Arch. Biochem. Biophys.* 722, 109236. doi:10.1016/j.abb.2022.109236

Li, P., Zhang, R., Wang, M., Chen, Y., Chen, Z., Ke, X., et al. (2022b). Baicalein prevents fructose-induced hepatic steatosis in rats: in the regulation of fatty acid *de novo* synthesis, fatty acid elongation and fatty acid oxidation. *Front. Pharmacol.* 13, 917329. doi:10.3389/fphar.2022.917329

Liu, D.-H., Luo, Y.-H., Liang, J.-C., and Huang, Y. k. (2010). Baicalin regulates lipid metabolism in high-fat feeding SD rat liver and the mechanism involves modulation of AMPK-alpha pathway. *J. hepatology* 18: 711–712. doi:10.3760/cma.j.issn.1007-3418.2010.09.017

Liu, W. J., Chen, W. W., Chen, J. Y., Sun, Y. B., Chang, D., Wang, C. X., et al. (2023). Baicalin attenuated metabolic dysfunction-associated fatty liver disease by suppressing oxidative stress and inflammation via the p62-Keap1-Nrf2 signalling pathway in db/db mice. *Phytother. Res.* 39, 1663–1678. doi:10.1002/ptr.8010

Liu, J., Yuan, Y., Gong, X., Zhang, L., Zhou, Q., Wu, S., et al. (2020). Baicalin and its nanoliposomes ameliorates nonalcoholic fatty liver disease via suppression of TLR4 signaling cascade in mice. *Int. Immunopharmacol.* 80, 106208. doi:10.1016/j.intimp.2020.106208

Long, M. T., Noureddin, M., and Gastroenterology, J. K. L. J. (2022). AGA clinical practice update: diagnosis and management of nonalcoholic fatty liver disease in lean individuals. *Expert Rev.* 163, 764–774.e761. doi:10.1053/j.gastro.2022.06.023

Lu, Q., Zhou, L., Wang, Z., Li, X., Ding, L., Qiu, Y., et al. (2022). Baicalin alleviate apoptosis via PKC-MAPK pathway in porcine peritoneal mesothelial cells induced by glaesserella parasuis. *Molecules* 27, 5083. doi:10.3390/molecules27165083

Ma, M. Y., Niu, X. J., Wang, Q., Wang, S. M., Li, X., and Zhang, S. H. (2023). Evidence and possible mechanism of Scutellaria baicalensis and its bioactive compounds for hepatocellular carcinoma treatment. *Ann. Med.* 55, 2247004. doi:10.1080/07853890.2023.2247004

Malhotra, J. D., Miao, H., Zhang, K., Wolfson, A., Pennathur, S., Pipe, S. W., et al. (2008). Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. *Proc. Natl. Acad. Sci. U. S. A.* 105, 18525–18530. doi:10.1073/pnas.0809677105

Metabolizm, W. R. J. Ż. C. I. (2012). Non-alcoholic fatty liver disease - pathogenesis and management. 39: 309–319.

Miao, L., Zhang, X., Zhang, H., Cheong, M. S., Chen, X., Farag, M. A., et al. (2024). Baicalin ameliorates insulin resistance and regulates hepatic glucose metabolism via activating insulin signaling pathway in obese pre-diabetic mice. *Phytomedicine* 124, 155296. doi:10.1016/j.phymed.2023.155296

Miralles-Linares, F., Puerta-Fernandez, S., Bernal-Lopez, M. R., Tinahones, F. J., Andrade, R. J., and Gomez-Huelgas, R. (2012). Metformin-induced hepatotoxicity. *Diabetes Care* 35, e21. doi:10.2337/dc11-2306

Na, H.-Y., and Sciences, B.-C. L. J. I. J. O. M. (2019). Scutellaria baicalensis alleviates insulin resistance in diet-induced obese mice by modulating inflammation. *Int. J. Mol. Sci.* 20, 727. doi:10.3390/ijms20030727

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., et al. (2009). CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat. Med.* 15, 914–920. doi:10.1038/nm.1964

Nobili, V., Alisi, A., Newton, K. P., and Schwimmer, J. B. (2016). Comparison of the phenotype and approach to pediatric vs adult patients with nonalcoholic fatty liver disease. *Gastroenterology* 150, 1798–1810. doi:10.1053/j.gastro.2016.03.009

Noh, J. W., Kwon, O. J., and Lee, B. C. (2021). The immunomodulating effect of baicalin on inflammation and insulin resistance in high-fat-diet-induced obese mice. *Evid. Based Complement. Altern. Med.* 2021, 5531367. doi:10.1155/2021/5531367

Ohtani, N., Kamiya, T., and Kawada, N. (2023). Recent updates on the role of the gut-liver axis in the pathogenesis of NAFLD/NASH, HCC, and beyond. *Hepatol. Commun.* 7, e0241. doi:10.1097/HC9.0000000000000241

Orzechowska, B. U., Wrobel, G., Turlej, E., Jatczak, B., Sochocka, M., and Chaber, R. (2020). Antitumor effect of baicalin from the Scutellaria baicalensis radix extract in B-acute lymphoblastic leukemia with different chromosomal rearrangements. *Int. Immunopharmacol.* 79, 106114. doi:10.1016/j.intimp.2019.106114

Osborne, N., Leahy, C., Lee, Y.-K., Rote, P., Song, B. J., and Hardwick, J. P. (2022). CYP4V2 fatty acid omega hydroxylase, a druggable target for the treatment of metabolic associated fatty liver disease (MAFLD). *MAFLD* 195, 114841. doi:10.1016/j.bcp.2021.114841

Palma, R., Pronio, A., Romeo, M., Scognamiglio, F., Ventriglia, L., Ormando, V. M., et al. (2022). The role of insulin resistance in fueling NAFLD pathogenesis: from molecular mechanisms to clinical implications. *J. Clin. Med.* 11, 3649. doi:10.3390/jcm11133649

Pan, T. L., Wang, P. W., Huang, C. H., Leu, Y. L., Wu, T. H., Wu, Y. R., et al. (2015). Herbal formula, Scutellariae radix and Rhei rhizoma attenuate dimethylnitrosamine-induced liver fibrosis in a rat model. *Sci. Rep.* 5, 11734. doi:10.1038/srep11734

Park, C. H., Shin, M. R., An, B. K., Joh, H. W., Lee, J. C., Roh, S. S., et al. (2017). Heat-processed scutellariae radix protects hepatic inflammation through the amelioration of oxidative stress in lipopolysaccharide-induced mice. *Am. J. Chin. Med.* 45, 1233–1252. doi:10.1142/S0192415X17500689

Pierantonelli, I., and Transplantation, G. S.-B. J. (2019). Nonalcoholic fatty liver disease: basic pathogenetic mechanisms in the progression from NAFLD to NASH. *Transplantation* 103: e1-e13. doi:10.1097/TP.0000000000002480

Portillo-Sanchez, P., Bril, F., Lomonaco, R., Barb, D., Orsak, B., Bruder, J. M., et al. (2019). Effect of pioglitazone on bone mineral density in patients with nonalcoholic steatohepatitis: a 36-month clinical trial. *J. Diabetes* 11, 223–231. doi:10.1111/1753-0407.12833

Qiao, H., Han, H., Hong, D., Ren, Z., Chen, Y., and Zhou, C. (2011). Protective effects of baicalin on carbon tetrachloride induced liver injury by activating PPARγ and inhibiting TGFβ1. *Pharm. Biol.* 49, 38–45. doi:10.3109/13880209.2010.493179

Qu, W., Ma, T., Cai, J., Zhang, X., Zhang, P., She, Z., et al. (2021). Liver fibrosis and MAFLD: from molecular aspects to novel pharmacological strategies. *Front. Med. (Lausanne)* 8, 761538. doi:10.3389/fmed.2021.761538

Rada, P., GonzáLEZ-RodríGUEZ, Á., GarcíA-MonzóN, C., and M Valverde, Á. (2020). Understanding lipotoxicity in NAFLD pathogenesis: is CD36 a key driver? *Cell Death Dis.* 11 (9), 802. doi:10.1038/s41419-020-03003-w

Rives, C., Fougerat, A., Ellero-Simatos, S., Loiseau, N., Guillou, H., Gamet-Payrastre, L., et al. (2020). Oxidative stress in NAFLD: role of nutrients and food contaminants. *Biomolecules* 10, 1702. doi:10.3390/biom10121702

Samuel, V. T., Liu, Z.-X., Qu, X., Elder, B. D., Bilz, S., Befroy, D., et al. (2004). Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J. Biol. Chem.* 279, 32345–32353. doi:10.1074/jbc.M313478200

Semanticscholar, I. E. C. J. (2019). *Scutellaria baicalensis is useful in the treatment of cancer and liver disease*.

Shao, B. Z., Xu, Z. Q., Han, B. Z., Su, D. F., and Liu, C. (2015). NLRP3 inflammasome and its inhibitors: a review. *Front. Pharmacol.* 6, 262. doi:10.3389/fphar.2015.00262

Shi, L., Hao, Z., Zhang, S., Wei, M., Lu, B., Wang, Z., et al. (2018). Baicalein and baicalin alleviate acetaminophen-induced liver injury by activating Nrf2 antioxidative pathway: the involvement of ERK1/2 and PKC. *Biochem. Pharmacol.* 150, 9–23. doi:10.1016/j.bcp.2018.01.026

Shi, L., Zhang, S., Huang, Z., Hu, F., Zhang, T., Wei, M., et al. (2020). Baicalin promotes liver regeneration after acetaminophen-induced liver injury by inducing NLRP3 inflammasome activation. *Free Radic. Biol. Med.* 160, 163–177. doi:10.1016/j.freeradbiomed.2020.05.012

Shin, N. R., Gu, N., Choi, H. S., and Kim, H. (2020). Combined effects of Scutellaria baicalensis with metformin on glucose tolerance of patients with type 2 diabetes via gut microbiota modulation. *Am. J. Physiol. Endocrinol. Metab.* 318, E52–e61. doi:10.1152/ajpendo.00221.2019

Singal, A. G., Manjunath, H., Yopp, A. C., Beg, M. S., Marrero, J. A., Gopal, P., et al. (2014). The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *a meta-analysis* 109, 325–334. doi:10.1038/ajg.2013.476

Son, S. H., Kang, J., Ahn, M., Nam, S., Jung, Y. W., Lee, K. Y., et al. (2021). Synthesis and biochemical evaluation of baicalein prodrugs. *Pharmaceutics* 13, 1516. doi:10.3390/pharmaceutics13091516

Song, P., Tobe, R. G., Inagaki, Y., Kokudo, N., Hasegawa, K., Sugawara, Y., et al. (2012). The management of hepatocellular carcinoma around the world: a comparison of guidelines from 2001 to 2011. *Liver Int.* 32, 1053–1063. doi:10.1111/j.1478-3231.2012.02792.x

Stefan, N., HäRING, H.-U., and Cusi, K. (2019). Non-alcoholic fatty liver disease: causes. *Diagnosis, Cardiometabolic Consequences, Treat. Strategies* 7, 313–324. doi:10.1016/S2213-8587(18)30154-2

Sun, H., Che, Q. M., Zhao, X., and Pu, X. P. (2010). Antifibrotic effects of chronic baicalein administration in a CCl4 liver fibrosis model in rats. *Eur. J. Pharmacol.* 631, 53–60. doi:10.1016/j.ejphar.2010.01.002

Sun, W., Liu, P., Wang, T., Wang, X., Zheng, W., and Li, J. (2019). Baicalein reduces hepatic fat accumulation by activating AMPK in oleic acid-induced HepG2 cells and high-fat diet-induced non-insulin-resistant mice. *Food Funct.* 11, 711–721. doi:10.1039/c9fo02237f

Szabo, G., Gastroenterology, J. P. J. N. R., and Hepatology, H. (2015). Inflammasome activation and function in liver disease. *Nat. Rev. Gastroenterol. Hepatol.* 12: 387–400. doi:10.1038/nrgastro.2015.94

Tauil, R. B., Golono, P. T., DE Lima, E. P., de Alvares Goulart, R., Guiguer, E. L., Bechara, M. D., et al. (2024). Metabolic-associated fatty liver disease: the influence of oxidative stress, inflammation, mitochondrial dysfunctions, and the role of polyphenols. *Pharm. (Basel)* 17, 1354. doi:10.3390/ph17101354

Veracruz, N., Hameed, B., Saab, S., and Wong, R. J. (2021). The association between nonalcoholic fatty liver disease and risk of cardiovascular disease, stroke, and extrahepatic cancers. *Stroke, Extrahepatic Cancers* 11, 45–81. doi:10.1016/j.jceh.2020.04.018

Violi, F., and Cangemi, R. (2010). Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N. Engl. J. Med.* 363, 1185–1186. doi:10.1056/NEJMc1006581

Vozarova, B., Stefan, N., Lindsay, R., Saremi, A., Pratley, R. E., Bogardus, C., et al. (2002). High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51, 1889–1895. doi:10.2337/diabetes.51.6.1889

Wang, T., Jiang, H., Cao, S., Chen, Q., Cui, M., Wang, Z., et al. (2017). Baicalin and its metabolites suppresses gluconeogenesis through activation of AMPK or AKT in insulin resistant HepG-2 cells. *Eur. J. Med. Chem.* 141, 92–100. doi:10.1016/j.ejmech.2017.09.049

Wang, Y., Tai, Y.-L., Zhao, D., Zhang, Y., Yan, J., Kakiyama, G., et al. (2021). Berberine prevents disease progression of nonalcoholic steatohepatitis through modulating multiple pathways. *Cells* 10, 210. doi:10.3390/cells10020210

Wang, Y., Yang, J., Li, X., and Wang, J. (2012). The metabolism of baicalin in rat and the biological activities of the metabolites. *Evid. Based Complement. Altern. Med.* 2012, 404529. doi:10.1155/2012/404529

Wei, S., Zhang, M., Yu, Y., Lan, X., Yao, F., Yan, X., et al. (2016). Berberine attenuates development of the hepatic gluconeogenesis and lipid metabolism disorder in type 2 diabetic mice and in palmitate-incubated HepG2 cells through suppression of the HNF-4α Mir122 pathway. *PLoS One* 11, e0152097. doi:10.1371/journal.pone.0152097

Wei, Y., Guo, J., Zheng, X., Wu, J., Zhou, Y., Yu, Y., et al. (2014). Preparation, pharmacokinetics and biodistribution of baicalin-loaded liposomes. *Int. J. Nanomedicine* 9, 3623–3630. doi:10.2147/IJN.S66312

XI, Y. L., Li, H. X., Chen, C., Liu, Y. Q., Lv, H. M., Dong, S. Q., et al. (2016). Baicalin attenuates high fat diet-induced insulin resistance and ectopic fat storage in skeletal muscle, through modulating the protein kinase B/Glycogen synthase kinase 3 beta pathway. *Chin. J. Nat. Med.* 14, 48–55. doi:10.3724/SP.J.1009.2016.00048

Xiang, H., Lei, H., Liu, Z., Liu, Y., Li, Y., Qiu, Y., et al. (2021). Network pharmacology and molecular docking analysis on molecular targets: mechanisms of baicalin and baicalein against hyperuricemic nephropathy. *Toxicol. Appl. Pharmacol.* 424, 115594. doi:10.1016/j.taap.2021.115594

Xiang, Y., Long, Y., Yang, Q., Zheng, C., Cui, M., Ci, Z., et al. (2020). Pharmacokinetics, pharmacodynamics and toxicity of Baicalin liposome on cerebral ischemia reperfusion injury rats via intranasal administration. *Brain Res.* 1726, 146503. doi:10.1016/j.brainres.2019.146503

Xin, H. G., Zhang, B. B., Wu, Z. Q., Hang, X. F., Xu, W. S., Ni, W., et al. (2014). Treatment with baicalein attenuates methionine-choline deficient diet-induced non-alcoholic steatohepatitis in rats. *Eur. J. Pharmacol.* 738, 310–318. doi:10.1016/j.ejphar.2014.06.006

Xu, W., Niu, Y., Ai, X., Xia, C., Geng, P., Zhu, H., et al. (2022). Liver-targeted nanoparticles facilitate the bioavailability and anti-HBV efficacy of baicalin *in vitro* and *in vivo*. *Biomedicines* 10, 900. doi:10.3390/biomedicines10040900

Yan, B. F., Wang, Y., Wang, W. B., Ding, X. J., Wei, B., Liu, S. J., et al. (2023). Huangqin decoction mitigates hepatic inflammation in high-fat diet-challenged rats by inhibiting TLR4/NF-κB/NLRP3 pathway. *J. Ethnopharmacol.* 303, 115999. doi:10.1016/j.jep.2022.115999

Yang, J., Xiang, D., Xiang, D., He, W., Liu, Y., Lan, L., et al. (2020). Baicalin protects against 17α-ethinylestradiol-induced cholestasis via the sirtuin 1/hepatic nuclear receptor-1α/farnesoid X receptor pathway. *Front. Pharmacol.* 10, 1685. doi:10.3389/fphar.2019.01685

Yan, P., Liu, J., Huang, Y., Yi, T., Zhang, H., Dai, G., et al. (2024). Baicalin enhances antioxidant, inflammatory defense, and microbial diversity of yellow catfish (*Pelteobagrus fulvidraco*) infected with Aeromonas hydrophila. *Front. Microbiol.* 15, 1465346. doi:10.3389/fmicb.2024.1465346

Yan, X., Zhang, Y., Peng, Y., and Li, X. (2022). The water extract of Radix scutellariae, its total flavonoids and baicalin inhibited CYP7A1 expression, improved bile acid, and glycolipid metabolism in T2DM mice. *J. Ethnopharmacol.* 293, 115238. doi:10.1016/j.jep.2022.115238

Yoon, E.-J., Lee, M. Y., Choi, B. I., Lim, K. J., Hong, S. Y., and Park, D. (2020). Pharmaceutical advantages of GenoTX-407, A combination of extracts from Scutellaria baicalensis root and magnolia officinalis bark. *Antioxidants (Basel).* 9, 1111. doi:10.3390/antiox9111111

You, Y. P., Yan, L., Ke, H. Y., Li, Y. P., Shi, Z. J., Zhou, Z. Y., et al. (2024). Baicalin inhibits PANoptosis by blocking mitochondrial Z-DNA formation and ZBP1-PANoptosome assembly in macrophages. *Acta Pharmacol. Sin.* 46, 430–447. doi:10.1038/s41401-024-01376-8

Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., and Wymer, M. (2016). Global Epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 64: 73–84. doi:10.1002/hep.28431

Younossi, Z. M., Otgonsuren, M., Henry, L., Venkatesan, C., Mishra, A., Erario, M., et al. (2015). Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 62, 1723–1730. doi:10.1002/hep.28123

Younossi, Z., Anstee, Q. M., Marietti, M., Hardy, T., Henry, L., Eslam, M., et al. (2018). Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 15, 11–20. doi:10.1038/nrgastro.2017.109

Yu, C. L., Yang, S. F., Hung, T. W., Lin, C. L., Hsieh, Y. H., and Chiou, H. L. (2019). Inhibition of eIF2α dephosphorylation accelerates pterostilbene-induced cell death in human hepatocellular carcinoma cells in an ER stress and autophagy-dependent manner. *Cell Death Dis.* 10, 418. doi:10.1038/s41419-019-1639-5

Zaghloul, R. A., Zaghloul, A. M., and EL-Kashef, D. H. (2022). Hepatoprotective effect of Baicalin against thioacetamide-induced cirrhosis in rats: targeting NOX4/NF-κB/NLRP3 inflammasome signaling pathways. *Life Sci.* 295, 120410. doi:10.1016/j.lfs.2022.120410

Zhang, L., Li, C., Lin, G., Krajcsi, P., and Zuo, Z. (2011). Hepatic metabolism and disposition of baicalein via the coupling of conjugation enzymes and transporters-*in vitro* and *in vivo* evidences. *Aaps J.* 13, 378–389. doi:10.1208/s12248-011-9277-6

Zhang, Z., Ma, G., Xue, C., Sun, H., Wang, Z., Xiang, X., et al. (2019). Establishment of rat liver microsome-hydrogel system for *in vitro* phase II metabolism and its application to study pharmacological effects of UGT substrates. *Drug Metab. Pharmacokinet.* 34, 141–147. doi:10.1016/j.dmpk.2019.01.005

Zhao, X. Y., Xiong, X., Liu, T., Mi, L., Peng, X., Rui, C., et al. (2018). Long noncoding RNA licensing of obesity-linked hepatic lipogenesis and NAFLD pathogenesis. *Nat. Commun.* 9, 2986. doi:10.1038/s41467-018-05383-2

Zhao, L., Wei, Y., Huang, Y., He, B., Zhou, Y., and Fu, J. (2013). Nanoemulsion improves the oral bioavailability of baicalin in rats: *in vitro* and *in vivo* evaluation. *Int. J. Nanomedicine* 8, 3769–3779. doi:10.2147/IJN.S51578

Zhao, Q., Zhang, Y., Wang, G., Hill, L., Weng, J-K., Chen, X-Y., et al. (2016). A specialized flavone biosynthetic pathway has evolved in the medicinal plant. *Scutellaria Baicalensis.* doi:10.1126/sciadv.1501780

Zhao, S., Huang, M., Yan, L., Zhang, H., Shi, C., and Liu, J. (2022). Exosomes derived from baicalin-pretreated mesenchymal stem cells alleviate hepatocyte ferroptosis after acute liver injury via the keap1-NRF2 pathway. *Oxid. Med. Cell Longev.* 2022, 8287227. doi:10.1155/2022/8287227

Zhao, W., Liu, L., Wang, Y., Mao, T., and Li, J. (2016). Effects of a combination of puerarin, baicalin and berberine on the expression of proliferator-activated receptor-γ and insulin receptor in a rat model of nonalcoholic fatty liver disease. *Exp. Ther. Med.* 11, 183–190. doi:10.3892/etm.2015.2846

Zhi, H., Jin, X., Zhu, H., Li, H., Zhang, Y., Lu, Y., et al. (2019). Exploring the effective materials of flavonoids-enriched extract from Scutellaria baicalensis roots based on the metabolic activation in influenza A virus induced acute lung injury. *Injury* 177, 112876. doi:10.1016/j.jpba.2019.112876

Zhong, X., and Liu, H. (2018). Baicalin attenuates diet induced nonalcoholic steatohepatitis by inhibiting inflammation and oxidative stress via suppressing JNK signaling pathways. *Biomed. Pharmacother.* 98, 111–117. doi:10.1016/j.biopha.2017.12.026

Zhou, Y., Wang, L., Gong, Z. P., Li, Y. T., Jin, Y., Zheng, L., et al. (2024). Comparative study on excretion of Shuganning Injection and Scutellariae Radix extract in bile, urine, and feces of rats. *Zhongguo Zhong Yao Za Zhi* 49, 809–818. doi:10.19540/j.cnki.cjcmm.20231106.201

Zhu, H., Qian, Z., He, F., Liu, M., Pan, L., Zhang, Q., et al. (2013). Novel pharmacokinetic studies of the Chinese formula Huang-Lian-Jie-Du-Tang in MCAO rats. *Phytomedicine* 20, 767–774. doi:10.1016/j.phymed.2012.11.012

Zhu, W., Jin, Z., Yu, J., Liang, J., Yang, Q., Li, F., et al. (2016). Baicalin ameliorates experimental inflammatory bowel disease through polarization of macrophages to an M2 phenotype. *Int. Immunopharmacol.* 35, 119–126. doi:10.1016/j.intimp.2016.03.030

Zou, J., Yan, C., and Wan, J. B. (2022). Red yeast rice ameliorates non-alcoholic fatty liver disease through inhibiting lipid synthesis and NF-κB/NLRP3 inflammasome-mediated hepatic inflammation in mice. *Chin. Med.* 17, 17. doi:10.1186/s13020-022-00573-z

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Targeting the gut microbiota and lipid metabolism: potential mechanisms of natural products for the treatment of non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is a chronic progressive liver disease with overnutrition and insulin resistance (IR) as the main etiologic factors. Hepatic lipid accumulation is a central factor contributing to this cascade of changes. Consequently, therapeutic interventions that target hepatic lipid metabolism and inflammatory response pathways hold considerable promise for the treatment of NAFLD. Furthermore, there is a close link between the gut microbiota (GM) and host health. GM and its metabolites can rely on multiple complex pathways to be deeply involved in the occurrence and development of NAFLD, which is associated with a variety of mechanisms. This makes it difficult to achieve satisfactory therapeutic efficacy of drugs targeting a single specific mechanism. In this context, natural products have the advantage of intervening in multiple targets and high safety. Consequently, an increasing number of researchers are considering natural products as a potential breakthrough point for the treatment of NAFLD. Notably, natural products influence intestinal mucosal permeability and metabolite production by regulating the abundance of beneficial flora in GM, which in turn regulates lipid metabolism to reduce hepatic steatosis and inhibit the progression of NAFLD. This paper reviews the research progress of natural products intervening in NAFLD through GM and its metabolites and lipid metabolism that has emerged in recent years, aiming to provide a basis for future natural product interventions in NAFLD.

KEYWORDS

gut microbiota, lipid metabolism, natural products, NAFLD, probiotics

1 Introduction

Non-alcoholic fatty liver disease (NAFLD), also known as metabolic dysfunction-associated fatty liver disease, MAFLD, is a term that has undergone a name change that has been advocated by multiple societies, led by the American Association for the Study of Liver Diseases, in 2023 (Rinella et al., 2023). This nomenclature change remains contentious due to its exclusion of patients with alcohol consumption, which is a

significant proportion of individuals affected by fatty liver disease (Kim et al., 2023; Kokkorakis et al., 2023). This article still uses the old name, NAFLD.

The incidence of NAFLD exhibits geographical variation. Current global estimates posit that NAFLD affects 32.4% of the global population, with an escalating prevalence that is of significant concern on an annual basis (Riazi et al., 2022). NAFLD has the potential to progress to other liver diseases, such as NASH and irreversible liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Additionally, it is closely related to cardiovascular and cerebrovascular diseases, metabolic syndrome, as well as chronic kidney disease (CKD) and a high incidence of extrahepatic malignancies (Thomas et al., 2024). This has a significant impact on the quality of life and long-term health of patients, and also places a considerable burden on the global healthcare system, suggesting the need for early intervention in NAFLD.

At present, Resmetirom is the only drug that has been approved by the FDA for the treatment of NASH, and it is notable that it can cause adverse effects (Keam, 2024). Concurrently, other clinical first-line drugs, such as SGLT-2 inhibitors, PPAR-γ agonists, GLP-1R agonists, and statins, while correcting the metabolic dysfunctions associated with NAFLD progression, also induce adverse effects including genitourinary infections, gastrointestinal reactions, worsening of heart failure, and osteoporosis (Hameed et al., 2023; Park et al., 2023; Wang Z. et al., 2023; Yang T. et al., 2024). In contrast, the therapeutic effects of vitamin E have been observed to be effective only in specific patient populations, including those possessing genetic variants of haptoglobin as well as genotypes of fatty acid desaturase 1/2 (FADS1/FADS2) (Banini et al., 2019). These approaches are insufficient to treat the increasing number of patients with NAFLD, and the urgent need exists to identify other effective therapeutic avenues.

Gut microbiota (GM) represents one of the most substantial microbial reservoirs within the human body (Pouwels et al., 2022), comprising approximately 10–100 trillion microorganisms in the gut of a typical adult (Younossi et al., 2018). These microorganisms play important roles in the processes of digestion and the maintenance of homeostasis of glucose/lipid metabolism (Paternostro and Trauner, 2022; Tilg et al., 2021). Dysregulation of GM has been demonstrated to result in disorders of glucose/lipid metabolism, inducing insulin resistance (IR) within the body, leading to abnormalities in fatty acids (FAs), triglyceride (TG), and cholesterol (TC), and causing hepatic steatosis. The metabolites of GM, such as bile acids (BAs), Short-chain fatty acids (SCFAs) and Trimethylamine N-oxide (TMAO), have been shown to be closely related to the energy metabolism of the organism (Caussy and Loomba, 2018). It is imperative to emphasise the significance of GM in the treatment of NAFLD. Traditional Chinese medicine (TCM) boasts numerous advantages, including multiple pathways of action, abundant targets, and low toxicity. TCM has demonstrated excellent potential in the treatment of NAFLD (Ji et al., 2022; Tan et al., 2023). Nevertheless, the absence of a definitive therapeutic mechanism hinders the advancement of TCM therapy for NAFLD. The exploration of natural products as a means to regulate lipid metabolism and intervene in NAFLD through GM and its metabolites is a promising avenue for further research.

2 Non-alcoholic fatty liver disease and dysfunctional lipid metabolism

The "multiple-hit" theory (Buzzetti et al., 2016) has gained widespread acceptance as the pathogenesis of NAFLD, proposing that the condition arises from the synergistic effect of environmental, dietary, lifestyle, epigenetic and other factors in individuals with a genetic predisposition (Juanola et al., 2021). The pathogenesis of NAFLD is the result of a combination of factors, but lipid metabolism disorders are still the core of NAFLD, and the liver, as an important lipid metabolising organ, greatly influences the lipid homeostasis in the organism (Böhm et al., 2013).

It is imperative to acknowledge the pivotal role of the balance between the rate of FAs accumulation and FAs degradation by hepatocytes in maintaining the low-fat state of the liver. The aforementioned balance encompasses the uptake of peripheral circulating free fatty acid (FFA), *de novo* lipogenesis (DNL), fatty acid oxidation (FAO), and entry into the bloodstream in the form of very low-density lipoproteins (V-LDL). These elements serve as the cornerstones for ensuring the balance of hepatic lipid metabolism (Paul et al., 2022). Conversely, an excess of FAs within hepatocytes leads to TG accumulation, which is a primary contributor to NAFLD (Santos-Baez and Ginsberg, 2021). A stable isotope tracer study (Tiwari and Siddiqi, 2012) demonstrated that the majority of TG accumulated in NAFLD (approximately 59%) originates from FFA produced by adipose tissue breakdown. Another significant source (Donnelly et al., 2005) is DNL synthesis (approximately 26.1%), and the remaining amount is derived from dietary intake (approximately 14.9%). This comprehensive analysis underscores the predominant role of FFA uptake from the circulation, along with NAL, as the pivotal source of TG accumulation within hepatocytes. As shown in Figure 1.

2.1 Key transporter proteins for free fatty acid uptake by the liver

Adipose tissue is the most significant TG storage site in the body. Stimulation of adipose tissue lipolysis to FAs results in entry into the peripheral circulation (Griffin et al., 2023). Hepatocytes rely on the uptake of FFA, with the uptake process mediated by fatty acid transporter proteins (FATP), fatty acid binding proteins (FABP), and human leukocyte differentiation antigen (CD36) (Canbay et al., 2007).

2.1.1 Fatty acid transportation protein

Fatty acid transporter protein (FATP) is a class of transmembrane transporter proteins that are primarily responsible for transporting long-chain fatty acids from the extracellular to the intracellular environment. FATP2/5 is the isoform of FATP distributed on the mammalian liver and is responsible for the uptake of extracellular FFA in the hepatocytes. It was found that knockdown of *Fatp2* in mice reduced the ability of hepatocytes to uptake peripheral circulating FFA by 40%, and *Fatp2*−/− mice did not develop hepatic steatosis compared to normal mice that also consumed high-fat diet (HFD) and already had developed NAFLD (Falcon et al., 2010).

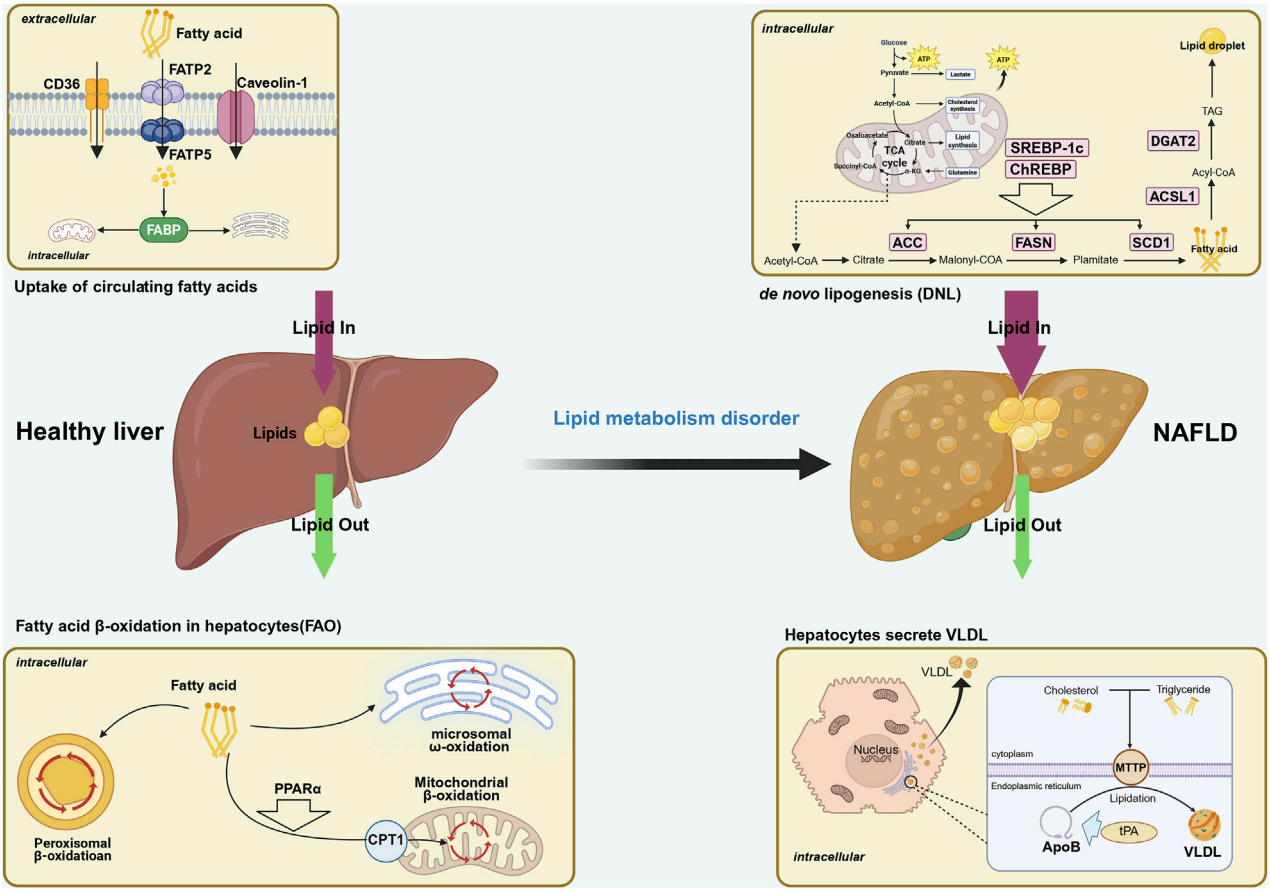

FIGURE 1
The thickness of the arrow represents the quantity in this figure. Possible mechanisms of the role of fatty acid metabolism in the development of NAFLD. Fatty acid metabolism is one of the important links in liver lipid metabolism, and its balance affects the progression of NAFLD. Fatty acid metabolism includes the uptake of circulating FFA, *de novo* lipogenesis (DNL), fatty acid β-oxidation (FAO), and the release of FFAs into the bloodstream in the form of very low-density lipoproteins (V-LDL). The first two increase the amount of fatty acids in the liver, while the latter two consume the amount of fatty acids in hepatocytes. When the increase in fatty acids is greater than the consumption, it will drive the development of NAFLD. NAFLD, non-alcoholic fatty liver disease; FATP, fatty acid transport protein; FABP, fatty acid-binding proteins; CAV-1, Caveolin-1; CD36, cluster of differentiation 36; FAO, fatty acid oxidation; TCA, tricarboxylic acid; FAS, fatty acid synthesis; DNL, *de novo* lipogenesis; ACC, acetyl-CoA carboxylase; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase1; Dgat2, diacylgycerol acyltransferase; ACSL, acyl-CoA synthetaselong chain family member; PPARα, peroxisomeproliferator-activated receptor alpha; CPT1, carnitine palmitoyltransferase 1; MTTP, microsomal triglyceride transfer protein; tPA, tissue plasminogen activator; VLDL, very low density lipoprotein.

Surprisingly, the knockdown of *Fatp5* also led to a reduction in the intrahepatic TG content, which was characterised by a decrease in the ability of hepatocytes to uptake long-chain FA and the activation of the NAL pathway (Doege et al., 2006). In comparison to normal subjects, FATP5 expression is notably elevated in the liver of NASH patients (Enooku et al., 2020), indicating that elevated FATP expression contributes to an increase in intrahepatic FA content, thereby promoting steatosis.

2.1.2 Fatty acid binding protein

Fatty acid binding protein (FABP) is present in the liver in the form of Liver FABP (L-FABP), which functions to transport lipotoxic FFA to the mitochondria to participate in FAO or to the endoplasmic reticulum to participate in TG synthesis. This process serves to reduce the damage to hepatocytes caused by lipotoxicity (Spann et al., 2006). In the absence of *L-fabp*, a significant accumulation of lipids has been observed in the liver of mice (Martin et al., 2015). NAFLD patients exhibit elevated L-FABP expression levels, which gradually decline as the condition progresses, thereby diminishing the liver cells' capacity to resist lipotoxicity. In comparison with the general population, NAFLD patients exhibit high specificity and sensitivity of L-FABP in the serum. Consequently, L-FABP in the serum has emerged as a promising diagnostic marker for NAFLD (Akbal et al., 2016). A positive correlation has been observed between the serum L-FABP levels and various indicators of NAFLD severity (Özenirler et al., 2013). This phenomenon may be attributed to L-FABP's capacity to influence FAs metabolism through peroxisomeproliferator-activated receptor α (PPARα) (Pawlak et al., 2015) and expedite the progression of NAFLD by promoting steatosis and activating hepatic stellate cells (HSC) (Newberry et al., 2012; Chen et al., 2013). Research has indicated that serum L-FABP levels can serve as a marker of liver cell damage in patients with NAFLD (Tanoglu and Beyazit, 2016; Lu et al., 2020). Notably, serum L-FABP levels have also been shown to predict survival rates across various stages of chronic liver disease, including hepatitis, cirrhosis, and

hepatocellular carcinoma (Eguchi and Iwasa, 2021). Furthermore, these levels have been observed to reflect the prognosis of hepatocellular carcinoma (HCC) of diverse etiologies (Eguchi et al., 2019).

2.1.3 Cluster of differentiation 36

Cluster of differentiation 36 (CD36) is a translocase enzyme (FAT) that primarily facilitates the uptake of long-chain FAs. Under normal circumstances, CD36 is expressed at low levels in the liver (Su and Abumrad, 2009). However, an environment with high fat content has been observed to induce high expression of CD36 in the cytoplasm of liver cells. This expression is not only increased but also driven from the cytoplasm to the cell membrane (Chabowski et al., 2013). This, in turn, has been shown to exacerbate FAs metabolic disorders and induce liver inflammation (Zhao et al., 2018). CD36 serves as a crucial link between FAs and long-chain acyl-CoA synthetase (ACSL) (Zhao et al., 2018). Inhibiting CD36 palmitoylation has been shown to drive FAT localization in the mitochondria, thereby promoting fatty acid oxidation. HFD has been observed to increase CD36 palmitoylation in the liver of mice, which in turn reduces the transport of FAs to ACSL1, leading to increased lipid accumulation (Zeng S. et al., 2022). This underscores the notion that the inhibition of CD36 palmitoylation may serve as a therapeutic strategy to delay the progression of NAFLD. Additionally, obesity has been found to be closely associated with CD36. Ob/ob mice exhibit elevated CD36 protein levels in their livers (Nassir et al., 2013), and the CD36 content in the livers of patients with grade III obesity (BMI ≥35) is positively associated with liver fat content (Greco et al., 2008). Research studies have demonstrated that the amount of CD36 in the liver cells of NAFLD patients is higher than that observed in normal individuals. Furthermore, the expression of CD36 in the liver can enhance the uptake of FFA by liver cells, thereby leading to TG accumulation (Sheedfar et al., 2014; Zhang et al., 2018). Notably, the study (Zhong et al., 2017) revealed that the absence of *Cd36* does not impact the liver's capacity for FFA uptake in murine models. Cardiomyocytes from subjects with *CD36* gene defects exhibited a complete loss of FFA uptake capacity due to the gene defect, while the uptake potential of liver cells was augmented (Yamashita et al., 2007). This evidence suggests that CD36 can drive the development and progression of NAFLD; however, the uptake of FFA by liver cells does not rely on CD36. CD36 is present in the peripheral circulation in the form of soluble CD36 (sCD36). Research studies (Handberg et al., 2012; Petta et al., 2013) have demonstrated that sCD36 can serve as a marker for the progression of fatty degeneration in the liver. The study (Rada et al., 2020) initially demonstrated that the plasma concentration of sCD36 can sensitively reflect the expression level of CD36 in the liver. Furthermore, an experiment using magnetic resonance spectroscopy to measure liver fat content (Heebøll et al., 2017) found that the concentration of circulating sCD36 was closely related to the level of intrahepatic lipids in NAFLD. Consequently, sCD36 in the blood emerges as a highly sensitive indicator of the severity of hepatocellular steatosis in patients with NAFLD.

2.1.4 Caveolin-1

Caveolin-1 (CAV-1) is a structural protein of the caveolae (Jiang et al., 2023), which is involved in lipid metabolism by specifically binding to signalling molecules (Fernandes and Oliveira-Brett, 2020). Upregulation of CAV-1 expression effectively reduced TG levels in the peripheral circulation of a rat model of HFD and decreased lipid deposition in the liver, alleviating the progression of NAFLD (Deng et al., 2024). The mechanism by which CAV-1 interferes with hepatic lipid metabolism is not yet fully defined, but significant progress has been made in this area. Disturbed iron metabolism has been identified as a significant contributor to hepatocyte death in NAFLD, where the accumulation of Fe^{2+} within the cells results in the generation of substantial amounts of Reactive Oxygen Species (ROS) via the Fenton reaction, thereby initiating cell death (Teschke, 2022). CAV-1 activates the hepatocyte FTL/FTH pathway and drives the conversion of Fe^{2+} to Fe^{3+}, which in turn inhibits oxidative stress in hepatocytes and ultimately alleviates liver injury during the course of NAFLD (Deng et al., 2023). CAV-1 inhibited the Akt/mtH pathway in the hepatocytes, and finally alleviated liver injury in the course of NAFLD. CAV-1 inhibited Akt/mtD, which was the most important factor in the metabolism of iron. CAV-1 has been shown to inhibit the Akt/mTOR pathway, thereby inducing lipid autophagy in NAFLD (Xue et al., 2020). In addition, the levels of Pink-1/Parkin content and autophagy-related proteins (LC3-II/I and Beclin-1) exhibited a positive correlation with CAV-1, while SREBP-1c content demonstrated a negative correlation with CAV-1 (Jiang et al., 2021). Upregulation of CAV-1 effectively activated the Pink-1/Parkin pathway-mediated mitochondrial autophagy, thereby inhibiting SREBP-1c expression and reducing cellular lipid accumulation. Researchers (Ding et al., 2018) successfully transfected plasmids overexpressing CAV-1 into HepG2 cells, thereby inducing an increase in intracellular TC efflux. Furthermore, *Cav-1* gene expression was found to be positively correlated with aortic endothelial cell ABCA1 levels, and negatively correlated with the level of cholesterol efflux from the aortic endothelial cells (Lin et al., 2007). This finding indicates that CAV-1 also affects cellular lipid metabolism by interfering with ABCA1 expression.

2.2 Hepatic *de novo* lipogenesis

Hepatic *de novo* lipogenesis (DNL) is another key mechanism for maintaining FA homeostasis in hepatocytes (Zeng H. et al., 2022), converting alternative carbon sources to FA through numerous enzymatic reactions, which are esterified and then stored in the liver as TG (Batchuluun et al., 2022). Typically, 2%–5% of the total amount of TG synthesized by the liver is derived from DNL (Diraison et al., 2003), and a high-carbon-water diet, obesity, and hyperinsulinemia increase this value to the 25%–30% range (Mk et al., 1993; Diraison et al., 1997; Siler et al., 1999), whereas starvation inhibits the DNL pathway (Cross et al., 2023). Consequently, the degree of DNL activity is closely related to the nutritional status of the organism. The study (Donnelly et al., 2005) utilised isotopes to examine the source of TG in the livers of patients with NAFLD, and found that 26% of the TG originated from the DNL pathway. The DNL pathway involves the conversion of acetyl-coenzyme A and malonyl-coenzyme A into fatty acids through a series of enzymatic reactions, including DNL, elongation, desaturation, and esterification (Hellerstein et al., 1996). Each step in the pathway is catalysed by specific enzymes, with the main enzymes responsible for *ab initio* synthesis being acetyl

coenzyme A carboxylase (ACC) and fatty acid synthase (FAs) (Yue et al., 2018). Stearoyl coenzyme A desaturase 1 (SCD1) is the regulatory enzyme for lengthening and desaturation (Zheng et al., 2021), while diacylglycerol acyltransferase (DGAT) and long-chain acetyl coenzyme A synthase 1 (ACSL1) are the regulatory enzymes for the esterification step (Filali-Mouncef et al., 2022). The process is primarily regulated by two key transcription factors, sterol regulatory element binding protein 1c (SREBP 1c) and carbohydrate regulatory element binding protein (ChREBP) (Linden et al., 2018), which are induced by insulin and glucose, respectively (Kawano and Cohen, 2013; Oosterveer and Schoonjans, 2014). Consequently, the present study aimed to review the effects of the DNL pathway on hepatic lipid metabolism, with a view to exploring clinical strategies for treating NAFLD by interfering with NAL.

2.2.1 Key transcription factors in *de novo* lipogenesis

2.2.1.1 SREBP-1c

SREBP-1c is one of the three SREBP isoforms (1a, 1c, 2) present in mammals (Eberlé et al., 2004). SREBP-1c is predominantly found in the liver and is exclusively responsible for the regulation of hepatic FA synthesis (Shimano and Sato, 2017). SREBP cleavage-activating protein (SCAP) is essential for activating the transcriptional activity of *Srebp* (Matsuda et al., 2001). Researchers (Horton et al., 2003) found that disrupting the transcriptional activity of SREBP by knocking out the *Scap* gene resulted in a near loss of lipid synthesis in mouse liver. The study (Jiang et al., 2022) exploited the fact that 25-hydroxyalcohol (25-HL) has a greater ability to sequester SCAP-SREBP, and by binding to insulin-inducible gene (INSIG) proteins, induced the coupling of INSIG to SCAP, resulting in the SREBP retention in the endoplasmic reticulum and inability to activate it, which in turn inhibits hepatic lipogenesis. These findings underscore the pivotal role of SREBP in hepatic lipid synthesis. As a member of the SREBP isoforms primarily implicated in hepatic FA synthesis, SREBP-1c activates the transcription of ACC1, FAS, and SCD1, thereby stimulating the DNL pathway and leading to the production of substantial quantities of FA, resulting in hepatic steatosis (Choi et al., 2014). SREBP-1c is closely associated with the progression of hepatic NAFLD (Badmus et al., 2022). SREBP-1c overexpression has been demonstrated to trigger hepatocyte lipid accumulation (Shimano et al., 1997). The hepatic deletion of SREBP-1c protein in ob/ob mice resulted in a 50% decrease in intrahepatic TG (Moon et al., 2012). Downregulation of SREBP-1c levels in mice by using antisense oligonucleotides was effective in reversing hepatic steatosis induced by HFD (Vitto et al., 2012). In addition, patatin-like phospholipase structural domain protein 3 (PNPLA3), which is closely related to NAFLD, can contribute to hepatic steatosis in several ways (Ericson et al., 2022). SREBP-1c upregulates the increased expression of the *Pnpla3* gene by binding to the PNPLA3 promoter, which in turn promotes lipid accumulation in the liver (Qiao et al., 2011). Furthermore, endoplasmic reticulum stress has been demonstrated to activate SREBP-1c (Ferré et al., 2021). The activator of transcription factor 6 (AFT6), the principal sensor of endoplasmic reticulum stress, exhibits analogous activation conditions to SREBP-1 (Ye et al., 2000). During endoplasmic reticulum stress, activated AFT6 activates SREBP-1c via the PERK-IRE1-eIF2α-ATF6 pathway, which in turn drives hepatocyte steatosis (Lee et al., 2012; Röhrl et al., 2014).

2.2.1.2 Carbohydrate regulatory element binding protein

Carbohydrate regulatory element binding protein (ChREBP) is a major regulator of DNL in the liver and is involved in glycolysis (Ishii et al., 2004), regulating the conversion of glucose to FA via the DNL pathway (Postic et al., 2007). ChREBP acts as a major glucose-responsive transcription factor (Yamashita et al., 2001), and high glucose status promotes the translocation of ChREBP into the nucleus and increases transcriptional activity (Li et al., 2006). It has been established that glucose, fructose, and even glucose derivatives (Iizuka et al., 2004) activate ChREBP expression. In turn, ChREBP is able to activate the expression of enzyme genes associated with DNL, such as ACC, FAS, and SCD1, thereby promoting lipid synthesis in the liver (Ishii et al., 2004). In addition, ChREBP is also involved in the maintenance of glucose homeostasis. To test the hypothesis that ChREBP deficiency causes a decrease in insulin sensitivity, researchers used a hyperinsulinemic euglycemic clamp to test insulin sensitivity in Liver-*Chrebp* KO mice (Jois et al., 2017). They found that only a reduction in exogenous glucose input ensured that the mice had blood glucose at basal levels, suggesting that *Chrebp* deficiency caused a decrease in insulin sensitivity.

Furthermore, the selective knockdown of *Chrebp* in hepatocytes of ob/ob mice significantly reduces lipid accumulation in hepatocytes and alleviates TG and FFA levels in the peripheral circulation (Dentin et al., 2006). This finding suggests that *CHREBP* knockdown is effective in reducing hepatic lipid accumulation by the DNL pathway. However, it should be noted that this does not necessarily imply that knockdown of *Chrebp* alone is beneficial to the organism. ChREBP also affects fibroblast growth hormone 21 (FGF21) expression in the liver (Iizuka et al., 2009).

The latter has been demonstrated to inhibit the body's sweet taste preference as well as sugar intake by acting on glutamatergic neurons in the ventral medial hypothalamus (Jensen-Cody et al., 2020). Moreover, ChREBP has been shown to promote the ubiquitination and subsequent degradation of nSREBP2, which in turn inhibits the biosynthesis of TC (Luo et al., 2020). ChREBP, a major component of the DNL pathway, which is responsible for the conversion of sugars into fats, is involved in a number of complex biological activities. While the knockdown of *Chrebp* can reduce the FA generated by the DNL pathway, it can also lead to other problems, indicating that direct inhibition/knockdown of *Chrebp* is not an effective solution to hepatic lipid accumulation.

2.2.2 Redirected synthesis of important regulatory enzymes

2.2.2.1 Acetyl coenzyme a carboxylase

Acetyl coenzyme A carboxylase (ACC) is the rate-limiting step in FA anabolism (Wang et al., 2022) and is biologically dependent (Packman and Whitney, 1990). Two isoforms of ACC have been identified in humans: ACC1 and ACC2 (Brownsey et al., 2006). The most significant difference between them is that ACC2 possesses an additional amino-terminal hydrophobic sequence, which is responsible for its ability to specifically anchor to the outer mitochondrial membrane (Abu-Elheiga et al., 2000). It has been established (Bianchi et al., 1990; Kim, 1997) that ACC1 functions as

the rate-limiting enzyme of the DNL process, localised in the cytoplasm and predominantly distributed in adipogenic tissues (including liver and adipose) (Kreuz et al., 2009). ACC2 is located in the mitochondrial membrane and is primarily responsible for the regulation of FAO, and the malonyl-coenzyme A variant produced by ACC2 has been shown to inhibit the activity of carnosine palmitoyltransferase 1 (CPT-1). This, in turn, inhibits the LCFA-CoAs transport to the mitochondria via CPT1 to participate in FAO (Hoy et al., 2021). Furthermore, the inhibition of ACC has been shown to alleviate hepatocellular lipid accumulation by down-regulating DNL as well as promoting FAO (Bourbeau and Bartberger, 2015). The study (Ross et al., 2020) found that oral administration of a hepatic ACC1/ACC2-targeted inhibitor (PF-05221304) to mice in a Western dietary model inhibited intrahepatic DNL, attenuated hepatic steatosis, and inhibited the activation process of hepatic stellate cells shifting to fibroblasts. In an experiment (Bates et al., 2020) using other ACC inhibitors (FIR) to intervene in HepG2 cells and mice, researchers found that the use of FIR was effective in reducing the DNL pathway and concomitantly augmenting FAO, and that this change was observed in in vivo and *in vitro* experiments. However, the opposite result of ACC deletion has also been observed, and it has been reported (Loomba et al., 2018) that deletion of ACC elevates circulating TG levels. The study found that knockdown of *Acc* significantly elevated plasma TG levels (200%) (Kim et al., 2017), and that *Acc* knockdown decreases the concentration of PUFA and thereby increases SREBP-1 activity, whereas restored-activated SREBP-1 catalyzes TG by activating the GPAT1 to catalyze TG synthesis and promote VLDL secretion into the circulation to trigger hyperlipidemia. *Acc−/−* mice with decreased lipoprotein lipase (LPL) activity have reduced TG clearance leading to hyperlipidemia (Goedeke et al., 2018). *ACC* deletion has been shown to inhibit PPARα expression, which enhances LPL activity, and therefore, in order to avoid adverse effects, knockdown of *ACC* to treat NAFLD may need to be coupled with PPARα agonists. Clinical trials (Calle et al., 2021) also observed that ACC inhibitors elevated TG levels in patients' plasma, but the combination of lipid-lowering drugs/PPARα agonists would resolve the TG elevation associated with ACC inhibitors. Consequently, further discourse is necessary to ascertain whether *ACC* knockdown holds potential benefits for NAFLD patients.

2.2.2.2 Fatty acid synthase

Fatty acid synthase (FASN) is a protein composed of seven subunits (Long and Cravatt, 2011), which is responsible for catalyzing the synthesis of palmitic acid (PA) from acetyl coenzyme A and malonyl coenzyme A in a 7:1 ratio during DNL, and PA is then extended by very long chain fatty acid elongase 6 (ELOVL6) and desaturated by stearoyl coenzyme A desaturase 1 (SCD1) to produce oleic acid (Parlati et al., 2021). *Fasn* transcription is predominantly subject to regulation by SREBP1c (Postic and Girard, 2008). The feeding of a high-fat, high-sucrose diet (HFD) to liver-*Fasn* KO mice has been demonstrated to cause the development of hepatic steatosis (Chakravarthy et al., 2005). The high expression of FASN in the liver (Dorn et al., 2010) has been shown to result in the accumulation of malonyl coenzyme A, thereby inhibiting FAO. FASN has been identified as the rate-limiting enzyme in the final step of FA synthesis by the DNL pathway (Nguyen et al., 2008), which exerts a significant influence on the upper limit of the hepatic capacity of the FA derived from the DNL pathway (Dorn et al., 2010). Researchers (Zhang et al., 2020) used MicroRNA-103 to target and inhibit the expression of FASN, which effectively inhibited FA synthesis via the DNL pathway and attenuated hepatic lipid accumulation. FASN was also associated with bioIR, one of the high-risk factors for NAFLD (Chen et al., 2023). In the DNL pathway, FASN catalyses the production of palmitic acid (PA), diglycerides (DAG), and ceramides, which activate protein kinase C (PKC) and damage mitochondria and the endoplasmic reticulum through inhibition of phosphorylation of the IRS1/PI3K site (Zhou et al., 2022), ultimately causing IR (Palomer et al., 2018). Related experiments have also demonstrated that inducing ubiquitinated degradation of FASN effectively ameliorates hepatic lipid accumulation in NAFLD mice (Xu et al., 2024). This finding suggests that the inhibition of FASN may represent a promising therapeutic approach for the management of NAFLD. However, the knockdown of *Fasn* has been observed to result in a decrease in PA content (Kang et al., 2024). It has been established that PA activates inflammation through the TLR4-NFκB pathway in HSC cells and upregulates the expression of pro-fibrotic genes, exacerbating MASH progression (Dong et al., 2020).

2.2.2.3 Stearoyl coenzyme a desaturase 1

Stearoyl coenzyme A desaturase 1 (SCD1) is located in the endoplasmic reticulum (Heinemann and Ozols, 2003). SCD1 feeds DNL by converting saturated fatty acids (SFAs) to monounsaturated fatty acids (MUFAs), and is a key rate-limiting enzyme for DNL (Flowers and Ntambi, 2008). Deletion of the *Scd1* gene has been shown to inhibit TG production by the DNL pathway and to upregulate liver and brown adipose (BAT) cell oxidation (Dobrzyn et al., 2004), enhancing body thermogenesis (Lee et al., 2004). The knockdown of *Scd1* has been shown to inhibit ceramide biosynthesis (Dobrzyn et al., 2005), primarily due to the fact that SCD1 deletion causes a decrease in the expression level and activity of a key enzyme (serine palmitoyltransferase) required for ceramide synthesis, and a decrease in the synthesis of the substrate (palmitate) (Wang K. et al., 2020). The accumulation of ceramide has been demonstrated to induce lipotoxicity (Unger, 2002), whilst concurrently promoting lipid synthesis in hepatocytes (Wang et al., 2024). SCD1 deficiency has been observed to promote the phosphorylation of AMP in combination with AMPK (Blázquez et al., 2001), which in turn reduces malonyl coenzyme A synthesis by inhibiting ACC. This, in turn, has been shown to increase CPT1 activity and facilitate the transport of FA to the mitochondria to participate in FAO (Longo et al., 2019). In summary, the suppression of SCD1 expression has been shown to inhibit the expression of genes involved in DNL while concomitantly upregulating the expression of genes associated with FAO (Ntambi et al., 2002). However, it should be noted that this does not automatically imply that the suppression of SCD1 is beneficial to human health. It is important to note that excess lipids can contribute to the development of various metabolic diseases; however, essential lipids remain vital components of the body's biometabolism (Sen et al., 2013). A study (Piccinin et al., 2019) found that the maintenance of the health of Liver-*Scd1*-KO mice is dependent on the dietary supplementation of oleic acid deficiency caused by SCD1 deletion, without which the body may

suffer severe liver injury. Although SCD1 deletion inhibits the synthesis of TGs, it also leads to insufficient synthesis of MUFA as well as the accumulation of SFA, which in turn leads to ER stress and inflammation, and ultimately, to liver injury (Flowers et al., 2006; 2008). It has been established (Rizki et al., 2006) that MUFA synthesised by SCD1 in the DNL pathway confers a protective effect on the liver in numerous instances. This is attributable to the fact that the absence of SCD1 results in the accumulation of lipids that are more toxic than MUFA in the liver. Conversely, the supplementation of SCD1 has been shown to reduce the amount of lipids with greater toxicity in the liver (Piccinin et al., 2019). In addition, SCD1 protects the liver by inhibiting iron death, and SCD1 inhibits iron death by down-regulating lipid peroxide production that induces iron death, which promotes NAFLD (Liu et al., 2021; Chen et al., 2022). The relationship between SCD1 and iron death may be a novel target for the future treatment of NAFLD.

2.2.2.4 Diacylglycerol acyl-transferase 2

Diacylglycerol acyl-transferase 2 (DGAT2) is the catalytic enzyme for the final step in the conversion of diacylglycerol to TAG, and includes two isoforms, DGAT1 and DGAT2 (Yen et al., 2008). DGAT2, which is abundantly expressed in the liver, primarily uses fatty acids from the DNL pathway to synthesize TG (Parlati et al., 2021), and researchers (Gluchowski et al., 2019) found that *Dgat2* deletion downregulated hepatic expression of DNL-related genes and significantly reduced hepatic TAG levels (by 70%) in NAFLD mice. The whole-body TG content of *Dgat2*−/− mice was only 10% of that of wild-type mice, with almost undetectable TG concentrations in the liver (Stone et al., 2004). The present study investigates the efficacy of specific knockdown of *Dgat2* in the liver of ob/ob mice in reducing NAFLD severity (Chen et al., 2002). These results suggest that the inhibition of DGAT2 may represent a significant intervention strategy for NAFLD, given its ability to influence TG synthesis through multiple pathways. Firstly, the inhibition of DGAT2 expression has been demonstrated to impede the TG esterification process. Secondly, DGAT2 deficiency has been shown to decrease the level of SREBP-1c transcription (Rong et al., 2024), which is responsible for FA synthesis. It is noteworthy that SREBP-2 remains unaffected in these circumstances. Since SREBP is initially localized to the endoplasmic reticulum membrane, it binds to SREBP cleavage-activating protein (SCAP) to form a stable complex, which is cleaved in order to form a mature SREBP. Inhibition of DGAT2 caused phosphatidylethanolamine (PE) enrichment in the endoplasmic reticulum (ER), blocking the cleavage of SREBP-1 independently of Insigs, which in turn inhibited SREBP-1 activation and suppressed TG synthesis by hepatocytes via the DNL pathway (Rong et al., 2024).

2.2.2.5 Acyl-CoA synthetase long chain family member 1

Acyl-CoA synthetase long chain family member (ACSL) plays a crucial role in fatty acid metabolism and lipid homeostasis by catalyzing the synthesis of acyl coenzyme A (Acyl-CoAs) from FFA. There are five different isoforms of ACSLs in the human body, of which ACSL1 is the predominant isoform, contributing 50% of the hepatic ACSLs activity (Dong et al., 2023). The subcellular location of ACSL1 dictates its function (Soupene and Kuypers, 2008). When localized in the mitochondria, ACSL1 facilitates the role of acyl-CoAs in fatty acid oxidation (FAO). Conversely, when ACSL1 is localized in the endoplasmic reticulum, it contributes to the TANK-binding kinase 1 (TBK1) (Huh et al., 2020). TBK1 is a serine/threonine protein that acts as an effector of inflammatory signaling in adipocytes and hepatocytes. In addition, TBK1 functions as a scaffolding protein that binds to ACSL1, thereby driving ACSL1 localization to mitochondria to enhance FAO. A study on Alzheimer's disease (AD) (Haney et al., 2024) found that *ACSL1* is the most important lipid synthesis gene for the formation of LD from TG in microglia in brain tissue, and overexpression of ACSL1 induced the synthesis of LD from TG in brain tissue, and the inhibition of ACSL1 attenuated the accumulation of LD in brain tissue, but further studies are needed to find out whether it also has such a role in the liver. Sortilin, a key regulator of the subcellular distribution of ACSL1 (Yang M. et al., 2024), has been shown to promote the translocation of mitochondrial ACSL1 to the nuclear endosome/lysosome. In addition, Consumption of sortilin has been observed to increase mitochondrial ACSL1 in adipocytes, thereby promoting the browning of white adipose tissue (WAT) and, consequently, reducing hepatic lipid deposition (Stanford et al., 2013). Lysine acetylation has been identified as a regulatory mark in almost all enzymes involved in FA anabolism (Zhao et al., 2010), and site mutation experiments have confirmed that acetylation at the specific sites K407 and K425 on the ACSL1 protein enhances its enzyme activity (Frahm et al., 2011; Chen Z. et al., 2018). However, there have been no experimental studies investigating the effect of acetylated ACSL1 on the NAFLD effects. The present study hypothesises that ACSL1 acetylation can be regulated by SIRT to enhance ACSL1 activity, to promote FAO, and ultimately affect NAFLD progression. This may be a novel strategy for future intervention in NAFLD.

2.3 Fatty acid oxidation in hepatocytes

Fatty acid oxidation (FAO) is accomplished intracellularly in mitochondria, peroxisomes, and microsomes on the endoplasmic reticulum (ER) (Dixon et al., 2021). It is important to note that there is variability in the FAs, as well as the catalytic enzymes involved in FAO at different subcellular levels. Mitochondria are the most prominent site of FAO (Adeva-Andany et al., 2019). In this process, FA is initially activated in the cytosol by lipoyl coenzyme A synthase, resulting in the formation of lipoyl coenzyme A. Carnitine palmitoyltransferase 1 (CPT1) then traps this lipoyl coenzyme A, forming lipoyl carnitine, which subsequently contributes to the process of FAO (Neuschwander-Tetri, 2010). CPT1 has been identified as the key rate-limiting enzyme in the mitochondrial FAO pathway (Fontaine et al., 2012), and it has been demonstrated that interference with the translocation function of CPT-1 can inhibit FAO (Abu-Elheiga et al., 2000).

Researchers (Weber et al., 2020) found that adeno-associated virus serotype 9 (AAV9) is the most potent AAV in gene therapy targeting the liver, and combining AAV9 with a heterodimer of human CPT1A (hCPT1a.m.) to form AAV9-hCPT1a.m., and injecting AAV9-hCPT1a.m. intravenously into the tails of mice with a model of NAFLD, this resulted in a significant increase in

liver fatty acid oxidation (FAO) and a reduction in hepatic steatosis induced by HFD. The observed outcomes may be attributed to the ability of AAV9-hCPT1a.m. to generate mutants that enhance CPT1 activity in the mouse liver. Enhancement of CPT1 is effective in promoting FAO and thus attenuating hepatic lipid accumulation. However, this does not imply that enhancing CPT1 expression is an effective strategy for treating NAFLD. Study (Fondevila et al., 2022) found that CPT1A was highly expressed in patients with liver fibrosis and activated HSC in mice, which was positively correlated with the degree of liver fibrosis, and in fibrotic hepatocytes, CPT1A overexpression increased FAO, which stimulated the production of ROS, and ultimately the activation of HSC, whereas the inhibition/specific knockdown of *CPT1* blocked the activation of HSC, which then interfered with the progression of liver fibrosis. In summary, in early NAFLD, promoting CPT1 expression helps to promote FAO to reduce intrahepatic lipids, while enhancing CPT1 accelerates the process of hepatic fibrosis when NAFLD shifts to hepatic fibrosis, thus intervening CPT1 at different stages of NALFD may reap completely opposite results.

2.4 Very low density lipoprotein secretion by hepatocytes

FA that is not utilised by FAO is esterified to TG, which is subsequently exported from the liver as very low density lipoprotein (VLDL). In this process, apolipoprotein B (ApoB) is the structural scaffold on which VLDL is built. During ApoB lipidation, VLDL translocates TG, TC, and phospholipids to ApoB by virtue of microsomal triglyceride transfer protein (MTTP) to assemble into spherical particles (Hussain et al., 2012). ApoB in turn secretes assembled VLDL into the circulation (Sparks et al., 2011).

MTTP plays a crucial role in the process of ApoB lipidation, a process which is essential for the acquisition of lipoprotein biosynthetic function and stability. In the absence of MTTP, the unique sequence features of ApoB render it susceptible to reversal of translocation and subsequent proteasomal degradation (Zhang et al., 2025). *Mttp* ± mice were fed a standard diet, and oil red O staining of their livers revealed the presence of numerous intracellular lipid droplets in liver cells (Hussain and Bakillah, 2008). Liver-*Mttp*-KO mice exhibited a 40% decrease in serum TG content and a 50% decrease in TC content compared to WT mice, despite increased liver TG and TC content and hepatocyte-enriched lipid droplets (Hussain et al., 2012). In addition, MTTP-mediated ApoB lipidation is also subject to regulation by intracellular tissue-type plasminogen activator of fibrinolysis (tPA/PLAT), and intrahepatocyte tPA does not affect MTTP protein expression levels (Dai et al., 2023). tPA acts directly on ApoB to block the ApoB-MTP interaction, thereby inhibiting MTTP-mediated neutral lipid transfer and ApoB lipidation. Hepatocyte tPA expression has been shown to be negatively correlated with TC and TG concentrations in mouse serum (Dai et al., 2023). Plasminogen activator inhibitor 1 (PAI-1) is a serine protease inhibitor that binds to tPA in hepatocytes. PAI-1 binds to hepatocyte tPA, blocks the inhibitory effect of tPA on ApoB, and promotes the assembly and secretion of VLDL. In summary, interfering with MTTP-mediated VLDL assembly and secretion based on the interaction between tPA, PAI-1, and apoB not only interferes with atherosclerotic cardiovascular disease (CVD), but may also be a potential new strategy for the treatment of NAFLD.

3 Crosstalk between gut microbiota, and its metabolites, and lipid metabolism pathways in non-alcoholic fatty liver disease

The GM constitutes the largest microbial population in the human body, comprising approximately 100 trillion microorganisms. The GM maintains host metabolic homeostasis by consuming exogenous food or endogenous host substances to produce a variety of metabolites, which in turn interact with the host. The bidirectional interaction between the GM and the liver is termed the gut-hepatic axis, and the two interact with each other via the portal circulation. Alterations in GM composition, function, and metabolite profiles have the capacity to disrupt host-microbe homeostasis (Wu et al., 2021). GM dysregulation has been shown to result in increased gastrointestinal permeability, lipopolysaccharide translocation, immune activation, and altered BAs signalling, which in turn contributes to the development of NAFLD, MASH (Anstee et al., 2019). The portal circulation facilitates the entry of toxic substances produced by the GM into the liver, thereby exposing it to the metabolites generated by the gut microbiota. This has been shown to be a direct trigger for metabolic disorders and degenerative necrosis of hepatocytes (Song and Zhang, 2022). A range of colony-specific metabolites (including BAs, SCFAs, branched-chain amino acids, TAMO) have been implicated in the pathogenesis of metabolic disorders (Bauer et al., 2022). Intervention with GM and its metabolites has also been recognised as an important breakthrough for targeted therapy of NAFLD (A et al., 2021). As shown in Figure 2.

3.1 Gut microbiota composition, abundance changes and non-alcoholic fatty liver disease

Four of the most common types of bacteria are present in the intestinal tract of healthy adults, including *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* are the most dominant bacterial phyla in the human gut (Shapira, 2016). With respect to abundance, the *Bacteroidetes* and *Firmicutes* are the most prevalent, followed by *Proteobacteria*, *Fusobacteria*, *Tenericutes*, *Actinobacteria* and *Verrucomicrobia*. Collectively, they constitute 90% of the total human gut microbiota (Gomaa, 2020). GM are dynamically changing collections of communities, and these microbial communities are correlated with the host's age, health status, diet, and lifestyle. A significant disparity in the compositional structure, as well as the abundance of GM, has been observed between patients with NAFLD and healthy populations, and this discrepancy has been termed intestinal microecological dysbiosis (Quesada-Vázquez et al., 2022). In a seminal study, GM from healthy and NAFLD mice was transplanted into the intestines of two groups of germ-free mice. The results indicated that GM from NAFLD mouse sources elevated the risk of NAFLD in germ-free mice (Le Roy et al., 2013). The compositional structure and

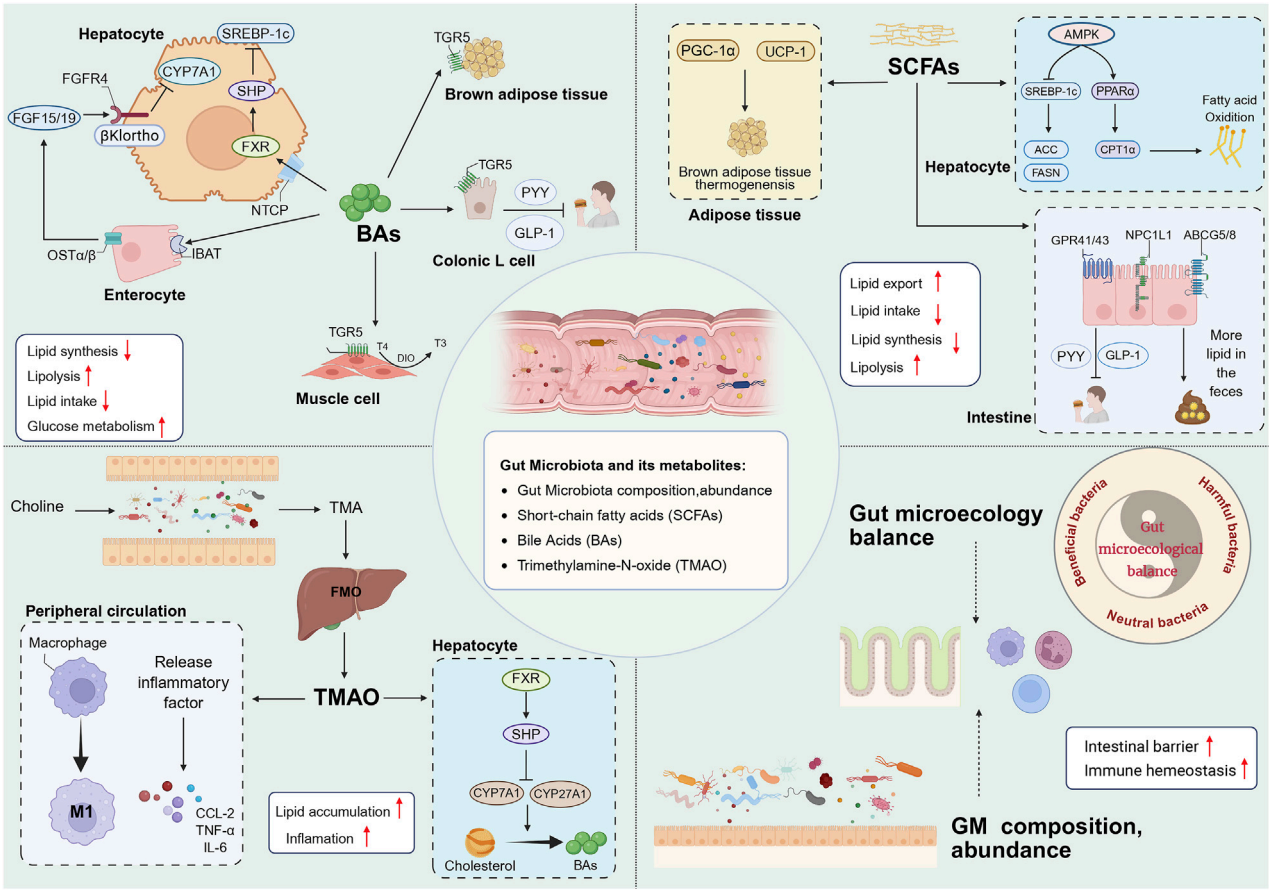

FIGURE 2
BAs contribute to host metabolism in various organs through FXR and TGR5. BAs synthesis in the liver and glucose metabolism are regulated by the intestinal FXR-FGF15/19 signal, and BAs also affect lipid synthesis in the liver through the FXR-SHP signal pathway. In addition, BAs enhance host energy metabolism through TGR5, including driving BAT thermogenesis; promoting the conversion of inactive thyroxine (T4) to active thyroid hormone (T3) in skeletal muscle to increase energy consumption; and promoting the release of GLP-1 and PYY by colon L cells to improve IR and suppress appetite. SCFAs promote BAT thermogenesis by activating PGC-1α and UCP-1 in adipose tissue. Secondly, SCFAs activate AMPK in the liver, on the one hand, SCFAs can downregulate DNL by inhibiting SREBP-1c and thereby reducing the expression of ACC and FASN, and on the other hand, they can promote FAO by activating PPARα and thereby upregulating the expression of CPT1α. In addition, SCFAs can reduce intake by activating GPR41/43 in the intestine to release PYY and GLP-1, and reduce lipid uptake through ABCG5/8 and NPC1L1, thereby increasing lipid excretion in the feces. TMAO enhances the pro-inflammatory polarization of macrophages and the release of inflammatory factors. TMAO inhibits the conversion of TC to BAs through the liver FXR/SHP signaling pathway. The balance of the intestinal microecology and the diversity of GM help to stabilize the intestinal mucosal barrier and immune system. BAs, bile acids; FXR, farnesoid X receptor; TGR5, G proteincoupled bile acid receptor; FGF15/19, fibroblast growth factor 15/19; FGFR4, fibroblast growth factor receptor 4; NTCP, sodium dependent taurocholate co-transporting polypeptide; OSTα/β, organic solute transporter subunit α/β; DIO2, Type II iodothyronine deionidinase; SHP, small heterodimer partner; BAT, brown adipose tissue; GLP-1, glucagon-like peptide-1; PYY, peptide YY; SCFAs, short chain fatty acid; PGC-1α, Peroxisome proliferator-activated receptor-γ coactivator-1α; UCP-1, Uncoupling protein 1; AMPK, AMP-activated protein kinase; SREBP-1c, sterol regulatory element-binding protein-1c; ACC, acetyl-co carboxylase; FASN, fatty acid synthase; PPARα, Peroxisome proliferator-activated receptor α; CPT1α, carnitine palmitoyltransferase-1 α; GPR41/43, G protein-coupled receptors 41/43; ABCG5/8, ATP-binding cassette transporter G5/8; NPC1L1, Niemann-Pick type C1 like1; TMAO, Trimethylamine oxide; TC, cholesterol; GM, gut microbiota.

abundance of GM are differentially characterised at different stages of NAFLD (Mouzaki et al., 2013).

Researchers conducted a comparative analysis of the GM of NAFLD patients and the normal population (Spencer et al., 2011). They revealed that the GM of NAFLD patients exhibited a higher abundance of Gram-negative bacteria, with the abundance of the *Bacteroidetes*, which belongs to Gram-negative bacteria, increasing by 20%, while the abundance of *Firmicutes*, a predominant member of Gram-positive bacteria, decreased by 24%. The ratio of *Bacteroidetes* to *Firmicutes* is elevated in NAFLD patients (Wang et al., 2016). Specifically, the relative abundance of *Ruminococcaceae*, which are known to produce SCFAs as part of *Firmicutes*, exhibited a marked reduction in GM. The abundance of *Escherichia*, *Prevotella*, and *Streptococcus* increased, while *Faecalibacterium, Clostridium, Bacteroides and Lactobacillus* are lacking in NAFLD patients (Li et al., 2021). In patients with MASH, the proportion of *Clostridium coccoides* in the intestine was found to be significantly higher (Zhu et al., 2013). The severity of NAFLD is closely related to GM. For instance, an elevated abundance of *Bacteroides* has been identified as an independent risk factor for the severity of NASH, while the progression of liver fibrosis is closely associated with the presence of *Ruminococcus* (Boursier et al., 2016). In patients with liver fibrosis, a decline in the numbers of *Enterococcus faecalis* and *Faecalibacterium prausnitzii* (*F. prausnitzii*) has been observed, and the butyrate

produced by these two flora has been shown to contribute to the maintenance of intestinal barrier function (Kwan et al., 2022).

In addition, GM can influence NAFLD through BAs metabolism. It is well known that microbial modifications of GM origin are essential for enterohepatic recycling of BAs, and that the synthesis of BAs, the size and compositional structure of the bile acid pool are dependent on GM.3-succinimidylated cholic acid (3-sucCA) is the primary bile acid, one of the types of BAs (CA), mainly from *Bacteroides uniformis*, and 3-sucCA levels are negatively correlated with NAFLD severity (Nie et al., 2024). Supplementation with *Bacteroides uniformis* has been shown to significantly ameliorate hepatic steatosis, as well as the degree of inflammation and fibrosis in MASH mice. A study (Leung et al., 2022) was conducted in which metagenomic metabolomics analysis was performed on fecal samples from 90 patients with NAFLD and 90 healthy individuals. The results indicated that, at the genus level, *Methanobrevibacter*, *Phascolarctobacterium*, and *Slackia* were independent risk factors for NAFLD, independent of obesity. At the species level, *Dorea formicigenerans* (*D. Formicigenerans*) was identified as an independent risk factor for NAFLD, independent of obesity. Gut barrier dysfunction has been identified as a pivotal factor in the progression of NAFLD (Lechner et al., 2020), characterised by the disruption of the intestinal mucosal barrier, thereby facilitating the passage of deleterious substances such as GM metabolites, bacteria, and enterogenous LPS through the portal system. This, in turn, results in the exacerbation of the hepatic inflammatory response, leading to liver injury and fibrosis (Ferro et al., 2020). Consequently, the modulation of the gut microbiota has emerged as a promising therapeutic approach for NAFLD management (Li et al., 2022). The restoration of GM ecological balance through probiotics, prebiotics, and fecal microbiota transplantation (FMT) has emerged as a novel therapeutic strategy to enhance NAFLD treatment (Carpi et al., 2022). In addition, engineered bacteria have emerged as a novel class of biotherapeutics, wherein the genetic material of bacteria is deliberately modified through genetic engineering to generate bacterial metabolites that are conducive to the control of disease progression (Canale et al., 2021). The experimental findings support these propositions, as researchers discovered that supplementation of probiotics to the gut significantly suppressed hepatic steatosis as well as intestinal inflammation in NAFLD mice (Ma et al., 2013). A meta-analysis (Sharpton et al., 2019) encompassing 1,252 patients also determined that supplementation with probiotics or synbiotics exhibited a strong correlation with enhanced liver function, diminished liver stiffness values (LSM), and the alleviation of hepatic steatosis in patients diagnosed with NAFLD.

3.2 Gut microbiota metabolites

3.2.1 Trimethylamine N-oxide

Trimethylamine N-oxide (TMAO) is primarily derived from dietary choline, which is converted to trimethylamine (TMA) via the GM and subsequently generates TMAO by hepatic plus monooxygenase enzymes (FMOs) (Subramaniam and Fletcher, 2018). TMAO drives NAFLD through a variety of mechanisms (Wang M. et al., 2023). TMAO can increase the serum levels of the inflammatory cytokine C-C motif chemokine ligand 2 (CCL2) and pro-inflammatory factors (TNF-α, IL-6) in hepatocytes (Rohrmann et al., 2016; Hosseinkhani et al., 2021). Secondly, TMAO can cause intestinal barrier damage and drive macrophage M1 polarization, which in turn aggravates liver inflammation (Nian et al., 2024).

TMAO inhibits the conversion of TC to BAs by activating the FXR-SHP signaling pathway to downregulate CYP7A1 and CYP27A1 expression (Aron-Wisnewsky et al., 2020), which ultimately aggravates lipid accumulation in the liver. The concentration of TMAO has been shown to be correlated with the incidence and severity of NAFLD, as well as with total NAFLD mortality. A cohort study encompassing 5292 subjects (Flores-Guerrero et al., 2021). The study found that serum TMAO levels were positively associated with all-cause mortality in patients with NAFLD, and that TMAO worsened the health status of these patients. However, TMAO did not affect all-cause mortality in non-NAFLD patients. Furthermore, TMAO has been demonstrated to inhibit pancreatic β-cell function, promote β-cell differentiation and apoptosis (Kong et al., 2024), and increase the risk of NAFLD (Rohm et al., 2022). A positive correlation between TMAO content in feces and the degree of hepatic steatosis in mice, which was closely related to the process of pro-inflammatory polarization of macrophages driven by TMAO (Nian et al., 2024). TMAO is a risk factor driving the progression of NAFLD, and inhibiting the synthesis of TMAO can effectively alleviate the development of NAFLD (Corbin and Zeisel, 2012). Consequently, a scientific strategy has been proposed to intervene in NAFLD by inhibiting TMAO synthesis through GM structural remodeling (Arias et al., 2020).

3.2.2 Short-chain fatty acids

Short-chain fatty acids (SCFAs) are metabolites released during the conversion of carbohydrates to monosaccharides by the human GM, mainly including acetate, propionate, and butyrate (Zhang et al., 2019). SCFAs inhibit the progression of NAFLD through the gut-hepatic axis (Chen X.-F. et al., 2020). SCFAs regulate the transcription of key enzymes of hepatic lipid metabolism (FASN, SREBP-1) to influence affect lipid synthesis (Fushimi et al., 2006; Hong et al., 2021). SCFAs have been demonstrated to promote the expression of the rate-limiting enzyme CYP7A1, thereby facilitating the conversion of TC to BA (Guan et al., 2022). In addition, SCFAs have been shown to upregulate the expression of ATP-binding cassette transporter protein A1 (ABCA1). Furthermore, it has been demonstrated that SCFAs enhance the output of TC by up-regulating the expression of ATP-binding cassette transporter proteins G5 and G8 (ABCG5/8), whilst concomitantly inhibiting the expression of ileal Niemann-Pick C1-like 1 (NPC1L1) to reduce TC uptake (He and You, 2020). This ultimately results in a reduction in body lipid accumulation. Butyrate ameliorated hepatic steatosis in HFD-fed mice, this amelioration was closely related to butyrate's promotion of hepatic ABCA1-mediated cholesterol efflux (Du et al., 2020). In addition, butyrate was found to be associated with the inhibition of intestinal NPC1L1 expression and the upregulation of ABCG5/8 expression in mice (Chen Y. et al., 2018). SCFAs have been shown to improve insulin resistance (IR) by activating G-coupled protein receptors 41/43 (GPR41/43) in the intestine to promote the release of the gastrointestinal peptide hormones tyrosine peptide (PYY) and glucagon-like peptide 1 (GLP-1)

from L-cells and reduce lipid intake by suppressing appetite (Psichas et al., 2015; Christiansen et al., 2018).

Furthermore, SCFAs bind to GPR41/43 in the liver, which has been shown to inhibit the expression of lipid-producing genes in the liver by upregulating PPARα expression and activating the AMPK pathway. In addition, this binding has been demonstrated to enhance mitochondrial function to induce the fatty acid oxidation (FAO) of liver fat, thereby increasing lipid consumption, inhibiting liver steatosis, and preventing the development of NAFLD (Hong et al., 2021). SCFAs have also been shown to inhibit cholesterol synthesis in the liver and reduce plasma cholesterol concentrations (Haghikia et al., 2022). Conversely, SCFAs have been observed to downregulate ACC and FASN expression through the AMPK-SREBP-1c pathway, thereby impeding lipid synthesis in hepatocytes (Sun C. et al., 2023). Furthermore, SCFAs have been shown to promote lipid metabolism by increasing CPT1 expression through the PPARα pathway (Kondo et al., 2009). SCFAs have been shown to promote the "browning" of white adipose tissue (WAT) (Sahuri-Arisoylu et al., 2016) and to enhance brown adipose tissue (BAT) thermogenesis and fat oxidation by increasing the expression of peroxisome activator-activated receptor gamma coactivator 1α (pgc-1α) and uncoupling protein 1 (UCP-1) (den Besten et al., 2013). Furthermore, study (Du et al., 2021) has demonstrated that SCFAs can also mediate microRNAs (miRNAs) to regulate gene expression and thereby intervene in the progression of NAFLD, such as via the action of microRNA-378a.

3.2.3 Bile acids

Primary BAs (PBA) are synthesised by the liver from TC and are stored in the gallbladder. Following the ingestion of food, PBA enters the intestine where it is converted into secondary BAs (SBA) by the action of intestinal flora. Of the SBA, 5% is excreted with faeces, while the remaining 95% is reintroduced to the liver via the ileocecal bile acid transporter protein (IBAT), thus forming the enterohepatic cycle of BAs. BAs metabolism has been identified as the predominant pathway of TC consumption in the liver, accounting for 90% of total daily TC consumption (de Aguiar Vallim et al., 2013). BAs biosynthesis has been identified as the major pathway of TC metabolism. An imbalance in cholesterol homeostasis results in intrahepatic TC accumulation, which in turn induces NAFLD, and an imbalance in cholesterol homeostasis is characterised by activation of cholesterol biosynthesis, increase in cholesterol de-esterification, and attenuation of cholesterol export and bile acid synthesis pathways (Henkel et al., 2018). Consequently, the activation of BAs biosynthesis has emerged as a promising therapeutic approach to mitigate NAFLD (Perino et al., 2021). Secondly, accumulated TCs activate Kupffer cells (KCs) and stellate cells (HSCs), triggering mitochondrial dysfunction and endoplasmic reticulum stress, which ultimately drives NAFLD development (Ioannou, 2016). Secondly, BAs have been demonstrated to affect NAFLD by modulating lipid metabolism. In the enterohepatic circulation, BAs primarily influence energy metabolism via the Farnesoid X Receptor (FXR) and G-protein coupled receptor 5 (TGR5) (Fiorucci and Distrutti, 2022). Specifically, FXR has been shown to inhibit hepatic lipid synthesis. Firstly, FXR activation by BAs induces Small Heterodimer Partner (SHP) expression and thus inhibits the activation of SREBP-1c, a key regulator of lipid synthesis genes, to suppress DNL (Adorini and Trauner, 2023). Conversely, FXR has been shown to promote hepatic lipid metabolism. Activation of FXR by BAs induces FGF15/19, which then binds to hepatic FGFR4/β-Klotho, thereby facilitating increased FAO and glucose metabolism (Adorini and Trauner, 2023). In addition, BAs activation of FXR has been demonstrated to promote FAO by inducing PPARα expression (Pineda Torra et al., 2003). Furthermore, FXR has been shown to accelerate cholesterol and triglyceride clearance via Scavenger Receptor Class B Type I (SR-BI), Syndecan-1 (SDC1), and Very Low-Density Lipoprotein Receptor (VLDLR) (Fiorucci et al., 2020). A recent study (Clifford et al., 2021) also found that activation of hepatic FXR by BAs specifically inhibited the expression of lipid synthesis genes Scd1, Dgat2 and Lpin1 in the liver. Notably, this effect was observed to be independent of the FXR-SHP-SREBP-1c pathway. In addition, BAs have been shown to inhibit intestinal absorption of lipids and thereby reduce intrahepatic lipid levels, an effect that is greatly dependent on the intestinal FXR. FXR agonists have been widely used in the treatment of NAFLD, such as obeticholic acid (OCA), and the efficacy of OCA in the treatment of NAFLD has also been demonstrated (Younossi et al., 2019).

Activation of TGR5 by BAs stimulates the secretion of PYY and GLP-1 from intestinal L cells via the cAMP signalling pathway, thereby improving insulin resistance (IR) and suppressing appetite, and consequently reducing lipid intake (Wahlström et al., 2016). Concurrently, activated TGR5 stimulates the process of brown adipose tissue (BAT) thermogenesis and thyroid hormone (T3) production in skeletal muscle, thereby increasing energy expenditure. Furthermore, researchers have demonstrated that activation of TGR5 by BAs acts on the signal-regulated kinase (ERK)/mitochondrial dynamin-related protein 1 (Drp1) pathway, which in turn drives WAT browning as well as increasing FAO (Velazquez-Villegas et al., 2018). A significant increase in conjugated 12α-hydroxylated (12α-OH) BAs, including taurodeoxycholic acid (TDCA) and glycodeoxycholic acid (GDCA), was observed in the livers of patients with hepatic fibrosis and mice (Xie et al., 2021). The combination of 12α-OH BA and TGR5 increased the expression of hepatic fibrosis-related proteins (α-SMA, TGF-β, COL I and PDGF) expression. It is noteworthy that serum BAs was more sensitive to alterations in liver disease than fecal BAs (Chen W. et al., 2020), which also predicts that serum BA may be a better reflection of disease changes than fecal BA.

4 Active metabolites in natural products modulate gut microbiota-lipid metabolism communication in non-alcoholic fatty liver disease

The use of natural products as a complementary therapy has garnered increased attention. It is well established that natural products comprise intricate chemical metabolites, with the capacity to act on numerous targets to elicit a therapeutic response. In recent years, there has been a discernible rise in research endeavors exploring the therapeutic potential of natural products in the context of GM-related diseases. This prompts the question of whether natural products can improve NAFLD by

TABLE 1 Mechanism of natural products targeting the GM-lipid metabolism in the treatment of NAFLD.

Active metabolites	Natural sources	Experimental model	Dose; Duration; grouping	Regulation of GM and its metabolism	Targeting lipid metabolism	Refs
Resveratrol (RSV)	*Polygonum cuspidatum Sieb. Et Zucc*	6-week-old male C57BL/6J mice	RSV 0.5% in diet; 8 weeks; grouping: (1) LFD group, (2) HFD group, (3) HFR group (HFD + RSV 0.5% in diet)	*Lactobacillus*↓, *Bifidobacterium*↓, *Enterococcus*↓	Reduces intestinal SR-B1 protein expression and increases fatty acid β-oxidation	Pang et al. (2023)
		6-week-old male C57BL/6J mice	RSV 300 mg/kg/d; 16 weeks; grouping: (1) NCD group, (2) HFD group, (3) RSV group (HFD +300 mg/kg/d RSV)	*Firmicutes* ↓, *Actinobacteria*↓, *Verrucomicrobia* ↑; *Blautia* ↓, *Lactobacillus* ↓; *Akkermansia Muciniphila* (*A. muciniphila*) ↑; 4-HPA ↑	Activation of the SIRT 1 pathway regulates brown fat and thermogenesis	Wang et al. (2025)
		Male SD rats	RSV 50 mg/kg/d, 100 mg/kg; 6 weeks; grouping: (1) NCD group, (2) HFD group, (3) L-Rsv group (HFD +50 mg/kg/d RSV), (4) H-RSV group (HFD +100 mg/kg/d RSV)	gut microbiota diversity ↑, SCFAs ↑, LPS ↓	No specific mechanism	Chen et al. (2020b)
		5-week-old male C57BL/6J mice	RSV 300 mg/kg; 16 weeks; grouping: (1) SD group, (2) HFD group, (3) HFDR group (HFD +300 mg/kg/d RSV)	SCFA-producing bacteria ↑	No specific mechanism	Wang et al. (2020b)
Curcumin (Cur)	*Curcuma longa* L	6-week-old SD male rat	Cur 100 mg/kg/d, Antibiotic (Abx) comprises vancomycin (0.25 g/L), eomycin sulfate (0.5 g/L), metronidazole (0.5 g/L), ampicillin (0.5 g/L); for 12 weeks; grouping: (1) NASH group, (2) Cur group, (3) NASH + Abx group, (4) Cur + Abx group	gut microbiota diversity ↑, tetrahydrocurcumin (THC) ↑	THC improves the function of LSECs through the NF-κB and PI3K/AKT/HIF-1α signaling pathways, indirectly reducing the fat degeneration and damage of hepatocytes	Wu et al. (2023)
		Human	Cur 500 mg/day; 24 days; grouping: (1) control group (placebo), (2) Cur group (500 mg/kg/d Cur)	*Firmicutes*/*Bacteroidetes* ↓, *Bacteroidetes* ↑	Activated by TGR5 to promote lipid metabolism	He et al. (2024)
		4-week-old SD male rat	Cur 200 mg/kg/d; 4 weeks; grouping: (1) NCD group, (2) HFD group, (3) Cur group (HFD +200 mg/kg/d Cur)	*Blautia* ↑, *Allobaculum* ↑; *Ruminococcus*↓, *Coprococcus*↓, *Mucispirillum* ↓; LPS ↓	Reduces lipid deposition and inhibits liver inflammation	Feng et al. (2017)
Chlorogenic acid (CGA)	*Lonicera japonica* Thunb	C57BL/6 mice; *Fxr*−/− mice	1.34 mg/kg/day CGA, 19 weeks, Grouping: (1) ND, (2) HFD, (3) HFD + CGA, (4) HFD + ADW, (5) HFD + ADW + CGA 1.34 mg/kg/day CGA; 4 weeks; grouping: (1) ND group, (2) HFD group, (3) CGA group (HFD +1.34 mg/kg/d CGA), (4) CGA group (HFD +10 mg/kg/d OCA)	*Bacteroidetes* ↑, *Verrucomicrobia* ↑, *Tenericutes* ↑; *Lachnospiraceae* ↓; *Roseburia* ↓, *Desulfovibrio* ↓	Regulates gut bile acid metabolism, promotes cholesterol metabolism and bile acid excretion regulated by FXR to improve lipid accumulation in the liver	Li et al. (2023)
		6-week-old male C57BL/6J mice	CGA 60 mg/kg/d; 12 weeks; grouping: (1) control group, (2) CGA group, (3) HFD group, (4) CGA + HFD group	*Bifidobacterium* ↑, *Escherichia* ↓	Increases Occulin and ZO-1 tight junction proteins on the intestinal mucosa, reduces the level of inflammatory factors in the serum, and inhibits the activation of the TLR4 signaling pathway	Shi et al. (2021)

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TABLE 1 (*Continued*) Mechanism of natural products targeting the GM-lipid metabolism in the treatment of NAFLD.

Active metabolites	Natural sources	Experimental model	Dose; Duration; grouping	Regulation of GM and its metabolism	Targeting lipid metabolism	Refs
		4-week-old male Kunming mice	CGA 200 mg/kg/d, 400 mg/kg/d; 12 weeks; grouping: (1) normal control group (saline 0.4 mL/d), (2) high L-carnitine control group (3% L-carnitine), (3) L-CGA (3% L-carnitine and 200 mg/kg/d CGA), (4) H-CGA (3% L-carnitine and 4200 mg/kg/d CGA)	*Akkermansia* ↑, *Bacteroides* ↑ *Erysipelatoclostridium* ↓, *Faecalibaculum* ↓; TMAO ↓, SCFAs ↑	CGA reverses elevated blood lipids and liver inflammatory factors in mice	Zhang et al. (2021)
		8-week-old male C57BL/6J mice	1.34 mg/kg/d CGA; 4 weeks; grouping: (1) control control group, (2) HFD group, (3) HFD + CGA (geniposide 90 mg/kg/d and CGA 1.34 mg/kg/d), (4) HFD + QHD (10 mL/kg/d), (5) HFD + NaB (200 mg/kg/d)	gut microbiota diversity ↑, tight junction proteins ↑	Reduces signaling of endotoxin and infiltration of Kupffer cells	Peng et al. (2018)
Berberine (BBR)	*Coptis chinensis* Franch	8-week-old male C57BL/6J mice	BBR 200 mg/kg/d; 8 weeks; grouping: (1) NCD group, (2) HFD group, (3) HFD + BBR (200 mg/kg/d)	*Blautia producta* ↑, *Clostridiales bacterium_VE202_06* ↑, *Akkermansia muciniphila* ↑	Upregulates LDLR expression in the liver, promoting the uptake of LDL by the liver	Yang et al. (2022)
		Male beagle dog	BBR 50 mg/kg, 7 days, grouping: (1) BBR by single (50 mg/kg), (2) multiple doses (50 mg/kg/d); 7 days	butyrate-producing bacteria↑	Butyrate enters the bloodstream and exerts a lipid-lowering effect	Feng et al. (2018)
		HepG2 cell	15 μM BBR; 24 h; grouping: (1) control, (2) 15 μm M3 + 2.5 μM PD98059, (4) 15 μm A8 + 2.5 μM PD98059, (4) BBR	No specific mechanism	Inhibit PCSK9 expression	Cao et al. (2019)
Betaine	*Beta vulgaris* L	9-week-old male C57BL/6J mice	LFD for 18 weeks; 18 weeks; grouping: (1) HFD for 18 weeks, (2) HFD 9 weeks + LFD 9 weeks; (3) HFD 9 weeks + (HFD + unprocessed rye bran) 9weeks, (4) HFD 9 weeks + (HFD + bioprocessed rye bran) 9 weeks, (5) HFD 9 weeks + (HFD + unprocessed wheat aleurone) 9 weeks, (6) HFD 9 weeks + (HFD + bioprocessed wheat aleurone), (7) LFD for 18 weeks	*Coriobacteriaceae* ↑; *Akkermansia* ↑, *Bifidobacterium* ↑, *Lactobacillus* ↑, *Ruminococcu* ↑	No specific mechanism	Koistinen et al. (2019)
		9-week-old C57BL/6J mice	1% wt/vol betaine; From the start of pregnancy until the offspring mice reach 3 weeks of age; grouping: (1) standard diet, (2) HFD, (3) HFD +1% wt/vol betaine	*Desulfovibrio* ↓, *Ruminococcus* ↓, *Bacteroides* ↑, *Parabacteroides* ↑, SCFAs ↑	Increases the mRNA expression of Pparα, Cpt1α, and Fatp2 to promote lipid metabolism	Sun et al. (2023b)
Quercetin (Que)	Scutellaria baicalensis Georgi	7-week-old male C57BL/6J mice	Que 100 mg/kg/d; 10 weeks; grouping: (1) normal chow diet, (2) normal chow diet + Que, (3) HFD, (4) HFD + Que	*A.muciniphila* ↑, indole-3-lactic acid (ILA) ↑	FTO/m6A/YTHDF2/ CYP8B1 pathway promotes the conversion of TC to BA, which in turn activates FXR to inhibit lipid synthesis	Liu et al. (2025)

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TABLE 1 (*Continued*) Mechanism of natural products targeting the GM-lipid metabolism in the treatment of NAFLD.

Active metabolites	Natural sources	Experimental model	Dose; Duration; grouping	Regulation of GM and its metabolism	Targeting lipid metabolism	Refs
		C57BL/6J mice	Que 50 mg/kg/day +100 μL/10 g body weight of 0.15% carboxymethylcellulose sodium; 7 weeks; control group: (1) Con group (carboxymethylcellulose sodium), (2) MetS group (carboxymethylcellulose sodium), (3) MetSQ group (Que)	*Lactobacillus* ↑	Promotes the synthesis of non-12α-hydroxylated BA in serum and stimulates thermogenesis in adipose tissue	Zhu et al. (2024)
		7-week-old male C57BL/6J mice	0.05% quercetin; 16 weeks; grouping: (1) Control, (2) Control + quercetin, (3) HFD, (4) HFD + quercetin	*Clostridia* ↑, *Bacilli* ↑, *Deltaproteobacteria* ↑, *Akkermansia* ↑, *Erysipelotrichi* ↓, *Betaproteobacteria* ↓	Suppresses the expression of genesinvolved in *de novo* lipogenesis	Porras et al. (2017)
Silymarin	Silybum marianum (L.) Gaertn	5-week-old male C57BL/6J	1% Silymarin; 12 weeks; grouping: (1) HFD, (2) HFD + Silymarin, (3) HFD +30–40 μM/kg/day B12	*Akkermansia* ↑, *Blautia* ↑	Activates the liver's fatty acid degradation pathway, thereby reducing fat production and enhancing fatty acid oxidation	Sun et al. (2023c)
		Huamn	silymarin 103.2 mg/d; 24 weeks; grouping: (1) silymarin group, (2) placebo group	gut microbiota diversity ↑, *Oscillospiraceae* ↑, SCFA ↑	Regulates bile acid metabolism and promotes lipid metabolism	Jin et al. (2024)
Astragalus polysaccharides (APS)	Astragalus membranaceus Bunge	4-week-old male C57BL/6J	4% APS; 12 weeks; grouping: (1) NCD, (2) HFD, (3) HFD +4% APS	*Firmicutes* ↓, *Bacteroidetes* ↑, SCFAs ↑	Inhibits the expression of glucokinase, CD36 and FASN in liver tissue, promotes the expression of CPT-1α and PPAR-α in the liver, and ultimately inhibits FA synthesis and promotes FAO	Hong et al. (2021)
		SPF male SD rat	200 mg/kg/d mAPS extracts; 4 weeks; grouping: (1) control group, (2) HFD group, (3) mAPS gruop, (4) HFD + mAPS group, (5) HFD + BER (300 mg/kg/)	*Firmicutes*/*Bacteroidetes (F/B)* ↓, *Proteobacteria* ↑; *Epsilonbacteria* ↑	Enhances AMPK and PPAR-α expression and reduces SREBP-1 expression, the SCFA-GPR41/43 signaling pathway	Zhong et al. (2022)
Ginkgo bilobaSeed Polysaccharide (GBSP)	Ginkgo biloba L	8-week-old male C57BL/6J	GBSP 100 mg/kg/d, 200 mg/kg/d; 12 weeks; grouping: (1) Control diet + isotonic saline, (2) HFD + isotonic saline, (3) HFD +100 mg/kg/d GBSP, (4) HFD +200 mg/kg/d GBSP	*Akkermansia* ↑, *Romboutsia* ↑, *Bacteroides* ↑,*Lactobacillus* ↑; SCFAs↑	Activates the AMPK/ACC signaling pathway to produce 3,4-dihydroxyphenylpropionic acid (DHPPA) to inhibit lipid synthesis	Liang et al. (2025)
Tanshinone (Tan)	Salvia miltiorrhiza bunge	(a)*Caenorhabditis elegans*; (b)6-8-week-old male C57BL/6J	(a) Tan 25, 50, 100 μM; 25 days; control group: (1) NCD group, (2) HFD group, (3) HFD +100 μM Orlistat, (4) HFD + Tan 25 μM, (5) HFD + Tan 50 μM, (6) HFD + Tan 100 μM (b) Salvia miltiorrhiza ethanol extract 15 g/kg/d; 8 weeks; grouping: (1) NCD, (2) HFD, (3) HFD +0.2 g/kg Metformin, (4) HFD + Salvia miltiorrhiza ethanol extract	*Firmicutes* ↓, *Actinobacteria* ↓, *Bacteroidota* ↑, *Verrucomicrobiota* ↑	Upregulates *TFEB* expression and promotes lipid metabolism in the liver	Zheng et al. (2024)
Diammonium glycyrrhizinate (DG)	Glycyrrhiza uralensis Fisch ex DC	4-week-old male C57BL/6J	DG 150 mg/kg on alternate days; 14 weeks; control group: (1) blank control (NCD group), (2) negative	*Firmicutes*/*Bacteroidetes* ↓; *Lactobacillus* ↑, *Desulfovibrio* ↓;	No specific mechanism	Li et al. (2018)

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TABLE 1 (*Continued*) Mechanism of natural products targeting the GM-lipid metabolism in the treatment of NAFLD.

Active metabolites	Natural sources	Experimental model	Dose; Duration; grouping	Regulation of GM and its metabolism	Targeting lipid metabolism	Refs
			control group (HFD + placebo on alternate days), (3) DG group (HFD + DG 150 mg/kg on alternate days)	*Ruminococcaceae* ↑, *Lachnospiraceae* ↑; gut microbiota diversity ↑, SCFAs ↑, tight junction proteins↑, goblet cells ↑		
Ginsenosides (GS)	Panax ginseng C. A. Mey	6-week-old male C57BL/6J	GS 100 mg/kg, 200 mg/kg; 12 weeks; grouping: (1) ND group (normal chow diet), (2) HFD group, (3) GS-L group (100 mg/kg), (4) GS-H group (200 mg/kg)	*Bacteroidetes* ↑, *Firmicutes* to *Bacteroidetes* ratio (F/B) ↓; *Parabacteroides* ↑, *Akkermansia* ↑, *Helicobacter* ↓; *Muribaculaceae* ↑, *Lachnospiraceae* ↓	Promotes the liver lipolysis gene (Cpt-1a) and inhibits the lipogenesis genes (Srebp-1c, Fas, Acc-1) to improve liver lipid accumulation	Liang et al. (2021)
		8-week-old male C57BL/6J	(a) GS 47.5 mg/kg, GP 466 mg/kg; 7 weeks; grouping: (1) ND group, (2) HFD group, (3) GP group, (4) GS group (b) 0.1 mL/10 g/d; 4 weeks; grouping: (1) HFD group (saline), (2) FGP group (HFD + FMT from GP mice,0.1 mL/10 g/d), (3) FGS group (HFD + FMT from GS mice,0.1 mL/10 g/d)	Sulfurospirillum ↑, *Bacteroides* ↑, Bifidobacterium ↑, SCFAs ↑	Activation of the SCFA-GLP-1/PYY signaling pathway and intestinal gluconeogenesis	Luo et al. (2024)
Platycodin (PD)	Platycodon grandiflorus (Jacq.) A. DC.	8-week-old male C57BL/6J	(a) 375 mg/kg/d, 1125 mg/kg/d; 12 weeks; grouping: (1) ND; (2) ND + PRE-H; (3) HFD; (4) HFD + PRE-H; (5) HFD + PRE-L (b) 0.2 mL/d, 11 weeks; grouping: (1) Don-HFD, (2) Don-HFD + PRE; (3)Rec-HFD; (4)Rec-HFD + PRE	A.*muciniphila* ↑, tight junction protein 1↑, occludin protein gene Ocln↑	Reduces JNK/IRS phosphorylation in the liver and activates the PI3K/PIP3/Akt insulin signaling pathway	Luo et al. (2023)

targeting and intervening in GM-lipid metabolism. A substantial body of research has already yielded results. As shown in Table 1.

4.1 Phenols

4.1.1 Resveratrol

Resveratrol (RSV)(300 mg/kg/day by gavage, for 16 weeks) is a stilbenoid polyphenol that is enriched in red wine, grapes and pineapple nectar. RSV can ameliorate hepatic steatosis by repairing the HFD-injured intestinal mucosal barrier, decreasing the abundance of harmful bacteria in the GM, and increasing the abundance of SCFA-producing bacteria (Wang P. et al., 2020). RSV (300 mg/kg/day, for 16 weeks) significantly enriches the GM-derived metabolite 4-hydroxyphenylacetic acid (4-HPA) by modulating the structure of GM, which in turn activates the SIRT 1 pathway to modulate adipose tissue browning and thermogenesis to attenuate obesity-associated symptoms and inflammation in HFD-fed mice. Changes in GM include, at the phylum level, decreasing the abundance of *Firmicutes* and *Actinobacteria*, increasing the abundance of *Verrucomicrobia*; at the genus level, inhibiting the HFD-induced reduction of *Blautia* and decreased the relative abundance of *Lactobacillus*; and at the species level, increased abundance of *Akkermansia Muciniphila* (*A. muciniphila*) (Wang et al., 2025). RSV (0.5% in diet, for 8 weeks) has been shown to inhibit FXR-induced SR-B1 protein expression in the mouse intestine by modulating the composition of GM and its bile acid metabolites (Pang et al., 2023). This modulation not only reduces intestinal coeliac secretion but also upregulates the expression of fatty acid FAO-related genes including *Acadm*, *Ehhadh* and *Cpt1a*. Furthermore, RSV (50 mg/kg/day, 100 mg/kg/day,for 6 weeks) has been shown to enhance the synthesis of SCFAs, reduce LPS production, strengthen intestinal barrier integrity, and inhibit intestinal inflammation, thereby ameliorating the progression of NASH by remodeling the GM structure in a study of SD rats induced with HFD (Chen M. et al., 2020). These results suggest that RSV can intervene in the progression of NAFLD through GM and GM metabolites.

4.1.2 Curcumin

Curcumin (Cur), a polyphenolic phytochemical derived from *Curcuma longa* L, has been shown to improve insulin sensitivity, lower blood lipids, and act as an antioxidant (Slika and Patra, 2020). It has been hypothesised that Cur can intervene in the progression of a variety of diseases by modulating the structure of the GM, which in turn intervenes in the progression of several diseases, including

NAFLD (Scazzocchio et al., 2020). Supplementation of rats with Cur (100 mg/kg/day for 12 weeks) has been shown to enhance liver sinusoidal endothelial cells (LSECs) function via the NF-κB and PI3K/AKT/HIF-1α signaling pathways, thereby indirectly mitigating hepatic cell steatosis and damage (Wu et al., 2023). An RCT study (He et al., 2024) that included 80 patients with NAFLD. The subjects were randomly divided into two groups and administered Cur (500 mg/kg/d) and placebo, respectively. The duration of the trial was 24 days. In comparison with the placebo, Cur supplementation led to a substantial reduction in liver fat content, BMI, blood lipid levels, and blood glucose levels in patients with NAFLD. The therapeutic effect was associated with the modulation of GM-mediated BAs metabolism and the promotion of BAs receptor TGR5 activation to increase GLP-1 secretion. Furthermore, a separate study (Feng et al., 2017) demonstrated that Cur (200 mg/kg/d, for 4 weeks) reversed the effects of HFD on GM in rats and improved the degree of hepatic steatosis. Cur supplementation has been shown to increase the abundance of SCFA-secreting bacteria (at the genus level), including *Blautia* and *Allobaculum*, while concomitantly inhibiting the growth of bacteria (at the phylum level) associated with the progression of obesity and diabetes, such as *Ruminococcus*, *Coprococcus*, and *Mucispirillum*. Furthermore, Cur has been demonstrated to reduce GM-derived LPS production, thereby promoting the expression of tight junction proteins, occludin and ZO-1, to enhance the intestinal mucosal barrier.

4.1.3 Chlorogenic acid

Chlorogenic acid (CGA) is one of the important active metabolites in *Lonicera japonica* Thunb (Mahboob et al., 2016; Tajik et al., 2017). The present study (Li et al., 2023) evaluated the effects of supplemental CGA (1.34 mg/kg/day for 4 weeks) on NASH mice under various conditions, including NASH mice, antibiotic-treated NASH mice, and *Fxr*−/− NASH mice. The results demonstrated that liver function and lipid levels decreased in NASH mice, while liver function and lipid levels in antibiotic-treated NASH mice and *Fxr*−/− NASH mice remained unchanged before and after CGA intervention. This finding indicates that the depletion of gut bacteria induced by antibiotics can counteract the therapeutic effect of CGA on NASH, thereby suggesting that the efficacy of CGA in treating NASH is contingent upon FXR functionality. The mechanism of action of CGA involves the modulation of intestinal bacterial metabolism, which is associated with alterations in the composition of GM. These alterations include an increase in the abundance of *Bacteroidetes*, *Verrucomicrobia*, and *Tenericutes* at the phylum level, and a decrease in the abundance of *Lachnospiraceae* at the family level. At the genus level, there was a decrease in the abundance of *Roseburia*, and *Desulfovibrio*. Furthermore, CGA increased the expression of FXR, SHP, and BSEP in hepatocytes, thereby promoting FXR-regulated cholesterol metabolism and bile acid excretion, thus enhancing liver function and reducing lipid levels in MASH mice. Additionally, CGA increased BAs excretion, leading to improved hepatic lipid accumulation. In addition, CGA supplementation (60 mg/kg/d for 12 weeks) elevated insulin sensitivity in mice with HFD-induced NAFLD, increased the abundance of *Bifidobacterium* and decreased the abundance of *Escherichia* in GM, and inhibited activation of the TLR4 signalling pathway. This was achieved by increasing the levels of the tight junction proteins Occludin and ZO-1 in the intestinal mucosa and by decreasing the levels of inflammatory factors in the serum (Shi et al., 2021). Other study (Zhang et al., 2021) supplemented mice fed L-carnitine with different doses of CGA (200 mg/kg/d and 400 mg/kg/d). The results demonstrated that, in comparison with the negative control group (supplemented with saline), CGA significantly ameliorated L-carnitine-induced liver damage, including a reduction in hepatitis, steatosis, and oxidative stress. The therapeutic effect of CGA manifested in a dose-dependent manner. CGA exerts its therapeutic effects by inhibiting intestinal TMAO synthesis and reshaping the intestinal microbiota. Their findings revealed that, at the genus level, the abundance of *Akkermansia* and *Bacteroides* significantly increased, while the abundance of *Erysipelatoclostridium* and *Faecalibaculum* decreased in the intestinal microbiota. Intestinal-derived TMAO was reduced and SCFA levels were elevated in the colon. CGA reversed elevated lipids and hepatic inflammatory factors in mice. Furthermore, the administration of CGA (1.34 mg/kg/d) to mice with NAFLD led to an augmentation in the expression of tight junction proteins within the intestinal mucosa. Concurrently, this intervention resulted in the inhibition of tight junction structure degradation and a reduction in the levels of LPS derived from the intestine in NAFLD mice. This effect was achieved via the RhoA/ROCK signaling pathway, thereby intervening in the progression of NAFLD (Peng et al., 2018).

4.2 Alkaloids

4.2.1 Berberine

Berberine (BBR) is an isoquinoline alkaloid isolated from Rhizoma Coptidis (*Coptis chinensis* Franch.), which has been shown to have beneficial lipid-lowering properties (Feng et al., 2015). Supplementing HFD-induced mice with BBR (200 mg/kg/d) can selectively act on the beneficial intestinal bacterium *Blautia producta*, which in turn upregulates LDLR expression in hepatocytes to increase hepatic uptake of LDL, and increases the abundance of *Lautia spp*. To stimulate the production of SCFAs, thus lowering TC, and effectively ameliorating HFD-induced hyperlipidemia (HLP) (Yang et al., 2022). BBR has been shown to effectively ameliorate the effects of HFD on hyperlipidaemia by promoting the growth of beneficial butyrate-producing bacteria in the intestinal microflora. These bacteria then enter the bloodstream, where they can exert a lipid-lowering effect (Feng et al., 2018). BBR has also been observed to inhibit the PCSKP and the PCSKF via ERK signalling as well as the ubiquitin-proteasome pathway to inhibit the expression of PCSK9 (Dong et al., 2015; Cao et al., 2019), a liver-derived serine protease that binds to LDLR and contributes to the elevation of serum LDL-C levels (Seidah et al., 2014). BBR has been observed to promote the phosphorylation of AMPK in HepG2 cells, which in turn has been shown to reduce the expression of genes related to lipid biosynthesis, such as *FAS*, *GPAT*, and *ACC*, and consequently reduce blood lipid levels (Cao et al., 2013).

4.2.2 Betaine

Betaine, an alkaloid isolated from the molasses of sugar beets (*Beta vulgaris* L) (Du et al., 2018), has been shown to ameliorate

hepatic lipid accumulation in both humans and mice induced by HFD (Abdelmalek et al., 2009). Study (Koistinen et al., 2019) has demonstrated that the supplementation of betaine has been found to increase the abundance of *Coriobacteriaceae* at the family level; at the genus level, it has been found to increase the abundance of *Akkermansia*, *Bifidobacterium*, *Lactobacillus*, and *Ruminococcu*, which has been demonstrated to benefit host health. Researchers (Wu et al., 2020) demonstrated that betaine reduces intestinal damage and intestinal permeability, thereby limiting the entry of intestinal-derived LPS into the systemic circulation, and consequently inhibits the LPS/MAPK/NF-κB signaling pathway release of pro-inflammatory cytokines, including TNF-α and IL-1β, and ameliorate the restriction of IRS-1 and PPARα expression by inflammatory factors, while promoting lipid metabolism as well as attenuating hepatic lipid accumulation (Stienstra et al., 2010; Alipourfard et al., 2019). Supplementation of betaine to mothers not only ameliorated the hepatic lipid accumulation in the mother's own liver but also attenuated the hepatic lipidosis in the offspring caused by the maternal HFD (Sun L. et al., 2023). The study also examined the process of lipid degeneration in the offspring due to the maternal maternal HFD. This outcome was associated with betaine's capacity to enhance maternal intestinal flora disruption and augment beneficial intestinal metabolites. This included a decrease in the abundance of *Desulfovibrio*, *Ruminococcus*, and an increase in the abundance of *Bacteroides* and *Parabacteroides*, as well as an increase in the concentration of SCFAs in the feces, without significant changes in the levels of BAs and trimethylamine oxide. These changes have been shown to have a significant impact on the expression of lipid metabolism-related genes in the liver, including increased mRNA expression of *Pparα*, *Cpt1α*, and *Fatp2*.

4.3 Flavonoids

4.3.1 Quercetin

Quercetin (QUE) is an important plant metabolite of *Scutellaria baicalensis* Georgi. QUE supplementation has been shown to reduce TG and TC levels in mice fed an HDF, with this effect being dose-dependent (Wang T. et al., 2023). QUE has also been demonstrated to decrease the degree of hepatic steatosis in mice. QUE often requires GM to exert its probiotic function, albeit indirectly. According to the findings of recent research (Liu et al., 2025), the administration of QUE (100 mg/kg/d) to mice maintained on HFD has been demonstrated to have a substantial impact on the enrichment of probiotic *A. muciniphila* in GM. The metabolic product indole-3-lactic acid (ILA) produced by *A. muciniphila* activates the FTO/m6A/YTHDF2/CYP8B1 pathway, which facilitates the conversion of TC to BA. This, in turn, activates FXR, thereby inhibiting lipid synthesis. The study (Zhu et al., 2024) established control group (Con) and metabolic syndrome (MetS) model by subcutaneous injection of saline or sodium glutamate (3 mg/g). The MetS mouse were further subdivided into MetS and MetSQ subgroups, which were administered 0.15% sodium carboxymethylcellulose and QUE (50 mg/kg/d), respectively. Compared with Con group and MetS group, the lipid levels and the degree of hepatic steatosis in MetSQ mice were significantly reduced. Que supplementation has been demonstrated to regulate GM structure, thereby enriching the population of *Lactobacillus*. That has been shown to promote the synthesis of non-12α-hydroxylated bile acids, such as ursodeoxycholic acid and lithocholic acid. These bile acids subsequently bind to TGR5 on adipocytes, thereby activating BAT and inducing WAT browning. This, in turn, enhances thermogenesis mediated by mitochondrial uncoupling protein 1 (UCP1), leading to improvements in metabolic dysfunction. Another study (Porras et al., 2017) found that oral QUE (0.05% (wt/wt)) altered GM, which in turn regulated the expression of genes involved in lipid metabolism, including *Lxrα*, *Srebp-1c*, *Cd36*, *Fabp1*, *C/ebpα*, and *Foxa1*. The study also found that QUE reversed impaired intestinal SCFA synthesis and inhibited TLR-4-mediated hepatic inflammation, which ultimately ameliorated NAFLD. In a randomised study of 41 patients with NAFLD, the study was completed. In a randomised, double-blind clinical trial (Li et al., 2024), patients suffering from NAFLD were treated with QUE (500 mg/day) over a period of 12 consecutive weeks. This treatment resulted in a significant reduction in intrahepatic lipid content.

4.3.2 Silymarin

Silymarin, a flavonolignan metabolite extracted from the seeds of *Silybum marianum* (L.) Gaertn, is composed primarily of the isomers silybin, silydianin, and silychristin. Silymarin has been shown to have lipid-lowering and antioxidant effects, with the potential to improve NAFLD (Saller et al., 2001; Abenavoli et al., 2010; Vargas-Mendoza et al., 2014). A study (Sun W.-L. et al., 2023) revealed that silymarin supplementation for a period of 12 weeks led to enhancements in hepatic lipid metabolism in obese rats. Whole-genome shotgun (WGS) and targeted metabolomics studies on a subset of rat fecal DNA samples demonstrated that silymarin supplementation effectively increased the abundance of *Akkermansia* and *Blautia* in the rat intestinal. Furthermore, silymarin's lipid-lowering effects were found to be associated with an increase in B12-synthesizing bacteria within the GM. In an RCT (Jin et al., 2024), 83 patients with NAFLD were randomly assigned to two groups, receiving either a placebo or silymarin (103.2 mg/day). Following a 24-week period of observation, the results indicated that silymarin administration led to a reduction in liver stiffness and an enhancement in liver function. Additionally, there was an observed increase in GM's diversity. Specifically, the abundance of *Oscillospiraceae* in the intestine exhibited a marked increase. This bacterium has been linked to a reduced risk of NAFLD and increased SCFAs production (Zhao et al., 2022; He et al., 2023), suggesting that silymarin may play a role in the management of NAFLD by influencing the composition of the GM.

4.4 Polysaccharides

4.4.1 Astragalus polysaccharides

Astragalus Polysaccharides (APS), an active metabolite of *Astragalus membranaceus* Bunge has been shown to be effective in attenuating metabolic disorders induced by HFD, including decreasing the extent of hepatic steatosis, inhibiting body mass gain, and improving insulin resistance (Liu et al., 2020). In order to investigate the mechanism of APS treatment for NAFLD, researchers (Hong et al., 2021) used metagenomic sequencing and non-targeted metabolomics analysis to supplement the effects

of APS (4%) on HFD mice. In comparison with the ND group and the HFD group, supplementation with 4% APS significantly altered the GM and metabolites in mice. Including decreasing the abundance of *Firmicutes* and increasing the abundance of *Bacteroidetes* as well as the synthesis of SCFAs. Furthermore, APS has been shown to suppress the expression of glucokinase (GK), CD36, and FASN in hepatic tissues, while promoting the hepatic mRNA expression of CPT-1α and PPAR-α, thereby inhibiting FA synthesis and promoting FAO. In addition, adipogenesis and lipolysis are both reduced by APS, which activates AMPK, upregulates PPAR-α, and downregulates SREBP-1c levels (Sun et al., 2014). Researchers (Song et al., 2024) conducted a study in which they found that APS was degraded to SCFA by GM. This degradation subsequently significantly enhanced intestinal integrity and stimulated GPCR43 expression. The promotion of GPCR43 expression was found to stimulate GLP-1 secretion and inhibit NAFLD progression by controlling blood glucose. Furthermore, APS has been demonstrated to enhance GM diversity, increasing the abundance of beneficial bacteria such as *Dubosiella* and *Monoglobus*, and decreasing the abundance of harmful bacteria such as *Escherichia* and *Acinetobacter*. Furthermore, the study (Zhong et al., 2022) administered 200 mg/kg/day of mAPS extracts to HFD mice. The results demonstrated that, in comparison with mice subjected to model group mice (only fed HFD), the administration of mAPS extracts significantly mitigated hepatic lipid accumulation and inflammation, as well as reduced blood lipid levels, induced by an HFD. The results also indicated that supplementation with mAPS extracts enhanced the expression of AMPK and PPAR-α, and reduced the expression of SREBP-1. Furthermore, the therapeutic effects of mAPS extracts were associated with the SCFA-GPR41/43 signaling pathway. In addition, mAPS extracts were found to remodel GM, including at the phylum level, where the application of mAPS extracts resulted in an increase in the abundance of *Proteobacteria* and a decrease in the ratio of *Firmicutes* to *Bacteroidetes*. At the class level, the abundance of *Epsilonbacteria* also exhibited a significant increase.

4.4.2 Ginkgo biloba seed polysaccharide

Ginkgo seeds have a long history in both medicine and food production. Ginkgo biloba (*Ginkgo biloba* L) Seed Polysaccharide (GBSP) is a polysaccharide that is isolated and purified from ginkgo seeds. In murine models of non-alcoholic fatty liver disease (NAFLD), administered at doses of 100 or 200 mg per kilogram of body weight, GBSP has been observed to attenuate liver steatosis, a condition characterized by an accumulation of fat in the liver, induced by HFD (Liang et al., 2025). This effect appears to be the result of a multifaceted regulatory mechanism involving several pathways. GBSP has been shown to significantly increase the abundance of *Akkermansia*, *Romboutsia*, *Lactobacillus*, and *Bacteroides*, as well as to activate the AMPK/ACC signaling pathway, thereby inhibiting lipid synthesis through the production of 3,4-dihydroxyphenylpropionic acid (DHPPA).

4.5 Terpenoids

4.5.1 Tanshinone

In the domain of traditional Chinese medicine, *Salvia miltiorrhiza* bunge (Dan shen) has a long history of utilization as an herbal remedy for the treatment of NAFLD. A pivotal metabolite of this medicinal approach is tanshinone (Tan), a crucial active metabolite in Danshen. The knockout of the *Tfeb* gene has been demonstrated to induce lipid accumulation in adipocytes. The present study (Zheng et al., 2024) investigated the effects of Tan at varying concentrations (25 μM, 50 μM, 100 μM) on *Caenorhabditis elegans* (*C. elegans*) induced by HFD. Model group (fed HFD), blank control (normal diet), and positive control (HFD and 100 μM Orlistat) were established. The results demonstrated that Tan induced nuclear translocation of the TFEB homolog HLH-30 in *C. elegans* and reduced fat accumulation, with the lipid-lowering effect of 100 μM Tan being comparable to that of the positive control. Subsequently, researchers administered an ethanol extract of Salvia miltiorrhiza (primarily Tan) to HFD mice via oral gavage (15 g/kg/day for 2 weeks) and established three groups: model group (HFD), blank control group (normal diet), and positive control group (HFD and 0.2 g/kg Metformin). The extract containing abundant Tan has been shown to reduce the abundance of *Firmicutes* and *Actinobacteria*, and increase the abundance of *Bacteroidota* and *Verrucomicrobiota*, thereby improving lipid accumulation in the liver (Zheng et al., 2024). That suggests that Tan treatment for NALFD may be a viable option.

The study (Shou et al., 2025) found that free cholesterol (FC) has a significant impact on the severity of NAFLD, exacerbating the accumulation of triglycerides in the liver by increasing ROS levels, damaging lysosomes, and inhibiting lipophagy. Dihydrotanshinone I (DhT) is a prominent metabolite of tanshinones. Supplementation with DhT has been shown to reduce liver lipids in mice with NAFLD. However, the knockdown of liver *Pparα* negated this effect, and no significant changes in GM or metabolites were observed before or after the intervention with DhT. This observation indicates that DhT activates PPARα pathway, leading to a reduction in ROS, which in turn promotes lipophagy. Notably, this effect is observed to be independent of GM.

4.5.2 Diammonium glycyrrhizinate

Diammonium glycyrrhizinate (DG) is a triterpene saponin metabolite that is extracted from the root of *Glycyrrhiza uralensis* Fisch ex DC. It is currently a first-line pharmaceutical agent for the treatment of inflammation and protection of the liver (Sun et al., 2019). The study (Li et al., 2018) randomly divided normal mice into three groups: blank control group (fed normal diet), negative control group (fed HFD and placebo), and DG group (fed HFD and 150 mg/kg DG). After 2 weeks, the DG group exhibited a marked decrease in body weight, as well as hepatic steatosis and inflammation, when compared with the placebo group. These enhancements are attributable to DG's capacity to enhance gut microbiota diversity in GM. The observed alterations encompass a decline in the *Firmicutes*/*Bacteroidetes* ratio at the phylum level. At the genus level, the relative abundance of probiotics such as *Lactobacillus* increased, while the relative abundance of LPS-producing bacteria such as *Desulfovibrio* decreased. At the family level, there was an increase in bacteria producing SCFAs, including *Ruminococcaceae* and *Lachnospiraceae*. Concurrently, DG has been observed to facilitate the restoration of intestinal mucosal barrier function by augmenting the expression of tight junction proteins and goblet cells, while concurrently stimulating mucin secretion.

4.6 Saponins

4.6.1 Ginsenosides

In traditional Chinese medicine, *Panax ginseng* C. A. Mey is regarded as the "king of botanical drugs", and ginsenosides (GS) have been characterized as important metabolites of *Panax ginseng* C. A. Mey (Yin et al., 2021). In the study (Luo et al., 2024), the isolation of ginsenosides (GS) and ginsenoside polysaccharides (GP) from dried ginseng slices was conducted. Two groups of mice were fed HFD and supplemented with GS (47.5 mg/kg/d) or GP (466 mg/kg/d), respectively. In comparison with the mice subjected to HFD, supplementation with GS or GP effectively intervened in the development of obesity induced by an HFD. In order to investigate the hypothesis that GS intervenes in obesity through GM, researchers transplanted GM from GS-supplemented mice into another group of mice subjected to an HFD. A blank control group was set up for comparison. The results (Luo et al., 2024) demonstrate that GS and GP can intervene in obesity induced by HFD, and this effect is mediated by GM. Specially GS has been demonstrated to selectively enrich species such as *Sulfurospirillum*, *Bacteroides*, and *Bifidobacterium* within the intestinal tract. Concurrently, *Bacteroides* and *Bifidobacterium* have been observed to facilitate the synthesis of SCFAs. Furthermore, GS has been shown to enhance obesity by stimulating the SCFA-GLP-1/PYY signaling pathway and intestinal gluconeogenesis. In the present study (Liang et al., 2021), the effects of ginsenoside extract on HFD-induced hepatic steatosis and metabolic endotoxemia in mice were investigated. Mice were divided into three groups: normal diet (ND) group, HFD group, and experimental group that received different doses of ginsenoside extract (100 mg/kg or 200 mg/kg). Compared with ND mice and HFD mice, ginsenoside extract significantly alleviated HFD-induced hepatic steatosis and metabolic endotoxemia, and improved liver function and intestinal barrier function. The therapeutic effect exhibited a dose-dependent relationship. This therapeutic effect is attributable to the influence of ginsenoside extract on GM. At the phylum level, ginsenoside extract led to a significant increase in the abundance of *Bacteroidetes* and a concomitant reduction in the *Firmicutes* to *Bacteroidetes* ratio (F/B). At the genus level, ginsenoside extract has been shown to promote the proliferation of *Parabacteroides* and *Akkermansia*, which synthesize SCFAs and regulate metabolic disorders, while inhibiting the prevalence of harmful bacteria *Helicobacter*. At the family level, ginsenoside extract has been shown to promote the prevalence of beneficial bacteria *Muribaculaceae*, while reversing the increase of harmful bacteria *Lachnospiraceae*. Concurrently, ginsenoside extract mitigates liver inflammation by impeding the activation of the NF-κB/IκB signaling pathway. GS has also been observed to enhance liver lipid accumulation by promoting the expression of genes involved in liver lipolysis (*Cpt-1a*) and inhibiting the expression of genes associated with lipogenesis (*Srebp-1c*, *Fas*, *Acc-1*) (Fang and Judd, 2018).

4.6.2 Platycodin

In East Asia, *Platycodon grandiflorus* (*Platycodon grandiflorus* (Jacq.) A. DC.) applications include use as both food and medicine. Platycodin D (PD) is a triterpene saponin metabolite extracted from the roots of *Platycodon grandiflorus*. For NAFLD, the potential of PD to reduce the risk of disease by promoting bile acid biosynthesis has been demonstrated (Kim et al., 2024). Secondly, PD has been shown to improve liver steatosis by downregulating the expression of intestinal lipid uptake proteins (CD36, NPC1L1, and ApoB) (Tang et al., 2024) and upregulating the expression of hepatic lipolysis proteins (CPT1, HSL, and UCP2) (Hwang et al., 2019).

Conversely, PD has been shown to inhibit excessive gluconeogenesis induced by HFD through the AMPK-PCK1-G6Pase signaling pathway, activate the AMPK-ACC-CPT-1 signaling pathway to reduce fatty acid biosynthesis, and increase fatty acid oxidation to reduce liver lipids (Shen et al., 2023). *In vitro* experiments have also demonstrated the protective effect of PD on NAFLD, which may involve reducing the level of oxidative stress induced by palmitic acid in AML-12 cells and enhancing mitochondrial function (Wen et al., 2022). PRE (*Platycodon grandiflorus* root extract) is an extract derived from the decoction of *Platycodon grandiflorus*, containing multiple Platycodin metabolites (Platycodin C, D2, D3, J). A study (Luo et al., 2023) used different doses of PRE (375 mg/kg or 1125 mg/kg) to intervene in mice fed HFD. The results demonstrated that both low and high doses of PRE effectively alleviated symptoms of MetS induced by HFD, including reducing the severity of hepatic steatosis, lowering lipid levels, and improving insulin sensitivity. In order to validate the therapeutic effect of PRE and its relationship with GM, researchers transplanted intestinal microbiota from PRE-treated mice into pseudo-germ-free mice (previously treated with antibiotics) induced by HFD. The results demonstrated that the therapeutic efficacy of PRE in MetS is closely associated with GM. PRE has been demonstrated to promote the enrichment of *A. muciniphila*, thereby activating the downstream PI3K/PIP3/Akt insulin signaling pathway and improving MetS. MetS is a key risk factor for the progression of NAFLD. It is imperative to acknowledge that the experimental design employed PRE, not PD alone. Although UPLC-LTQ-Orbitrap MS analysis indicated that PD was dominant in PRE, the contribution of PD to the therapeutic effects of PRE requires further investigation.

5 Discussion and perspective

Despite the fact that the pathogenesis of NAFLD has not yet been elucidated, some progress has been made in the study of its pathogenesis. There is a broad consensus in the scientific community that lipid metabolism disorders play a pivotal role in the development of NAFLD, as evidenced by the traditional "second-strike" theory and the current mainstream "multiple-strike" theory (Santos-Baez and Ginsberg, 2021). Consequently, addressing lipid metabolism disorders is imperative for the treatment of NAFLD.

GM, a complex microbial ecosystem within the human body, influences the balance between disease and illness. The present study established that GM and its metabolites interact with hepatic lipid metabolism through the "gut-liver axis" (Han et al., 2023). This finding suggests that GM and its metabolites may have a role in the prevention and intervention of NAFLD. It is important to note that GM is highly sensitive to external factors, and a variety of metabolites, including those of natural origin, can trigger dynamic changes in GM structure and function. Consequently,

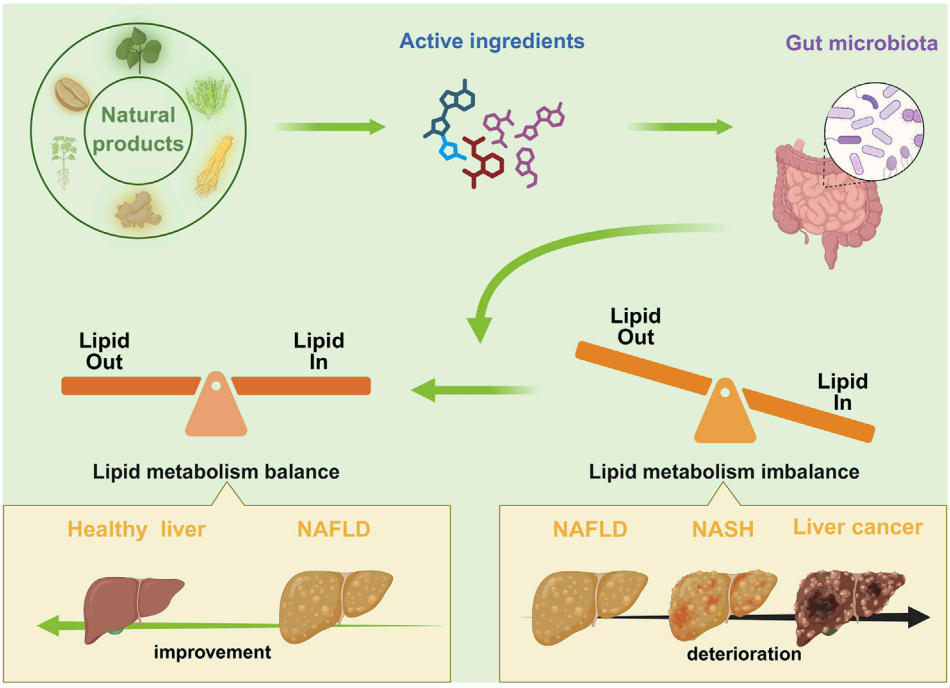

FIGURE 3
An overview of the therapeutic effects of natural products on NAFLD through regulation of glucose and lipid metabolism. Imbalances in liver lipid metabolism (the accumulation of lipids in the liver exceeds their consumption) can trigger the progression of NAFLD. Natural products regulate liver imbalances through the gut microbiota, promoting lipid metabolism balance in the liver, which helps reverse the progression of NAFLD. NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

the promotion of beneficial GM remodeling through therapeutic interventions holds considerable promise in the treatment of NAFLD. Probiotics have been shown to possess NAFLD therapeutic capabilities by modulating lipid metabolism, inhibiting inflammatory responses, and maintaining intestinal mucosal barrier integrity (Ji et al., 2019).

However, it is important to acknowledge the limitations of studies examining direct probiotic transplantation as a treatment for NAFLD. Firstly, the specific molecular biological mechanisms by which probiotics affect lipid metabolism in the pathogenesis of NAFLD have not been fully elucidated. Secondly, significant inter-individual differences in GM, colonization resistance of host intrinsic flora to foreign probiotics, and survival stability of transplanted probiotics act as obstacles to direct probiotic supplementation. Conversely, natural products have been shown to promote natural growth by supplying the nutrients required for the proliferation of host-intrinsic probiotics, thereby reducing interference with the GM ecosystem (Guo et al., 2022). This approach is hypothesized to be safer and to exhibit reduced colonization resistance. These findings (Koistinen et al., 2019; Yang et al., 2022; Sun W.-L. et al., 2023; Zhu et al., 2024) also confirm the therapeutic efficacy of natural product-derived metabolites in NAFLD. Consequently, the utilisation of natural products to indirectly modulate GM structure and thereby intervene in NAFLD emerges as a potential option. As shown in Figure 3.

Nevertheless, contemporary research endeavors concerning the impact of natural products and their metabolites on hepatic lipid metabolism through GM and its metabolites continue to encounter significant challenges. First, there is a paucity of studies that specifically examine the molecular mechanisms by which GM influences hepatic lipid metabolism. Secondly, the synthesis mechanisms of GM metabolites remain unclear, and the mechanisms by which natural product derivatives influence GM metabolites also require further elucidation. It is imperative to investigate the mechanisms by which GM metabolites regulate the expression of genes associated with lipid metabolism through epigenetic modifications. Furthermore, there is a necessity to explore methodologies that can be employed to overcome the heterogeneity in treatment efficacy that is caused by differences in GM composition among individuals. A significant challenge in the field pertains to the limited water solubility, poor intestinal absorption rates, and substantial first-pass metabolism exhibited by numerous metabolites of botanical drugs, which collectively result in considerably diminished bioavailability. A major challenge in this field is the heterogeneity of the studies, which is characterized by significant variations in dosage, treatment duration, and evaluation criteria. This heterogeneity poses a significant obstacle to the comparison of results across studies. In order to address these challenges, researchers must collect large-scale gut microbiome data and combine it with multi-omics technologies to analyze the potential associations between GM characteristics and lipid metabolism. Furthermore, subsequent studies ought to prioritize the investigation of the particular mechanisms that govern the interactions between GM and lipid metabolism within the body. This will facilitate the development of precise strategies for

modulating lipid metabolism through GM reprogramming. Such research not only reveals the impact of individual gut microbiome differences on health status and treatment sensitivity but also assists clinicians in developing personalized treatment plans based on individual GM profiles and lipid metabolism states. Furthermore, advancements in technology have led to innovations that enhance bioavailability. These include the utilization of liposomes, polymer nanoparticles, and other encapsulation technologies to improve solubility and targeting, as well as conducting structural modifications to enhance stability and absorption rates. These technological advancements have proven effective in improving bioavailability.

In conclusion, natural products hold considerable promise in the treatment of diseases and merit further investigation. Natural products have been shown to regulate lipid metabolism in NAFLD by affecting GM and metabolites, thereby intervening in the progression of NAFLD. The potential of natural products in driving NAFLD therapy by targeting GM and lipid metabolism is significant, as it not only enriches the theoretical underpinnings of the gut-hepatic axis, but also deepens our understanding of natural products.

Author contributions

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Conflict of interest

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

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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References

Abdelmalek, M. F., Sanderson, S. O., Angulo, P., Soldevila-Pico, C., Liu, C., Peter, J., et al. (2009). Betaine for nonalcoholic fatty liver disease: results of a randomized placebo-controlled trial. *Hepatol. Balt. Md* 50, 1818–1826. doi:10.1002/hep.23239

Abenavoli, L., Capasso, R., Milic, N., and Capasso, F. (2010). Milk thistle in liver diseases: past, present, future. *Phytother. Res. PTR* 24, 1423–1432. doi:10.1002/ptr.3207

Abu-Elheiga, L., Brinkley, W. R., Zhong, L., Chirala, S. S., Woldegiorgis, G., and Wakil, S. J. (2000). The subcellular localization of acetyl-CoA carboxylase 2. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1444–1449. doi:10.1073/pnas.97.4.1444

Adeva-Andany, M. M., Carneiro-Freire, N., Seco-Filgueira, M., Fernández-Fernández, C., and Mouriño-Bayolo, D. (2019). Mitochondrial β-oxidation of saturated fatty acids in humans. *Mitochondrion* 46, 73–90. doi:10.1016/j.mito.2018.02.009

Adorini, L., and Trauner, M. (2023). FXR agonists in NASH treatment. *J. Hepatol.* 79, 1317–1331. doi:10.1016/j.jhep.2023.07.034

Agus, A., Clément, K., and Sokol., H. (2021). Gut microbiota-derived metabolites as central regulators in metabolic disorders. *Gut* 70, 1174–1182. doi:10.1136/gutjnl-2020-323071

Akbal, E., Koçak, E., Akyürek, Ö., Köklü, S., Batgi, H., and Şenes, M. (2016). Liver fatty acid-binding protein as a diagnostic marker for non-alcoholic fatty liver disease. *Wien. Klin. Wochenschr.* 128, 48–52. doi:10.1007/s00508-014-0680-8

Alipourfard, I., Datukishvili, N., and Mikeladze, D. (2019). TNF-A downregulation modifies insulin receptor substrate 1 (IRS-1) in metabolic signaling of diabetic insulin-resistant hepatocytes. *Mediat. Inflamm.* 2019, 3560819. doi:10.1155/2019/3560819

Anstee, Q. M., Reeves, H. L., Kotsiliti, E., Govaere, O., and Heikenwalder, M. (2019). From NASH to HCC: current concepts and future challenges. *Nat. Rev. Gastroenterol. Hepatol.* 16, 411–428. doi:10.1038/s41575-019-0145-7

Arias, N., Arboleya, S., Allison, J., Kaliszewska, A., Higarza, S. G., Gueimonde, M., et al. (2020). The relationship between choline bioavailability from diet, intestinal microbiota composition, and its modulation of human diseases. *Nutrients* 12, 2340. doi:10.3390/nu12082340

Aron-Wisnewsky, J., Vigliotti, C., Witjes, J., Le, P., Holleboom, A. G., Verheij, J., et al. (2020). Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat. Rev. Gastroenterol. Hepatol.* 17, 279–297. doi:10.1038/s41575-020-0269-9

Badmus, O. O., Hillhouse, S. A., Anderson, C. D., Hinds, T. D., and Stec, D. E. (2022). Molecular mechanisms of metabolic associated fatty liver disease (MAFLD): functional analysis of lipid metabolism pathways. *Clin. Sci. Lond. Engl. 1979* 136, 1347–1366. doi:10.1042/CS20220572

Banini, B. A., Cazanave, S. C., Yates, K. P., Asgharpour, A., Vincent, R., Mirshahi, F., et al. (2019). Haptoglobin 2 allele is associated with histologic response to vitamin E in subjects with nonalcoholic steatohepatitis. *J. Clin. Gastroenterol.* 53, 750–758. doi:10.1097/MCG.0000000000001142

Batchuluun, B., Pinkosky, S. L., and Steinberg, G. R. (2022). Lipogenesis inhibitors: therapeutic opportunities and challenges. *Nat. Rev. Drug Discov.* 21, 283–305. doi:10.1038/s41573-021-00367-2

Bates, J., Vijayakumar, A., Ghoshal, S., Marchand, B., Yi, S., Kornyeyev, D., et al. (2020). Acetyl-CoA carboxylase inhibition disrupts metabolic reprogramming during hepatic stellate cell activation. *J. Hepatol.* 73, 896–905. doi:10.1016/j.jhep.2020.04.037

Bauer, K. C., Littlejohn, P. T., Ayala, V., Creus-Cuadros, A., and Finlay, B. B. (2022). Nonalcoholic fatty liver disease and the gut-liver Axis: exploring an undernutrition perspective. *Gastroenterology* 162, 1858–1875.e2. doi:10.1053/j.gastro.2022.01.058

Bianchi, A., Evans, J. L., Iverson, A. J., Nordlund, A. C., Watts, T. D., and Witters, L. A. (1990). Identification of an isozymic form of acetyl-CoA carboxylase. *J. Biol. Chem.* 265, 1502–1509. doi:10.1016/s0021-9258(19)40045-8

Blázquez, C., Geelen, M. J., Velasco, G., and Guzmán, M. (2001). The AMP-activated protein kinase prevents ceramide synthesis *de novo* and apoptosis in astrocytes. *FEBS Lett.* 489, 149–153. doi:10.1016/s0014-5793(01)02089-0

Böhm, T., Berger, H., Nejabat, M., Riegler, T., Kellner, F., Kuttke, M., et al. (2013). Food-derived peroxidized fatty acids may trigger hepatic inflammation: a novel hypothesis to explain steatohepatitis. *J. Hepatol.* 59, 563–570. doi:10.1016/j.jhep.2013.04.025

Bourbeau, M. P., and Bartberger, M. D. (2015). Recent advances in the development of acetyl-CoA carboxylase (ACC) inhibitors for the treatment of metabolic disease. *J. Med. Chem.* 58, 525–536. doi:10.1021/jm500695e

Boursier, J., Mueller, O., Barret, M., Machado, M., Fizanne, L., Araujo-Perez, F., et al. (2016). The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatol. Balt. Md* 63, 764–775. doi:10.1002/hep.28356

Brownsey, R. W., Boone, A. N., Elliott, J. E., Kulpa, J. E., and Lee, W. M. (2006). Regulation of acetyl-CoA carboxylase. *Biochem. Soc. Trans.* 34, 223–227. doi:10.1042/BST20060223

Buzzetti, E., Pinzani, M., and Tsochatzis, E. A. (2016). The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 65, 1038–1048. doi:10.1016/j.metabol.2015.12.012

Calle, R. A., Amin, N. B., Carvajal-Gonzalez, S., Ross, T. T., Bergman, A., Aggarwal, S., et al. (2021). ACC inhibitor alone or co-administered with a DGAT2 inhibitor in patients with non-alcoholic fatty liver disease: two parallel, placebo-controlled, randomized phase 2a trials. *Nat. Med.* 27, 1836–1848. doi:10.1038/s41591-021-01489-1

Canale, F. P., Basso, C., Antonini, G., Perotti, M., Li, N., Sokolovska, A., et al. (2021). Metabolic modulation of tumours with engineered bacteria for immunotherapy. *Nature* 598, 662–666. doi:10.1038/s41586-021-04003-2

Canbay, A., Bechmann, L., and Gerken, G. (2007). Lipid metabolism in the liver. *Z. Gastroenterol.* 45, 35–41. doi:10.1055/s-2006-927368

Cao, S., Xu, P., Yan, J., Liu, H., Liu, L., Cheng, L., et al. (2019). Berberrubine and its analog, hydroxypropyl-berberrubine, regulate LDLR and PCSK9 expression via the ERK signal pathway to exert cholesterol-lowering effects in human hepatoma HepG2 cells. *J. Cell. Biochem.* 120, 1340–1349. doi:10.1002/jcb.27102

Cao, S., Zhou, Y., Xu, P., Wang, Y., Yan, J., Bin, W., et al. (2013). Berberine metabolites exhibit triglyceride-lowering effects via activation of AMP-activated protein kinase in Hep G2 cells. *J. Ethnopharmacol.* 149, 576–582. doi:10.1016/j.jep.2013.07.025

Carpi, R. Z., Barbalho, S. M., Sloan, K. P., Laurindo, L. F., Gonzaga, H. F., Grippa, P. C., et al. (2022). The effects of probiotics, prebiotics and synbiotics in non-alcoholic fat liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH): a systematic review. *Int. J. Mol. Sci.* 23, 8805. doi:10.3390/ijms23158805

Caussy, C., and Loomba, R. (2018). Gut microbiome, microbial metabolites and the development of NAFLD. *Nat. Rev. Gastroenterol. Hepatol.* 15, 719–720. doi:10.1038/s41575-018-0058-x

Chabowski, A., Żendzian-Piotrowska, M., Konstantynowicz, K., Pankiewicz, W., Mikłosz, A., Łukaszuk, B., et al. (2013). Fatty acid transporters involved in the palmitate and oleate induced insulin resistance in primary rat hepatocytes. *Acta Physiol. oxf. Engl.* 207, 346–357. doi:10.1111/apha.12022

Chakravarthy, M. V., Pan, Z., Zhu, Y., Tordjman, K., Schneider, J. G., Coleman, T., et al. (2005). "New" hepatic fat activates PPARalpha to maintain glucose, lipid, and cholesterol homeostasis. *Cell Metab.* 1, 309–322. doi:10.1016/j.cmet.2005.04.002

Chen, A., Tang, Y., Davis, V., Hsu, F.-F., Kennedy, S. M., Song, H., et al. (2013). Liver fatty acid binding protein (L-Fabp) modulates murine stellate cell activation and diet-induced nonalcoholic fatty liver disease. *Hepatol. Balt. Md* 57, 2202–2212. doi:10.1002/hep.26318

Chen, H. C., Smith, S. J., Ladha, Z., Jensen, D. R., Ferreira, L. D., Pulawa, L. K., et al. (2002). Increased insulin and leptin sensitivity in mice lacking acyl CoA:diacylglycerol acyltransferase 1. *J. Clin. Invest.* 109, 1049–1055. doi:10.1172/JCI14672

Chen, J., Li, X., Ge, C., Min, J., and Wang, F. (2022). The multifaceted role of ferroptosis in liver disease. *Cell Death Differ.* 29, 467–480. doi:10.1038/s41418-022-00941-0

Chen, M., Hou, P., Zhou, M., Ren, Q., Wang, X., Huang, L., et al. (2020a). Resveratrol attenuates high-fat diet-induced non-alcoholic steatohepatitis by maintaining gut barrier integrity and inhibiting gut inflammation through regulation of the endocannabinoid system. *Clin. Nutr. Edinb. Scotl.* 39, 1264–1275. doi:10.1016/j.clnu.2019.05.020

Chen, Q., Wang, Y., Jiao, F., Shi, C., Pei, M., Wang, L., et al. (2020b). Betaine inhibits Toll-like receptor 4 responses and restores intestinal microbiota in acute liver failure mice. *Sci. Rep.* 10, 21850. doi:10.1038/s41598-020-78935-6

Chen, T., Dalton, G., Oh, S.-H., Maeso-Diaz, R., Du, K., Meyers, R. A., et al. (2023). Hepatocyte smoothened activity controls susceptibility to insulin resistance and nonalcoholic fatty liver disease. *Cell. Mol. Gastroenterol. Hepatol.* 15, 949–970. doi:10.1016/j.jcmgh.2022.12.008

Chen, W., Wei, Y., Xiong, A., Li, Y., Guan, H., Wang, Q., et al. (2020c). Comprehensive analysis of serum and fecal bile acid profiles and interaction with gut microbiota in primary biliary cholangitis. *Clin. Rev. Allergy Immunol.* 58, 25–38. doi:10.1007/s12016-019-08731-2

Chen, X.-F., Chen, X., and Tang, X. (2020d). Short-chain fatty acid, acylation and cardiovascular diseases. *Clin. Sci. Lond. Engl. 1979* 134, 657–676. doi:10.1042/CS20200128

Chen, Y., Xu, C., Huang, R., Song, J., Li, D., and Xia, M. (2018a). Butyrate from pectin fermentation inhibits intestinal cholesterol absorption and attenuates atherosclerosis in apolipoprotein E-deficient mice. *J. Nutr. Biochem.* 56, 175–182. doi:10.1016/j.jnutbio.2018.02.011

Chen, Z., Luo, L., Chen, R., Hu, H., Pan, Y., Jiang, H., et al. (2018b). Acetylome profiling reveals extensive lysine acetylation of the fatty acid metabolism pathway in the diatom phaeodactylum tricornutum. *Mol. Cell. Proteomics MCP* 17, 399–412. doi:10.1074/mcp.RA117.000339

Choi, Y.-J., Shin, H.-S., Choi, H. S., Park, J.-W., Jo, I., Oh, E.-S., et al. (2014). Uric acid induces fat accumulation via generation of endoplasmic reticulum stress and SREBP-1c activation in hepatocytes. *Lab. Investig. J. Tech. Methods Pathol.* 94, 1114–1125. doi:10.1038/labinvest.2014.98

Christiansen, C. B., Gabe, M. B. N., Svendsen, B., Dragsted, L. O., Rosenkilde, M. M., and Holst, J. J. (2018). The impact of short-chain fatty acids on GLP-1 and PYY secretion from the isolated perfused rat colon. *Am. J. Physiol. Gastrointest. Liver Physiol.* 315, G53-G65–G65. doi:10.1152/ajpgi.00346.2017

Clifford, B. L., Sedgeman, L. R., Williams, K. J., Morand, P., Cheng, A., Jarrett, K. E., et al. (2021). FXR activation protects against NAFLD via bile-acid-dependent reductions in lipid absorption. *Cell Metab.* 33, 1671–1684.e4. doi:10.1016/j.cmet.2021.06.012

Corbin, K. D., and Zeisel, S. H. (2012). Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. *Curr. Opin. Gastroenterol.* 28, 159–165. doi:10.1097/MOG.0b013e32834e7b4b

Cross, E., Dearlove, D. J., and Hodson, L. (2023). Nutritional regulation of hepatic *de novo* lipogenesis in humans. *Curr. Opin. Clin. Nutr. Metab. Care* 26, 65–71. doi:10.1097/MCO.0000000000000914

Dai, W., Zhang, H., Lund, H., Zhang, Z., Castleberry, M., Rodriguez, M., et al. (2023). Intracellular tPA-PAI-1 interaction determines VLDL assembly in hepatocytes. *Science* 381, eadh5207. doi:10.1126/science.adh5207

de Aguiar Vallim, T. Q., Tarling, E. J., and Edwards, P. A. (2013). Pleiotropic roles of bile acids in metabolism. *Cell Metab.* 17, 657–669. doi:10.1016/j.cmet.2013.03.013

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D.-J., and Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* 54, 2325–2340. doi:10.1194/jlr.R036012

Deng, G., Li, J., Huang, M., Li, Y., Shi, H., Wu, C., et al. (2024). Erchen decoction alleviates the progression of NAFLD by inhibiting lipid accumulation and iron overload through Caveolin-1 signaling. *J. Ethnopharmacol.* 319, 117320. doi:10.1016/j.jep.2023.117320

Deng, G.-H., Wu, C.-F., Li, Y.-J., Shi, H., Zhong, W.-C., Hong, M.-K., et al. (2023). Caveolin-1 is critical for hepatic iron storage capacity in the development of nonalcoholic fatty liver disease. *Mil. Med. Res.* 10, 53. doi:10.1186/s40779-023-00487-3

Dentin, R., Benhamed, F., Hainault, I., Fauveau, V., Foufelle, F., Dyck, J. R. B., et al. (2006). Liver-specific inhibition of ChREBP improves hepatic steatosis and insulin resistance in ob/ob mice. *Diabetes* 55, 2159–2170. doi:10.2337/db06-0200

Ding, S., Kang, J., Tong, L., Lin, Y., Liao, L., and Gao, B. (2018). Erchen decoction ameliorates lipid metabolism by the regulation of the protein CAV-1 and the receptors VLDLR, LDLR, ABCA1, and SRB1 in a high-fat diet rat model. *Evid.-Based Complement. Altern. Med. ECAM* 2018, 5309490. doi:10.1155/2018/5309490

Diraison, F., Moulin, P., and Beylot, M. (2003). Contribution of hepatic *de novo* lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab.* 29, 478–485. doi:10.1016/s1262-3636(07)70061-7

Diraison, F., Pachiaudi, C., and Beylot, M. (1997). Measuring lipogenesis and cholesterol synthesis in humans with deuterated water: use of simple gas chromatographic/mass spectrometric techniques. *J. Mass Spectrom. JMS* 32, 81–86. doi:10.1002/(SICI)1096-9888(199701)32:1<81::AID-JMS454>3.0.CO;2-2

Dixon, E. D., Nardo, A. D., Claudel, T., and Trauner, M. (2021). The role of lipid sensing nuclear receptors (PPARs and LXR) and metabolic lipases in obesity, diabetes and NAFLD. *Genes* 12, 645. doi:10.3390/genes12050645

Dobrzyn, A., Dobrzyn, P., Lee, S.-H., Miyazaki, M., Cohen, P., Asilmaz, E., et al. (2005). Stearoyl-CoA desaturase-1 deficiency reduces ceramide synthesis by downregulating serine palmitoyltransferase and increasing beta-oxidation in skeletal

muscle. *Am. J. Physiol. Endocrinol. Metab.* 288, E599–E607. doi:10.1152/ajpendo.00439.2004

Dobrzyn, P., Dobrzyn, A., Miyazaki, M., Cohen, P., Asilmaz, E., Hardie, D. G., et al. (2004). Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. *Proc. Natl. Acad. Sci. U. S. A.* 101, 6409–6414. doi:10.1073/pnas.0401627101

Doege, H., Baillie, R. A., Ortegon, A. M., Tsang, B., Wu, Q., Punreddy, S., et al. (2006). Targeted deletion of FATP5 reveals multiple functions in liver metabolism: alterations in hepatic lipid homeostasis. *Gastroenterology* 130, 1245–1258. doi:10.1053/j.gastro.2006.02.006

Dong, B., Li, H., Singh, A. B., Cao, A., and Liu, J. (2015). Inhibition of PCSK9 transcription by berberine involves down-regulation of hepatic HNF1α protein expression through the ubiquitin-proteasome degradation pathway. *J. Biol. Chem.* 290, 4047–4058. doi:10.1074/jbc.M114.597229

Dong, H., Zhong, W., Zhang, W., Hao, L., Guo, W., Yue, R., et al. (2023). Loss of long-chain acyl-CoA synthetase 1 promotes hepatocyte death in alcohol-induced steatohepatitis. *Metabolism* 138, 155334. doi:10.1016/j.metabol.2022.155334

Dong, Z., Zhuang, Q., Ning, M., Wu, S., Lu, L., and Wan, X. (2020). Palmitic acid stimulates NLRP3 inflammasome activation through TLR4-NF-κB signal pathway in hepatic stellate cells. *Ann. Transl. Med.* 8, 168. doi:10.21037/atm.2020.02.21

Donnelly, K. L., Smith, C. I., Schwarzenberg, S. J., Jessurun, J., Boldt, M. D., and Parks, E. J. (2005). Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J. Clin. Invest.* 115, 1343–1351. doi:10.1172/JCI23621

Dorn, C., Riener, M.-O., Kirovski, G., Saugspier, M., Steib, K., Weiss, T. S., et al. (2010). Expression of fatty acid synthase in nonalcoholic fatty liver disease. *Int. J. Clin. Exp. Pathol.* 3, 505–514.

Du, J., Shen, L., Tan, Z., Zhang, P., Zhao, X., Xu, Y., et al. (2018). Betaine supplementation enhances lipid metabolism and improves insulin resistance in mice fed a high-fat diet. *Nutrients* 10, 131. doi:10.3390/nu10020131

Du, J., Zhang, P., Luo, J., Shen, L., Zhang, S., Gu, H., et al. (2021). Dietary betaine prevents obesity through gut microbiota-drived microRNA-378a family. *Gut Microbes* 13, 1–19. doi:10.1080/19490976.2020.1862612

Du, Y., Li, X., Su, C., Xi, M., Zhang, X., Jiang, Z., et al. (2020). Butyrate protects against high-fat diet-induced atherosclerosis via up-regulating ABCA1 expression in apolipoprotein E-deficiency mice. *Br. J. Pharmacol.* 177, 1754–1772. doi:10.1111/bph.14933

Eberlé, D., Hegarty, B., Bossard, P., Ferré, P., and Foufelle, F. (2004). SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* 86, 839–848. doi:10.1016/j.biochi.2004.09.018

Eguchi, A., Hasegawa, H., Iwasa, M., Tamai, Y., Ohata, K., Oikawa, T., et al. (2019). Serum liver-type fatty acid-binding protein is a possible prognostic factor in human chronic liver diseases from chronic hepatitis to liver cirrhosis and hepatocellular carcinoma. *Hepatol. Commun.* 3, 825–837. doi:10.1002/hep4.1350

Eguchi, A., and Iwasa, M. (2021). The role of elevated liver-type fatty acid-binding proteins in liver diseases. *Pharm. Res.* 38, 89–95. doi:10.1007/s11095-021-02998-x

Enooku, K., Tsutsumi, T., Kondo, M., Fujiwara, N., Sasako, T., Shibahara, J., et al. (2020). Hepatic FATP5 expression is associated with histological progression and loss of hepatic fat in NAFLD patients. *J. Gastroenterol.* 55, 227–243. doi:10.1007/s00535-019-01633-2

Ericson, E., Bergenholm, L., Andréasson, A.-C., Dix, C. I., Knöchel, J., Hansson, S. F., et al. (2022). Hepatic patatin-like phospholipase domain-containing 3 levels are increased in I148M risk allele carriers and correlate with NAFLD in humans. *Hepatol. Commun.* 6, 2689–2701. doi:10.1002/hep4.2032

Falcon, A., Doege, H., Fluitt, A., Tsang, B., Watson, N., Kay, M. A., et al. (2010). FATP2 is a hepatic fatty acid transporter and peroxisomal very long-chain acyl-CoA synthetase. *Am. J. Physiol. Endocrinol. Metab.* 299, E384–E393. doi:10.1152/ajpendo.00226.2010

Fang, H., and Judd, R. L. (2018). Adiponectin regulation and function. *Compr. Physiol.* 8, 1031–1063. doi:10.1002/cphy.c170046

Feng, R., Shou, J.-W., Zhao, Z.-X., He, C.-Y., Ma, C., Huang, M., et al. (2015). Transforming berberine into its intestine-absorbable form by the gut microbiota. *Sci. Rep.* 5, 12155. doi:10.1038/srep12155

Feng, R., Zhao, Z.-X., Ma, S.-R., Guo, F., Wang, Y., and Jiang, J.-D. (2018). Gut microbiota-regulated pharmacokinetics of berberine and active metabolites in beagle dogs after oral administration. *Front. Pharmacol.* 9, 214. doi:10.3389/fphar.2018.00214

Feng, W., Wang, H., Zhang, P., Gao, C., Tao, J., Ge, Z., et al. (2017). Modulation of gut microbiota contributes to curcumin-mediated attenuation of hepatic steatosis in rats. *Biochim. Biophys. Acta Gen. Subj.* 1861, 1801–1812. doi:10.1016/j.bbagen.2017.03.017

Fernandes, I. P. G., and Oliveira-Brett, A. M. (2020). Caveolin proteins electrochemical oxidation and interaction with cholesterol. *Bioelectrochemistry Amst. Neth.* 133, 107451. doi:10.1016/j.bioelechem.2019.107451

Ferré, P., Phan, F., and Foufelle, F. (2021). SREBP-1c and lipogenesis in the liver: an update1. *Biochem. J.* 478, 3723–3739. doi:10.1042/BCJ20210071

Ferro, D., Baratta, F., Pastori, D., Cocomello, N., Colantoni, A., Angelico, F., et al. (2020). New insights into the pathogenesis of non-alcoholic fatty liver disease: gut-derived lipopolysaccharides and oxidative stress. *Nutrients* 12, 2762. doi:10.3390/nu12092762

Filali-Mouncef, Y., Hunter, C., Roccio, F., Zagkou, S., Dupont, N., Primard, C., et al. (2022). The ménage à trois of autophagy, lipid droplets and liver disease. *Autophagy* 18, 50–72. doi:10.1080/15548627.2021.1895658

Fiorucci, S., Biagioli, M., Sepe, V., Zampella, A., and Distrutti, E. (2020). Bile acid modulators for the treatment of nonalcoholic steatohepatitis (NASH). *Expert Opin. Investig. Drugs* 29, 623–632. doi:10.1080/13543784.2020.1763302

Fiorucci, S., and Distrutti, E. (2022). Linking liver metabolic and vascular disease via bile acid signaling. *Trends Mol. Med.* 28, 51–66. doi:10.1016/j.molmed.2021.10.005

Flores-Guerrero, J. L., Post, A., van Dijk, P. R., Connelly, M. A., Garcia, E., Navis, G., et al. (2021). Circulating trimethylamine-N-oxide is associated with all-cause mortality in subjects with nonalcoholic fatty liver disease. *Liver Int. Off. J. Int. Assoc. Study Liver* 41, 2371–2382. doi:10.1111/liv.14963

Flowers, M. T., Groen, A. K., Oler, A. T., Keller, M. P., Choi, Y., Schueler, K. L., et al. (2006). Cholestasis and hypercholesterolemia in SCD1-deficient mice fed a low-fat, high-carbohydrate diet. *J. Lipid Res.* 47, 2668–2680. doi:10.1194/jlr.M600203-JLR200

Flowers, M. T., Keller, M. P., Choi, Y., Lan, H., Kendziorski, C., Ntambi, J. M., et al. (2008). Liver gene expression analysis reveals endoplasmic reticulum stress and metabolic dysfunction in SCD1-deficient mice fed a very low-fat diet. *Physiol. Genomics* 33, 361–372. doi:10.1152/physiolgenomics.00139.2007

Flowers, M. T., and Ntambi, J. M. (2008). Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism. *Curr. Opin. Lipidol.* 19, 248–256. doi:10.1097/MOL.0b013e3282f9b54d

Fondevila, M. F., Fernandez, U., Heras, V., Parracho, T., Gonzalez-Rellan, M. J., Novoa, E., et al. (2022). Inhibition of carnitine palmitoyltransferase 1A in hepatic stellate cells protects against fibrosis. *J. Hepatol.* 77, 15–28. doi:10.1016/j.jhep.2022.02.003

Fontaine, M., Dessein, A.-F., Douillard, C., Dobbelaere, D., Brivet, M., Boutron, A., et al. (2012). A novel mutation in CPT1A resulting in hepatic CPT deficiency. *JIMD Rep.* 6, 7–14. doi:10.1007/8904_2011_94

Frahm, J. L., Li, L. O., Grevengoed, T. J., and Coleman, R. A. (2011). Phosphorylation and acetylation of acyl-CoA synthetase- I. *J. Proteomics Bioinform* 4, 129–137. doi:10.4172/jpb.1000180

Fushimi, T., Suruga, K., Oshima, Y., Fukiharu, M., Tsukamoto, Y., and Goda, T. (2006). Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. *Br. J. Nutr.* 95, 916–924. doi:10.1079/bjn20061740

Gluchowski, N. L., Gabriel, K. R., Chitraju, C., Bronson, R. T., Mejhert, N., Boland, S., et al. (2019). Hepatocyte deletion of triglyceride-synthesis enzyme acyl CoA: diacylglycerol acyltransferase 2 reduces steatosis without increasing inflammation or fibrosis in mice. *Hepatol. Balt. Md* 70, 1972–1985. doi:10.1002/hep.30765

Goedeke, L., Bates, J., Vatner, D. F., Perry, R. J., Wang, T., Ramirez, R., et al. (2018). Acetyl-CoA carboxylase inhibition reverses NAFLD and hepatic insulin resistance but promotes hypertriglyceridemia in rodents. *Hepatol. Balt. Md* 68, 2197–2211. doi:10.1002/hep.30097

Gomaa, E. Z. (2020). Human gut microbiota/microbiome in health and diseases: a review. *Ant. Van Leeuwenhoek* 113, 2019–2040. doi:10.1007/s10482-020-01474-7

Greco, D., Kotronen, A., Westerbacka, J., Puig, O., Arkkila, P., Kiviluoto, T., et al. (2008). Gene expression in human NAFLD. *Am. J. Physiol. Gastrointest. Liver Physiol.* 294, G1281–G1287. doi:10.1152/ajpgi.00074.2008

Griffin, J. D., Buxton, J. M., Culver, J. A., Barnes, R., Jordan, E. A., White, A. R., et al. (2023). Hepatic Activin E mediates liver-adipose inter-organ communication, suppressing adipose lipolysis in response to elevated serum fatty acids. *Mol. Metab.* 78, 101830. doi:10.1016/j.molmet.2023.101830

Guan, B., Tong, J., Hao, H., Yang, Z., Chen, K., Xu, H., et al. (2022). Bile acid coordinates microbiota homeostasis and systemic immunometabolism in cardiometabolic diseases. *Acta Pharm. Sin. B* 12, 2129–2149. doi:10.1016/j.apsb.2021.12.011

Guo, X., Yin, X., Liu, Z., and Wang, J. (2022). Non-alcoholic fatty liver disease (NAFLD) pathogenesis and natural products for prevention and treatment. *Int. J. Mol. Sci.* 23, 15489. doi:10.3390/ijms232415489

Haghikia, A., Zimmermann, F., Schumann, P., Jasina, A., Roessler, J., Schmidt, D., et al. (2022). Propionate attenuates atherosclerosis by immune-dependent regulation of intestinal cholesterol metabolism. *Eur. Heart J.* 43, 518–533. doi:10.1093/eurheartj/ehab644

Hameed, I., Hayat, J., Marsia, S., Samad, S. A., Khan, R., Siddiqui, O. M., et al. (2023). Comparison of sodium-glucose cotransporter-2 inhibitors and thiazolidinediones for management of non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Clin. Res. Hepatol. Gastroenterol.* 47, 102111. doi:10.1016/j.clinre.2023.102111

Han, H., Jiang, Y., Wang, M., Melaku, M., Liu, L., Zhao, Y., et al. (2023). Intestinal dysbiosis in nonalcoholic fatty liver disease (NAFLD): focusing on the gut-liver axis. *Crit. Rev. Food Sci. Nutr.* 63, 1689–1706. doi:10.1080/10408398.2021.1966738

Handberg, A., Højlund, K., Gastaldelli, A., Flyvbjerg, A., Dekker, J. M., Petrie, J., et al. (2012). Plasma sCD36 is associated with markers of atherosclerosis, insulin resistance and fatty liver in a nondiabetic healthy population. *J. Intern. Med.* 271, 294–304. doi:10.1111/j.1365-2796.2011.02442.x

Haney, M. S., Pálovics, R., Munson, C. N., Long, C., Johansson, P. K., Yip, O., et al. (2024). APOE4/4 is linked to damaging lipid droplets in Alzheimer's disease microglia. *Nature* 628, 154–161. doi:10.1038/s41586-024-07185-7

He, C., Wang, W., Wei, G., Wang, Y., Wei, Y., Wang, J., et al. (2023). Sodium alginate combined with oxymatrine ameliorates CCl4-induced chemical hepatic fibrosis in mice. *Int. Immunopharmacol.* 125, 111144. doi:10.1016/j.intimp.2023.111144

He, Y., Chen, X., Li, Y., Liang, Y., Hong, T., Yang, J., et al. (2024). Curcumin supplementation alleviates hepatic fat content associated with modulation of gut microbiota-dependent bile acid metabolism in patients with nonalcoholic simple fatty liver disease: a randomized controlled trial. *Am. J. Clin. Nutr.* 120, 66–79. doi:10.1016/j.ajcnut.2024.05.017

He, Y.-J., and You, C.-G. (2020). The potential role of gut microbiota in the prevention and treatment of lipid metabolism disorders. *Int. J. Endocrinol.* 2020, 8601796. doi:10.1155/2020/8601796

Heebøll, S., Poulsen, M. K., Ornstrup, M. J., Kjær, T. N., Pedersen, S. B., Nielsen, S., et al. (2017). Circulating sCD36 levels in patients with non-alcoholic fatty liver disease and controls. *Int. J. Obes.* 41, 262–267. doi:10.1038/ijo.2016.223

Heinemann, F. S., and Ozols, J. (2003). Stearoyl-CoA desaturase, a short-lived protein of endoplasmic reticulum with multiple control mechanisms. *Prostagl. Leukot. Essent. Fat. Acids* 68, 123–133. doi:10.1016/s0952-3278(02)00262-4

Hellerstein, M. K., Schwarz, J. M., and Neese, R. A. (1996). Regulation of hepatic *de novo* lipogenesis in humans. *Annu. Rev. Nutr.* 16, 523–557. doi:10.1146/annurev.nu.16.070196.002515

Henkel, J., Alfine, E., Saín, J., Jöhrens, K., Weber, D., Castro, J. P., et al. (2018). Soybean oil-derived poly-unsaturated fatty acids enhance liver damage in NAFLD induced by dietary cholesterol. *Nutrients* 10, 1326. doi:10.3390/nu10091326

Hong, Y., Sheng, L., Zhong, J., Tao, X., Zhu, W., Ma, J., et al. (2021). Desulfovibrio vulgaris, a potent acetic acid-producing bacterium, attenuates nonalcoholic fatty liver disease in mice. *Gut Microbes* 13, 1–20. doi:10.1080/19490976.2021.1930874

Horton, J. D., Shah, N. A., Warrington, J. A., Anderson, N. N., Park, S. W., Brown, M. S., et al. (2003). Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. U. S. A.* 100, 12027–12032. doi:10.1073/pnas.1534923100

Hosseinkhani, F., Heinken, A., Thiele, I., Lindenburg, P. W., Harms, A. C., and Hankemeier, T. (2021). The contribution of gut bacterial metabolites in the human immune signaling pathway of non-communicable diseases. *Gut Microbes* 13, 1–22. doi:10.1080/19490976.2021.1882927

Hoy, A. J., Nagarajan, S. R., and Butler, L. M. (2021). Tumour fatty acid metabolism in the context of therapy resistance and obesity. *Nat. Rev. Cancer* 21, 753–766. doi:10.1038/s41568-021-00388-4

Huh, J. Y., Reilly, S. M., Abu-Odeh, M., Murphy, A. N., Mahata, S. K., Zhang, J., et al. (2020). TANK-binding kinase 1 regulates the localization of acyl-CoA synthetase ACSL1 to control hepatic fatty acid oxidation. *Cell Metab.* 32, 1012–1027. doi:10.1016/j.cmet.2020.10.010

Hussain, M. M., and Bakillah, A. (2008). New approaches to target microsomal triglyceride transfer protein. *Curr. Opin. Lipidol.* 19, 572–578. doi:10.1097/MOL.0b013e328312707c

Hussain, M. M., Rava, P., Walsh, M., Rana, M., and Iqbal, J. (2012). Multiple functions of microsomal triglyceride transfer protein. *Nutr. Metab.* 9, 14. doi:10.1186/1743-7075-9-14

Hwang, K.-A., Hwang, Y.-J., Im, P. R., Hwang, H.-J., Song, J., and Kim, Y.-J. (2019). Platycodon grandiflorum extract reduces high-fat diet-induced obesity through regulation of adipogenesis and lipogenesis pathways in mice. *J. Med. Food* 22, 993–999. doi:10.1089/jmf.2018.4370

Iizuka, K., Bruick, R. K., Liang, G., Horton, J. D., and Uyeda, K. (2004). Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proc. Natl. Acad. Sci. U. S. A.* 101, 7281–7286. doi:10.1073/pnas.0401516101

Iizuka, K., Takeda, J., and Horikawa, Y. (2009). Glucose induces FGF21 mRNA expression through ChREBP activation in rat hepatocytes. *FEBS Lett.* 583, 2882–2886. doi:10.1016/j.febslet.2009.07.053

Ioannou, G. N. (2016). The role of cholesterol in the pathogenesis of NASH. *Trends Endocrinol. Metab. Tem.* 27, 84–95. doi:10.1016/j.tem.2015.11.008

Ishii, S., Iizuka, K., Miller, B. C., and Uyeda, K. (2004). Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription. *Proc. Natl. Acad. Sci. U. S. A.* 101, 15597–15602. doi:10.1073/pnas.0405238101

Jensen-Cody, S. O., Flippo, K. H., Claflin, K. E., Yavuz, Y., Sapouckey, S. A., Walters, G. C., et al. (2020). FGF21 signals to glutamatergic neurons in the ventromedial hypothalamus to suppress carbohydrate intake. *Cell Metab.* 32, 273–286. doi:10.1016/j.cmet.2020.06.008

Ji, L., Li, Q., He, Y., Zhang, X., Zhou, Z., Gao, Y., et al. (2022). Therapeutic potential of traditional Chinese medicine for the treatment of NAFLD: a promising drug Potentilla discolor Bunge. *Acta Pharm. Sin. B* 12, 3529–3547. doi:10.1016/j.apsb.2022.05.001

Ji, Y., Yin, Y., Li, Z., and Zhang, W. (2019). Gut microbiota-derived components and metabolites in the progression of non-alcoholic fatty liver disease (NAFLD). *Nutrients* 11, 1712. doi:10.3390/nu11081712

Jiang, S.-Y., Yang, X., Yang, Z., Li, J.-W., Xu, M.-Q., Qu, Y.-X., et al. (2022). Discovery of an insulin-induced gene binding compound that ameliorates nonalcoholic steatohepatitis by inhibiting sterol regulatory element-binding protein-mediated lipogenesis. *Hepatol. Balt. Md* 76, 1466–1481. doi:10.1002/hep.32381

Jiang, W., Wang, J., Xue, W., Xin, J., Shi, C., Wen, J., et al. (2021). Caveolin-1 attenuates acetaminophen aggravated lipid accumulation in alcoholic fatty liver by activating mitophagy via the Pink-1/Parkin pathway. *Eur. J. Pharmacol.* 908, 174324. doi:10.1016/j.ejphar.2021.174324

Jiang, X., Wu, S., and Hu, C. (2023). A narrative review of the role of exosomes and caveolin-1 in liver diseases and cancer. *Int. Immunopharmacol.* 120, 110284. doi:10.1016/j.intimp.2023.110284

Jin, Y., Wang, X., Chen, K., Chen, Y., Zhou, L., Zeng, Y., et al. (2024). Silymarin decreases liver stiffness associated with gut microbiota in patients with metabolic dysfunction-associated steatotic liver disease: a randomized, double-blind, placebo-controlled trial. *Lipids Health Dis.* 23, 239. doi:10.1186/s12944-024-02220-y

Jois, T., Chen, W., Howard, V., Harvey, R., Youngs, K., Thalmann, C., et al. (2017). Deletion of hepatic carbohydrate response element binding protein (ChREBP) impairs glucose homeostasis and hepatic insulin sensitivity in mice. *Mol. Metab.* 6, 1381–1394. doi:10.1016/j.molmet.2017.07.006

Juanola, O., Martínez-López, S., Francés, R., and Gómez-Hurtado, I. (2021). Non-alcoholic fatty liver disease: metabolic, genetic, epigenetic and environmental risk factors. *Int. J. Environ. Res. Public. Health* 18, 5227. doi:10.3390/ijerph18105227

Kang, J., Wu, J., Liu, Q., Jiang, H., Li, W., Li, Y., et al. (2024). FASN regulates STING palmitoylation via malonyl-CoA in macrophages to alleviate sepsis-induced liver injury. *Biochim. Biophys. Acta Mol. Basis Dis.* 1870, 167299. doi:10.1016/j.bbadis.2024.167299

Kawano, Y., and Cohen, D. E. (2013). Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J. Gastroenterol.* 48, 434–441. doi:10.1007/s00535-013-0758-5

Keam, S. J. (2024). Resmetirom: first approval. *Drugs* 84, 729–735. doi:10.1007/s40265-024-02045-0

Kim, C.-W., Addy, C., Kusunoki, J., Anderson, N. N., Deja, S., Fu, X., et al. (2017). Acetyl CoA carboxylase inhibition reduces hepatic steatosis but elevates plasma triglycerides in mice and humans: a bedside to bench investigation. *Cell Metab.* 26, 576. doi:10.1016/j.cmet.2017.08.011

Kim, G.-A., Moon, J. H., and Kim, W. (2023). Critical appraisal of metabolic dysfunction-associated steatotic liver disease: implication of Janus-faced modernity. *Clin. Mol. Hepatol.* 29, 831–843. doi:10.3350/cmh.2023.0277

Kim, K. H. (1997). Regulation of mammalian acetyl-coenzyme A carboxylase. *Annu. Rev. Nutr.* 17, 77–99. doi:10.1146/annurev.nutr.17.1.77

Kim, W., Baek, W. H., Yun, S. H., Lee, H., Kim, M. J., Lee, S.-Y., et al. (2024). Platycodi radix extract prevents hepatic steatosis by enhancing bile acid synthesis in a high-fat diet-induced fatty liver mouse model. *Nutrients* 16, 893. doi:10.3390/nu16060893

Koistinen, V. M., Kärkkäinen, O., Borewicz, K., Zarei, I., Jokkala, J., Micard, V., et al. (2019). Contribution of gut microbiota to metabolism of dietary glycine betaine in mice and *in vitro* colonic fermentation. *Microbiome* 7, 103. doi:10.1186/s40168-019-0718-2

Kokkorakis, M., Boutari, C., Katsiki, N., and Mantzoros, C. S. (2023). From non-alcoholic fatty liver disease (NAFLD) to steatotic liver disease (SLD): an ongoing journey towards refining the terminology for this prevalent metabolic condition and unmet clinical need. *Metabolism* 147, 155664. doi:10.1016/j.metabol.2023.155664

Kondo, T., Kishi, M., Fushimi, T., and Kaga, T. (2009). Acetic acid upregulates the expression of genes for fatty acid oxidation enzymes in liver to suppress body fat accumulation. *J. Agric. Food Chem.* 57, 5982–5986. doi:10.1021/jf900470c

Kong, L., Zhao, Q., Jiang, X., Hu, J., Jiang, Q., Sheng, L., et al. (2024). Trimethylamine N-oxide impairs β-cell function and glucose tolerance. *Nat. Commun.* 15, 2526. doi:10.1038/s41467-024-46829-0

Kreuz, S., Schoelch, C., Thomas, L., Rist, W., Rippmann, J. F., and Neubauer, H. (2009). Acetyl-CoA carboxylases 1 and 2 show distinct expression patterns in rats and humans and alterations in obesity and diabetes. *Diabetes Metab. Res. Rev.* 25, 577–586. doi:10.1002/dmrr.997

Kwan, S.-Y., Jiao, J., Joon, A., Wei, P., Petty, L. E., Below, J. E., et al. (2022). Gut microbiome features associated with liver fibrosis in Hispanics, a population at high risk for fatty liver disease. *Hepatol. Balt. Md* 75, 955–967. doi:10.1002/hep.32197

Lechner, S., Yee, M., Limketkai, B. N., and Pham, E. A. (2020). Fecal microbiota transplantation for chronic liver diseases: current understanding and future direction. *Dig. Dis. Sci.* 65, 897–905. doi:10.1007/s10620-020-06100-0

Lee, J.-S., Zheng, Z., Mendez, R., Ha, S.-W., Xie, Y., and Zhang, K. (2012). Pharmacologic ER stress induces non-alcoholic steatohepatitis in an animal model. *Toxicol. Lett.* 211, 29–38. doi:10.1016/j.toxlet.2012.02.017

Lee, S.-H., Dobrzyn, A., Dobrzyn, P., Rahman, S. M., Miyazaki, M., and Ntambi, J. M. (2004). Lack of stearoyl-CoA desaturase 1 upregulates basal thermogenesis but causes hypothermia in a cold environment. *J. Lipid Res.* 45, 1674–1682. doi:10.1194/jlr.M400039-JLR200

Le Roy, T., Llopis, M., Lepage, P., Bruneau, A., Rabot, S., Bevilacqua, C., et al. (2013). Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut* 62, 1787–1794. doi:10.1136/gutjnl-2012-303816

Leung, H., Long, X., Ni, Y., Qian, L., Nychas, E., Siliceo, S. L., et al. (2022). Risk assessment with gut microbiome and metabolite markers in NAFLD development. *Sci. Transl. Med.* 14, eabk0855. doi:10.1126/scitranslmed.abk0855

Li, F., Ye, J., Shao, C., and Zhong, B. (2021). Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and Meta-analysis. *Lipids Health Dis.* 20, 22. doi:10.1186/s12944-021-01440-w

Li, H., Cheng, S., Huo, J., Dong, K., Ding, Y., Man, C., et al. (2022). Lactobacillus plantarum J26 alleviating alcohol-induced liver inflammation by maintaining the intestinal barrier and regulating MAPK signaling pathways. *Nutrients* 15, 190. doi:10.3390/nu15010190

Li, H., Xi, Y., Xin, X., Feng, Q., and Hu, Y. (2023). Geniposide plus chlorogenic acid reverses non-alcoholic steatohepatitis via regulation of gut microbiota and bile acid signaling in a mouse model *in vivo*. *Front. Pharmacol.* 14, 1148737. doi:10.3389/fphar.2023.1148737

Li, M. V., Chang, B., Imamura, M., Poungvarin, N., and Chan, L. (2006). Glucose-dependent transcriptional regulation by an evolutionarily conserved glucose-sensing module. *Diabetes* 55, 1179–1189. doi:10.2337/db05-0822

Li, N., Cui, C., Xu, J., Mi, M., Wang, J., and Qin, Y. (2024). Quercetin intervention reduced hepatic fat deposition in patients with nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled crossover clinical trial. *Am. J. Clin. Nutr.* 120, 507–517. doi:10.1016/j.ajcnut.2024.07.013

Li, Y., Liu, T., Yan, C., Xie, R., Guo, Z., Wang, S., et al. (2018). Diammonium glycyrrhizinate protects against nonalcoholic fatty liver disease in mice through modulation of gut microbiota and restoration of intestinal barrier. *Mol. Pharm.* 15, 3860–3870. doi:10.1021/acs.molpharmaceut.8b00347

Liang, S., Yao, Z., Chen, J., Qian, J., Dai, Y., and Li, H. (2025). Structural characterization of a α-d-glucan from Ginkgo biloba seeds and its protective effects on non-alcoholic fatty liver disease in mice. *Carbohydr. Polym.* 349, 123022. doi:10.1016/j.carbpol.2024.123022

Liang, W., Zhou, K., Jian, P., Chang, Z., Zhang, Q., Liu, Y., et al. (2021). Ginsenosides improve nonalcoholic fatty liver disease via integrated regulation of gut microbiota, inflammation and energy homeostasis. *Front. Pharmacol.* 12, 622841. doi:10.3389/fphar.2021.622841

Lin, Y.-C., Ma, C., Hsu, W.-C., Lo, H.-F., and Yang, V. C. (2007). Molecular interaction between caveolin-1 and ABCA1 on high-density lipoprotein-mediated cholesterol efflux in aortic endothelial cells. *Cardiovasc. Res.* 75, 575–583. doi:10.1016/j.cardiores.2007.04.012

Linden, A. G., Li, S., Choi, H. Y., Fang, F., Fukasawa, M., Uyeda, K., et al. (2018). Interplay between ChREBP and SREBP-1c coordinates postprandial glycolysis and lipogenesis in livers of mice. *J. Lipid Res.* 59, 475–487. doi:10.1194/jlr.M081836

Liu, B., Yi, W., Mao, X., Yang, L., and Rao, C. (2021). Enoyl coenzyme A hydratase 1 alleviates nonalcoholic steatohepatitis in mice by suppressing hepatic ferroptosis. *Am. J. Physiol. Endocrinol. Metab.* 320, E925–E937. doi:10.1152/ajpendo.00614.2020

Liu, J., Liu, Y., Huang, C., He, C., Yang, T., Ren, R., et al. (2025). Quercetin-driven Akkermansia muciniphila alleviates obesity by modulating bile acid metabolism via an ILA/m6A/CYP8B1 signaling. *Adv. Sci. Weinh. Baden-Wurtt. Ger.* 12, e2412865. doi:10.1002/advs.202412865

Liu, Y.-L., Zhang, Q.-Z., Wang, Y.-R., Fu, L.-N., Han, J.-S., Zhang, J., et al. (2020). Astragaloside IV improves high-fat diet-induced hepatic steatosis in nonalcoholic fatty liver disease rats by regulating inflammatory factors level via TLR4/NF-κB signaling pathway. *Front. Pharmacol.* 11, 605064. doi:10.3389/fphar.2020.605064

Long, J. Z., and Cravatt, B. F. (2011). The metabolic serine hydrolases and their functions in mammalian physiology and disease. *Chem. Rev.* 111, 6022–6063. doi:10.1021/cr200075y

Longo, R., Peri, C., Cricrì, D., Coppi, L., Caruso, D., Mitro, N., et al. (2019). Ketogenic diet: a new light shining on old but gold biochemistry. *Nutrients* 11, 2497. doi:10.3390/nu11102497

Loomba, R., Kayali, Z., Noureddin, M., Ruane, P., Lawitz, E. J., Bennett, M., et al. (2018). GS-0976 reduces hepatic steatosis and fibrosis markers in patients with nonalcoholic fatty liver disease. *Gastroenterology* 155, 1463–1473. doi:10.1053/j.gastro.2018.07.027

Lu, Y.-C., Chang, C.-C., Wang, C.-P., Hung, W.-C., Tsai, I.-T., Tang, W.-H., et al. (2020). Circulating fatty acid-binding protein 1 (FABP1) and nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. *Int. J. Med. Sci.* 17, 182–190. doi:10.7150/ijms.40417

Luo, H.-Y., Fang, J., Zhang, W.-H., Chan, K.-C., Chan, Y.-M., Dong, C.-X., et al. (2024). Dissecting the anti-obesity components of ginseng: how ginseng polysaccharides and ginsenosides target gut microbiota to suppress high-fat diet-induced obesity. *J. Adv. Res.* S2090-1232 (24), 00558–7. doi:10.1016/j.jare.2024.12.002

Luo, J., Yang, H., and Song, B.-L. (2020). Mechanisms and regulation of cholesterol homeostasis. *Nat. Rev. Mol. Cell Biol.* 21, 225–245. doi:10.1038/s41580-019-0190-7

Luo, Z., Xu, W., Yuan, T., Shi, C., Jin, T., Chong, Y., et al. (2023). Platycodon grandiflorus root extract activates hepatic PI3K/PIP3/Akt insulin signaling by enriching gut Akkermansia muciniphila in high fat diet fed mice. *Phytomedicine Int. J. Phytother. Phytopharm.* 109, 154595. doi:10.1016/j.phymed.2022.154595

Ma, Y.-Y., Li, L., Yu, C.-H., Shen, Z., Chen, L.-H., and Li, Y.-M. (2013). Effects of probiotics on nonalcoholic fatty liver disease: a meta-analysis. *World J. Gastroenterol.* 19, 6911–6918. doi:10.3748/wjg.v19.i40.6911

Mahboob, T., Azlan, A.-M., Tan, T.-C., Samudi, C., Sekaran, S. D., Nissapatorn, V., et al. (2016). Anti-encystment and amoebicidal activity of *Lonicera japonica* Thunb. and its major constituent chlorogenic acid *in vitro*. *Asian pac. J. Trop. Med.* 9, 866–871. doi:10.1016/j.apjtm.2016.07.008

Martin, G. G., Atshaves, B. P., Landrock, K. K., Landrock, D., Schroeder, F., and Kier, A. B. (2015). Loss of L-FABP, SCP-2/SCP-x, or both induces hepatic lipid accumulation in female mice. *Arch. Biochem. Biophys.* 580, 41–49. doi:10.1016/j.abb.2015.06.009

Matsuda, M., Korn, B. S., Hammer, R. E., Moon, Y. A., Komuro, R., Horton, J. D., et al. (2001). SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. *Genes Dev.* 15, 1206–1216. doi:10.1101/gad.891301

Mk, H., Ra, N., and Jm, S. (1993). Model for measuring absolute rates of hepatic *de novo* lipogenesis and reesterification of free fatty acids. *Am. J. Physiol.* 265, E814–E820. doi:10.1152/ajpendo.1993.265.5.E814

Moon, Y.-A., Liang, G., Xie, X., Frank-Kamenetsky, M., Fitzgerald, K., Koteliansky, V., et al. (2012). The Scap/SREBP pathway is essential for developing diabetic fatty liver and carbohydrate-induced hypertriglyceridemia in animals. *Cell Metab.* 15, 240–246. doi:10.1016/j.cmet.2011.12.017

Mouzaki, M., Comelli, E. M., Arendt, B. M., Bonengel, J., Fung, S. K., Fischer, S. E., et al. (2013). Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatol. Balt. Md* 58, 120–127. doi:10.1002/hep.26319

Nassir, F., Adewole, O. L., Brunt, E. M., and Abumrad, N. A. (2013). CD36 deletion reduces VLDL secretion, modulates liver prostaglandins, and exacerbates hepatic steatosis in ob/ob mice. *J. Lipid Res.* 54, 2988–2997. doi:10.1194/jlr.M037812

Neuschwander-Tetri, B. A. (2010). Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatol. Balt. Md* 52, 774–788. doi:10.1002/hep.23719

Newberry, E. P., Kennedy, S. M., Xie, Y., Luo, J., Crooke, R. M., Graham, M. J., et al. (2012). Decreased body weight and hepatic steatosis with altered fatty acid ethanolamide metabolism in aged L-Fabp -/- mice. *J. Lipid Res.* 53, 744–754. doi:10.1194/jlr.M020966

Nguyen, P., Leray, V., Diez, M., Serisier, S., Le Bloc'h, J., Siliart, B., et al. (2008). Liver lipid metabolism. *J. Anim. Physiol. Anim. Nutr.* 92, 272–283. doi:10.1111/j.1439-0396.2007.00752.x

Nian, F., Chen, Y., Xia, Q., Zhu, C., Wu, L., and Lu, X. (2024). Gut microbiota metabolite trimethylamine N-oxide promoted NAFLD progression by exacerbating intestinal barrier disruption and intrahepatic cellular imbalance. *Int. Immunopharmacol.* 142, 113173. doi:10.1016/j.intimp.2024.113173

Nie, Q., Luo, X., Wang, K., Ding, Y., Jia, S., Zhao, Q., et al. (2024). Gut symbionts alleviate MASH through a secondary bile acid biosynthetic pathway. *Cell* 187, 2717–2734.e33. doi:10.1016/j.cell.2024.03.034

Ntambi, J. M., Miyazaki, M., Stoehr, J. P., Lan, H., Kendziorski, C. M., Yandell, B. S., et al. (2002). Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11482–11486. doi:10.1073/pnas.132384699

Oosterveer, M. H., and Schoonjans, K. (2014). Hepatic glucose sensing and integrative pathways in the liver. *Cell. Mol. Life Sci. CMLS* 71, 1453–1467. doi:10.1007/s00018-013-1505-z

Özenirler, S., Degertekin, C. K., Erkan, G., Elbeğ, Ş., Tuncer, C., Kandilc, U., et al. (2013). Serum liver fatty acid binding protein shows good correlation with liver histology in NASH. *Hepatogastroenterology* 60, 1095–1100. doi:10.5754/hge11949

Packman, S., and Whitney, S. (1990). Fatty acid transport in multiple carboxylase deficiency fibroblasts. *J. Inherit. Metab. Dis.* 13, 716–720. doi:10.1007/BF01799574

Palomer, X., Pizarro-Delgado, J., Barroso, E., and Vázquez-Carrera, M. (2018). Palmitic and oleic acid: the yin and Yang of fatty acids in type 2 diabetes mellitus. *Trends Endocrinol. Metab. Tem.* 29, 178–190. doi:10.1016/j.tem.2017.11.009

Pang, J., Raka, F., Heirali, A. A., Shao, W., Liu, D., Gu, J., et al. (2023). Resveratrol intervention attenuates chylomicron secretion via repressing intestinal FXR-induced expression of scavenger receptor SR-B1. *Nat. Commun.* 14, 2656. doi:10.1038/s41467-023-38259-1

Park, M. J., Kim, H., Kim, M. G., and Kim, K. (2023). Comparison of glucagon-like peptide-1 receptor agonists and thiazolidinediones on treating nonalcoholic fatty liver disease: a network meta-analysis. *Clin. Mol. Hepatol.* 29, 693–704. doi:10.3350/cmh.2022.0330

Parlati, L., Régnier, M., Guillou, H., and Postic, C. (2021). New targets for NAFLD. *JHEP Rep. Innov. Hepatol.* 3, 100346. doi:10.1016/j.jhepr.2021.100346

Paternostro, R., and Trauner, M. (2022). Current treatment of non-alcoholic fatty liver disease. *J. Intern. Med.* 292, 190–204. doi:10.1111/joim.13531

Paul, B., Lewinska, M., and Andersen, J. B. (2022). Lipid alterations in chronic liver disease and liver cancer. *JHEP Rep. Innov. Hepatol.* 4, 100479. doi:10.1016/j.jhepr.2022.100479

Pawlak, M., Lefebvre, P., and Staels, B. (2015). Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J. Hepatol.* 62, 720–733. doi:10.1016/j.jhep.2014.10.039

Peng, J.-H., Leng, J., Tian, H.-J., Yang, T., Fang, Y., Feng, Q., et al. (2018). Geniposide and chlorogenic acid combination ameliorates non-alcoholic steatohepatitis involving the protection on the gut barrier function in mouse induced by high-fat diet. *Front. Pharmacol.* 9, 1399. doi:10.3389/fphar.2018.01399

Perino, A., Demagny, H., Velazquez-Villegas, L., and Schoonjans, K. (2021). Molecular physiology of bile acid signaling in health, disease, and aging. *Physiol. Rev.* 101, 683–731. doi:10.1152/physrev.00049.2019

Petta, S., Handberg, A., Marchesini, G., Cammà, C., Di Marco, V., Cabibi, D., et al. (2013). High sCD36 plasma level is associated with steatosis and its severity in patients with genotype 1 chronic hepatitis C. *J. Viral Hepat.* 20, 174–182. doi:10.1111/j.1365-2893.2012.01641.x

Piccinin, E., Cariello, M., De Santis, S., Ducheix, S., Sabbà, C., Ntambi, J. M., et al. (2019). Role of oleic acid in the gut-liver Axis: from diet to the regulation of its synthesis via stearoyl-CoA desaturase 1 (SCD1). *Nutrients* 11, 2283. doi:10.3390/nu11102283

Pineda Torra, I., Claudel, T., Duval, C., Kosykh, V., Fruchart, J.-C., and Staels, B. (2003). Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor. *Mol. Endocrinol. Balt. Md* 17, 259–272. doi:10.1210/me.2002-0120

Porras, D., Nistal, E., Martínez-Flórez, S., Pisonero-Vaquero, S., Olcoz, J. L., Jover, R., et al. (2017). Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease in mice is mediated by modulating intestinal microbiota imbalance and related gut-liver axis activation. *Free Radic. Biol. Med.* 102, 188–202. doi:10.1016/j.freeradbiomed.2016.11.037

Postic, C., Dentin, R., Denechaud, P.-D., and Girard, J. (2007). ChREBP, a transcriptional regulator of glucose and lipid metabolism. *Annu. Rev. Nutr.* 27, 179–192. doi:10.1146/annurev.nutr.27.061406.093618

Postic, C., and Girard, J. (2008). Contribution of *de novo* fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J. Clin. Invest.* 118, 829–838. doi:10.1172/JCI34275

Pouwels, S., Sakran, N., Graham, Y., Leal, A., Pintar, T., Yang, W., et al. (2022). Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. *BMC Endocr. Disord.* 22, 63. doi:10.1186/s12902-022-00980-1

Psichas, A., Sleeth, M. L., Murphy, K. G., Brooks, L., Bewick, G. A., Hanyaloglu, A. C., et al. (2015). The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int. J. Obes. 2005* 39, 424–429. doi:10.1038/ijo.2014.153

Qiao, A., Liang, J., Ke, Y., Li, C., Cui, Y., Shen, L., et al. (2011). Mouse patatin-like phospholipase domain-containing 3 influences systemic lipid and glucose homeostasis. *Hepatol. Balt. Md* 54, 509–521. doi:10.1002/hep.24402

Quesada-Vázquez, S., Bone, C., Saha, S., Triguero, I., Colom-Pellicer, M., Aragonès, G., et al. (2022). Microbiota dysbiosis and gut barrier dysfunction associated with non-alcoholic fatty liver disease are modulated by a specific metabolic cofactors' combination. *Int. J. Mol. Sci.* 23, 13675. doi:10.3390/ijms232213675

Rada, P., González-Rodríguez, Á., García-Monzón, C., and Valverde, Á. M. (2020). Understanding lipotoxicity in NAFLD pathogenesis: is CD36 a key driver? *Cell Death Dis.* 11, 802. doi:10.1038/s41419-020-03003-w

Riazi, K., Azhari, H., Charette, J. H., Underwood, F. E., King, J. A., Afshar, E. E., et al. (2022). The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol. Hepatol.* 7, 851–861. doi:10.1016/S2468-1253(22)00165-0

Rinella, M. E., Lazarus, J. V., Ratziu, V., Francque, S. M., Sanyal, A. J., Kanwal, F., et al. (2023). A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatol. Balt. Md* 78, 1966–1986. doi:10.1097/HEP.0000000000000520

Rizki, G., Arnaboldi, L., Gabrielli, B., Yan, J., Lee, G. S., Ng, R. K., et al. (2006). Mice fed a lipogenic methionine-choline-deficient diet develop hypermetabolism coincident with hepatic suppression of SCD-1. *J. Lipid Res.* 47, 2280–2290. doi:10.1194/jlr.M600198-JLR200

Rohm, T. V., Meier, D. T., Olefsky, J. M., and Donath, M. Y. (2022). Inflammation in obesity, diabetes, and related disorders. *Immunity* 55, 31–55. doi:10.1016/j.immuni.2021.12.013

Röhrl, C., Eigner, K., Winter, K., Korbelius, M., Obrowsky, S., Kratky, D., et al. (2014). Endoplasmic reticulum stress impairs cholesterol efflux and synthesis in hepatic cells. *J. Lipid Res.* 55, 94–103. doi:10.1194/jlr.M043299

Rohrmann, S., Linseisen, J., Allenspach, M., von Eckardstein, A., and Müller, D. (2016). Plasma concentrations of trimethylamine-N-oxide are directly associated with dairy food consumption and low-grade inflammation in a German adult population. *J. Nutr.* 146, 283–289. doi:10.3945/jn.115.220103

Rong, S., Xia, M., Vale, G., Wang, S., Kim, C.-W., Li, S., et al. (2024). DGAT2 inhibition blocks SREBP-1 cleavage and improves hepatic steatosis by increasing phosphatidylethanolamine in the ER. *Cell Metab.* 36, 617–629.e7. doi:10.1016/j.cmet.2024.01.011

Ross, T. T., Crowley, C., Kelly, K. L., Rinaldi, A., Beebe, D. A., Lech, M. P., et al. (2020). Acetyl-CoA carboxylase inhibition improves multiple dimensions of NASH pathogenesis in model systems. *Cell. Mol. Gastroenterol. Hepatol.* 10, 829–851. doi:10.1016/j.jcmgh.2020.06.001

Sahuri-Arisoylu, M., Brody, L. P., Parkinson, J. R., Parkes, H., Navaratnam, N., Miller, A. D., et al. (2016). Reprogramming of hepatic fat accumulation and "browning" of adipose tissue by the short-chain fatty acid acetate. *Int. J. Obes. 2005* 40, 955–963. doi:10.1038/ijo.2016.23

Saller, R., Meier, R., and Brignoli, R. (2001). The use of silymarin in the treatment of liver diseases. *Drugs* 61, 2035–2063. doi:10.2165/00003495-200161140-00003

Santos-Baez, L. S., and Ginsberg, H. N. (2021). Nonalcohol fatty liver disease: balancing supply and utilization of triglycerides. *Curr. Opin. Lipidol.* 32, 200–206. doi:10.1097/MOL.0000000000000756

Scazzocchio, B., Minghetti, L., and D'Archivio, M. (2020). Interaction between gut microbiota and curcumin: a new key of understanding for the health effects of curcumin. *Nutrients* 12, 2499. doi:10.3390/nu12092499

Seidah, N. G., Awan, Z., Chrétien, M., and Mbikay, M. (2014). PCSK9: a key modulator of cardiovascular health. *Circ. Res.* 114, 1022–1036. doi:10.1161/CIRCRESAHA.114.301621

Sen, S., Jumaa, H., and Webster, N. J. G. (2013). Splicing factor SRSF3 is crucial for hepatocyte differentiation and metabolic function. *Nat. Commun.* 4, 1336. doi:10.1038/ncomms2342

Shapira, M. (2016). Gut microbiotas and host evolution: scaling up symbiosis. *Trends Ecol. Evol.* 31, 539–549. doi:10.1016/j.tree.2016.03.006

Sharpton, S. R., Maraj, B., Harding-Theobald, E., Vittinghoff, E., and Terrault, N. A. (2019). Gut microbiome-targeted therapies in nonalcoholic fatty liver disease: a systematic review, meta-analysis, and meta-regression. *Am. J. Clin. Nutr.* 110, 139–149. doi:10.1093/ajcn/nqz042

Sheedfar, F., Sung, M. M., Aparicio-Vergara, M., Kloosterhuis, N. J., Miquilena-Colina, M. E., Vargas-Castrillón, J., et al. (2014). Increased hepatic CD36 expression with age is associated with enhanced susceptibility to nonalcoholic fatty liver disease. *Aging* 6, 281–295. doi:10.18632/aging.100652

Shen, Q., Zhong, Y.-T., Liu, X.-X., Hu, J.-N., Qi, S.-M., Li, K., et al. (2023). Platycodin D ameliorates hyperglycaemia and liver metabolic disturbance in HFD/STZ-induced type 2 diabetic mice. *Food Funct.* 14, 74–86. doi:10.1039/d2fo03308a

Shi, A., Li, T., Zheng, Y., Song, Y., Wang, H., Wang, N., et al. (2021). Chlorogenic acid improves NAFLD by regulating gut microbiota and GLP-1. *Front. Pharmacol.* 12, 693048. doi:10.3389/fphar.2021.693048

Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S., and Goldstein, J. L. (1997). Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J. Clin. Invest.* 99, 846–854. doi:10.1172/JCI119248

Shimano, H., and Sato, R. (2017). SREBP-regulated lipid metabolism: convergent physiology - divergent pathophysiology. *Nat. Rev. Endocrinol.* 13, 710–730. doi:10.1038/nrendo.2017.91

Shou, J.-W., Ma, J., Wang, X., Li, X.-X., Chen, S.-C., Kang, B.-H., et al. (2025). Free cholesterol-induced liver injury in non-alcoholic fatty liver disease: mechanisms and a therapeutic intervention using dihydrotanshinone I. *Adv. Sci. Weinh. Baden-Wurtt. Ger.* 12, e2406191. doi:10.1002/advs.202406191

Siler, S. Q., Neese, R. A., and Hellerstein, M. K. (1999). *De novo* lipogenesis, lipid kinetics, and whole-body lipid balances in humans after acute alcohol consumption. *Am. J. Clin. Nutr.* 70, 928–936. doi:10.1093/ajcn/70.5.928

Slika, L., and Patra, D. (2020). Traditional uses, therapeutic effects and recent advances of curcumin: a mini-review. *Mini Rev. Med. Chem.* 20, 1072–1082. doi:10.2174/1389557520666200414161316

Song, Q., and Zhang, X. (2022). The role of gut-liver Axis in gut microbiome dysbiosis associated NAFLD and NAFLD-HCC. *Biomedicines* 10, 524. doi:10.3390/biomedicines10030524

Song, Q., Zou, J., Li, D., Cheng, S. W., Li, K. L. S., Yang, X., et al. (2024). Gastrointestinal metabolism of Astragalus membranaceus polysaccharides and its related hypoglycemic mechanism based on gut microbial transformation. *Int. J. Biol. Macromol.* 280, 135847. doi:10.1016/j.ijbiomac.2024.135847

Soupene, E., and Kuypers, F. A. (2008). Mammalian long-chain acyl-CoA synthetases. *Exp. Biol. Med. Maywood N. J.* 233, 507–521. doi:10.3181/0710-MR-287

Spann, N. J., Kang, S., Li, A. C., Chen, A. Z., Newberry, E. P., Davidson, N. O., et al. (2006). Coordinate transcriptional repression of liver fatty acid-binding protein and microsomal triglyceride transfer protein blocks hepatic very low density lipoprotein secretion without hepatosteatosis. *J. Biol. Chem.* 281, 33066–33077. doi:10.1074/jbc.M607148200

Sparks, J. D., Chamberlain, J. M., O'Dell, C., Khatun, I., Hussain, M. M., and Sparks, C. E. (2011). Acute suppression of apo B secretion by insulin occurs independently of MTP. *Biochem. Biophys. Res. Commun.* 406, 252–256. doi:10.1016/j.bbrc.2011.02.028

Spencer, M. D., Hamp, T. J., Reid, R. W., Fischer, L. M., Zeisel, S. H., and Fodor, A. A. (2011). Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. *Gastroenterology* 140, 976–986. doi:10.1053/j.gastro.2010.11.049

Stanford, K. I., Middelbeek, R. J. W., Townsend, K. L., An, D., Nygaard, E. B., Hitchcox, K. M., et al. (2013). Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. *J. Clin. Invest.* 123, 215–223. doi:10.1172/JCI62308

Stienstra, R., Saudale, F., Duval, C., Keshtkar, S., Groener, J. E. M., van Rooijen, N., et al. (2010). Kupffer cells promote hepatic steatosis via interleukin-1beta-dependent suppression of peroxisome proliferator-activated receptor alpha activity. *Hepatol. Balt. Md* 51, 511–522. doi:10.1002/hep.23337

Stone, S. J., Myers, H. M., Watkins, S. M., Brown, B. E., Feingold, K. R., Elias, P. M., et al. (2004). Lipopenia and skin barrier abnormalities in DGAT2-deficient mice. *J. Biol. Chem.* 279, 11767–11776. doi:10.1074/jbc.M311000200

Su, X., and Abumrad, N. A. (2009). Cellular fatty acid uptake: a pathway under construction. *Trends Endocrinol. Metab. Tem.* 20, 72–77. doi:10.1016/j.tem.2008.11.001

Subramaniam, S., and Fletcher, C. (2018). Trimethylamine N-oxide: breathe new life. *Br. J. Pharmacol.* 175, 1344–1353. doi:10.1111/bph.13959

Sun, C., Li, A., Wang, H., Ma, J., and Hou, J. (2023a). Positive regulation of acetate in adipocyte differentiation and lipid deposition in obese mice. *Nutrients* 15, 3736. doi:10.3390/nu15173736

Sun, L., Tan, X., Liang, X., Chen, H., Ou, Q., Wu, Q., et al. (2023b). Maternal betaine supplementation mitigates maternal high fat diet-induced NAFLD in offspring mice through gut microbiota. *Nutrients* 15, 284. doi:10.3390/nu15020284

Sun, W.-L., Hua, S., Li, X.-Y., Shen, L., Wu, H., and Ji, H.-F. (2023c). Microbially produced vitamin B12 contributes to the lipid-lowering effect of silymarin. *Nat. Commun.* 14, 477. doi:10.1038/s41467-023-36079-x

Sun, W.-M., Wang, Y.-P., Duan, Y.-Q., Shang, H.-X., and Cheng, W.-D. (2014). Radix Hedysari polysaccharide suppresses lipid metabolism dysfunction in a rat model of non-alcoholic fatty liver disease via adenosine monophosphate-activated protein kinase pathway activation. *Mol. Med. Rep.* 10, 1237–1244. doi:10.3892/mmr.2014.2327

Sun, Z.-G., Zhao, T.-T., Lu, N., Yang, Y.-A., and Zhu, H.-L. (2019). Research progress of glycyrrhizic acid on antiviral activity. *Mini Rev. Med. Chem.* 19, 826–832. doi:10.2174/1389557519666190119111125

Tajik, N., Tajik, M., Mack, I., and Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. *Eur. J. Nutr.* 56, 2215–2244. doi:10.1007/s00394-017-1379-1

Tan, Y., Huang, Z., Liu, Y., Li, X., Stalin, A., Fan, X., et al. (2023). Integrated serum pharmacochemistry, 16S rRNA sequencing and metabolomics to reveal the material basis and mechanism of Yinzhihuang granule against non-alcoholic fatty liver disease. *J. Ethnopharmacol.* 310, 116418. doi:10.1016/j.jep.2023.116418

Tang, X., Yang, Y., Peng, W., Xu, M., Fan, Q., Li, F., et al. (2024). Platycodon D reduces obesity and non-alcoholic fatty liver disease induced by a high-fat diet through inhibiting intestinal fat absorption. *Front. Pharmacol.* 15, 1412453. doi:10.3389/fphar.2024.1412453

Tanoglu, A., and Beyazit, Y. (2016). Liver fatty acid-binding protein may be a useful marker for non-alcoholic fatty liver disease but obesity is a major concern. *Wien. Klin. Wochenschr.* 128, 304. doi:10.1007/s00508-016-0951-7

Teschke, R. (2022). Aluminum, arsenic, beryllium, cadmium, chromium, cobalt, copper, iron, lead, mercury, molybdenum, nickel, platinum, thallium, titanium, vanadium, and zinc: molecular aspects in experimental liver injury. *Int. J. Mol. Sci.* 23, 12213. doi:10.3390/ijms232012213

Thomas, J. A., Kendall, B. J., El-Serag, H. B., Thrift, A. P., and Macdonald, G. A. (2024). Hepatocellular and extrahepatic cancer risk in people with non-alcoholic fatty liver disease. *Lancet Gastroenterol. Hepatol.* 9, 159–169. doi:10.1016/S2468-1253(23)00275-3

Tilg, H., Adolph, T. E., and Moschen, A. R. (2021). Multiple parallel hits hypothesis in nonalcoholic fatty liver disease: revisited after a decade. *Hepatol. Balt. Md* 73, 833–842. doi:10.1002/hep.31518

Tiwari, S., and Siddiqi, S. A. (2012). Intracellular trafficking and secretion of VLDL. *Arterioscler. Thromb. Vasc. Biol.* 32, 1079–1086. doi:10.1161/ATVBAHA.111.241471

Unger, R. H. (2002). Lipotoxic diseases. *Annu. Rev. Med.* 53, 319–336. doi:10.1146/annurev.med.53.082901.104057

Vargas-Mendoza, N., Madrigal-Santillán, E., Morales-González, A., Esquivel-Soto, J., Esquivel-Chirino, C., García-Luna Y González-Rubio, M., et al. (2014). Hepatoprotective effect of silymarin. *World J. Hepatol.* 6, 144–149. doi:10.4254/wjh.v6.i3.144

Velazquez-Villegas, L. A., Perino, A., Lemos, V., Zietak, M., Nomura, M., Pols, T. W. H., et al. (2018). TGR5 signalling promotes mitochondrial fission and beige remodelling of white adipose tissue. *Nat. Commun.* 9, 245. doi:10.1038/s41467-017-02068-0

Vitto, M. F., Luz, G., Luciano, T. F., Marques, S. O., Souza, D. R., Pinho, R. A., et al. (2012). Reversion of steatosis by SREBP-1c antisense oligonucleotide did not improve hepatic insulin action in diet-induced obesity mice. *Horm. Metab. Res. Horm. Stoffwechselforsch. Horm. Metab.* 44, 885–890. doi:10.1055/s-0032-1321819

Wahlström, A., Sayin, S. I., Marschall, H.-U., and Bäckhed, F. (2016). Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab.* 24, 41–50. doi:10.1016/j.cmet.2016.05.005

Wang, B., Jiang, X., Cao, M., Ge, J., Bao, Q., Tang, L., et al. (2016). Altered fecal microbiota correlates with liver biochemistry in nonobese patients with non-alcoholic fatty liver disease. *Sci. Rep.* 6, 32002. doi:10.1038/srep32002

Wang, K., Li, C., Lin, X., Sun, H., Xu, R., Li, Q., et al. (2020a). Correction: targeting alkaline ceramidase 3 alleviates the severity of nonalcoholic steatohepatitis by reducing oxidative stress. *Cell Death Dis.* 11, 191. doi:10.1038/s41419-020-2396-1

Wang, M., Li, X. S., Wang, Z., de Oliveira Otto, M. C., Lemaitre, R. N., Fretts, A., et al. (2023a). Trimethylamine N-oxide is associated with long-term mortality risk: the multi-ethnic study of atherosclerosis. *Eur. Heart J.* 44, 1608–1618. doi:10.1093/eurheartj/ehad089

Wang, P., Wang, J., Li, D., Ke, W., Chen, F., and Hu, X. (2020b). Targeting the gut microbiota with resveratrol: a demonstration of novel evidence for the management of hepatic steatosis. *J. Nutr. Biochem.* 81, 108363. doi:10.1016/j.jnutbio.2020.108363

Wang, P., Wang, R., Zhao, W., Zhao, Y., Wang, D., Zhao, S., et al. (2025). Gut microbiota-derived 4-hydroxyphenylacetic acid from resveratrol supplementation prevents obesity through SIRT1 signaling activation. *Gut Microbes* 17, 2446391. doi:10.1080/19490976.2024.2446391

Wang, P., Zhang, S.-Y., Dong, Y., Zeng, G., Liu, H., Wang, X., et al. (2024). Adipose ADM2 ameliorates NAFLD via promotion of ceramide catabolism. *Acta Pharm. Sin. B* 14, 4883–4898. doi:10.1016/j.apsb.2024.09.010

Wang, T., Liu, L., Deng, J., Jiang, Y., Yan, X., and Liu, W. (2023b). Analysis of the mechanism of action of quercetin in the treatment of hyperlipidemia based on metabolomics and intestinal flora. *Food Funct.* 14, 2112–2127. doi:10.1039/d2fo03509j

Wang, Y., Yu, W., Li, S., Guo, D., He, J., and Wang, Y. (2022). Acetyl-CoA carboxylases and diseases. *Front. Oncol.* 12, 836058. doi:10.3389/fonc.2022.836058

Wang, Z., Du, H., Zhao, Y., Ren, Y., Ma, C., Chen, H., et al. (2023c). Response to pioglitazone in non-alcoholic fatty liver disease patients with vs. without type 2 diabetes: a meta-analysis of randomized controlled trials. *Front. Endocrinol.* 14, 1111430. doi:10.3389/fendo.2023.1111430

Weber, M., Mera, P., Casas, J., Salvador, J., Rodríguez, A., Alonso, S., et al. (2020). Liver CPT1A gene therapy reduces diet-induced hepatic steatosis in mice and highlights potential lipid biomarkers for human NAFLD. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* 34, 11816–11837. doi:10.1096/fj.202000678R

Wen, X., Wang, J., Fan, J., Chu, R., Chen, Y., Xing, Y., et al. (2022). Investigating the protective effects of Platycodin D on non-alcoholic fatty liver disease in a palmitic acid-induced *in vitro* model. *J. Vis. Exp. JoVE*. doi:10.3791/64816

Wu, J., He, C., Bu, J., Luo, Y., Yang, S., Ye, C., et al. (2020). Betaine attenuates LPS-induced downregulation of Occludin and Claudin-1 and restores intestinal barrier function. *BMC Vet. Res.* 16, 75. doi:10.1186/s12917-020-02298-3

Wu, J., Li, M., Huang, N., Guan, F., Luo, H., Chen, L., et al. (2023). Curcumin alleviates high-fat diet-induced nonalcoholic steatohepatitis via improving hepatic endothelial function with microbial biotransformation in rats. *J. Agric. Food Chem.* 71, 10338–10348. doi:10.1021/acs.jafc.3c01067

Wu, L., Feng, J., Li, J., Yu, Q., Ji, J., Wu, J., et al. (2021). The gut microbiome-bile acid axis in hepatocarcinogenesis. *Biomed. Pharmacother. Biomedecine Pharmacother.* 133, 111036. doi:10.1016/j.biopha.2020.111036

Xie, G., Jiang, R., Wang, X., Liu, P., Zhao, A., Wu, Y., et al. (2021). Conjugated secondary 12α-hydroxylated bile acids promote liver fibrogenesis. *EBioMedicine* 66, 103290. doi:10.1016/j.ebiom.2021.103290

Xu, S., Wu, X., Wang, S., Xu, M., Fang, T., Ma, X., et al. (2024). TRIM56 protects against nonalcoholic fatty liver disease by promoting the degradation of fatty acid synthase. *J. Clin. Invest.* 134, e166149. doi:10.1172/JCI166149

Xue, W., Wang, J., Jiang, W., Shi, C., Wang, X., Huang, Y., et al. (2020). Caveolin-1 alleviates lipid accumulation in NAFLD associated with promoting autophagy by inhibiting the Akt/mTOR pathway. *Eur. J. Pharmacol.* 871, 172910. doi:10.1016/j.ejphar.2020.172910

Yamashita, H., Takenoshita, M., Sakurai, M., Bruick, R. K., Henzel, W. J., Shillinglaw, W., et al. (2001). A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver. *Proc. Natl. Acad. Sci. U. S. A.* 98, 9116–9121. doi:10.1073/pnas.161284298

Yamashita, S., Hirano, K., Kuwasako, T., Janabi, M., Toyama, Y., Ishigami, M., et al. (2007). Physiological and pathological roles of a multi-ligand receptor CD36 in atherogenesis; insights from CD36-deficient patients. *Mol. Cell. Biochem.* 299, 19–22. doi:10.1007/s11010-005-9031-4

Yang, M., Ge, J., Liu, Y.-L., Wang, H.-Y., Wang, Z.-H., Li, D.-P., et al. (2024a). Sortilin-mediated translocation of mitochondrial ACSL1 impairs adipocyte thermogenesis and energy expenditure in male mice. *Nat. Commun.* 15, 7746. doi:10.1038/s41467-024-52218-4

Yang, T., Zhou, Y., and Cui, Y. (2024b). Urinary tract infections and genital mycotic infections associated with SGLT-2 inhibitors: an analysis of the FDA Adverse Event Reporting System. *Expert Opin. Drug Saf.* 23, 1035–1040. doi:10.1080/14740338.2023.2288897

Yang, Y.-N., Wang, Q.-C., Xu, W., Yu, J., Zhang, H., and Wu, C. (2022). The berberine-enriched gut commensal Blautia producta ameliorates high-fat diet (HFD)-induced hyperlipidemia and stimulates liver LDLR expression. *Biomed. Pharmacother. Biomedecine Pharmacother.* 155, 113749. doi:10.1016/j.biopha.2022.113749

Ye, J., Rawson, R. B., Komuro, R., Chen, X., Davé, U. P., Prywes, R., et al. (2000). ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol. Cell* 6, 1355–1364. doi:10.1016/s1097-2765(00)00133-7

Yen, C.-L. E., Stone, S. J., Koliwad, S., Harris, C., and Farese, R. V. (2008). Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. *J. Lipid Res.* 49, 2283–2301. doi:10.1194/jlr.R800018-JLR200

Yin, X., Hu, H., Shen, X., Li, X., Pei, J., and Xu, J. (2021). Ginseng omics for ginsenoside biosynthesis. *Curr. Pharm. Biotechnol.* 22, 570–578. doi:10.2174/1389201021666200807113723

Younossi, Z., Anstee, Q. M., Marietti, M., Hardy, T., Henry, L., Eslam, M., et al. (2018). Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 15, 11–20. doi:10.1038/nrgastro.2017.109

Younossi, Z. M., Ratziu, V., Loomba, R., Rinella, M., Anstee, Q. M., Goodman, Z., et al. (2019). Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet lond. Engl.* 394, 2184–2196. doi:10.1016/S0140-6736(19)33041-7

Yue, Y., Zhang, L., Zhang, X., Li, X., and Yu, H. (2018). *De novo* lipogenesis and desaturation of fatty acids during adipogenesis in bovine adipose-derived mesenchymal stem cells. *Vitro Cell. Dev. Biol. Anim.* 54, 23–31. doi:10.1007/s11626-017-0205-7

Zeng, H., Qin, H., Liao, M., Zheng, E., Luo, X., Xiao, A., et al. (2022a). CD36 promotes *de novo* lipogenesis in hepatocytes through INSIG2-dependent SREBP1 processing. *Mol. Metab.* 57, 101428. doi:10.1016/j.molmet.2021.101428

Zeng, S., Wu, F., Chen, M., Li, Y., You, M., Zhang, Y., et al. (2022b). Inhibition of fatty acid translocase (FAT/CD36) palmitoylation enhances hepatic fatty acid β-oxidation by increasing its localization to mitochondria and interaction with long-chain acyl-CoA synthetase 1. *Antioxid. Redox Signal.* 36, 1081–1100. doi:10.1089/ars.2021.0157

Zhang, L., Wang, X., and Chen, X.-W. (2025). The biogenesis and transport of triglyceride-rich lipoproteins. *Trends Endocrinol. Metab. Tem.* 36, 262–277. doi:10.1016/j.tem.2024.07.015

Zhang, M., Tang, Y., Tang, E., and Lu, W. (2020). MicroRNA-103 represses hepatic *de novo* lipogenesis and alleviates NAFLD via targeting FASN and SCD1. *Biochem. Biophys. Res. Commun.* 524, 716–722. doi:10.1016/j.bbrc.2020.01.143

Zhang, P., Ge, Z., Wang, H., Feng, W., Sun, X., Chu, X., et al. (2018). Prolactin improves hepatic steatosis via CD36 pathway. *J. Hepatol.* 68, 1247–1255. doi:10.1016/j.jhep.2018.01.035

Zhang, X., Shi, L., Chen, R., Zhao, Y., Ren, D., and Yang, X. (2021). Chlorogenic acid inhibits trimethylamine-N-oxide formation and remodels intestinal microbiota to alleviate liver dysfunction in high L-carnitine feeding mice. *Food Funct.* 12, 10500–10511. doi:10.1039/d1fo01778k

Zhang, Z., Tang, H., Chen, P., Xie, H., and Tao, Y. (2019). Demystifying the manipulation of host immunity, metabolism, and extraintestinal tumors by the gut microbiome. *Signal Transduct. Target. Ther.* 4, 41. doi:10.1038/s41392-019-0074-5

Zhao, H., Gao, X., Liu, Z., Zhang, L., Fang, X., Sun, J., et al. (2022). Sodium alginate prevents non-alcoholic fatty liver disease by modulating the gut-liver Axis in high-fat diet-fed rats. *Nutrients* 14, 4846. doi:10.3390/nu14224846

Zhao, L., Zhang, C., Luo, X., Wang, P., Zhou, W., Zhong, S., et al. (2018). CD36 palmitoylation disrupts free fatty acid metabolism and promotes tissue inflammation in non-alcoholic steatohepatitis. *J. Hepatol.* 69, 705–717. doi:10.1016/j.jhep.2018.04.006

Zhao, S., Xu, W., Jiang, W., Yu, W., Lin, Y., Zhang, T., et al. (2010). Regulation of cellular metabolism by protein lysine acetylation. *Science* 327, 1000–1004. doi:10.1126/science.1179689

Zheng, L., Li, B., Yuan, A., Bi, S., Puscher, H., Liu, C., et al. (2024). TFEB activator tanshinone IIA and derivatives derived from Salvia miltiorrhiza Bge. Attenuate hepatic steatosis and insulin resistance. *J. Ethnopharmacol.* 335, 118662. doi:10.1016/j.jep.2024.118662

Zheng, Z.-G., Zhu, S.-T., Cheng, H.-M., Zhang, X., Cheng, G., Thu, P. M., et al. (2021). Discovery of a potent SCAP degrader that ameliorates HFD-induced obesity, hyperlipidemia and insulin resistance via an autophagy-independent lysosomal pathway. *Autophagy* 17, 1592–1613. doi:10.1080/15548627.2020.1757955

Zhong, M., Yan, Y., Yuan, H., A, R., Xu, G., Cai, F., et al. (2022). Astragalus mongholicus polysaccharides ameliorate hepatic lipid accumulation and inflammation as well as modulate gut microbiota in NAFLD rats. *Food Funct.* 13, 7287–7301. doi:10.1039/d2fo01009g

Zhong, S., Zhao, L., Wang, Y., Zhang, C., Liu, J., Wang, P., et al. (2017). Cluster of differentiation 36 deficiency aggravates macrophage infiltration and hepatic inflammation by upregulating monocyte chemotactic protein-1 expression of hepatocytes through histone deacetylase 2-dependent pathway. *Antioxid. Redox Signal.* 27, 201–214. doi:10.1089/ars.2016.6808

Zhou, K., Chen, Q., Chen, J., Liang, D., Feng, W., Liu, M., et al. (2022). Spatiotemporal regulation of insulin signaling by liquid-liquid phase separation. *Cell Discov.* 8, 64. doi:10.1038/s41421-022-00430-1

Zhu, L., Baker, S. S., Gill, C., Liu, W., Alkhouri, R., Baker, R. D., et al. (2013). Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatol. Balt. Md* 57, 601–609. doi:10.1002/hep.26093

Zhu, X., Dai, X., Zhao, L., Li, J., Zhu, Y., He, W., et al. (2024). Quercetin activates energy expenditure to combat metabolic syndrome through modulating gut microbiota-bile acids crosstalk in mice. *Gut Microbes* 16, 2390136. doi:10.1080/19490976.2024.2390136

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Bletilla striata polysaccharides ameliorate metabolic-associated fatty liver disease by decreasing the NLRP3 inflammasome and pyroptosis

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Background: The role of nucleotide-binding oligomerization domain-like receptors containing pyrin domain 3 (NLRP3) inflammasome and pyroptosis in the inflammatory microenvironment of metabolic-associated fatty liver disease (MASLD) has been posited as crucial. *Bletilla striata* polysaccharides (BSPs), extracted from the tubers of *Bletilla striata (Thunb.) Rchb.f.*, exhibit significant anti-inflammatory properties. However, their potential protective effects on MASLD and their role in regulating pyroptosis remain unclear.

Objectives: This study investigates the efficacy of BSP-1, a purified metabolite isolated from crude BSPs, on MASLD by evaluating its ability to modulate the NLRP3/caspase-1/GSDMD signaling pathway.

Methods: To simulate MASLD *in vivo* and *in vitro*, high-fat diet (HFD)-induced rat models and free fatty acid (FFA)-stimulated HepG2 cells were used. Serum indicators and histopathological staining were employed to assess liver injury and lipid deposition. Additionally, enzyme-linked immunosorbent assay (ELISA), immunohistochemistry (IHC), immunofluorescence, real-time quantitative polymerase chain reaction (RT-qPCR), and western blotting (WB) analysis were conducted to examine the NLRP3/caspase-1/GSDMD pathway and related cytokine levels.

Results: BSP-1 significantly ameliorates alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), and triglyceride (TG) levels in both rat serum and HepG2 cells. Furthermore, BSP-1 reduces inflammatory factors interleukin (IL)-1β and IL-18, while improving pathological changes in rat liver tissue. Mechanistically, BSP-1 regulates the expression of pyroptosis-related proteins and mRNAs in the NLRP3/caspase-1/GSDMD pathway, thereby protecting against MASLD.

Discussion: BSP-1 may represent a promising therapeutic agent for MASLD treatment by inhibiting the NLRP3/caspase-1/GSDMD signaling pathway.

KEYWORDS

***Bletilla striata* polysaccharide, metabolic-associated fatty liver disease, nod-like receptor protein 3, pyroptosis, inflammasome**

1 Introduction

Metabolic-associated fatty liver disease (MASLD) is a chronic liver condition closely linked to metabolic disturbances. Its histological progression typically advances from simple steatosis to nonalcoholic steatohepatitis, and potentially to liver fibrosis and hepatocellular carcinoma (Kalligeros et al., 2024). Currently, MASLD is recognized as the most prevalent chronic liver disease worldwide, affecting approximately 37.8% of adults globally (Riazi et al., 2022). Due to the stigma associated with the term nonalcoholic fatty liver disease (NAFLD), a series of Delphi surveys resulted in the establishment of new criteria for MASLD that encompass 99% of patients previously diagnosed with NAFLD. Consequently, research data related to NAFLD remain relevant to MASLD (Hagström et al., 2024; Song et al., 2024). As our understanding of MASLD pathogenesis evolves, the widely accepted "multiple hit model" clarifies that after the initial "first hit" of fat accumulation driven by obesity and insulin resistance, the liver undergoes changes influenced by chronic inflammatory pathways, interactions among various organs and tissues (including adipose tissue, pancreas, intestine, cardiovascular system, and kidneys), genetic factors, lifestyle interactions, and broader metabolic dysfunction (Tilg et al., 2021; Targher et al., 2024).

The dynamic balance between cell proliferation and death is a fundamental aspect of both physiological and pathological processes within the body. Pyroptosis, one of the programmed cell death pathways, is characterized by cell swelling, membrane perforation, and the release of cellular contents. In normal physiological contexts, pyroptosis plays a crucial role in the host's defense against pathogens (Liu et al., 2021). Conversely, in pathological conditions, the excessive cytokine storm and inflammatory response induced by pyroptosis-triggered by inflammasomes and executed by gasdermin proteins-can lead to significant tissue damage and multi-organ dysfunction (Rao et al., 2022). Studies have identified the nucleotide-binding oligomerization domain-like receptors containing pyrin domain 3 (NLRP3) inflammasome, composed of NLRP3, leucine-rich repeats, and a pyrin domain, as a key inflammatory mediator that can be initiated and activated by pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), or cytokines involved in immune and inflammatory responses. Activated NLRP3 inflammasomes recruit the downstream molecule caspase-1 through the "bridge" ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), thereby triggering the maturation of pro-inflammatory cytokines (interleukin (IL)-1β and interleukin (IL)-18) and the processing of gasdermin D (GSDMD) to induce the release of IL-1β and IL-18 and cell pyroptosis (Fu and Wu, 2023). In recent years, with increasing attention to the pathogenesis of MASLD, the pyroptosis-related NLRP3/caspase-1/GSDMD pathway has been recognized as closely associated with the pathogenesis of MASLD (Carvalho Ribeiro and Szabo, 2022). Some natural products, such as penthorum chinense pursh extract, green tea epigallocatechin gallate, have shown to treat MASLD by regulating the pyroptosis-related NLRP3/caspase-1/GSDMD pathway (Luo et al., 2024; Zhang et al., 2025). Therefore, inhibiting the conduction of the pyroptosis pathway may represent a potential therapeutic target for delaying the progression of this disease.

Bletilla striata (Thunb.) Rchb.f. tubers (Orchidaceae), a well-known medicinal orchid, has been extensively utilized in traditional Chinese botanical drug for millennia and is reckoned for its efficacy in astringing to stop bleeding, alleviating swelling, and promoting tissue regeneration (He et al., 2017). It exhibits significant health-improving effects in treating digestive tract mucosal injuries, ulcers, hemorrhages, bruises, and burns (Xu et al., 2019). Polysaccharides, the primary bioactive metabolites in *Bletilla striata*, possess various pharmacological properties, including antioxidant, antibacterial, antifibrotic, antiglycation, anticancer, and immunomodulatory activities (Zhu et al., 2023). Meanwhile, BSP has a good regulatory effect on inflammatory response and liver protective effect. BSP can improve liver fibrosis by regulating the TLR2/TLR4-MyD88-NF-κB signaling pathway (Jiang et al., 2023). Additionally, it can also be used as an anti-inflammatory drug to improve acute respiratory distress syndrome (ARDS) by regulating the NLRP3 inflammasome and pyroptosis (Wu et al., 2024). Our previous research has confirmed that crude BSPs enhance the levels of intestinal cellular tight junction proteins, zonula occludens-1 (ZO-1) and occludin both *in vivo* and *in vitro*, while also modulate the expression of inflammatory cytokines (interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α)) in thioacetamide (TAA)-induced liver cirrhosis rats and lipopolysaccharide (LPS)-induced injury in intestinal epithelial cells, thereby providing a therapeutic strategy for treating inflammation-related diseases induced by gut-derived PAMPs (Luo et al., 2018; Luo, et al., 2019). Hpowever, the potential of BSPs to ameliorate MASLD by regulating the NLRP3 inflammasome and pyroptosis are unknown.

Therefore, in the present investigation, we isolated and purified BSP-1 from crude BSPs using DEAE-Sepharose Fast Flow ion-exchange chromatography and Sephacryl S-100 gel filtration chromatography. We then assessed the impact of BSP-1 on MASLD by evaluating its ability to regulate the expression of pyroptosis-related NLRP3/caspase-1/GSDMD pathway in both high-fat diet (HFD)-induced rat models *in vivo* and free fatty acid (FFA)-induced HepG2 cells *in vitro*. The findings will provide further evidence for the regulative effects and mechanisms of BSP-1 on MASLD.

2 Materials and methods

2.1 Reagents and antibodies

The HFD was purchased from Wuhan Chunyuhong Experimental Animal Feed Co., Ltd. (56.3% basic feed +20% sucrose +12% lard +5% egg yolk powder +3% milk powder +2% soybean oil +1.5% cholesterol +0.2% sodium cholate). The normal diet was purchased from Henan Huanyu Hekang Biotechnology Co., LTD. (50% corn +22% soybean meal +9% bran +9% flour +7% fish meal +2.5% bone meal +0.5% salt +0.5% vitamin/mineral premix). Kits for measuring alanine aminotransferase (ALT, #C001-a), aspartate aminotransferase (AST, #C002-a), total cholesterol (TC, #C048-a), and triglyceride (TG, #C019-a) levels were provided by Changchun Huili Biotechnology Co., Ltd. (Changchun, China). Enzyme-linked immunosorbent assay (ELISA) kits for IL-18 (#MM-0194R2) and IL-1β (#MM-0047R2) were obtained from Jiangsu Meimian Industrial Company, Ltd. (Jiangsu, China). Anti-β-actin (#66009-1-Ig) was purchased from Wuhan Sanying Biotechnology Co., Ltd. (Wuhan, China). An antibody against NLRP3 (#TU269693) was obtained from Abmart Shanghai Co., Ltd.

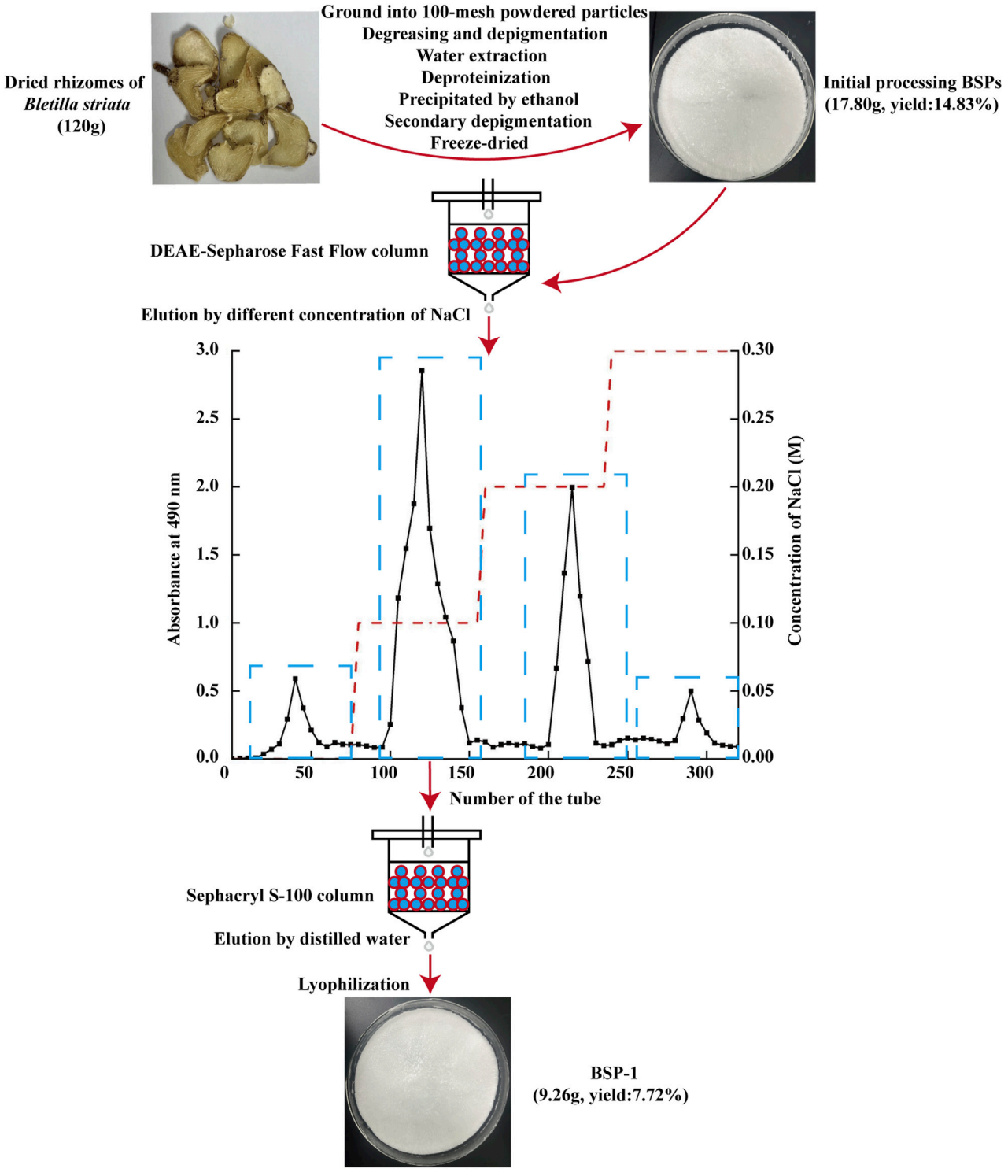

FIGURE 1
The extraction and purification of BSP-1.

(Shanghai, China). An antibody against caspase-1 (#AF4005) was obtained from Affinity Biosciences (Cincinnati, OH). An antibody against GSDMD (#10137S) was obtained from ImmunoWay Biotechnology Company (Plano, TX, United States). An antibody against apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) (#A1170) was obtained from Abclonal (Wuhan, China). HRP-conjugated goat anti-mouse secondary antibody (#BA1051) and HRP-conjugated goat anti-rabbit secondary antibody (#BA1054) were purchased from Wuhan Boster Biotechnology Co., Ltd. (Wuhan, China). Total RNA extraction reagent (#R401-01), HiScript II Q RT SuperMix (#R223-01) and the ChamQ SYBR qPCR Master Mix

Kit (#Q311-02) were purchased from Vazyme Biotech Co., Ltd. (Nanjing, China).

2.2 Extraction and purification of BSP-1

The extraction, isolation, and purification of BSP-1 were performed in accordance with established protocols (Ji et al., 2020). Initially, air-dried *B. striata* were obtained from Hubei Provincal Hospital of Traditional Chinese Medicine (Wuhan, China). The material was ground into 100-mesh powdered particles and extracted three times with distilled water at a ratio of 1:15 (W/V) at 90°C for 3 h each time. The three filtrates were collected and mixed, followed by defatting, decolorization, overnight alcohol precipitation at 4°C, and protein removal using the Sevage method. Following these steps, the filtrate was collected through centrifugation, dialyzed with ultrapure water for 48 h, and then lyophilized to yield BSPs. Ultimately, BSP-1 was isolated and purified through DEAE-Sepharose Fast Flow ion exchange chromatography and Sephacryl S-100 gel filtration chromatography, and stored at 4°C (Figure 1).

2.3 Characterization of BSP-1

2.3.1 Determination of polysaccharide content and ultraviolet (UV) spectroscopy

The phenol-sulfuric acid method was employed to detect the polysaccharide content in BSP-1, with three replicate measurements taken and their average value calculated. Additionally, BSP-1 at a concentration of 0.1 mg/mL was scanned and assayed for impurity content within the range of 200–400 nm using a UV-Visible spectrophotometer, with distilled water serving as the reference.

2.3.2 Determination of relative molecular weight

To determine the relative molecular weight of BSP-1, 2 mg of BSP-1 was dissolved in 0.2 M NaCl aqueous solution. A 20 μL aliquot of a 5 mg/mL BSP-1 solution was loaded onto a TSK-gel G-3000PWXL stainless steel chromatography column for high-performance gel permeation chromatography analysis. The mobile phase consisted of 0.2 M NaCl aqueous solution at a flow rate of 0.6 mL/min, and the column temperature was maintained at 40°C.

2.3.3 Monosaccharide composition analysis

1 mg of BSP-1 was dissolved in 1 mL of hydrochloric acid-methanol solution, and the mixture was subjected to a constant-temperature metal bath at 80°C under nitrogen gas for 16 h. After the hydrochloric acid-methanol was dried using a nitrogen blow dryer, 1 mL of 2 M trifluoroacetic acid was added, and the reaction was carried out at 120°C for 1 h, followed by drying again. Subsequent to acid hydrolysis, 500 μL of 0.3 M NaOH, 500 μL of 0.5 M 1-phenyl-3-methyl-5-pyrazolone -methanol, 100 μL of 0.3 M HCl, and 700 μL of dichloromethane were added sequentially for dissolution, water bath treatment, extraction, and phase separation. After derivatization of the monosaccharides, high-performance liquid chromatography (HPLC) was employed to detect the monosaccharide composition of BSP-1.

2.4 Grouping of experimental animals

Five-week-old male Sprague-Dawley (SD) rats (License No.: SYXK [Hubei] 2023-0067), obtained from the Hubei Provincial Center for Disease Control and Prevention (Wuhan, China), underwent an initial 1-week acclimatization period. The rats were housed in a temperature-controlled room at 25°C ± 1°C with controlled humidity, subjected to a natural light-dark cycle, and had free access to food and water. All experimental protocols used in this study were approved by the Research Ethics Committee of Hubei University of Chinese Medicine (Approval No.: HUCMS00308242) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

The rats were randomly divided into five groups (n = 10 rats per group): a normal group, a model group, and low-dose, medium-dose, and high-dose BSP-1 groups. Except for the control group (fed a normal diet), the model group and BSP-1 groups were subjected to a 12-week HFD to induce a model of MASLD (Farage et al., 2023). During the final 4 weeks of the study, the rats in the BSP-1 groups were gavaged with 100 mg/kg/d, 200 mg/kg/d, and 400 mg/kg/d of BSP-1, respectively, while the normal and model groups received an equal volume of saline daily (Qiu et al., 2023). The body weights of the rats were measured weekly during the experimental period. At the end of the experiment, the rats were anesthetized with isoflurane, and blood was collected from the abdominal aorta for serum separation by centrifugation. The liver tissues of the euthanized rats were collected, rinsed with saline, blotted dry, weighed, photographed, and subjected to subsequent analysis. The study procedure is illustrated in Figure 2A.

2.5 Histopathological examination of rat liver tissue

Liver tissues from rats in each group were fixed in 4% paraformaldehyde for 24 h, followed by dehydration, paraffin embedding, and sectioning. The slides were then stained with hematoxylin and eosin (H&E) and Oil Red, and observed and photographed under a biological microscope.

2.6 Cells and culture

HepG2 cells, obtained from Procell of Wuhan (CL-0103) and verified by STR analysis, were cultured in DMEM containing 10% fetal bovine serum and 1% penicillin-streptomycin. Prior to further research, the cells were maintained in a 37°C incubator with 5% CO_2. The research steps are illustrated in Figure 3B.

2.7 CCK8 assay in cells

HepG2 cells were seeded into 96-well plates at a density of 5 × 10^3 cells per well and cultured in a 37°C incubator with 5% CO_2

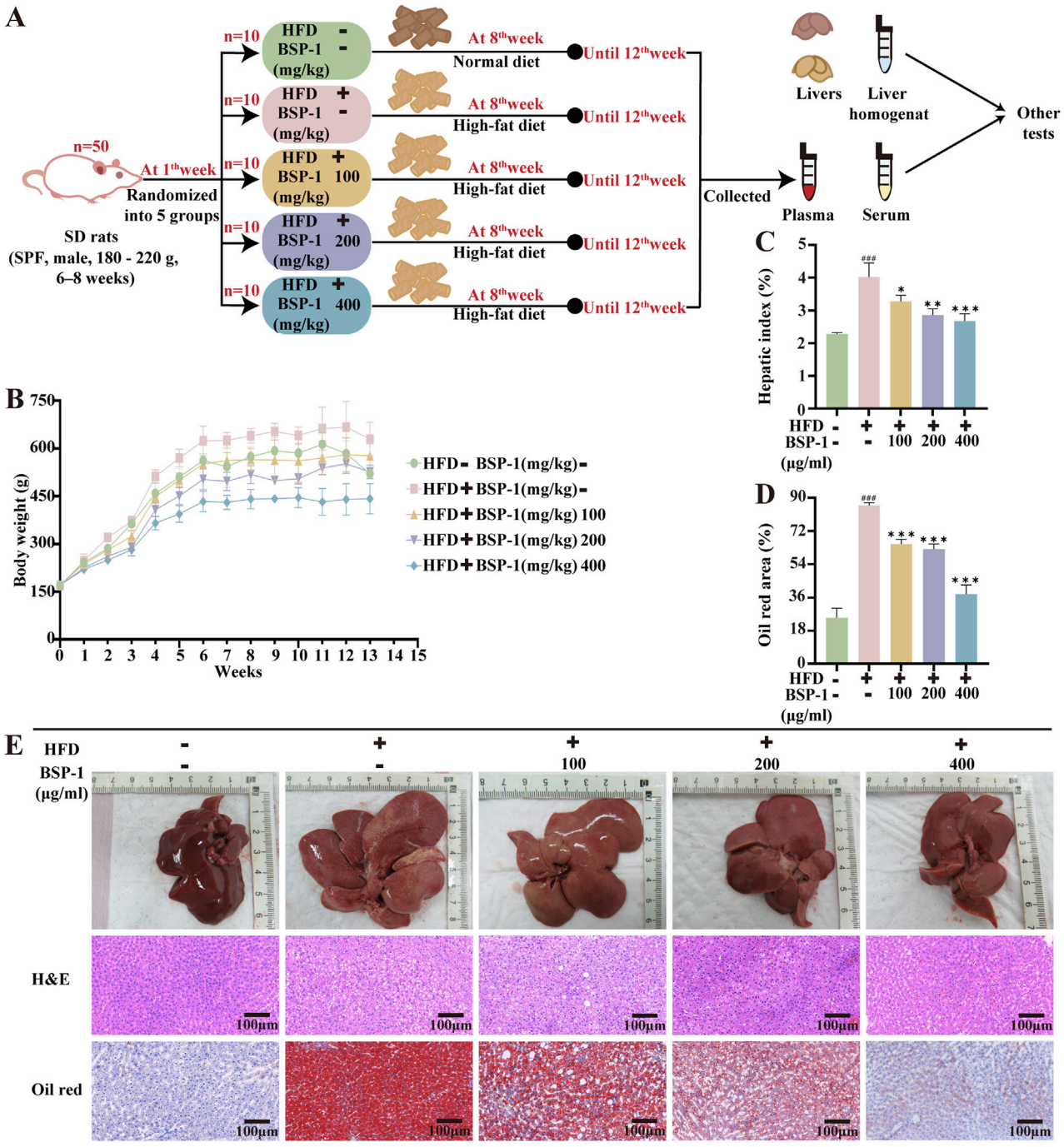

FIGURE 2
Brief steps of *in vivo* experiments, observation and recording of rat body weight, and pathological changes in liver tissue. **(A)** Grouping of experimental Animals. **(B)** The body weight of rats. **(C)** The hepatic index of rats. **(D)** The oil red area of liver tissue in rats. **(E)** The effect of BSP-1 on liver tissue in rats (anatomical observations and H&E stainning, Oil red stainning, 100 ×). The data are shown as the means ± SDs. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, vs. the model group.

according to different grouping and cell treatments. The groups for assessing the toxicity of BSP-1 on HepG2 cells after 24 h and 48 h of treatment were as follows: normal group, BSP-1 groups (25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, 800 μg/mL, 1,000 μg/mL). The groups for assessing the toxicity of BSP-1 on FFA-induced HepG2 cells after 24 h of treatment were as follows: normal group, FFA group, FFA + BSP-1 groups (25 μg/mL, 50 μg/mL, 100 μg/mL). After cell culture, 10 μL of CCK8 was added to each well, and the plates were incubated at 37°C for 1–4 h before measuring the absorbance of each well using a microplate reader.

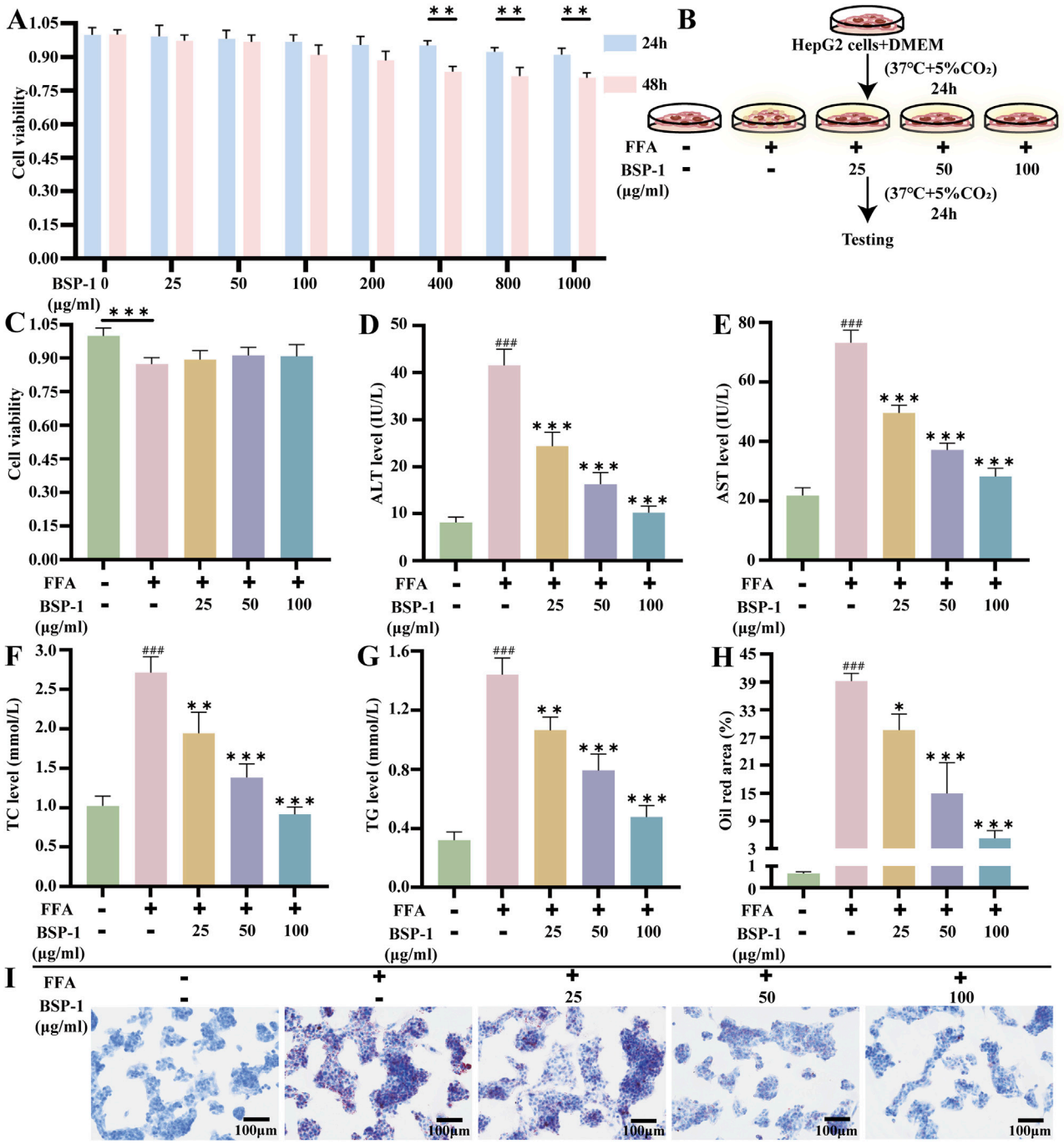

FIGURE 3
Brief steps *in vitro* experiments, cytotoxicity test of BSP-1, detection of liver function and TC, TG levels, and Oil red staining in HepG2 cells. **(A)** CCK8 assay in HepG2 cells. **(B)** HepG2 cells and culture. **(C)** CCK8 assay in HepG2 cells. The measurement of **(D)** ALT **(E)** AST **(F)** TC **(G)** TG in HepG2 cells. **(H)** The oil red area of liver tissue in HepG2 cells. **(I)** The effect of BSP-1 in HepG2 cells (Oil red stainning, 100 ×). The data are shown as the means ± SDs. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, vs. the model group.

2.8 Measurement of IL-1β and IL-18 in rat serum and HepG2 cells

Rat serum and HepG2 cells from each group, centrifuged at 3,000 rpm for 10 min at 4°C, were used to detect the levels of IL-1β and IL-18 by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

2.9 Measurement of TC and TG contents and liver function in rat serum and HepG2 cells

Rat serum and HepG2 cells from each group, centrifuged at 3,000 rpm for 10 min at 4°C, were used to detect the levels of ALT, AST, TC, and TG in the serum using commercially available kits according to the manufacturer's instructions.

TABLE 1 Primer sequence for qRT–PCR.

Gene	Forward (5′–3′)	Reverse (5′–3′)	Species
NLRP3	CTGCTGAAGTGGATCGAAGTG	TGCAAAAGGAAGAAACCACGT	Rat
GSDMD	CCAAAGCCGGAAGAAGATGG	ACTAAAGTCATGCCGCCTCT	Rat
caspase-1	AACTGAACAAAGAAGGTGGCG	GCAGATAATGAGGGCAAGACG	Rat
ASC	CTGTGCTTAGAGACATGGGCA	GTTGGTGGTCTCTGCACGAA	Rat
β-actin	TGACGTTGACATCCGTAAAGACC	GTGCTAGGAGCCAGGGCAGTAA	Rat
NLRP3	GTTTGACCCCGATGATGAGC	CTTGTGGATGGGTGGGTTTG	Homo
GSDMD	AAGACGGTCACCATCCCC	AAGGTCCTCTGCTTCTTATCC	Homo
caspase-1	GCACACGTCTTGCTCTCATTA	TTCACATCTACGCTGTACCCC	Homo
ASC	GATCCAGGCCCCTCCTCA	ACCAGGTAGGACTGGGACTC	Homo
β-actin	CCCTGGAGAAGAGCTACGAG	CGTACAGGTCTTTGCGGATG	Homo

2.10 RT-qPCR analysis of rat liver tissue and HepG2 cells

Total RNA was extracted from rat liver tissue and HepG2 cells using TRIzol reagent according to the manufacturer's instructions and stored at −80°C until use. The isolated RNA was then quantitated and reverse-transcribed into cDNA using a cDNA reverse transcription kit following the provided protocol. Real-time quantitative polymerase chain reaction (RT-qPCR) was performed using a real-time PCR instrument from Applied Biosystems (California, United States), and the mRNA levels of the target genes were normalized to the relative levels of β-actin using the $2^{-\Delta\Delta CT}$ method. The primer sequences used in this study are shown in Table 1.

2.11 Western blot (WB) analysis of rat liver tissue and HepG2 cells

Total protein was extracted from rat liver tissue and HepG2 cells using pre-cooled RIPA lysis buffer and quantitated using a BCA protein assay kit. The extracted protein supernatant was boiled for 10 min for denaturation, cooled to room temperature, and stored at −20°C until use. The total protein was separated by gel electrophoresis and transferred to a PVDF membrane. The primary antibody dilution ratios were as follows: NLRP3 (1:1000), ASC (1:1000), Cleaved caspase1 (1:1000), Cleaved Gasdermin D (1:1000), and β-Actin (1:10000). After incubation with HRP-labeled secondary antibodies diluted with TBST, immunoreactive bands were observed using a chemiluminescence method.

2.12 Immunohistochemical analysis of rat liver tissue

Rat liver tissue samples were sectioned, dewaxed, and subjected to antigen retrieval. The sections were treated with 3% H_2O_2 to inhibit endogenous peroxidase activity and then blocked with 5% normal goat serum at room temperature for 30 min to prevent non-specific binding signals. Diluted NLRP3 antibody (1:100) and GSDMD polyclonal antibody (1:100) were added as primary antibodies and incubated overnight at 4°C. After washing three times with PBS, HRP-labeled goat anti-rabbit/mouse secondary antibody was added and incubated at 37°C for 30 min. Freshly prepared DAB chromogenic solution and Mayer's hematoxylin were then added for staining. The slides were washed with PBS, dehydrated, mounted, and observed and photographed under a biological microscope.

2.13 Immunofluorescence of HepG2 cells

HepG2 cells were washed with PBS and fixed with 4% paraformaldehyde. The cells were then permeabilized with 0.5% Triton X-100 (prepared in PBS) at room temperature and washed again with PBS. Goat serum was added to block antibodies at room temperature to reduce non-specific binding signals. Diluted NLRP3 antibody (1:100) and GSDMD polyclonal antibody (1:100) were added as primary antibodies and incubated overnight at 4°C. After washing three times with PBS, fluorescence (Cy3)-labeled goat anti-rabbit IgG (1:400) secondary antibody was added and incubated at 37°C for 1 h. Finally, DAPI was added for incubation for 5 min, and the cells were mounted and observed and photographed under a fluorescence microscope.

2.14 Statistical analysis

The data from these experiments are presented as means ± standard deviations (SDs). All experimental data were analyzed using Image-Pro Plus 6.0 and GraphPad Prism 8.0. For multiple comparisons, one-way analysis of variance (ANOVA) was used, followed by the Tukey test. A p-value <0.05 was considered statistically significant.

3 Results

3.1 Characterization of BSP

The purified BSP-1 was found to contain a polysaccharide content of 96.12% ± 0.08%, as determined by the phenol-sulfuric

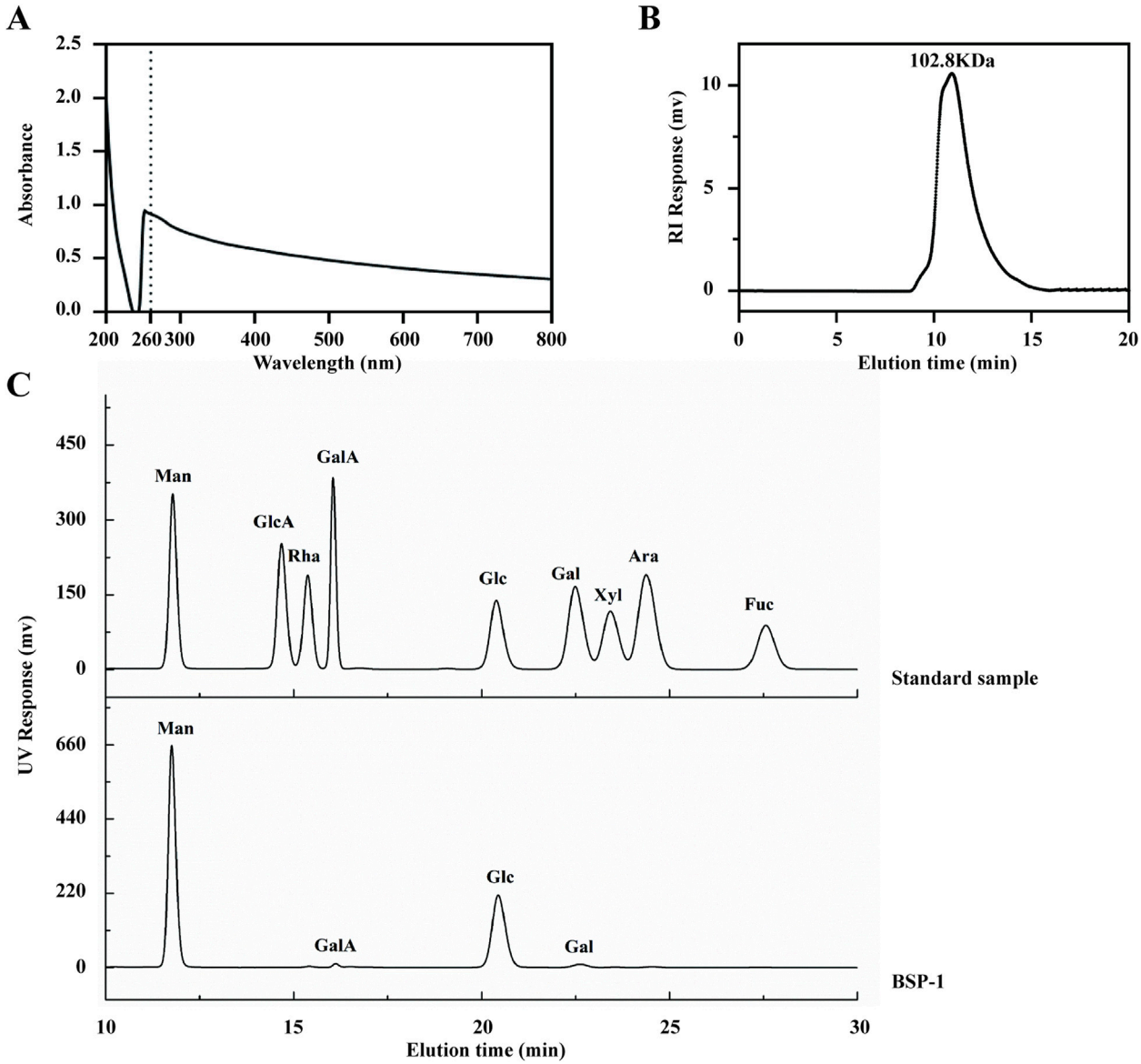

FIGURE 4
Characterization of BSP. **(A)** The UV Spectroscopy of BSP-1. **(B)** The average molecular weight of BSP-1. **(C)** The monosaccharide composition of BSP-1.

acid method. UV spectroscopy indicated that the composition was relatively homogeneous, with minimal other special impurities. The average molecular weight of BSP-1 was determined to be 102.8 kDa through high-performance gel permeation chromatography. HPLC analysis revealed that BSP is composed of mannose (Man), glucose (Glc), galactose (Gal), and galacturonic acid (GalA) in a molar ratio of 62.5:34.9:1.7:0.9 (Figures 4A–C).

3.2 Effect of BSP-1 on body weight and liver index in rats

To ascertain the impact of BSP-1 on MASLD *in vivo*, we observed that the body weights of rats in all groups gradually increased over time during the study period. Additionally, rats in the HFD group exhibited higher body weights and liver indices. However, after 4 weeks of BSP-1 intervention, the rate of weight gain was attenuated in all groups, with the most significant effect observed in the 400 mg/kg/d group. Anatomical observations showed that the livers of rats in the HFD group were more fragile and heterogeneous in texture, with a yellower color. Following BSP-1 intervention, the livers became smaller, redder, and showed a significant reduction in fatty degeneration (Figures 2B,C,E).

3.3 Effect of BSP-1 on liver tissue in rats and pathological tissue in HepG2 cells

Histopathological observations through H&E staining revealed severe hepatocyte degeneration and the formation of lipid vacuoles

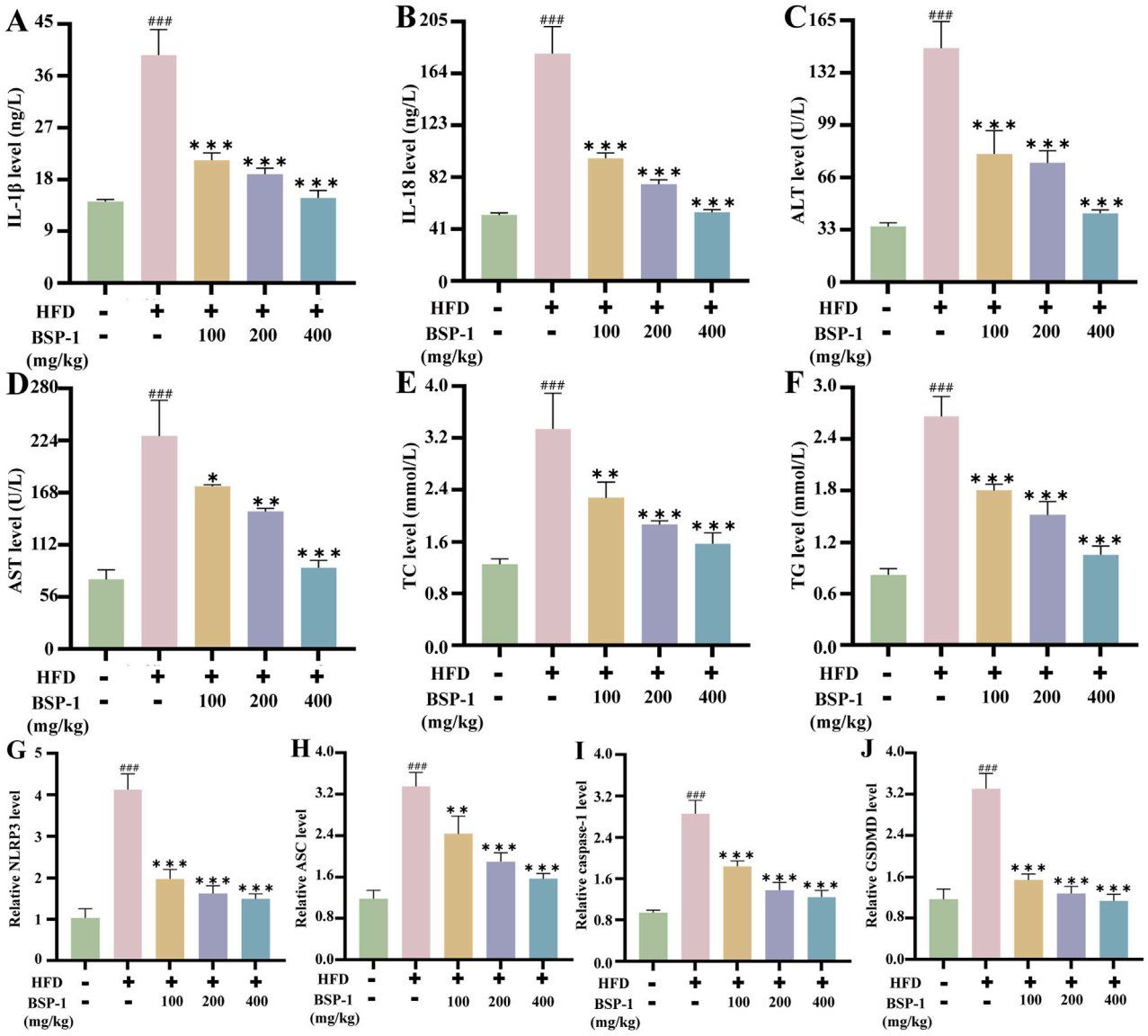

FIGURE 5
Measurement of IL-1β, IL-18, TC, TG, liver function in rat serum and qRT-PCR analysis in rat liver tissue. The measurement of **(A)** IL-1β **(B)** IL-18 **(C)** ALT **(D)** AST **(E)** TC **(F)** TG in rat serum. The mRNA levels of **(G)** the NLRP3 **(H)** the ASC **(I)** the caspase-1 **(J)** the GSDMD in rat liver tissue. The data are shown as the means ± SDs. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, vs. the model group.

in MASLD rats from the HFD group. This phenomenon decreased with increasing concentrations of BSP-1 intervention (Figure 2E). Consequently, we used Oil Red staining to observe the lipid droplet content in the HFD-induced rat groups and FAA-induced HepG2 cell groups, further validating this observation (Figures 2E, 3I).

3.4 Effects of BSP-1 on biochemical indicators in rat serum and HepG2 cells

By measuring biochemical indicators in rat serum and HepG2 cells, the impact of BSP-1 on liver tissue damage and lipid deposition in MASLD was observed (Figures 3D–G, 5C–F). Compared with the control group, the levels of ALT, AST, TC, and TG in rat serum and HepG2 cells were significantly elevated in the model group. In contrast, the levels of these biochemical indicators were lower in the rat serum and HepG2 cells of the various BSP-1-treated groups compared to the model group. Notably, the reductions in ALT, AST, TC, and TG levels were most significant in the MASLD rat serum of the 400 mg/kg/d group and in the HepG2 cells of the FFA + BSP-1 (100 μg/mL) group, suggesting that BSP-1 is non-hepatotoxic and can improve HFD-induced liver tissue damage and lipid accumulation in rats, as well as FFA-induced liver damage and lipid accumulation in HepG2 cells.

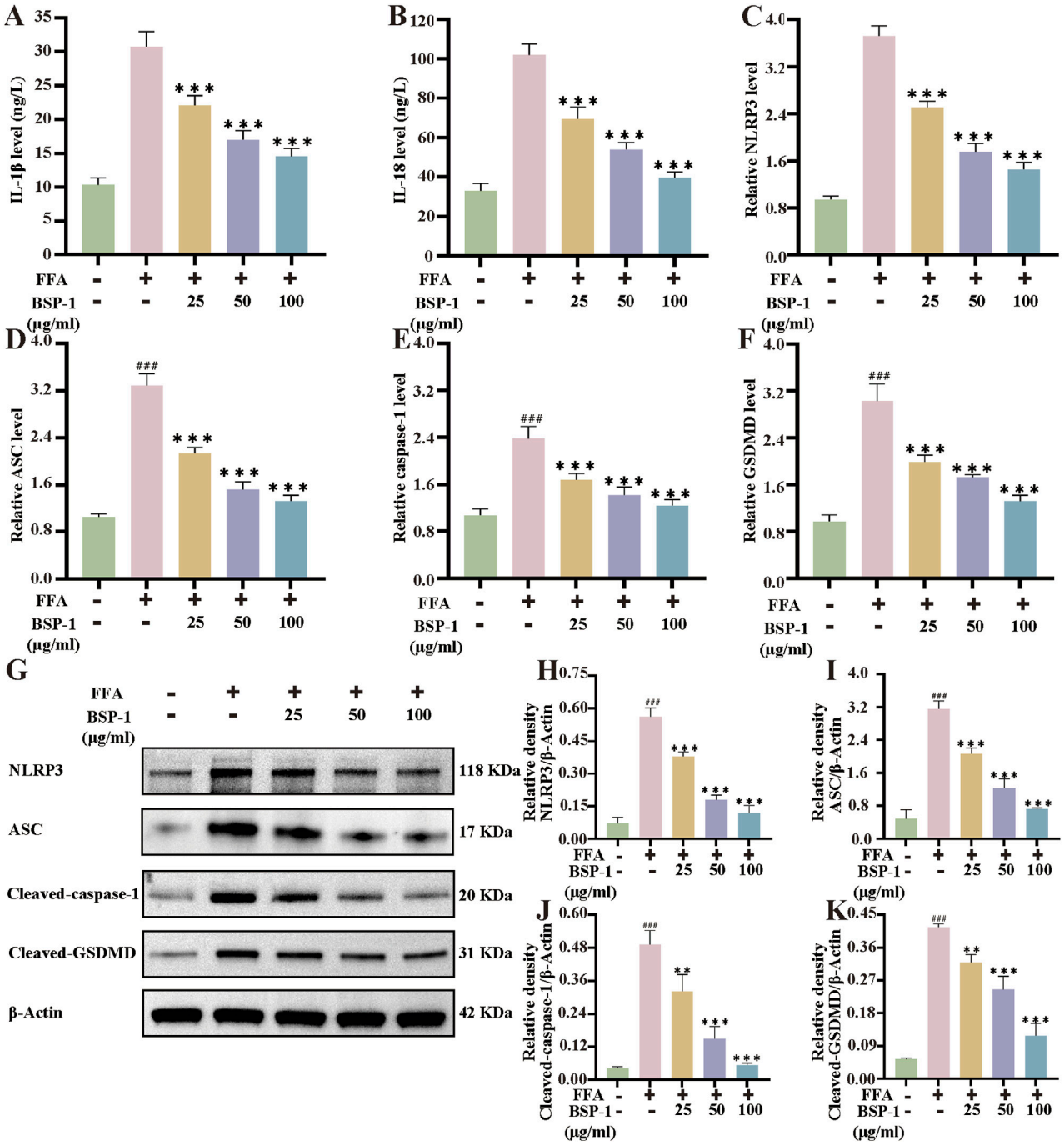

FIGURE 6
Measurement of IL-1β and IL-18, qRT-PCR and WB analysis in HepG2 cells. The measurement of **(A)** IL-1β and **(B)** IL-18 in HepG2 cells. The mRNA levels of **(C)** the NLRP3 **(D)** the ASC **(E)** the caspase-1 and **(F)** the GSDMD in HepG2 cells. **(G)** The WB analysis in HepG2 cells. **(H)** The NLRP3/β-Actin **(I)** the ASC/β-Actinof **(J)** the Cleaved-caspase-1/β-Actin and **(K)** the Cleaved-GSDMD/β-Actinof WB in HepG2 cells. The data are shown as the means ± SDs. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, vs. the model group.

3.5 Effects of BSP-1 on the expression of inflammatory cytokines in rat serum and HepG2 cells

We then investigated the impact of BSP-1 on inflammatory cytokines in MASLD (Figures 5A,B, 6A,B). The levels of IL-1β and IL-18 in rat serum and HepG2 cells were significantly higher in the model group compared to the control group. However, after treatment with different doses of BSP-1, the levels of IL-1β and IL-18 in rat serum and HepG2 cells were significantly reduced compared to the model group, indicating that BSP-1 treatment can effectively decrease the production of inflammatory cytokines in HFD-induced rat serum and FFA-induced HepG2 cells.

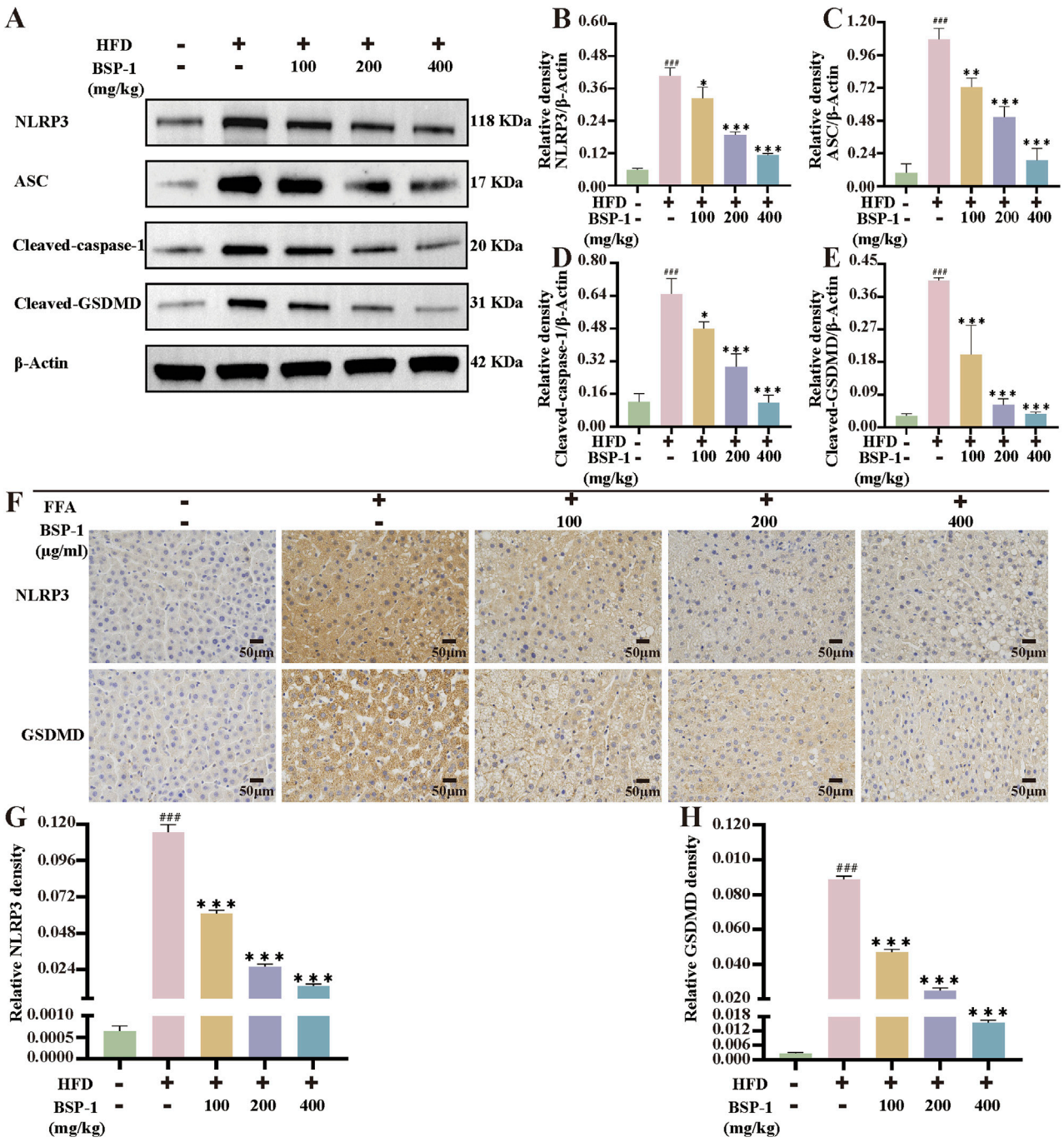

FIGURE 7
The WB analysis and immunohistochemical staining in rat liver tissue. **(A)** The WB analysis in rat liver tissue. **(B)** The NLRP3/β-Actin **(C)** the ASC/β-Actinof **(D)** the Cleaved-caspase-1/β-Actin and **(E)** the Cleaved-GSDMD/β-Actinof WB in rat liver tissue. **(F)** The evaluation of NLRP3 and GSDMD immunohistochemical staining (200 ×). The immunohistochemical density of **(G)** the NLRP3 and **(H)** the GSDMD in rat liver tissue. The data are shown as the means ± SDs. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, vs. the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, vs. the model group.

3.6 Effects of BSP-1 on the pyroptosis-related NLRP3/caspase-1/GSDMD pathway in rat liver tissue and HepG2 cells

To further understand the effects of BSP-1 on MASLD through *in vivo* and *in vitro* experiments, we investigated the expression of proteins related to the pyroptosis-related NLRP3/caspase-1/GSDMD pathway. After WB detection and semi-quantitative analysis, the expression of NLRP3, ASC, Cleaved-caspase-1, and Cleaved-GSDMD was significantly increased in the model group compared to the normal group in both rat liver tissue and HepG2 cells. However, after intervention with the BSP-1 group, the expression of these related proteins was downregulated (Figures 6G–K, 7A–E). Additionally, immunohistochemical and

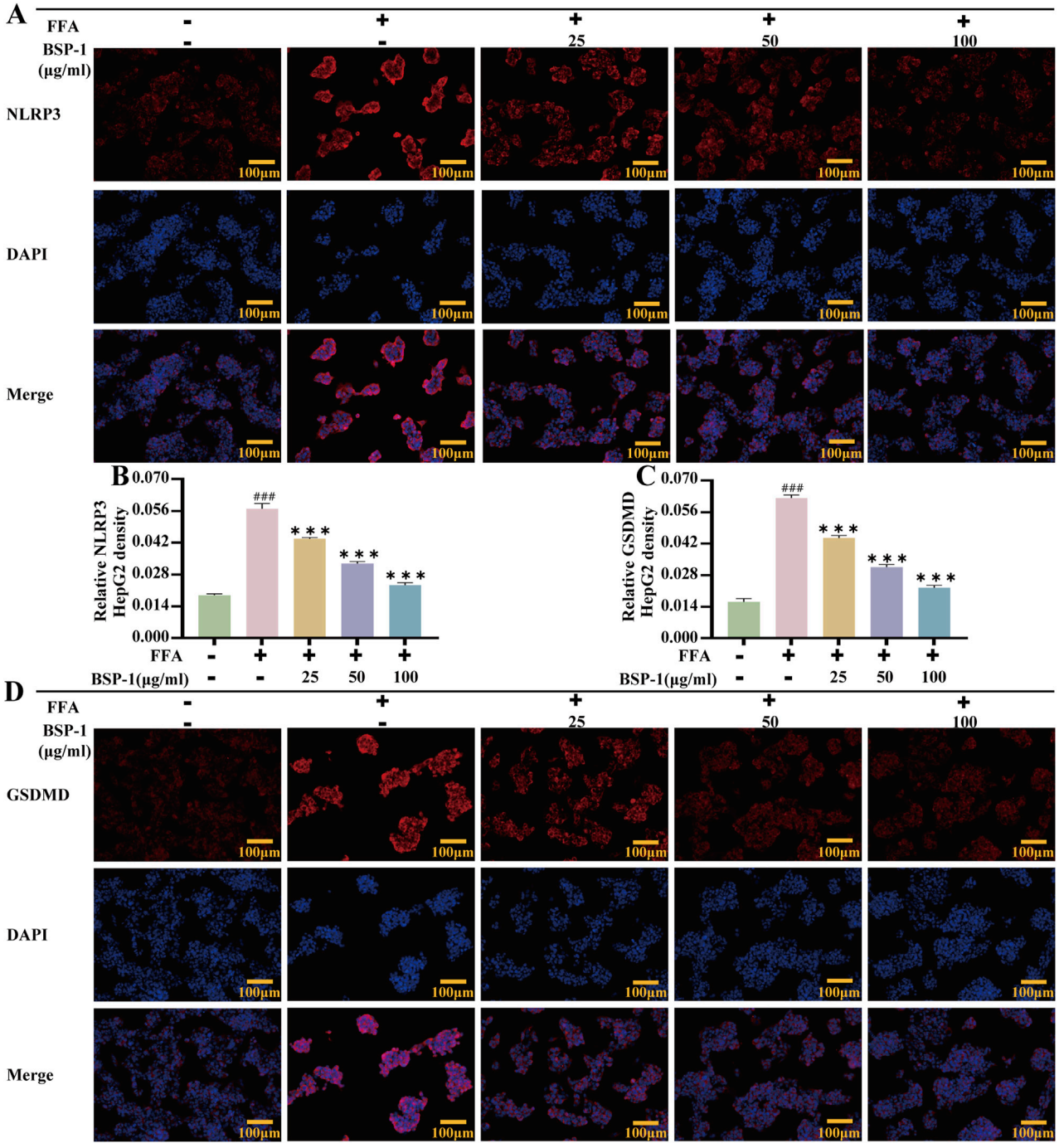

FIGURE 8
Immunofluorescence of HepG2 Cells. **(A)** The evaluation of NLRP3 immunofluorescence staining (100 ×). The immunohistochemical density of **(B)** the NLRP3 and **(C)** the GSDMD in HepG2 cells. **(D)** The evaluation of GSDMD immunofluorescence staining (100 ×). The data are shown as the means ± SDs. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$, vs. the control group; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, vs. the model group.

immunofluorescence experiments, along with semi-quantitative analysis, were conducted to confirm this phenomenon (Figures 7F–H, 8A–D). To detect whether the relevant target genes were transcribed into mRNA, qRT-PCR analysis was performed, revealing a positive correlation between the expression trends of NLRP3, ASC, caspase-1, GSDMD-related mRNA and their protein levels in each group (Figures 5G–J, 6C–F).

4 Discussion

In recent years, polysaccharides have emerged as a well-known treasury of new pharmaceutical resources, possessing potential therapeutic effects on various diseases. BSP has good biocompatibility, degradability and low toxicity, and possesses excellent pharmacological activity and clinical application value

(Zhu et al., 2023). Prior research has demonstrated that BSP ameliorates gut microbiota dysbiosis induced by a HFD in mice by reducing the Firmicutes/Bacteroidetes ratio, thereby mitigating abnormal weight gain (Zhang et al., 2024). Additionally, BSP exhibits the ability to alleviate hepatic fibrosis through the TLR2/TLR4-MyD88-NF-κB signaling pathway, ultimately achieving hepatic protection (Jiang et al., 2023). Although existing studies have shown that BSP has anti-inflammatory and liver-protecting effects, its specific therapeutic effect on MASLD has not been systematically studied. This study verified for the first time through *in vivo* and *in vitro* experiments the possibility of BSP-1 improving MASLD. To validate this hypothesis, we first extracted and purified the polysaccharide BSP-1 from the traditional Chinese medicine *Bletilla striata*. Subsequently, we induced MASLD in rats using a HFD and fatty degeneration in HepG2 cells with FFA, and administered BSP-1 to observe its regulatory effects on MASLD.

In the initial stages of our experiment, we extracted a novel neutral polysaccharide, BSP-1, which comprises Man, GalA, Glc, and Gal. Prior studies have demonstrated that polysaccharide-based therapeutics exhibit efficacy in improving MASLD. For instance, polysaccharides derived from Auricularia auricular-judae specifically target and activate the Toll-like receptor 4 (TLR4) on macrophages within the TLR4/NF-κB signaling pathway, which is situated upstream of the NLRP3 inflammasome. This activation enhances innate immune functions by promoting phagocytosis and cytokine production in macrophages (Tang et al., 2024). Similarly, the polysaccharide from *Gynostemma pentaphyllum*, upon degradation by intestinal microbiota, yields bioactive products that support the growth of beneficial gut flora such as *Akkermansia* and *Lactobacillus*. Concurrently, it downregulates the TLR2/NLRP3 pathway, thereby delaying the progression of MASLD in murine models (Yue et al., 2022). Furthermore, *Lycium barbarum* polysaccharide has been shown to mitigate liver injury in a methionine-choline deficient diet-induced steatohepatitis model by downregulating the NF-κB and NLRP3/6 pathways, thus exhibiting antioxidant and immunoregulatory properties (Xiao et al., 2018). Additionally, based on the monosaccharide composition of BSP, mannose intake has been reported to alleviate intrahepatic oxidative stress, inflammation, and fibrosis in thioacetamide-induced rats, thereby demonstrating hepatoprotective effects (Shaker et al., 2021). Besides monosaccharide composition, MW also significantly influences biological activity. The MW of BSP is 102.8 kDa. When compared to the MWs reported in previous studies, a lower MW is associated with reduced molecular volume, solubility, and viscosity, potentially indicating enhanced biological effects *in vivo* (Wang et al., 2019). Consequently, we further assessed the bioactivity of BSP concerning MASLD.

The disruption of the synthesis and excretion balance of free fatty acids and triglycerides in the liver leads to hepatic steatosis, which is crucial in the development of MASLD. The induction of HepG2 cells by FFA mixture with potential cytotoxicity and lipotoxicity (oleic acid to palmitic acid ratio of 2:1) represents an *in vitro* cell model that simulates benign chronic steatosis (Lee et al., 2019; Wang et al., 2022). SD rats fed a high-fat diet developed MASLD, accompanied by hepatic glycerol accumulation and hyperlipidemia. In our study, rats fed with HFD exhibited significant biochemical characteristics of MASLD, including hyperlipidemia and lipid accumulation in the liver. Significant lipid droplet aggregation was also observed in HepG2 cells induced by FFA. The non-specific clinical feature of MASLD is elevated liver transaminases (ALT and AST), which are positively correlated with most MASLD patients. Improving liver function damage and lipid metabolism disorders caused by MASLD has a positive effect on the treatment of MASLD. In this study, after treatment with BSP-1, elevated levels of TC, TG, and liver transaminases (ALT, AST) were significantly reduced, indicating that BSP-1 can alleviate liver cell damage caused by MASLD and improve lipid metabolism. Pathological section analysis also showed that BSP-1 treatment group showed a significant reduction in fat deposition and a alleviation of inflammatory infiltration. This further confirms the therapeutic effect of BSP-1 on MASLD and provides morphological evidence for subsequent molecular mechanism research.

MASLD is closely related to chronic inflammation of the liver. The NLRP3 inflammasome is essential for the progression of chronic inflammation and has recently become a potential new therapeutic target for MASLD (Chan et al., 2023; Hutchison et al., 2023). It is ubiquitously present in hepatic immune and parenchymal cells and effectively recognizes disturbances caused by PAMPs and DAMPs in the hepatic cellular milieu, thereby exerting innate immune functions (Sharma and Kanneganti, 2021). In general, the levels of the sensor NLRP3, the adapter ASC, and the effector caspase-1 within the multi-molecular protein complex NLRP3 inflammasome remain relatively stable and low. Although immune cells, upon sensing PAMPs and DAMPs, activate the NF-κB signaling pathway to increase the expression of NLRP3 genes, thereby elevating their numbers, post-translational modifications such as ubiquitination, phosphorylation, and sumoylation, along with interactions between NLRP3 and organelles, further regulate immune cells to prevent easy activation of the NLRP3 inflammasome in response to stimulation (Swanson et al., 2019). Once activated in macrophage- and monocyte-dominant liver sites, the NLRP3 inflammasome triggers the production of pro-inflammatory cytokines, lysosomal damage, and increased reactive oxygen species, further inducing the transcription of NLRP3 genes. Additionally, the prion-like polymerization of fibrils formed during the docking of the PYD domains of NLRP3 and ASC facilitates the recruitment of caspase-1 by the caspase recruitment domain of ASC. The activated NLRP3 inflammasome mainly induces pyroptosis by cleving GSDMD, allowing its N-terminal domain to bind to the cell membrane and form pores. Meanwhile, it promotes the secretion of extracellular active pro-inflammatory cytokines IL-1β and IL-18 (Hooftman et al., 2020), which intensifies the liver inflammatory response and accelerates the development of MASLD. In our study, we found that BSP-1 could effectively weaken the protein and mRNA expressions of NLRP3, ASC, caspase-1 and GSDMD, indicating that the NLRP3 inflammasome-related pathway was inhibited. In the rat model induced by HFD, immunohistochemical analysis also showed that BSP-1 could improve the expression of NLRP3 and GSDMD proteins in liver tissues. The same conclusion was reached by immunofluorescence detection of the expression of NLRP3 and GSDMD proteins in the HepG2 cells model induced by FFA. Furthermore, BSP-1 also inhibits the production of pro-inflammatory cytokines IL-1β and

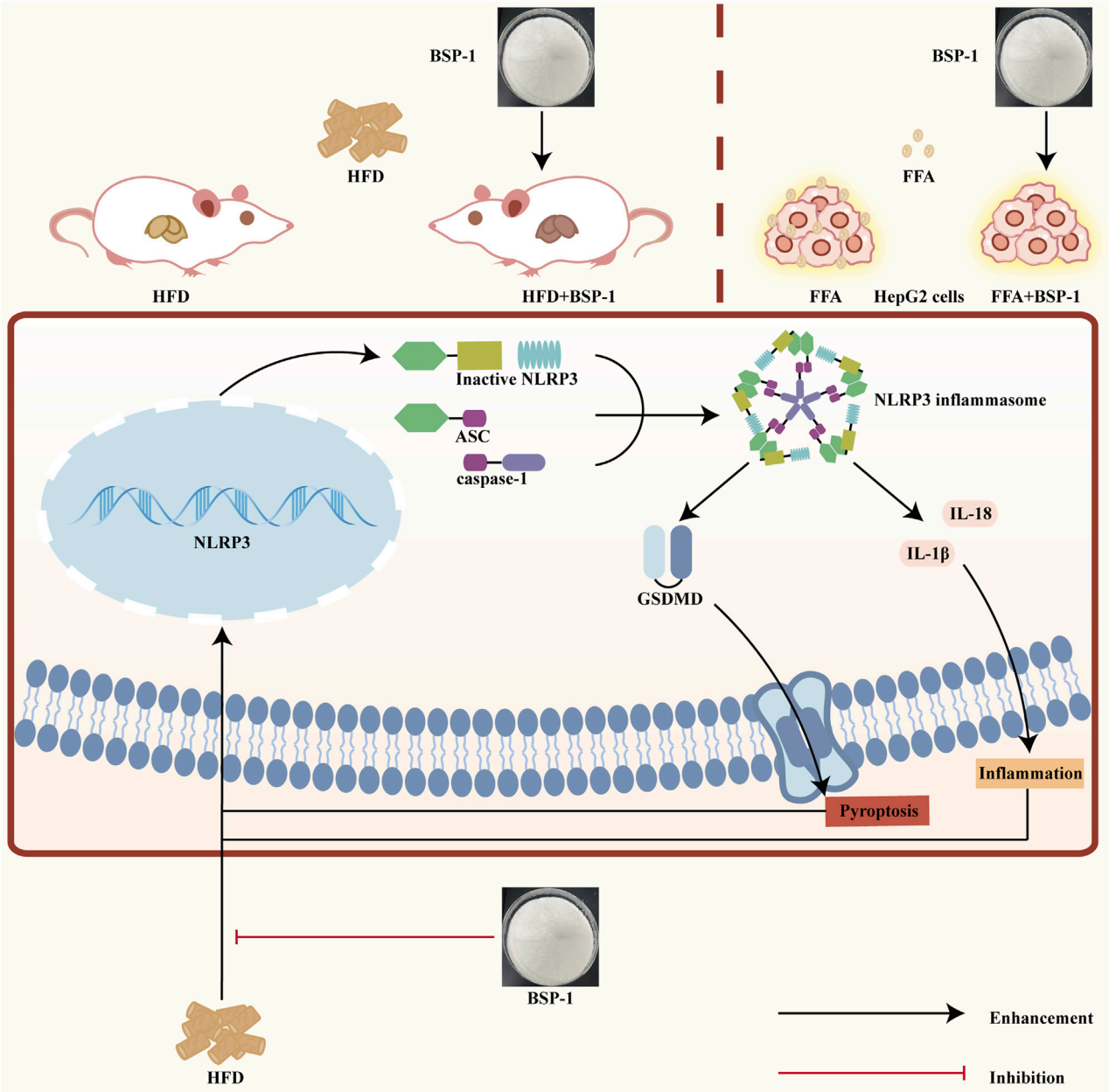

FIGURE 9
The impact of BSP-1 on MASLD by evaluating its ability to reduce the expression of pyroptosis-related NLRP3/caspase-1/GSDMD pathway in both HFD-induced rat models *in vivo* and FFA-induced HepG2 cells *in vitro*.

IL-18 in the HFD-induced rat and FFA-induced HepG2 cells, which further indicates its inhibitory effect on liver inflammation. Therefore, BSP-1 plays a role in alleviating MASLD by preventing steatosis, inflammation, and pyroptosis, which may be relate to the inhibition of NLRP3/caspase-1/GSDMD signaling pathway (Figure 9).

5 Conclusion

In conclusion, our research results show that BSP-1 can improve liver injury and steatosis caused by MASLD in both *in vitro* and *in vivo* experiments, this effect may be attributed to the regulation of the pyrother-related NLRP3/caspase-1/GSDMD pathway. This result provides evidence that BSP-1 has the potential to treat MASLD. However, the existing studies are still unclear whether BSP-1 exerts its effect through direct action on NLRP3 or other indirect mechanisms. In the future, the interaction mechanism between it and the NLRP3/caspase-1/GSDMD pathway should be further clarified. Meanwhile, further clarifying the molecular structure of BSP-1 is of great significance for studying its pharmacological activity and molecular mechanism. Clinical trials based on human MASLD patients will be carried out in

the future to verify the therapeutic potential of BSP-1. Therefore, as an emerging polysaccharide drug, BSP-1 still requires more in-depth and comprehensive research in the process of MASLD research.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal experimental procedures were ethically approved by the Research Ethical Committee of Hubei University of Traditional Chinese Medicine (Approval NO. HUCMS 00308242). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TY: Conceptualization, Investigation, Writing – original draft. JX: Data curation, Investigation, Methodology, Writing – original draft. WT: Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. XW: Investigation, Visualization, Writing – original draft. JL: Investigation, Visualization, Writing – original draft. FY: Project administration, Supervision, Validation, Writing – review and editing. LL: Funding acquisition, Project administration, Supervision, Writing – review and editing.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1563275/full#supplementary-material

References

Carvalho Ribeiro, M., and Szabo, G. (2022). Role of the inflammasome in liver disease. *Annu. Rev. Pathol.* 17 (1), 345–365. doi:10.1146/annurev-pathmechdis-032521-102529

Chan, W. K., Chuah, K. H., Rajaram, R. B., Lim, L. L., Ratnasingam, J., and Vethakkan, S. R. (2023). Metabolic dysfunction-associated steatotic liver disease (MASLD): a state-of-the-art review. *J. Obes. Metab. Syndr.* 32 (3), 197–213. doi:10.7570/jomes23052

Farage, A. E., Abdo, W., Osman, A., Abdel-Kareem, M. A., Hakami, Z. H., Alsulimani, A., et al. (2023). Betulin prevents high fat diet-induced non-alcoholic fatty liver disease by mitigating oxidative stress and upregulating Nrf2 and SIRT1 in rats. *Life. Sci.* 322, 121688. doi:10.1016/j.lfs.2023.121688

Fu, J., and Wu, H. (2023). Structural mechanisms of NLRP3 inflammasome assembly and activation. *Annu. Rev. Immunol.* 41 (1), 301–316. doi:10.1146/annurev-immunol-081022-021207

Hagström, H., Vessby, J., Ekstedt, M., and Shang, Y. (2024). 99% of patients with NAFLD meet MASLD criteria and natural history is therefore identical. *J. Hepatol.* 80 (2), e76–e77. doi:10.1016/j.jhep.2023.08.026

He, X., Wang, X., Fang, J., Zhao, Z., Huang, L., Guo, H., et al. (2017). *Bletilla striata*: medicinal uses, phytochemistry and pharmacological activities. *J. Ethnopharmacol.* 195, 20–38. doi:10.1016/j.jep.2016.11.026

Hooftman, A., Angiari, S., Hester, S., Corcoran, S. E., Runtsch, M. C., Ling, C., et al. (2020). The immunomodulatory metabolite itaconate modifies NLRP3 and inhibits inflammasome activation. *Cell. Metab.* 32 (3), 468–478. doi:10.1016/j.cmet.2020.07.016

Hutchison, A. L., Tavaglione, F., Romeo, S., and Charlton, M. (2023). Endocrine aspects of metabolic dysfunction-associated steatotic liver disease (MASLD): beyond insulin resistance. *J. Hepatol.* 79 (6), 1524–1541. doi:10.1016/j.jhep.2023.08.030

Ji, X., Yin, M., Nie, H., and Liu, Y. (2020). A review of isolation, chemical properties, and bioactivities of polysaccharides from *Bletilla striata*. *Biomed. Res. Int.* 2020, 5391379. doi:10.1155/2020/5391379

Jiang, G., Wang, B., Wang, Y., Kong, H., Wang, Y., Gao, P., et al. (2023). Structural characteristics of a novel *Bletilla striata* polysaccharide and its activities for the alleviation of liver fibrosis. *Carbohydr. Polym.* 313, 120781. doi:10.1016/j.carbpol.2023.120781

Kalligeros, M., Henry, L., and Younossi, Z. M. (2024). Metabolic dysfunction-associated steatotic liver disease and its link to cancer. *Metabolism* 160, 156004. doi:10.1016/j.metabol.2024.156004

Lee, M. R., Yang, H. J., Park, K. I., and Ma, J. Y. (2019). Lycopus lucidus Turcz. ex Benth. Attenuates free fatty acid-induced steatosis in HepG2 cells and non-alcoholic fatty liver disease in high-fat diet-induced obese mice. *Phytomedicine* 55, 14–22. doi:10.1016/j.phymed.2018.07.008

Liu, X., Xia, S., Zhang, Z., Wu, H., and Lieberman, J. (2021). Channelling inflammation: gasdermins in physiology and disease. *Nat. Rev. Drug. Discov.* 20 (5), 384–405. doi:10.1038/s41573-021-00154-z

Luo, L., Liu, Y., Cai, X., Wang, Y., Xue, J., Zhang, J., et al. (2019). *Bletilla striata* polysaccharides ameliorates lipopolysaccharide-induced injury in intestinal epithelial cells. *Saudi. J. Gastroenterol.* 25 (5), 302–308. doi:10.4103/sjg.SJG_520_18

Luo, L., Zhou, Z., Xue, J., Wang, Y., Zhang, J., Cai, X., et al. (2018). *Bletilla striata* polysaccharide has a protective effect on intestinal epithelial barrier disruption in TAA-induced cirrhotic rats. *Exp. Ther. Med.* 16 (3), 1715–1722. doi:10.3892/etm.2018.6430

Luo, R., Hu, Y., Wang, L., Ke, Z., Wang, W., Wang, P., et al. (2024). Penthorum chinense pursh extract ameliorates hepatic steatosis by suppressing pyroptosis via the

NLRP3/Caspase-1/GSDMD pathway. *Food Sci. Nutr.* 12 (7), 5176–5187. doi:10.1002/fsn3.4165

Qiu, B., Zeng, Y., Liu, J., Yuan, Z., and Liu, H. (2023). Study on the protective effect of *Bletilla striata* polysaccharide on intestinal mucosal inflammatory injury in rats with ulcerative colitis based on the NF-κB/NLRP3/caspase-1 pathway. *Chin. J. Immunol.* 39 (08), 1623. doi:10.3969/j.issn.1000-484X.2023.08.011

Rao, Z., Zhu, Y., Yang, P., Chen, Z., Xia, Y., Qiao, C., et al. (2022). Pyroptosis in inflammatory diseases and cancer. *Theranostics* 12, 4310–4329. doi:10.7150/thno.71086

Riazi, K., Azhari, H., Charette, J. H., Underwood, F. E., King, J. A., Afshar, E. E., et al. (2022). The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet. Gastroenterol. Hepatol.* 7 (9), 851–861. doi:10.1016/S2468-1253(22)00165-0

Shaker, M. E., Eisa, N. H., Elgaml, A., El-Mesery, A., El-Shafey, M., El-Dosoky, M., et al. (2021). Ingestion of mannose ameliorates thioacetamide-induced intrahepatic oxidative stress, inflammation and fibrosis in rats. *Life. Sci.* 286, 120040. doi:10.1016/j.lfs.2021.120040

Sharma, B. R., and Kanneganti, T. D. (2021). NLRP3 inflammasome in cancer and metabolic diseases. *Nat. Immunol.* 22 (5), 550–559. doi:10.1038/s41590-021-00886-5

Song, S. J., Lai, J. C. T., Wong, G. L. H., Wong, V. W. S., and Yip, T. C. F. (2024). Can we use old NAFLD data under the new MASLD definition? *J. Hepatol.* 80 (2), e54–e56. doi:10.1016/j.jhep.2023.07.021

Swanson, K. V., Deng, M., and Ting, J. P. (2019). The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat. Rev. Immunol.* 19 (8), 477–489. doi:10.1038/s41577-019-0165-0

Tang, J., He, Z., Zhang, B., Cheng, J., Qiu, W., Chen, X., et al. (2024). Structural properties, bioactivities, structure-activity relationships and bio-applications of polysaccharides from Auricularia auricula: a review. *Int. J. Biol. Macromol.* 280 (Pt 3), 135941. doi:10.1016/j.ijbiomac.2024.135941

Targher, G., Byrne, C. D., and Tilg, H. (2024). MASLD: a systemic metabolic disorder with cardiovascular and malignant complications. *Gut* 73 (4), 691–702. doi:10.1136/gutjnl-2023-330595

Tilg, H., Adolph, T. E., and Moschen, A. R. (2021). Multiple parallel hits hypothesis in nonalcoholic fatty liver disease: revisited after a decade. *J. Hepatol.* 73 (2), 833–842. doi:10.1002/hep.31518

Wang, Y., Chen, C., Chen, J., Sang, T., Peng, H., Lin, X., et al. (2022). Overexpression of NAG-1/GDF15 prevents hepatic steatosis through inhibiting oxidative stress-mediated dsDNA release and AIM2 inflammasome activation. *Redox. Biol.* 52, 102322. doi:10.1016/j.redox.2022.102322

Wang, Y., Han, S., Li, R., Cui, B., Ma, X., Qi, X., et al. (2019). Structural characterization and immunological activity of polysaccharides from the tuber of *Bletilla striata*. *Int. J. Biol. Macromol.* 122, 628–635. doi:10.1016/j.ijbiomac.2018.10.201

Wu, Q., Zhou, M., Chen, Y., Zhu, B., Zhou, F., Ye, X., et al. (2024). *Bletilla striata* polysaccharides protect against ARDS by modulating the NLRP3/caspase1/GSDMD and HMGB1/TLR4 signaling pathways to improve pulmonary alveolar macrophage pyroptosis. *J. Ethnopharmacol.* 319 (Pt 3), 117361. doi:10.1016/j.jep.2023.117361

Xiao, J., Wang, F., Liong, E. C., So, K. F., and Tipoe, G. L. (2018). *Lycium barbarum* polysaccharides improve hepatic injury through NFkappa-B and NLRP3/6 pathways in a methionine choline deficient diet steatohepatitis mouse model. *Int. J. Biol. Macromol.* 120 (Pt B), 1480–1489. doi:10.1016/j.ijbiomac.2018.09.151

Xu, D., Pan, Y., and Chen, J. (2019). Chemical constituents, pharmacologic properties, and clinical applications of *Bletilla striata*. *Front. Pharmacol.* 10, 1168. doi:10.3389/fphar.2019.01168

Yue, S. R., Tan, Y. Y., Zhang, L., Zhang, B. J., Jiang, F. Y., Ji, G., et al. (2022). *Gynostemma pentaphyllum* polysaccharides ameliorate non-alcoholic steatohepatitis in mice associated with gut microbiota and the TLR2/NLRP3 pathway. *Front. Endocrinol. (Lausanne)* 13, 885039. doi:10.3389/fendo.2022.885039

Zhang, J., Wang, S., Zhang, T., Zi, M., Wang, S., and Zhang, Q. (2025). Green tea epigallocatechin gallate attenuate metabolic dysfunction-associated steatotic liver disease by regulation of pyroptosis. *Lipids. Health. Dis.* 24 (1), 180. doi:10.1186/s12944-025-02596-5

Zhang, Q., Zeng, R., Tang, J., Jiang, X., and Zhu, C. (2024). The "crosstalk" between microbiota and metabolomic profile in high-fat-diet-induced obese mice supplemented with *Bletilla striata* polysaccharides and composite polysaccharides. *Int. J. Biol. Macromol.* 262 (Pt 1), 130018. doi:10.1016/j.ijbiomac.2024.130018

Zhu, Z., Liang, T., Dai, G., Zheng, J., Dong, J., Xia, C., et al. (2023). Extraction, structural-activity relationships, bioactivities, and application prospects of *Bletilla striata* polysaccharides as ingredients for functional products: a review. *Int. J. Biol. Macromol.* 245, 125407. doi:10.1016/j.ijbiomac.2023.125407

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Multi-omics reveals total flavones from Abelmoschus manihot (L.) Medik. [Malvaceae] ameliorate MAFLD via PI3K/AKT/mTOR-mediated autophagy

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Introduction: Metabolic-associated fatty liver disease (MAFLD) has emerged as a global health crisis, which is characterized by hepatic lipid accumulation, inflammation, and fibrosis. Currently, effective therapeutic strategies for MAFLD are still scarce.

Methods: This study aimed to explore the hepatoprotective effects and underlying mechanisms of total flavones from Abelmoschus manihot (L.) Medik. (Malvaceae), abbreviated as TFA, in the context of MAFLD. Ultra-high-performance liquid chromatography-quadrupole orbitrap mass spectrometry (UHPLC-QTOF-MS) was used to identify the metabolites in TFA. MAFLD mice induced by a high-fat diet were treated with TFA at doses of 50 and 100 mg/kg. Body weight gain, hepatic lipid accumulation, and serum levels of alanine transaminase (ALT), aspartate transaminase (AST), total cholesterol (TC), and triglycerides (TG) were determined. Histological analysis was performed to evaluate hepatic steatosis, fibrosis, as well as the levels of inflammatory cytokines (IL-6, TNF-α) and antioxidant markers (SOD, GSH). Transcriptomic and metabolomic analyses were carried out to explore the molecular mechanisms. In vitro studies were conducted in HepG2 cells, and the role of autophagy was investigated using the autophagy inhibitor 3-MA.

Results: Using UHPLC-QTOF-MS, 56 metabolites were identified in TFA, including hyperoside, rutin, and quercetin derivatives, which possess anti-lipidemic and anti-inflammatory properties. In MAFLD mice, TFA treatment significantly decreased body weight gain, hepatic lipid accumulation, and the serum levels of ALT, AST, TC, and TG. Histological analysis demonstrated that TFA alleviated hepatic steatosis and fibrosis, with decreased levels of inflammatory cytokines and increased antioxidant markers. Transcriptomic and metabolomic

analyses indicated that TFA regulated nucleotide metabolism, pyrimidine metabolism, and the PI3K/AKT/mTOR signaling pathway. In HepG2 cells, TFA inhibited palmitic acid/oleic acid-induced lipid deposition and the production of reactive oxygen species (ROS). Mechanistically, TFA activated autophagy through the inhibition of PI3K/AKT/mTOR phosphorylation, as demonstrated by the increased LC3II/I conversion and decreased p62 expression. The autophagy inhibitor 3-MA abolished the protective effects of TFA.

Discussion: Our findings suggest that TFA ameliorates MAFLD via promoting PI3K/AKT/mTOR-mediated autophagy. The metabolites identified in TFA might contribute to its multi-target therapeutic effects. Considering the limited treatment options for MAFLD, TFA exhibits great potential as a novel therapeutic agent for MAFLD intervention, thus justifying further preclinical and clinical investigations.

KEYWORDS

total flavonoids from *Abelmoschus manihot* (L.) Medik. [Malvaceae], metabolic associated fatty liver disease, PI3K/AKT/mTOR pathway, autophagy, inflammation, oxidative stress

1 Introduction

Metabolic Associated Fatty Liver Disease (MAFLD) has emerged as a global public health crisis, with a prevalence rate affecting nearly 25% of the global population (Miao et al., 2024; Cotter and Rinella, 2020). Furthermore, epidemiological studies have documented a consistent upward trend in MAFLD incidence rates worldwide (Younossi et al., 2023). MAFLD represents a complex clinicopathological syndrome characterized by excessive hepatic lipid accumulation, independent of alcohol consumption and other established hepatotoxic factors. Without timely intervention, MAFLD progression may culminate in severe complications including cirrhosis and hepatocellular carcinoma (Pais et al., 2016). Importantly, MAFLD has been identified as a significant risk factor for multiple systemic disorders, including type 2 diabetes mellitus, cardiovascular diseases, and chronic kidney disease (Byrne and Targher, 2015). Despite extensive research efforts, there remains an unmet clinical need for effective therapeutic strategies against MAFLD. Compounding this challenge, emerging evidence reveals the multifactorial pathogenesis of MAFLD, presenting significant obstacles for targeted drug development. Notably, natural metabolites have garnered increasing scientific interest for their potential hepatoprotective effects in MAFLD management (Yang et al., 2024; Sun et al., 2023a; Liu et al., 2025).

Flavonoids represent a ubiquitous class of phytochemicals distributed across various plant species, with particularly high concentrations in medicinal botanical drugs. Structural analysis reveals significant heterogeneity in flavonoid metabolites derived from distinct botanical sources. The structural complexity of flavonoids poses significant challenges in isolating individual metabolites with high purity. Consequently, researchers typically work with total flavonoid extracts containing multiple metabolites in varying proportions. Natural flavonoids demonstrate significant hepatoprotective efficacy, making them promising candidates for managing metabolic disorders. The hepatoprotective mechanisms primarily involve modulation of hepatic lipid metabolism through enhanced hyperlipolysis and fibrotic lipolysis, thereby mitigating oxidative stress-induced hepatocyte damage (Li et al., 2021; Wang et al., 2021; Fan et al., 2023a). Furthermore, flavonoids exhibit pleiotropic effects including anti-inflammatory, antioxidant, anti-apoptotic, and immunomodulatory properties (Lv et al., 2023; Ku et al., 2020). Extensive preclinical studies have consistently validated the hepatoprotective efficacy of flavonoid metabolites. However, the precise molecular mechanisms underlying flavonoid-mediated hepatoprotection remain incompletely characterized, warranting further investigation.

Autophagy represents an essential cellular catabolic process that critically regulates cellular homeostasis and functional integrity. Experimental evidence consistently demonstrates that impaired hepatocyte autophagy leads to marked intracellular lipid accumulation, independent of nutritional status (Shen et al., 2023; Gao et al., 2020). Conversely, chronic high-fat diet exposure induces progressive suppression of hepatic autophagy, culminating in hepatocyte injury and pathological steatosis. The PI3K/AKT/mTOR signaling axis has emerged as a central regulator of autophagic flux, attracting substantial research focus. mTOR inhibition triggers GSK-3β-mediated phosphorylation and activation of ULK1, initiating autophagosome formation. Pharmacological inhibition of PI3K/AKT/mTOR phosphorylation potently induces autophagic activity (Liu et al., 2020a; Sun et al., 2023b). This cascade consequently alleviates hepatocyte lipid overload and inflammatory signaling, thereby mitigating MAFLD pathogenesis.

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; α-SMA, α-smooth muscle actin; Col-1, Type I collagen; ELISA, Enzyme Linked Immunosorbent Assay; FBS, Fetal bovine serum; GO, Gene Ontology; HDL-C, High-density lipoprotein cholesterol; HE, Hematoxylin and eosin; HFD, High-fat diet; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC-MS, Liquid chromatography-mass spectrometry; LDL-C, Low-density lipoprotein cholesterol; MAFLD, Metabolic Associated Fatty Liver Disease; NCD, Normal chow diet; OA, leic acid; ORO, Oil Red O; PA, Palmitic acid; PBS, Phosphate-buffered saline; RT-qPCR, Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction; TC, Total cholesterol; TFA-H, Total flavonoids from Abelmoschus manihot high-dose; TFA-L, Total flavonoids from Abelmoschus manihot low-dose; TG, Triglycerides; UHPLC-Q-Orbitrap HRMS, Ultra-high-performance liquid chromatography-quadrupole orbitrap high-resolution mass spectrometry; WB, Western blot.

Abelmoschus manihot (L.) Medik. [Malvaceae] a pharmacologically active botanical drug in traditional Chinese medicine, has been widely utilized for its anti-inflammatory properties in clinical practice. Phytochemical analysis has identified total flavones of *A. manihot (L.) Medik.* [Malvaceae] (TFA) as the principal bioactive metabolites responsible for its therapeutic effects (Xue et al., 2023). Although TFA has demonstrated therapeutic potential in various metabolic and inflammatory disorders, its specific role in MAFLD remains unexplored (Diao et al., 2023; Tao et al., 2024; Zhou et al., 2022). Given the rising global prevalence of MAFLD and the limited efficacy of current treatment options, investigating TFA's effects on this condition could offer novel mechanistic insights and therapeutic opportunities. This investigation commenced with comprehensive phytochemical profiling to characterize the major metabolites of TFA. Through integrated *in vivo* and *in vitro* experimental approaches, we elucidated the hepatoprotective mechanism of TFA through PI3K/AKT/mTOR-mediated autophagic regulation in MAFLD. These findings establish TFA as a promising therapeutic candidate with translational potential for MAFLD intervention.

2 Materials and methods

2.1 Sample preparation

Abelmoschus manihot (L.) Medik. [Malvaceae] was collected from Wuxi County, Chongqing, China. The extraction of TFA from its flowers was conducted by the Department of Pharmacy at Chongqing University, China. The extraction of TFA was conducted through an optimized protocol combining high-speed homogenization with ultrasound-assisted extraction, a dual-phase approach that maximizes flavonoid yield while maintaining structural integrity of bioactive metabolites. The crude extract was subsequently purified using AB-8 macroporous adsorption resin, a weakly polar resin known for its efficacy in removing impurities and enriching target metabolites. The purification process consisted of adsorption and desorption steps to effectively isolate and concentrate the total flavonoids. The flavonoid-enriched solution was then concentrated under reduced pressure and dried to yield a fine, yellowish powder. The resulting powder, representing the purified total flavonoids, was stored at 4°C for subsequent analysis and experimental use. The entire extraction and purification procedure was meticulously optimized to ensure high purity and yield of the total flavonoids, which is crucial for subsequent biological and pharmacological studies.

2.2 TFA metabolite testing

Precisely 20.6 mg of powdered TCM sample was homogenized with 1.00 mL of 50% methanol in a 2 mL polypropylene centrifuge tube, followed by ultrasonication for 30 min. A 500 µL aliquot of the suspension was transferred to a pre-chilled 1.5 mL microcentrifuge tube and centrifuged at 4°C (12,000 rpm) for 10 min using a refrigerated centrifuge. The clarified supernatant (100 µL) was carefully transferred using a calibrated micropipette into a certified LC-MS injection vial with 250 µL glass insert.

Chromatographic separation was performed on a Vanquish UHPLC system (Thermo Fisher Scientific, Inc., Waltham, MA, United States) with ACQUITY UPLC® HSS T3 column (2.1 × 100 mm, 1.7 µm; Waters Corp., MA, United States). The mobile phase comprised (A) 0.1% formic acid and (B) acetonitrile at 0.3 mL/min flow rate, with column oven maintained at 40°C.

Mass spectrometry analysis was conducted on Q Exactive™ HF-X system (Q Exactive, Thermo Fisher Scientific, Inc., Waltham, MA, United States) with heated electrospray ionization (HESI-II) source. Ionization parameters: spray voltage 3.7 kV (positive)/3.5 kV (negative), capillary temp 320°C, sheath gas 30 psi, auxiliary gas 10 psi. High-purity nitrogen (99.999%) was used as sheath/auxiliary gas and collision gas (1.5 mTorr). Data acquisition was performed in "Full scan/dd-MS2" mode. Full scan parameters: resolution 7,000, AGC target 1×10^6, max injection time 50 m. dd-MS2 parameters: resolution 17,500, AGC 1×10^5, isolation window 2 m/z, stepped NCE 10/30/60 V, threshold 1×10^5.

2.3 Animals and treatment

Male C57BL/6J mice (6–8weeks, 18–22g) were obtained from Jiangsu Huachuang Xinnuo Pharmaceutical Technology Co., Ltd. (Jiangsu, China). Following a 7-day acclimatization with standard chow in specific pathogen-free (SPF) conditions, mice were randomly divided into four groups (n = 8/group): Control, Model, TFA-L (50 mg/kg), and TFA-H (100 mg/kg). Control mice received normal chow diet (NCD), while other groups were fed high-fat diet (HFD; 60% fat, D12492) for 16 weeks. Control and Model groups received vehicle, whereas TFA-L and TFA-H groups were administered TFA via daily oral gavage. The study protocol was approved by the Institutional Animal Care and Use Committee of Chongqing University Three Gorges Hospital (IACUC No. SXYYDW 2024-088).

2.4 Weight and biochemical analysis

Weekly body weight measurements were recorded using an electronic balanceby investigators blinded to group assignments. Following 12-h fasting (water *ad libitum*), mice were anesthetized with 2% isoflurane for terminal blood collection via cardiac puncture and liver tissue harvesting, which were immediately snap-frozen in liquid nitrogen. Serum biochemical parameters including TC (A111-2-1), TG (A110-1-1), LDL-C (A113-2-1), ALT (C009-2-1), and AST (C010-2-1) were quantified using commercial kits (Nanjing Jiancheng Bioengineering Institute) according to manufacturer's protocols.

2.5 Cell culture and treatment

HepG2 cells were maintained in DMEM (C11995500BT, Gibco) supplemented with 10% FBS (FSP500, ExCell Bio) and 1% penicillin-streptomycin (15,140,122, Gibco) at 37°C in a humidified 5% CO_2 incubator. For *in vitro* MAFLD modeling, cells were exposed to 0.25 mM palmitic acid (PA) and 0.5 mM oleic acid (OA) (KT004, Kunchuang) for 24 h (Wu et al., 2024). TFA

stock solution (100 mg/mL in DMEM) was prepared and stored at 4°C. Following MAFLD induction, cells were co-treated with TFA and PA/OA mixture for 24 h to assess therapeutic effects.

2.6 TFA cytotoxicity analysis in HepG2 cells

Cytotoxicity assessment was performed using Cell Counting Kit-8 (CCK-8, Dojindo Laboratories) according to the manufacturer's protocol. HepG2 cells were seeded at 3×10^3 cells/well in 96-well plates and cultured for 24 h to achieve 70%–80% confluence. Cells were treated with TFA (0–16 mg/mL) in serum-free medium for 24 h. Following treatment, 10 μL CCK-8 reagent was added per well and plates were incubated at 37°C, 5% CO_2 for 4 h. Absorbance was measured at 450 nm using a microplate reader.

2.7 Hematoxylin and eosin (H&E) staining

Mice liver tissues were fixed in 4% paraformaldehyde and then embedded in paraffin. Paraffin-embedded tissues were sectioned at 5 μm thickness using a rotary microtome (Leica RM2235) for hematoxylin-eosin (H&E) staining. Tissue sections were deparaffinized in xylene (2 × 5 min) and rehydrated through graded ethanol series (100%, 95%, 80%, 70%, each for 2 min). Sections were stained with Mayer's hematoxylin (5 min) followed by eosin Y (2 min) with intermediate washes in distilled water. Stained sections were dehydrated through ascending ethanol series (70%, 80%, 95%, 100%, each for 2 min) and cleared in xylene (2 × 5 min). Finally, the sections were mounted with neutral balsam and observed under a microscope for histological analysis.

2.8 RNA-sequencing analysis

Whole transcriptomics sequencing was performed using RNA sequencing technology. Total RNA was isolated from liver tissue using TRIzol Reagent (Thermo Fisher) followed by quality assessment with NanoDrop One (A260/A280 = 1.8–2.2). Sequencing libraries were prepared from 2 μg total RNA using NEBNext® Ultra II reagents, with 200–500 bp inserts selected by 0.6×AMPure XP bead purification prior to Illumina HiSeq X Ten sequencing. Raw reads were processed through HISAT2 alignment and featurecounts quantification. Differentially expressed genes were identified with $|\log2FC| \geq 0.25$ and FDR-adjusted $p < 0.05$ using DESeq2.

2.9 Liver untargeted metabolomics analysis

Liver samples underwent preprocessing through sequential metabolite extraction and purification, ensuring stability and representativeness of biological specimens. High-resolution liquid chromatography-mass spectrometry (LC-MS) was subsequently implemented to systematically characterize the metabolic profiles. Data-dependent acquisition (DDA) was employed to capture metabolite-specific fragmentation patterns, utilizing m/z differentials to generate paired primary and secondary mass spectra. Multivariate chemometric analyses—including principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA)—were conducted for data processing, quality control validation, and statistical evaluation. This analytical framework enabled identification of group-specific metabolite variations, pathway enrichment analysis (KEGG database), and mechanistic exploration of hepatic pathophysiology.

2.10 Oil Red O staining

HepG2 cells from experimental groups were sequentially washed thrice with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde at room temperature for 15 min. Fixed cells were subsequently incubated with freshly prepared Oil Red O solution for 30 min at room temperature to visualize lipid accumulation. Sequential graded washes were performed using 60% isopropanol (3 × 5 min) followed by PBS hydration (3 × 5 min) to remove unbound dye. For microscopic analysis, adherent cells were imaged using an optical microscope Leica DMi8. Lipid accumulation was quantified by threshold-based area segmentation in ImageJ with normalization to total cellular area, employing blinded analysis protocol.

2.11 ROS detection

HepG2 cells were seeded at 5×10^5 cells/well in 2 mL complete DMEM using 6-well culture plates and allowed to adhere for 12 h under standard conditions (37°C, 5% CO_2). Cells were exposed to predetermined interventions for 24 h. Cellular ROS levels were detected by loading 5 μM dihydroethidium (DHE) probe (diluted in serum-free medium) followed by 30 min incubation in darkness (CA1420, Solarbio). Post-staining, cells were subjected to three PBS washes (3 × 5 min) prior to fluorescence imaging using an Thermo M5000.

2.12 Determination of SOD and GSH

Cellular lysates were prepared by homogenization in ice followed by centrifugation (12,000 g, 10 min, 4°C) with subsequent collection of supernatant aliquots (200 μL) avoiding pellet contamination. Superoxide dismutase (SOD) activity and glutathione (GSH) levels were quantified using commercial assay kits (BC5165 and BC1175, respectively; Solarbio) following the manufacturer's protocols, with all measurements performed in triplicate.

2.13 Cellular autophagy vesicle assay

HepG2 cells in the logarithmic growth phase were plated in 6-well plates at a density of 3×10^5 cells/well and maintained for 24 h. Following 24 h treatment with various TFA concentrations, autophagy was evaluated using the Autophagy Staining Assay Kit (Beyotime, C3018S). The kit's fluorescent probe,

monodansylcadaverine (MDC), facilitated efficient autophagy detection. Fluorescence microscopy images were acquired and analyzed quantitatively for fluorescence intensity using ImageJ.

2.14 Enzyme linked immunosorbent assay (ELISA)

Cytokine quantification was performed using commercially available ELISA kits (MultiSciences Biotech Co.) for interleukin-6 (IL-6, Cat# EK206) and tumor necrosis factor-α (TNF-α, Cat# EK282), following manufacturer-recommended protocols. Briefly, 50 μL of appropriately diluted samples were dispensed into pre-coated 96-well microplates in triplicate. Following sample addition, 100 μL of horseradish peroxidase (HRP)-conjugated detection antibody was incubated with the samples at 37°C for 60 min in a humidified chamber. Post-substrate incubation, absorbance was measured at 450 nm using a SpectraMax i3x.

2.15 Real-time quantitative PCR analysis

Total RNA was isolated from mouse liver tissue and HepG2 cells using Trizol reagent (Cat. No. 15596026, Invitrogen) according to the manufacturer's instructions. Genomic DNA was eliminated from the RNA samples using DNase I, followed by reverse transcription to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Vazyme, R323-01). Relative mRNA expression levels were quantified using the QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, United States) with PowerUp SYBR Green Master Mix (Vazyme, Q711-02). Custom-designed PCR primers (Shanghai Sangon Biotech Co., Ltd.) were used for qPCR, and their sequences are provided in Supplementary Table S1.

2.16 Western blot

Protein lysates were prepared from liver tissue and HepG2 cells using ice-cold RIPA buffer (Beyotime, P0013B) supplemented with protease inhibitor cocktail (Solarbio, 329–98-6) and phosphatase inhibitors (Selleck, B15001), with protein concentration determined by BCA assay (Beyotime, P0010). Equal protein aliquots (30 μg/lane) were resolved on 10% SDS-PAGE gels and electrotransferred to PVDF membranes (0.45 μm, IPVH00010, Millipore). Membranes were blocked, followed by overnight incubation with primary antibodies (4°C) and 1 h incubation with HRP-conjugated secondary antibodies, with detection using ECL substrate (BL520A, Biosharp) on a Bio-Rad ChemiDoc. Protein expression levels were normalized to GAPDH (internal control) using ImageJ for densitometric analysis. Primary antibodies were obtained as follows: PI3K (AF6241), p-PI3K (AF3241), AKT (AF6261), p-AKT (AF0016), LC-3B (AF4650), p-mTOR (AF3308), and p62 (AF5384) from Affinity Biosciences; IL-6 (BS6419) and TNF-α (BS 1857) from Bioworld Technology. Antibody dilutions were optimized as: goat anti-mouse IgG-HRP (1:2000), goat anti-rabbit IgG-HRP (1:2000), PI3K (1:1000), p-PI3K (1:1000), AKT (1:1000), p-AKT (1:1000), LC-3B (1:1000), p-mTOR (1:1000), p62 (1:1000), IL-6 (1:1000), and TNF-α (1:1000) in blocking buffer.

2.17 Data analysis

The results from each group are presented as mean ± standard deviation. Differences between two groups were analyzed using the t-test, while differences among multiple groups were assessed using one-way analysis of variance (ANOVA). Statistical analyses were performed using SPSS 22.0, with a significance level set at $P < 0.05$.

3 Results

3.1 Determination of the effective metabolites of TFA by UHPLC-QTOF-MS

In this study, the TFA was extracted using high-speed homogenisation-ultrasonic-assisted liquid extraction and purified by removing impurities with AB-8 weakly polar macroporous adsorbent resin. The separated and enriched TFA concentrate was subsequently dried to obtain a brownish-yellow powder. As depicted in Figure 1; Supplementary Table S2, this powder was analyzed by ultra-high-performance liquid chromatography-quadrupole orbitrap high-resolution mass spectrometry (UHPLC-Q-Orbitrap HRMS). A total of 56 metabolites were detected and classified into flavonoids, coumarins and their derivatives, organooxygen compounds, prenol lipids, cinnamic acids and their derivatives, benzene and substituted derivatives. The main metabolites included hyperoside, rutin, isoquercitrin, quercetin-3-O-β-D-lucopyranoside, and myricetin 3-O-glucoside.

3.2 TFA improves body weight, lipid, and glucose metabolism in HFD-induced MAFLD mice

To evaluate the impact of TFA on MAFLD, HFD-induced MAFLD mice were used in the experiment. The results showed significant differences in the body weight gain curves among the four groups. By the fifth week, the body weight of mice fed with HFD was significantly higher than that of mice fed with NCD. However, the weight of the HFD + TFA-L group was significantly reduced after 10 weeks of treatment (Figure 2A). At the same time, the liver mass index was significantly reduced by the TFA-L and TFA-H intervention (Figure 2B). To assess the efficacy of TFA-L and TFA-H in treating HFD-induced liver injury, the levels of serum ALT and AST were measured. The results revealed that the levels of serum ALT and AST were remarkably reduced after TFA administration (Figures 2C,D).

To assess the effect of TFA on lipid and glucose metabolism in MAFLD mice, the lipid and glucose levels in the serum and liver of the mice were measured. Intraperitoneal insulin

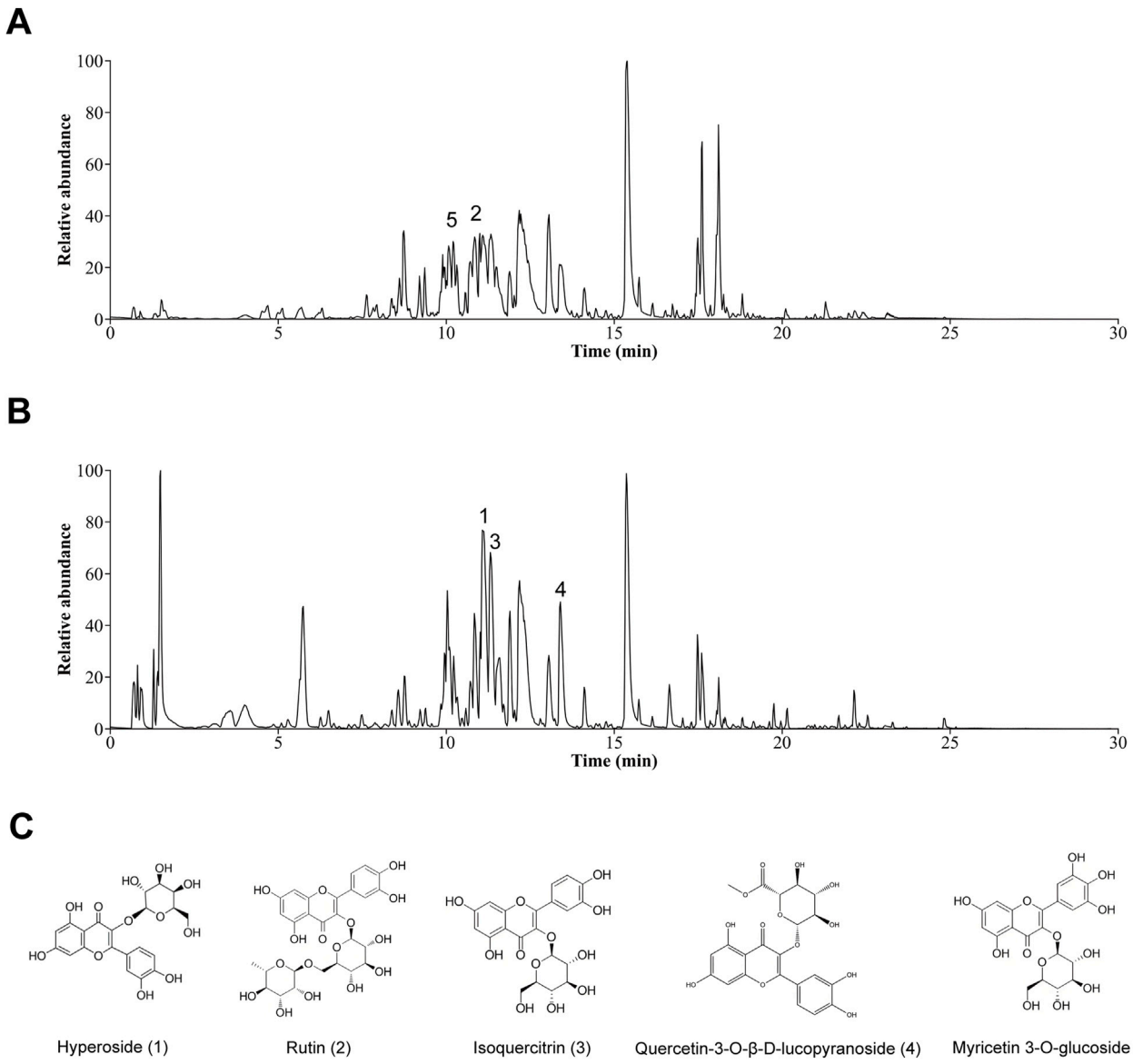

FIGURE 1
Graphs of TFA. **(A)** Plot of the basal peak of positive ion mode. **(B)** Plot of the basal peak of negative ion mode. **(C)** Structures of the five main chemical metabolites in TFA.

tolerance test and intraperitoneal glucose tolerance test were employed to determine the effect of TFA on stabilizing insulin homeostasis. The results demonstrated that TFA-L and TFA-H treatment could improve insulin resistance in MAFLD mice (Figures 2E–G). Oil Red O staining of liver tissue revealed that both TFA-L and TFA-H treatment significantly reduced hepatic lipid accumulation in HFD-fed mice (Figures 2H,I). In addition, the results indicated that HFD feeding significantly increased the levels of TC and TG in the serum and liver, while TFA-L and TFA-H treatment significantly decreased the elevated TC and TG levels (Figures 2J,K). After 16 weeks of HFD treatment, the level of LDL-C in the serum was promoted to increase (Figure 2L). In summary, these results suggest that TFA improve systemic lipid and glucose metabolism in MAFLD mice in a dose-dependent manner.

3.3 TFA alleviates liver injury in MAFLD mice

The liver injury in MAFLD is characterized by inflammation and fibrosis. To further evaluate the influence of TFA on hepatic steatosis and lipid accumulation in MAFLD mice, HE staining was utilized to evaluate the degree of steatosis within the liver tissue. The results indicated that following TFA-L and TFA-H treatment, the liver injury and hepatic steatosis in MAFLD mice were alleviated. Masson staining demonstrated the fibrosis of the liver tissue around the hepatic sinusoids in mice. After TFA-L and TFA-H treatment, the infiltration of inflammatory cells was reduced, and the liver fibrosis was ameliorated and was dose dependent (Figure 3A).

Additionally, immunohistochemical analysis demonstrated that TFA-L and TFA-H significantly decreased the expression of

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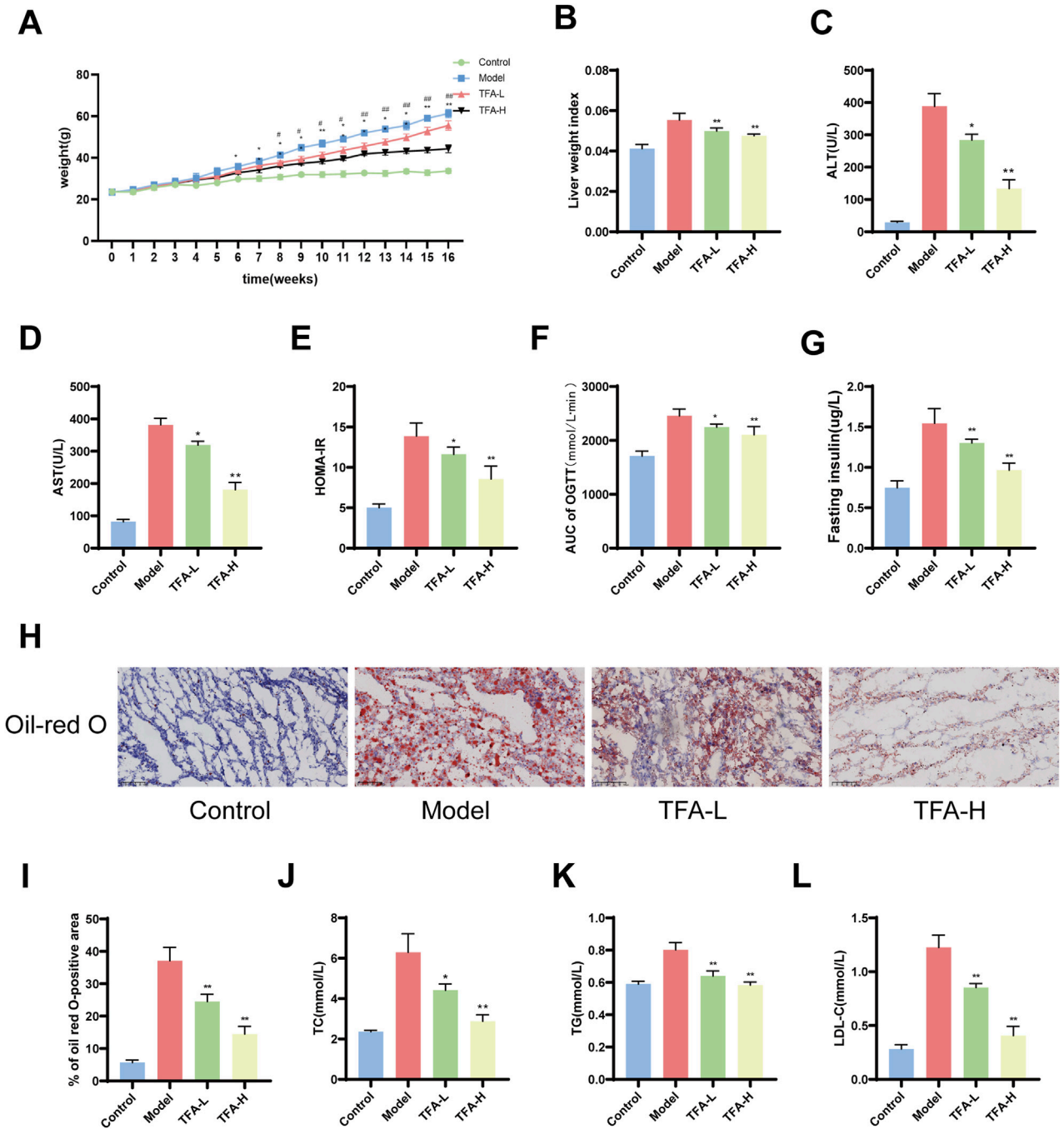

FIGURE 2
Effects of TFA on blood glucose and lipid levels in MAFLD mice. **(A)** Weekly body weight changes. **(B)** Liver weight index. **(C)** ALT levels. **(D)** AST levels. **(E)** Insulin resistance index. **(F)** Area under the curve for oral glucose tolerance test. **(G)** Fasting insulin levels. **(H)** Oil Red O staining of liver. **(I)** Percentage of areas stained positive for Oil Red O. **(J)** TC levels. **(K)** TG levels. **(L)** LDL-C levels. *$P < 0.05$ vs. Model. **$P < 0.01$ vs. Model.

α-smooth muscle actin (α-SMA) and type I collagen (Col-1) in mice liver tissues, indicating its ability to inhibit the progression of hepatic fibrosis (Figures 3B–D). Excessive inflammatory and oxidative stress damage exacerbates the progression of MAFLD, leading to increased hepatocyte injury and potentially triggering more severe liver conditions. Further studies revealed that TFA-L and TFA-H significantly reduced the levels of hepatic inflammatory cytokines, including IL-1β, IL-6, and TNF-α, indicating its potent anti-inflammatory effects (Figures 3E–G). Concurrently, TFA-L and TFA-H treatment markedly elevated the levels of antioxidant stress markers, SOD and GSH, suggesting that TFA alleviates oxidative stress damage by enhancing antioxidant capacity (Figures 3H,I).

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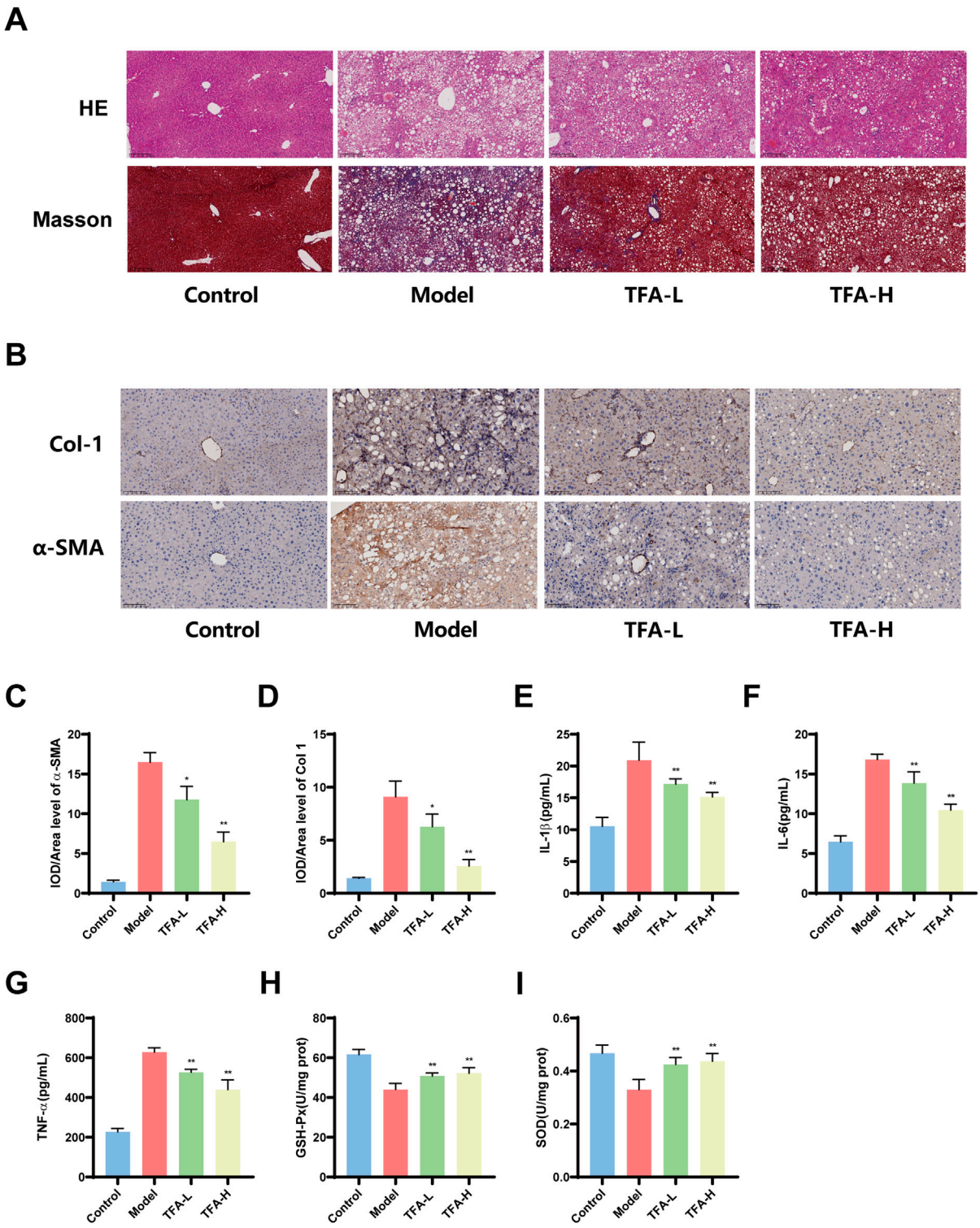

FIGURE 3
Effects of TFA on hepatic pathological injury, inflammatory levels, oxidative stress and fibrosis in MAFLD mice. **(A)** Representative images of HE and Masson staining of mice liver tissues. **(B)** IHC analysis of α-SMA and Col1 expression in mice liver tissues. **(C)** Quantification of α-SMA-positive area in liver tissues by IHC. **(D)** Quantification of Col1-positive area in liver tissues by IHC. **(E)** Hepatic IL-1β levels in mice. **(F)** Hepatic IL-6 levels in mice. **(G)** Hepatic TNF-α levels in mice. **(H)** Hepatic GSH-Px levels in mice. **(I)** Hepatic SOD levels in mice. $^{*}P < 0.05$ vs. Model. $^{**}P < 0.01$ vs. Model.

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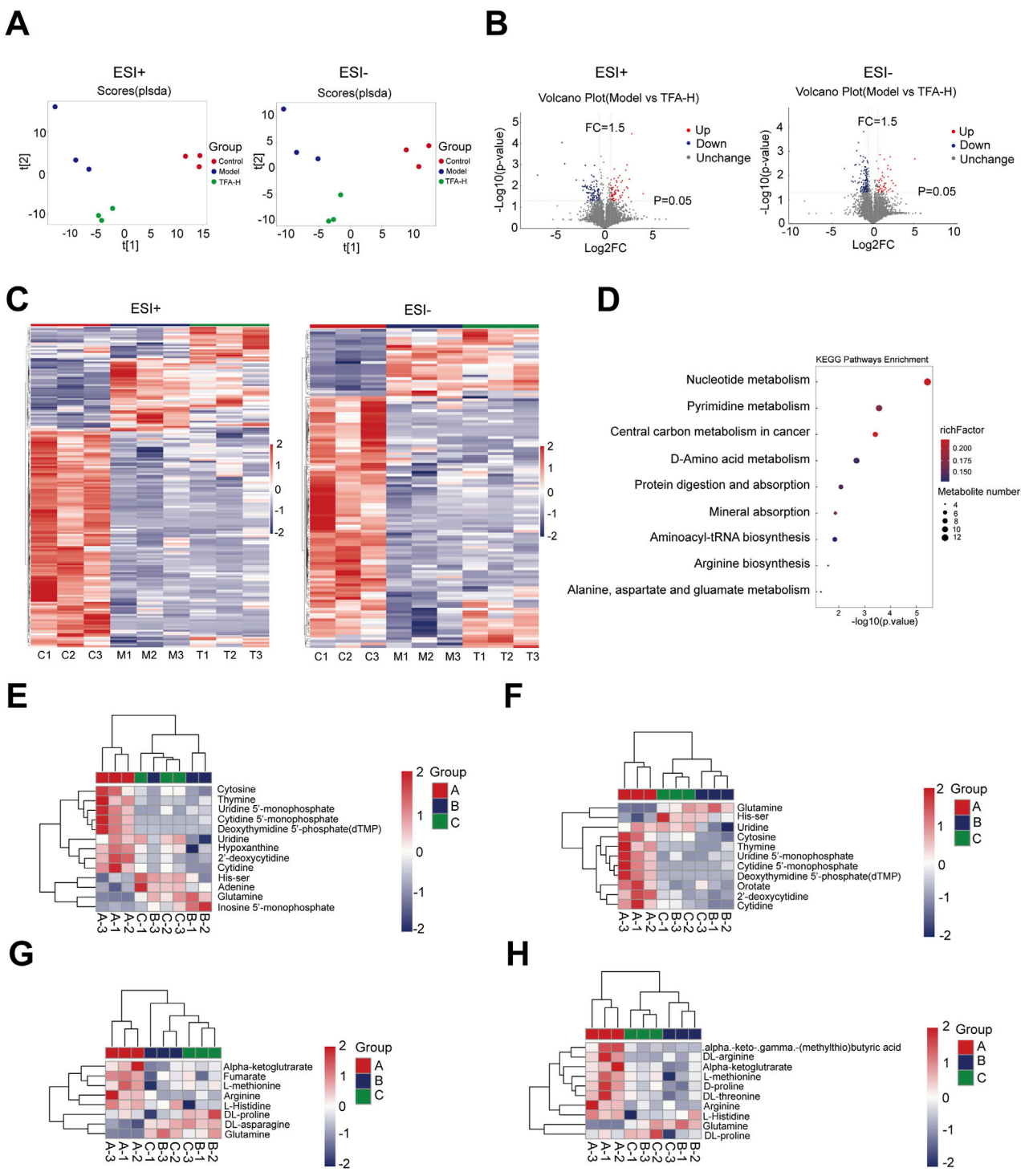

FIGURE 4
Analysis of differential metabolites of MAFLD by TFA. **(A)** OPLS-DA analyses with high experimental stability and reliability. **(B)** Differential metabolites between model and TFA-H groups. **(C)** Clustering heatmap of significant differential metabolites in each group. **(D)** KEGG enrichment analysis of differential metabolite metabolic pathways. **(E)** Nucleotide metabolism in each group. **(F)** Pyrimidine metabolism in each group. **(G)** Central carbon metabolism in cancer in each group. **(H)** D-Amino acid metabolism in each group.

3.4 Metabolomic analysis of different metabolites in TFA-treated MAFLD livers

This study employed untargeted metabolomics to analyze the hepatic metabolite profiles of mice in the control, model, and TFA-H-treated groups. OPLS-DA demonstrated high experimental stability and reliability (Figure 4A). Further analysis revealed that, following TFA-H treatment, 172 differential metabolites were identified in negative ion mode between the model and TFA-H groups, with

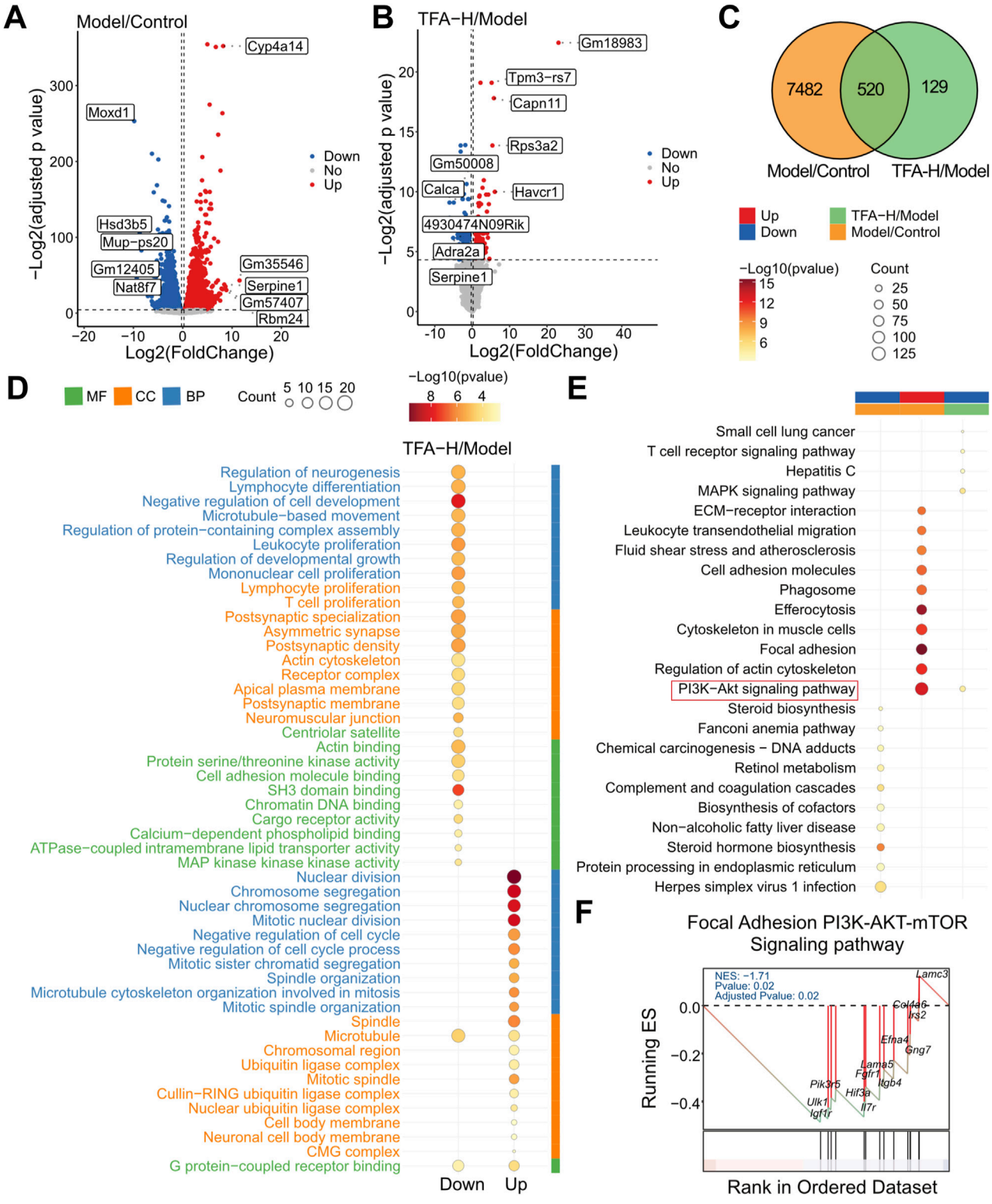

FIGURE 5
Transcriptomic analysis of TFA for MAFLD. **(A)** Differential genes in the normal and model groups. **(B)** Differential genes in the model and TFA-H groups. **(C)** Intersection analysis of differential genes by Wayne plot analysis. **(D)** GO analysis of differential genes in the model and TFA-H groups. **(E)** KEGG analysis of differentially expressed genes between the model and TFA-H groups. **(F)** GSEA analysis.

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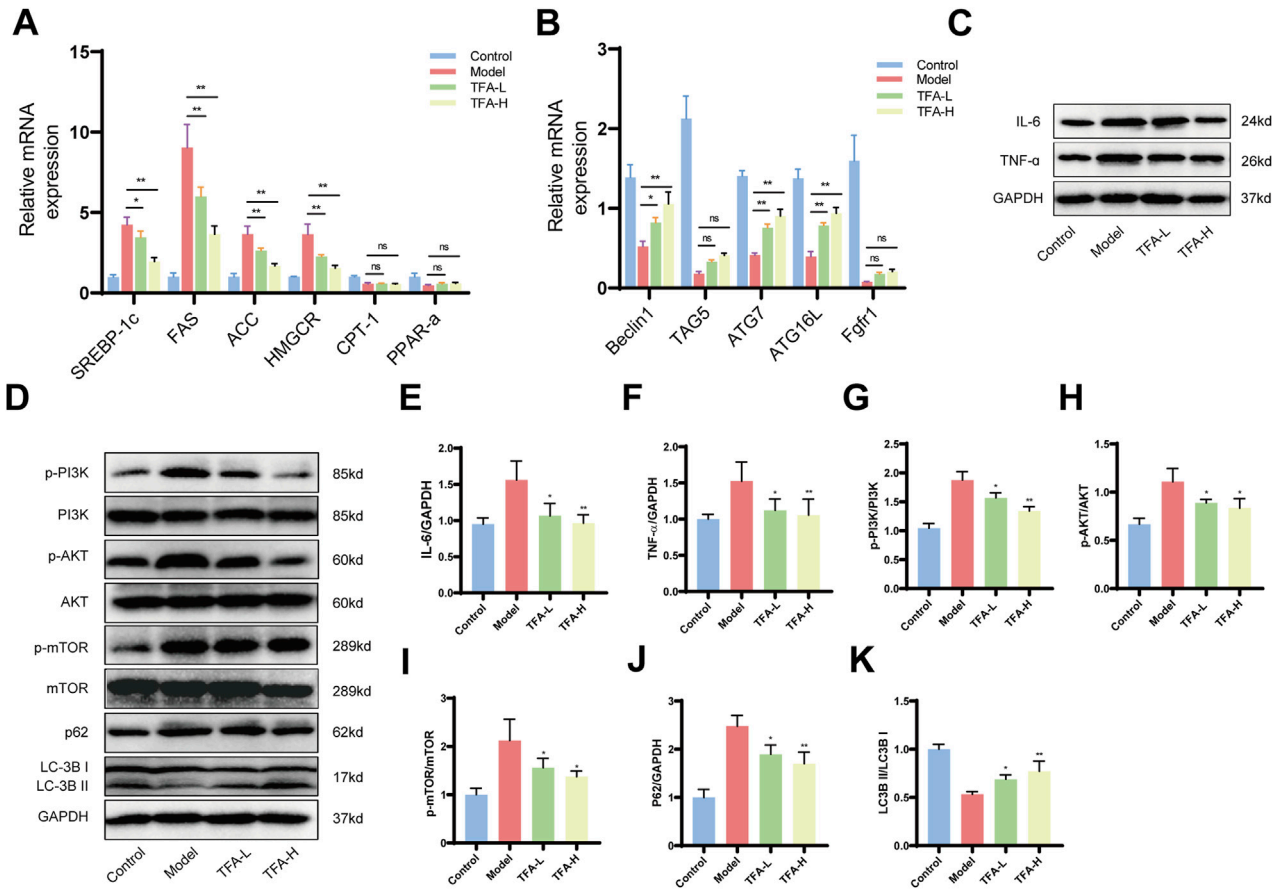

FIGURE 6
Effect of TFA on hepatic PI3K/AKT/mTOR signalling pathway and autophagy in MAFLD mice. **(A)** Effects of TFA on mRNA expression of genes related to lipid metabolism in mice liver. **(B)** Effects of TFA on mRNA expression of genes related to autophagy in mice liver. **(C)** Protein expression of IL-6 and TNF-α in mice liver. **(D)** Protein expression of p-PI3K, PI3K, p-AKT, AKT, p-mTOR, mTOR, LC-3B, and p62. **(E)** Relative protein quantification of IL-6. **(F)** Relative protein quantification of TNF-α. **(G)** Relative protein quantification of p-PI3K/PI3K. **(H)** Relative protein quantification of p-AKT/AKT. **(I)** Relative protein quantification of p-mTOR/mTOR. **(J)** Relative protein quantification of p62. **(K)** Relative protein quantification of LC3BII/I. *$P < 0.05$ vs. Model. **$P < 0.01$ vs. Model.

125 downregulated and 47 upregulated metabolites. In positive ion mode, 173 differential metabolites were identified, including 104 downregulated and 69 upregulated metabolites (Figure 4B). Additional analysis comparing the model and TFA-H groups identified differential metabolites between these two groups (Figure 4C). KEGG enrichment analysis revealed that the differential metabolites primarily affect Nucleotide metabolism, Pyrimidine metabolism, Central carbon metabolism in cancer and D-Amino acid metabolism (Figures 4D–H).

3.5 Transcriptomic analysis of differential genes in TFA-treated MAFLD livers

To further investigate the specific mechanisms by which TFA attenuates MAFLD, we performed transcriptome sequencing analyses to explore the molecular mechanisms. The differential genes among groups are presented in Figures 5A,B. When compared with the control group, 4,320 genes were upregulated and 3,682 genes were downregulated in the Model group. In contrast, relative to the Model group, 264 genes were over-expressed and 385 genes were under-expressed in the TFA-H group. Notably, 520 genes were identified as the common intersections among the three groups (Figure 5C). Subsequently, 649 differentially expressed genes between TFA-H and Model group, 8002 differentially expressed genes between Model and control group underwent Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Additionally, Gene Set Enrichment Analysis (GSEA) was conducted on these genes using MsigDB canonical pathways. As depicted in Figure 5D, the GO analysis revealed significant enrichment in the following molecular functions: G protein-coupled receptor binding, protein serine/threonine kinase activity and cell adhesion molecule binding. Additionally, the KEGG pathway analysis demonstrated significant enrichment in the PI3K/AKT signalling pathway, MAPK signalling pathway and T cell receptor signalling pathway (Figure 5E). Meanwhile, GESA analysis showed significant enrichment in the PI3K/AKT/mTOR signalling pathway (Figure 5F).

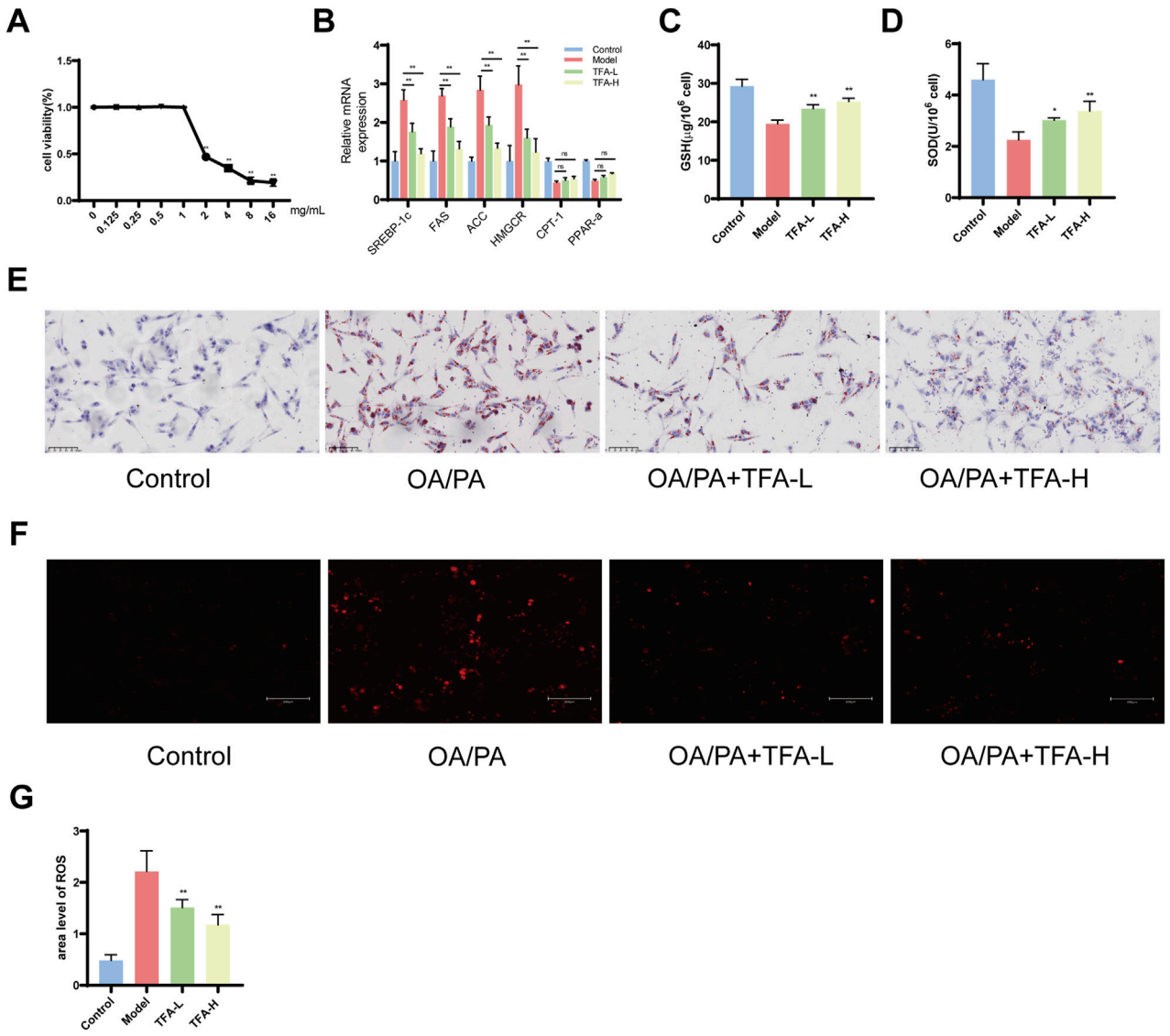

FIGURE 7
Effect of TFA on oxidative stress and inflammation levels in HepG2 cells. **(A)** Cell viability detected by CCK8. **(B)** The mRNA expression of genes related to lipid metabolism in HepG2 cells. **(C)** GSH content of HepG2 cells. **(D)** SOD content of HepG2 cells. **(E)** Oil red O staining. **(F)** ROS fluorescence staining. **(G)** ROS fluorescence area analysis. *$P < 0.05$ vs. Model. **$P < 0.01$ vs. Model.

3.6 TFA activates autophagy by modulating the PI3K/AKT/mTOR signalling pathway in liver tissue after treatment of MAFLD mice

MAFLD is characterized by lipid metabolism disorders, inflammatory responses and progressive fibrosis. To elucidate the effects of TFA on these processes, we examined the expression of genes related to lipid metabolism. TFA-L and TFA-H primarily suppressed the expression of lipid synthesis genes, such as SREBP-1c, FAS, ACC and HMGCR, while genes involved in lipid catabolism, including CPT-1 and PPAR-α, remained unaffected (Figure 6A). To assess whether TFA promotes autophagy, we analyzed autophagy-related genes, including Beclin1, ATG7, and ATG16L, which were significantly upregulated in TFA-L and TFA-H group (Figure 6B).

In addition, TFA-L and TFA-H treatment downregulated hepatic inflammatory cytokine-related proteins, mitigating inflammation in MAFLD (Figures 6C,E,F). In lipid metabolism, The PI3K/AKT/mTOR signaling pathway, closely linked to autophagy, plays a pivotal role in regulating lipid metabolism and inflammation. Further investigation into the PI3K/AKT/mTOR pathway revealed elevated phosphorylation levels of PI3K, AKT, and mTOR in the model group. TFA-L and TFA-H intervention enhanced the LC3I-to-LC3II conversion rate and LC3II expression while reducing the autophagy substrate p62 protein levels (Figures 6D,G–K). These findings collectively demonstrate that TFA activates autophagy in the liver tissues of MAFLD mice, providing mechanistic insights into its therapeutic potential.

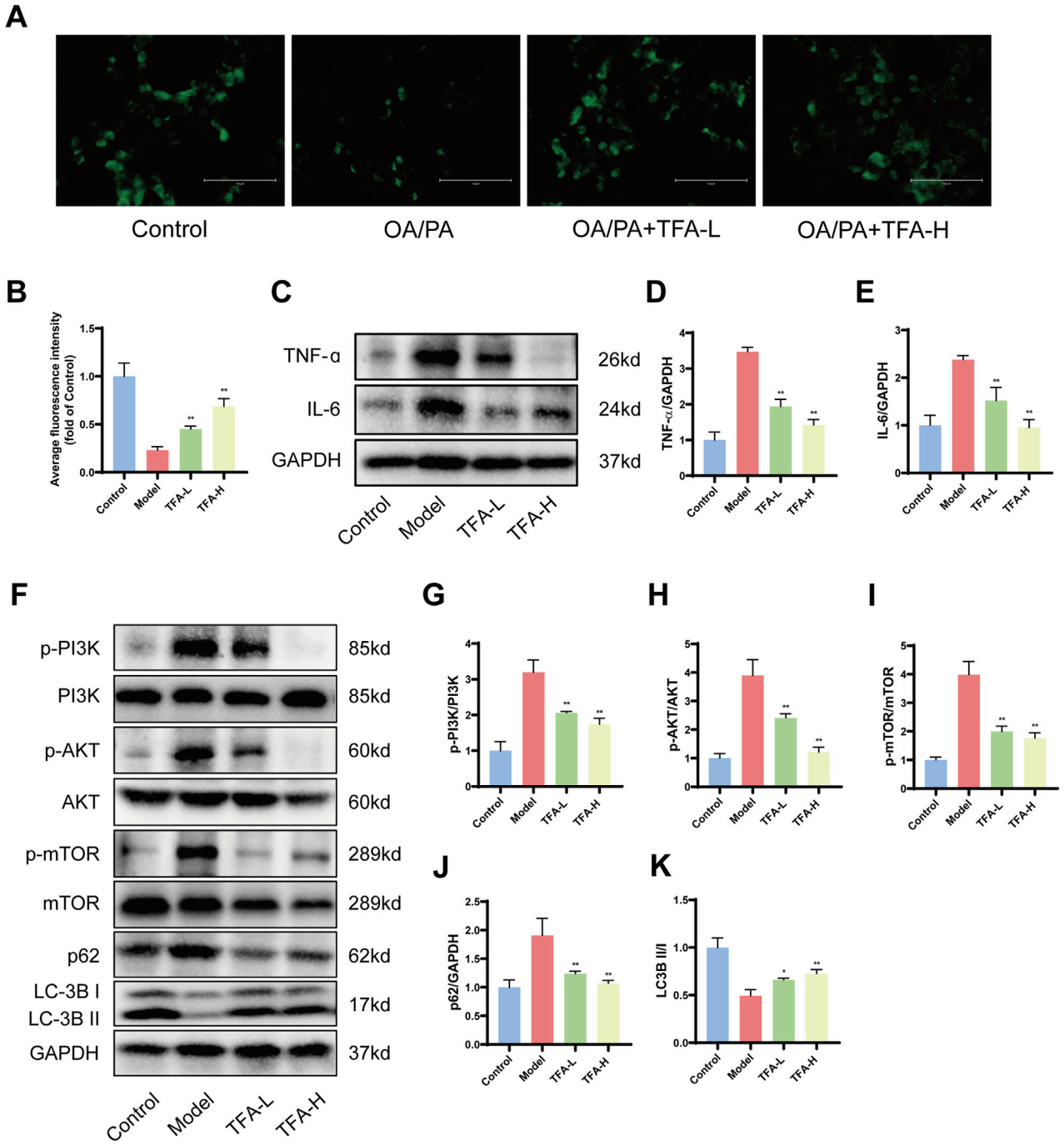

FIGURE 8
Effect of TFA on PI3K/AKT/mTOR signalling pathway and autophagy in HepG2 cells. **(A)** MDC fluorescence staining. **(B)** Average fluorescence intensity of MDC. **(C)** Protein expression of IL-6 and TNF-α in HepG2 cells. **(D)** Relative quantification of TNF-α. **(E)** Relative quantification of IL-6. **(F)** Protein expression of p-PI3K, PI3K, p-AKT, AKT, p-mTOR, mTOR, LC-3B, and p62 protein expression. **(G)** Relative quantification of p-PI3K/PI3K. **(H)** Relative quantification of p-AKT/AKT. **(I)** Relative protein quantification of p-mTOR/mTOR. **(J)** Relative protein quantification of p62. **(K)** Relative protein quantification of LC3BII/I. *$P < 0.05$ vs. Model. **$P < 0.01$ vs. Model.

3.7 Effect of TFA on lipid deposition and inflammatory response in HepG2 cells

Firstly, to ascertain the optimal concentration TFA on HepG2 cells, the CCK8 assay was employed to evaluate the effects of TFA at concentrations of 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/mL. Finally, a concentration of 0.5 mg/L (TFA-L group) and 1 mg/L (TFA-H group) was selected for the 24-h intervention as the subsequent treatment condition (Figure 7A). Subsequently, HepG2 cells were treated with a mixture of oleic acid (OA) and palmitic acid (PA) to establish the MAFLD cell model. To further examine the effects of TFA on lipid metabolism in HepG2 cells, we

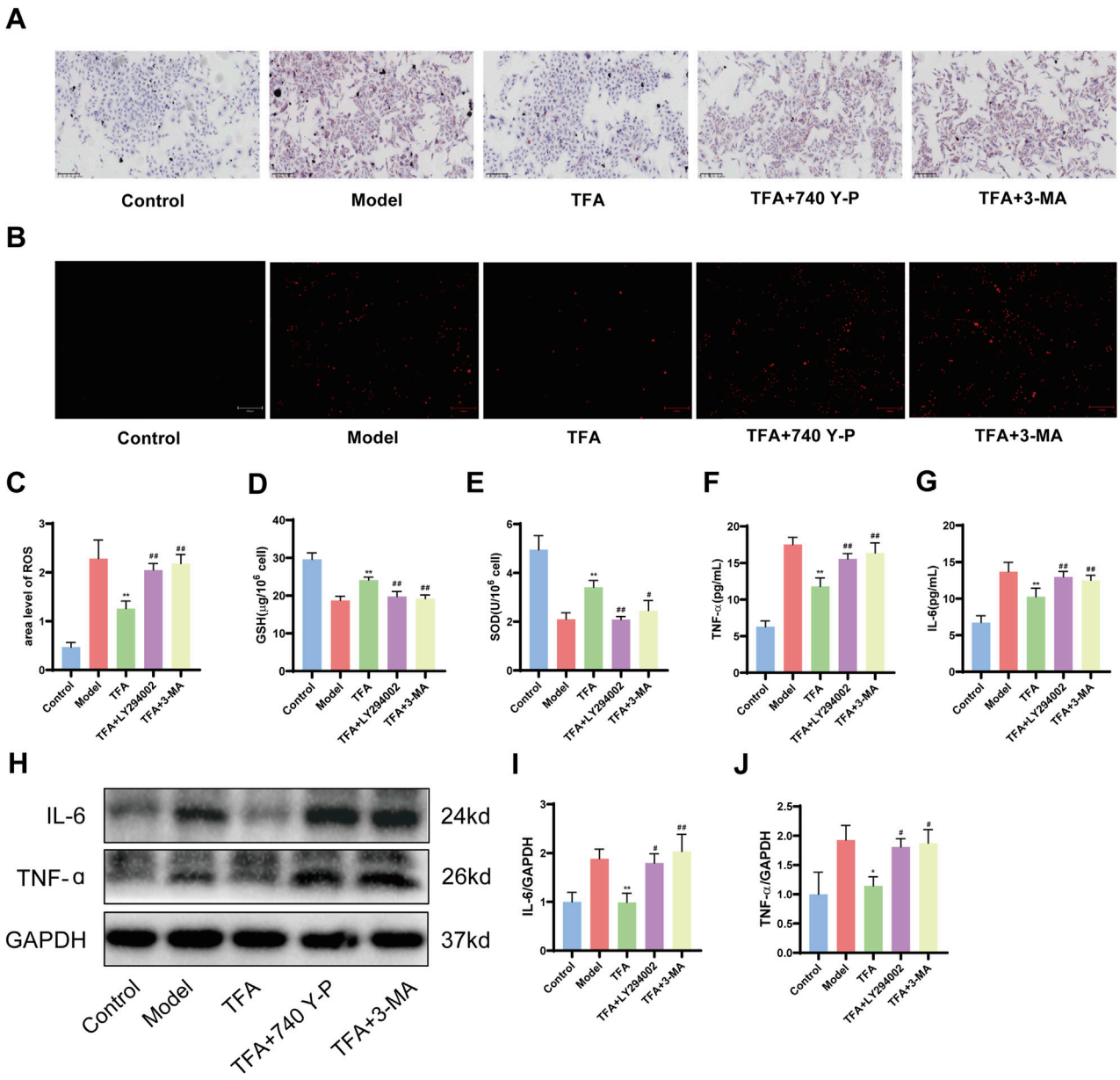

FIGURE 9
Effect of 740 Y-P (PI3K agonist) and 3-MA (autophagy inhibitor) on the protective effect of TFA. **(A)** Oil red O staining. **(B)** ROS fluorescence staining. **(C)** ROS fluorescence area analysis. **(D)** GSH content of HepG2 cells. **(E)** SOD content of HepG2 cells. **(F)** IL-6 level of HepG2 cell supernatants. **(G)** TNF-α level of HepG2 cell supernatants. **(H)** Protein expression of IL-6 and TNF-α in HepG2 cells. **(I)** Relative quantification of IL-6. **(J)** Relative quantification of TNF-α. $^{*}P < 0.05$ vs. Model. $^{**}P < 0.01$ vs. Model.

measured the expression of key lipid metabolism-related genes. The results showed that both TFA-L and TFA-H significantly downregulated key lipid synthesis-related genes, including SREBP-1c, FAS, ACC, and HMGCR. In contrast, TFA treatment had no significant effect on the expression of fatty acid oxidation-related genes (CPT-1 and PPAR-α) (Figure 7B). Meanwhile, TFA-L and TFA-H increased the levels of GSH and SOD in the cells (Figures 7C,D).

The Oil Red O staining demonstrated that after 24 h of treatment, TFA-L and TFA-H significantly mitigated lipid deposition (Figure 7E). Moreover, the ROS staining of HepG2 cells revealed that TFA-L and TFA-H remarkably decreased the levels of reactive oxygen species (Figures 7F,G). Collectively, the above-mentioned results indicated that TFA-L and TFA-H could reduce lipid deposition and oxidative stress damage in HepG2 cells.

3.8 TFA regulates the PI3K/AKT/mTOR signalling pathway to enhance autophagy in HepG2 cells

We investigated the effects of TFA-L and TFA-H on autophagy in HepG2 cells using MDC as a fluorescent probe. With increasing

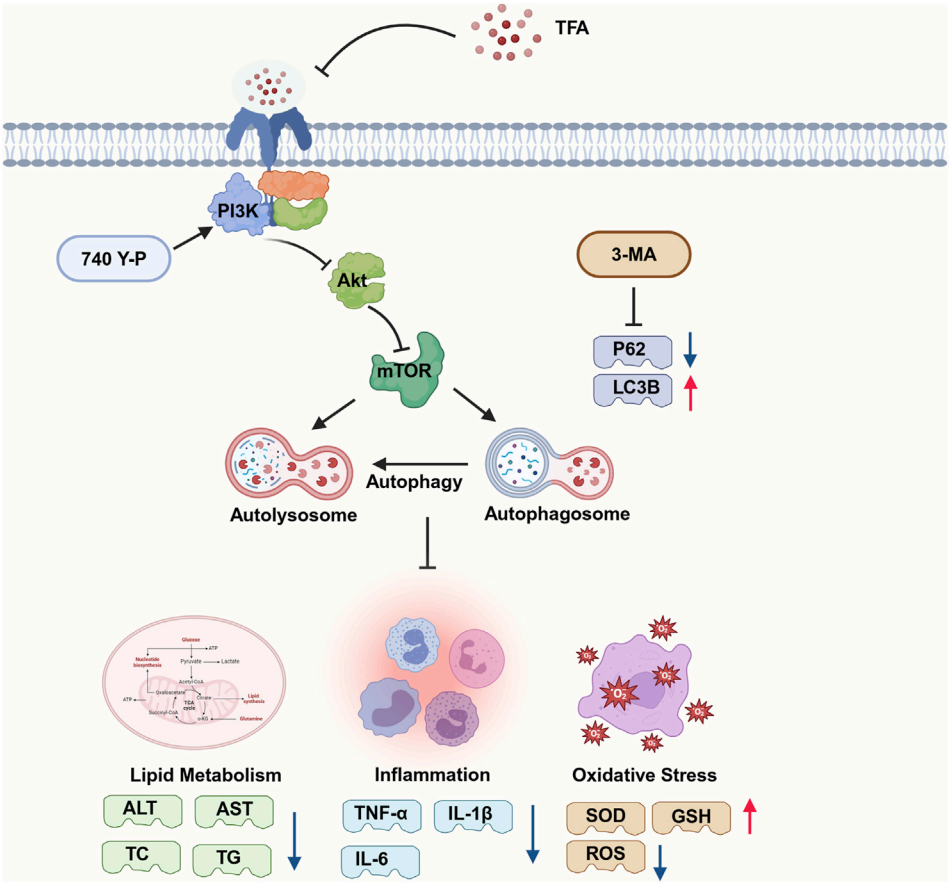

FIGURE 10
Mechanism of action of TFA in the treatment of MAFLD. Part of the picture provided by Biorender. TFA may activate autophagy by inhibiting PI3K/AKT/mTOR signaling pathway, thereby reducing lipid deposition and the release of inflammatory factors and achieving the goal of treating MAFLD. 3-MA inhibition of autophagy can downregulated the levels of SOD and GSH and upregulated the expression levels of IL-1β and IL-6. These results prove that the activation of autophagy is inversely related to the regulation of lipid deposition and inflammatory response.

drug concentrations, we observed a dose-dependent increase in autophagic vesicles compared to the model group (Figure 8A).

Following a 24 h intervention with OA and PA in HepG2 cells, the expression of IL-6, TNF-α, p-PI3K, p-AKT, and p-mTOR proteins was significantly upregulated in the model group. However, after 24 h of TFA treatment, the levels of IL-6, TNF-α, p-PI3K, p-AKT, and p-mTOR proteins were notably decreased. Upon TFA intervention, the expression of LC3II protein and the LC3II/I ratio were enhanced, while the expression of p62 protein was significantly reduced (Figures 8B–K). These findings indicated that the PI3K/AKT/mTOR signaling pathway was implicated in the treatment of MAFLD by TFA.

3.9 Inhibition of autophagy eliminates the protective effect of TFA on HepG2 cells

In order to explore the potential pivotal roles of autophagy and the PI3K/AKT/mTOR signaling pathway in the protective mechanism of TFA against MAFLD, a series of experimental interventions were carried out. OA/PA treated HepG2 cells were further exposed to the autophagy inhibitor 3-MA (5 mM) and the PI3K agonist 740 Y-P (20 μM) either in the presence or absence of TFA (Wang et al., 2024; Yan et al., 2022). As hypothesized, the co-addition of 3-MA and 740 Y-P exerted a significant inhibitory effect on the beneficial actions of TFA. Specifically, this led to a notable elevation in lipid accumulation within HepG2 cells (Figure 9A). Through the systematic assessment of ROS staining and the quantification of GSH and SOD levels, it was clearly demonstrated that the combined treatment with 3-MA and 740 Y-P markedly suppressed the antioxidant effects conferred by TFA (Figures 9B–E). To further analyze changes in inflammatory levels, we measured IL-6 and TNF-α levels in the cell supernatant using ELISA and assessed their protein expression in cells via western blot. The results revealed that the addition of 740 Y-P and 3-MA significantly attenuated the protective effects of TFA (Figures 9F–J).

4 Discussion

The global prevalence of MAFLD continues to rise, yet the development of clinically validated therapeutic interventions

remains an unmet medical need. MAFLD pathogenesis encompasses a multifactorial interplay of molecular mechanisms, including hepatic insulin resistance, impaired lipid homeostasis, mitochondrial oxidative stress, chronic inflammation, and gut-liver axis dysregulation. (Friedman et al., 2018; Xu et al., 2022). The multifactorial nature of MAFLD pathophysiology limits the therapeutic efficacy of single-target pharmacological approaches, necessitating the development of multi-modal intervention strategies. Consequently, there is growing emphasis on the development of pleiotropic therapeutic agents capable of simultaneously modulating multiple pathological pathways in MAFLD management. Phytochemicals have emerged as promising therapeutic candidates for MAFLD, owing to their inherent ability to modulate multiple molecular targets through synergistic mechanisms of action. Preclinical studies have demonstrated that bioactive metabolites, particularly flavonoids (e.g., quercetin), polyphenols (e.g., epigallocatechin gallate), and terpenoids (e.g., ursolic acid), exert hepatoprotective effects through coordinated regulation of lipid homeostasis, antioxidant defense systems, and inflammatory signaling pathways (He et al., 2023; Yu et al., 2024). Specifically, resveratrol has been shown to enhance insulin receptor substrate-1 phosphorylation, while curcumin modulates AMP-activated protein kinase signaling, both demonstrating significant attenuation of hepatic lipid accumulation in preclinical models (Ding et al., 2018; Yan et al., 2018). Elucidating the molecular mechanisms underlying the therapeutic effects of these phytochemicals not only advances our understanding of MAFLD pathophysiology but also informs the rational design of next-generation multi-target therapeutic agents. Future research directions should prioritize the systematic characterization of molecular signaling networks modulated by these metabolites, coupled with rigorous evaluation of their clinical efficacy through well-designed randomized controlled trials, to facilitate the translation of these findings into evidence-based MAFLD therapies.

The advent of ultra-high-performance liquid chromatography coupled with UHPLC-Q-Orbitrap HRMS has transformed the analysis of TCM matrices, achieving sub-ppm mass accuracy and enabling comprehensive metabolite profiling (Liu R. et al., 2020; Liu Z. et al., 2024; Bi et al., 2021). Employing UHPLC-Q-Orbitrap HRMS, we conducted a systematic chemical profiling of TFA, acquiring high-resolution MS/MS spectra in both positive and negative ionization modes to establish its comprehensive phytochemical inventory. A total of 56 metabolites were identified in TFA, primarily comprising flavonoids, coumarins and their derivatives, organic oxygen compounds, isoprenoid lipids, cinnamic acids and their derivatives, as well as benzene and its substituted derivatives. Notably, several metabolites with established efficacy in treating MAFLD were identified, including hyperoside, rutin, isoquercitrin, quercetin-3-O-β-D-glucopyranoside, and myricetin 3-O-glucoside (Wang, Sheng, 2021; Liu Y. et al., 2024; Yi et al., 2023; Jin et al., 2024). These flavonoids were identified as bioactive substances with potent anti-inflammatory and antioxidant properties. Specifically, hyperoside has been shown to ameliorate MAFLD by regulating cholesterol metabolism and bile acid metabolism and excretion (Wang, Sheng, 2021; Jang, 2022). Additionally, rutin can impede the progression of MAFLD by alleviating inflammation and upregulating the expression of genes related to fatty acid oxidation (Liu Y. et al., 2024; Tung et al., 2021). These findings not only elucidate the pharmacological activities of the major metabolites in TFA but also provide a robust theoretical basis for its potential application in the treatment of MAFLD.

The pathological progression of MAFLD is intricately linked to chronic inflammation and progressive fibrosis, driven by the activation of pro-inflammatory cytokines and extracellular matrix deposition (Chen and Zhao, 2024; Dong et al., 2023). Excessive inflammation, characterized by elevated levels of cytokines such as TNF-α and IL-6, and oxidative stress, marked by increased ROS and MDA, significantly contribute to hepatocyte damage and the progression to more severe liver conditions, including non-alcoholic steatohepatitis and cirrhosis (Clare et al., 2022; Qu et al., 2021). Our data demonstrate that TFA treatment significantly reduced hepatic inflammation and fibrosis, while enhancing antioxidant capacity in MAFLD mice. These results underscore the potential of TFA as a multi-target therapeutic agent for MAFLD, effectively addressing key pathological mechanisms, including lipid accumulation, inflammation, and oxidative stress. The multifaceted actions of TFA, targeting multiple pathways, make it a promising candidate for the development of novel, integrative therapies for MAFLD. Excessive lipid accumulation activates pro-inflammatory signaling in hepatocytes and recruits immune cells, thereby amplifying the inflammatory cascade (Wei et al., 2025; Zhao et al., 2024). The anti-inflammatory properties of TFA in hepatocytes may break this vicious cycle, potentially offering therapeutic benefits for metabolic dysfunction-associated steatotic liver disease.

Untargeted metabolomics analysis identified significant perturbations in hepatic metabolic pathways, particularly nucleotide metabolism, pyrimidine metabolism, central carbon metabolism, and D-amino acid metabolism in MAFLD progression. Dysregulation of nucleotide metabolism can lead to an energy imbalance in hepatocytes, impairing liver repair and regenerative capacity, and exacerbating hepatic injury and inflammatory responses (Caddeo and Romeo, 2024). The AKT/mTORC1 signaling pathway promotes pyrimidine and purine synthesis by activating ribonucleotide reductase M2. Furthermore, this pathway modulates one-carbon metabolism (folate cycle) to provide methyl donors required for nucleotide biosynthesis. Disruption of pyrimidine metabolism, further promotes hepatocyte damage and fibrotic progression (Petta et al., 2024). Aberrations in central carbon metabolism, such as increased lactate production and decreased NAD^+/NADH ratios, result in metabolic energy dysfunction, promoting lipid accumulation and oxidative stress, thereby aggravating hepatic inflammation and fibrosis (Patterson et al., 2016). Dysregulation of D-amino acid metabolism, disrupts hepatic metabolic homeostasis, contributing to lipid metabolic disorders and hepatocyte injury (Demirel et al., 2023). Following TFA treatment, the number of differential metabolites was significantly reduced, indicating that TFA partially restored metabolic balance in the livers of MAFLD mice. Improvements in lipid and lipid-like molecule metabolism, including reduced triglyceride content and increased phospholipid levels, suggest that TFA alleviates hepatic steatosis by modulating lipid metabolic pathways. Enrichment analysis of nucleotide and pyrimidine metabolism pathways

demonstrated that TFA mitigates MAFLD-associated hepatocyte injury by restoring pyrimidine metabolism and nucleotide metabolism.

The PI3K/AKT/mTOR axis serves as a central regulator of hepatic autophagy flux, with its dysregulation significantly contributing to MAFLD progression (Fan X. et al., 2023; Huang et al., 2021; Alshehade et al., 2022). Mechanistically, mTORC1 suppressing autophagosome initiation complex assembly (Kim and Guan, 2015). In MAFLD, hyperactivated PI3K/AKT signaling sustains mTORC1 activity, resulting in autophagic flux impairment and hepatic lipotoxicity (Lin et al., 2025; Chen and Lin, 2022; Wang et al., 2022). Activation of the PI3K/AKT signaling pathway enhances mTOR activity, which in turn suppresses autophagy, leading to increased hepatic lipid accumulation, insulin resistance, and inflammation. Conversely, inhibition of this pathway promotes autophagy, facilitating the degradation of lipid droplets and alleviating hepatic steatosis (Zhao et al., 2021). Recent studies have demonstrated that modulating the PI3K/AKT/mTOR axis can restore autophagic flux, improve lipid metabolism, and mitigate liver injury in MAFLD models (Tsuji et al., 2023). Transcriptomic profiling in this study suggests that TFA's therapeutic efficacy in MAFLD may be mediated through modulation of the PI3K/AKT signaling pathway. This was corroborated by *in vivo* and *in vitro* experiments showing that TFA significantly affects critical metabolites of the PI3K/AKT/mTOR pathway, thereby reestablishing autophagic function and alleviating MAFLD-associated pathologies.

Specifically, TFA treatment was shown to enhance the expression of autophagy-related genes, such as Beclin1, ATG7, and ATG16L, while reducing the phosphorylation levels of PI3K, AKT, and mTOR (Sundarraj et al., 2021). These molecular changes were accompanied by a significant increase in the LC3I-to-LC3II conversion ratio and a marked decrease in the levels of the autophagy substrate p62, indicative of enhanced autophagic flux (Kaizuka et al., 2016). By restoring autophagy, TFA effectively reduced lipid accumulation, suppressed inflammatory responses and attenuated fibrosis in MAFLD models. These findings provide robust experimental evidence and detailed molecular insights into the therapeutic potential of TFA for MAFLD, highlighting its multifaceted effects on lipid metabolism, inflammation, and fibrosis. Targeting the PI3K/AKT/mTOR pathway and its associated autophagic processes represents a promising strategy for developing multi-target therapies for MAFLD, addressing the complex and multifactorial nature of the disease. Further preclinical and clinical studies are warranted to explore the long-term efficacy, safety, and clinical applicability of TFA in the treatment of MAFLD.

5 Conclusion

Our study elucidates that TFA exerts its therapeutic effects on MAFLD by targeting the PI3K/AKT/mTOR signaling cascade, which leads to the induction of autophagy, ultimately enhancing lipid homeostasis, mitigating inflammatory responses, and alleviating oxidative damage (Figure 10). The results underscore the potential of TFA as a multi-target therapeutic strategy for MAFLD, offering a rationale for further clinical investigations and drug development efforts.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://doi.org/10.6084/m9.figshare.29436344.v3.

Ethics statement

The animal study was approved by the Medical Ethics Committee of Chongqing University Three Gorges Hospital. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CL: Methodology, Conceptualization, Data curation, Writing – original draft, Resources, Formal Analysis. LZ: Formal Analysis, Writing – original draft, Methodology, Resources. JH: Writing – original draft, Conceptualization, Project administration, Methodology, Formal Analysis, Supervision. HS: Resources, Writing – original draft, Investigation. ZL: Resources, Writing – original draft, Investigation. YW: Investigation, Resources, Writing – original draft. PS: Visualization, Writing – review and editing. YaX: Writing – review and editing, Visualization. YuX: Project administration, Formal Analysis, Conceptualization, Methodology, Writing – review and editing, Supervision. WS: Writing – review and editing, Conceptualization, Methodology, Formal Analysis, Project administration. MY: Formal Analysis, Project administration, Supervision, Writing – review and editing, Conceptualization.

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Conflict of interest

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

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Supplementary material

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References

Alshehade, S., Alshawsh, M. A., Murugaiyah, V., Asif, M., Alshehade, O., Almoustafa, H., et al. (2022). The role of protein kinases as key drivers of metabolic dysfunction-associated fatty liver disease progression: new insights and future directions. *Life Sci.* 305, 120732. doi:10.1016/j.lfs.2022.120732

Bi, Y., Han, X., Lai, Y., Fu, Y., Li, K., Zhang, W., et al. (2021). Systems pharmacological study based on UHPLC-Q-Orbitrap-HRMS, network pharmacology and experimental validation to explore the potential mechanisms of Danggui-Shaoyao-San against atherosclerosis. *J. Ethnopharmacol.* 278, 114278. doi:10.1016/j.jep.2021.114278

Byrne, C. D., and Targher, G. (2015). NAFLD: a multisystem disease. *J. Hepatol.* 62 (1 Suppl. l), S47–S64. doi:10.1016/j.jhep.2014.12.012

Caddeo, A., and Romeo, S. (2024). Precision medicine and nucleotide-based therapeutics to treat steatotic liver disease. *Clin. Mol. Hepatol.* 31, S76–S93. doi:10.3350/cmh.2024.0438

Chen, C. L., and Lin, Y. C. (2022). Autophagy dysregulation in metabolic associated fatty liver disease: a new therapeutic target. *Int. J. Mol. Sci.* 23 (17), 10055. doi:10.3390/ijms231710055

Chen, Y., and Zhao, X. (2024). The mediating role of insulin resistance in the association between inflammatory score and MAFLD: NHANES 2017-2018. *Immun. Inflamm. Dis.* 12 (10), e70035. doi:10.1002/iid3.70035

Clare, K., Dillon, J. F., and Brennan, P. N. (2022). Reactive oxygen species and oxidative stress in the pathogenesis of MAFLD. *J. Clin. Transl. Hepatol.* 10 (5), 939–946. doi:10.14218/JCTH.2022.00067

Cotter, T. G., and Rinella, M. (2020). Nonalcoholic fatty liver disease 2020: the state of the disease. *Gastroenterology* 158 (7), 1851–1864. doi:10.1053/j.gastro.2020.01.052

Demirel, M., Koktasoglu, F., Ozkan, E., Dulun Agac, H., Gul, A. Z., Sharifov, R., et al. (2023). Mass spectrometry-based untargeted metabolomics study of non-obese individuals with non-alcoholic fatty liver disease. *Scand. J. Gastroenterol.* 58 (11), 1344–1350. doi:10.1080/00365521.2023.2225667

Diao, Z., Yu, H., Wu, Y., Sun, Y., Tang, H., Wang, M., et al. (2023). Identification of the main flavonoids of abelmoschus manihot (L.) medik and their metabolites in the treatment of diabetic nephropathy. *Front. Pharmacol.* 14, 1290868. doi:10.3389/fphar.2023.1290868

Ding, L., Zhang, B., Li, J., Yang, L., and Wang, Z. (2018). Beneficial effect of resveratrol on alpha-naphthyl isothiocyanate-induced cholestasis *via* regulation of the FXR pathway. *Mol. Med. Rep.* 17 (1), 1863–1872. doi:10.3892/mmr.2017.8051

Dong, Q., Bao, H., Wang, J., Shi, W., Zou, X., Sheng, J., et al. (2023). Liver fibrosis and MAFLD: the exploration of multi-drug combination therapy strategies. *Front. Med. (Lausanne)* 10, 1120621. doi:10.3389/fmed.2023.1120621

Fan, C., Ling-Hu, A., Sun, D., Gao, W., Zhang, C., Duan, X., et al. (2023a). Nobiletin ameliorates hepatic lipid deposition, oxidative stress, and inflammation by mechanisms that involve the Nrf2/NF-κB axis in nonalcoholic fatty liver disease. *J. Agric. Food Chem.* 71 (50), 20105–20117. doi:10.1021/acs.jafc.3c06498

Fan, X., Jiao, G., Pang, T., Wen, T., He, Z., Han, J., et al. (2023b). Ameliorative effects of mangiferin derivative TPX on insulin resistance *via* PI3K/AKT and AMPK signaling pathways in human HepG2 and HL-7702 hepatocytes. *Phytomedicine* 114, 154740. doi:10.1016/j.phymed.2023.154740

Friedman, S. L., Neuschwander-Tetri, B. A., Rinella, M., and Sanyal, A. J. (2018). Mechanisms of NAFLD development and therapeutic strategies. *Nat. Med.* 24 (7), 908–922. doi:10.1038/s41591-018-0104-9

Gao, Y., Zhang, W., Zeng, L. Q., Bai, H., Li, J., Zhou, J., et al. (2020). Exercise and dietary intervention ameliorate high-fat diet-induced NAFLD and liver aging by inducing lipophagy. *Redox Biol.* 36, 101635. doi:10.1016/j.redox.2020.101635

He, Y., Wang, H., Lin, S., Chen, T., Chang, D., Sun, Y., et al. (2023). Advanced effect of curcumin and resveratrol on mitigating hepatic steatosis in metabolic associated fatty liver disease *via* the PI3K/AKT/mTOR and HIF-1/VEGF Cascade. *Biomed. Pharmacother.* 165, 115279. doi:10.1016/j.biopha.2023.115279

Huang, S. J., Chen, S. Q., Lin, Y., Yang, H. Y., Ran, J., Yan, F. F., et al. (2021). Maternal nicotine exposure aggravates metabolic associated fatty liver disease *via* PI3K/Akt signaling in adult offspring mice. *Liver Int.* 41 (8), 1867–1878. doi:10.1111/liv.14902

Jang, E. (2022). Hyperoside as a potential natural product targeting oxidative stress in liver diseases. *Antioxidants (Basel)* 11 (8), 1437. doi:10.3390/antiox11081437

Jin, H. L., Feng, X. Y., Feng, S. L., Dai, L., Zhu, W. T., and Yuan, Z. W. (2024). Isoquercitrin attenuates the progression of non-alcoholic steatohepatitis in mice by modulating galectin-3-mediated insulin resistance and lipid metabolism. *Phytomedicine* 123, 155188. doi:10.1016/j.phymed.2023.155188

Kaizuka, T., Morishita, H., Hama, Y., Tsukamoto, S., Matsui, T., Toyota, Y., et al. (2016). An autophagic flux probe that releases an internal control. *Mol. Cell.* 64 (4), 835–849. doi:10.1016/j.molcel.2016.09.037

Kim, Y. C., and Guan, K. L. (2015). mTOR: a pharmacologic target for autophagy regulation. *J. Clin. Invest.* 125 (1), 25–32. doi:10.1172/JCI73939

Ku, Y. S., Ng, M. S., Cheng, S. S., Lo, A. W., Xiao, Z., Shin, T. S., et al. (2020). Understanding the composition, biosynthesis, accumulation and transport of flavonoids in crops for the promotion of crops as healthy sources of flavonoids for human consumption. *Nutrients* 12 (6), 1717. doi:10.3390/nu12061717

Li, J., Wang, T., Liu, P., Yang, F., Wang, X., Zheng, W., et al. (2021). Hesperetin ameliorates hepatic oxidative stress and inflammation *via* the PI3K/AKT-Nrf2-ARE pathway in oleic acid-induced HepG2 cells and a rat model of high-fat diet-induced NAFLD. *Food Funct.* 12 (9), 3898–3918. doi:10.1039/d0fo02736g

Lin, S., Qiu, X., Fu, X., Zhang, S., Tang, C., Kuang, J., et al. (2025). SNRK modulates mTOR-autophagy pathway for liver lipid homeostasis in MAFLD. *Mol. Ther.* 33 (1), 279–296. doi:10.1016/j.ymthe.2024.11.016

Liu, B., Deng, X., Jiang, Q., Li, G., Zhang, J., Zhang, N., et al. (2020a). Scoparone improves hepatic inflammation and autophagy in mice with nonalcoholic steatohepatitis by regulating the ROS/P38/Nrf2 axis and PI3K/AKT/mTOR pathway in macrophages. *Biomed. Pharmacother.* 125, 109895. doi:10.1016/j.biopha.2020.109895

Liu, R., Su, C., Xu, Y., Shang, K., Sun, K., Li, C., et al. (2020b). Identifying potential active components of walnut leaf that action diabetes mellitus through integration of UHPLC-Q-Orbitrap HRMS and network pharmacology analysis. *J. Ethnopharmacol.* 253, 112659. doi:10.1016/j.jep.2020.112659

Liu, Y., Sun, Z., Dong, R., Liu, P., Zhang, X., Li, Y., et al. (2024b). Rutin ameliorated lipid metabolism dysfunction of diabetic NAFLD *via* AMPK/SREBP1 pathway. *Phytomedicine* 126, 155437. doi:10.1016/j.phymed.2024.155437

Liu, Y., Zhou, F., Zhao, H., Song, J., Song, M., Zhu, J., et al. (2025). Dimeric guaianolide sesquiterpenoids from the flowers of Chrysanthemum indicum ameliorate hepatic steatosis through mitigating SIRT1-mediated lipid accumulation and ferroptosis. *J. Adv. Res.* doi:10.1016/j.jare.2024.12.047

Liu, Z., Fu, H., Gan, Y., Ye, Y., Huang, B., Jiang, M., et al. (2024a). UHPLC-Q-Orbitrap HRMS and network analysis to explore the mechanisms of QiShenYiQi dripping pill for treating myocardial infarction. *Front. Pharmacol.* 15, 1443560. doi:10.3389/fphar.2024.1443560

Lv, H. W., Wang, Q. L., Luo, M., Zhu, M. D., Liang, H. M., Li, W. J., et al. (2023). Phytochemistry and pharmacology of natural prenylated flavonoids. *Arch. Pharm. Res.* 46 (4), 207–272. doi:10.1007/s12272-023-01443-4

Miao, L., Targher, G., Byrne, C. D., Cao, Y. Y., and Zheng, M. H. (2024). Current status and future trends of the global burden of MASLD. *Trends Endocrinol. Metab.* 35 (8), 697–707. doi:10.1016/j.tem.2024.02.007

Pais, R., Barritt, A. S. t., Calmus, Y., Scatton, O., Runge, T., Lebray, P., et al. (2016). NAFLD and liver transplantation: current burden and expected challenges. *J. Hepatol.* 65 (6), 1245–1257. doi:10.1016/j.jhep.2016.07.033

Patterson, R. E., Kalavalapalli, S., Williams, C. M., Nautiyal, M., Mathew, J. T., Martinez, J., et al. (2016). Lipotoxicity in steatohepatitis occurs despite an increase in

tricarboxylic acid cycle activity. *Am. J. Physiol. Endocrinol. Metab.* 310 (7), E484–E494. doi:10.1152/ajpendo.00492.2015

Petta, S., Targher, G., Romeo, S., Pajvani, U. B., Zheng, M. H., Aghemo, A., et al. (2024). The first MASH drug therapy on the horizon: current perspectives of resmetirom. *Liver Int.* 44 (7), 1526–1536. doi:10.1111/liv.15930

Qu, W., Ma, T., Cai, J., Zhang, X., Zhang, P., She, Z., et al. (2021). Liver fibrosis and MAFLD: from molecular aspects to novel pharmacological strategies. *Front. Med. (Lausanne)* 8, 761538. doi:10.3389/fmed.2021.761538

Shen, B., Wang, Y., Cheng, J., Peng, Y., Zhang, Q., Li, Z., et al. (2023). Pterostilbene alleviated NAFLD *via* AMPK/mTOR signaling pathways and autophagy by promoting Nrf2. *Phytomedicine* 109, 154561. doi:10.1016/j.phymed.2022.154561

Sun, C., Zhang, J., Hou, J., Hui, M., Qi, H., Lei, T., et al. (2023a). Induction of autophagy *via* the PI3K/Akt/mTOR signaling pathway by pueraria flavonoids improves non-alcoholic fatty liver disease in Obese mice. *Biomed. Pharmacother.* 157, 114005. doi:10.1016/j.biopha.2022.114005

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., et al. (2023b). Erratum to "IDF Diabetes Atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045" [diabetes res. Clin. Pract. 183 (2022) 109119]. *Diabetes Res. Clin. Pract.* 204, 110945. doi:10.1016/j.diabres.2023.110945

Sundarraj, K., Raghunath, A., Panneerselvam, L., and Perumal, E. (2021). Fisetin inhibits autophagy in HepG2 cells *via* PI3K/Akt/mTOR and AMPK pathway. *Nutr. Cancer.* 73 (11-12), 2502–2514. doi:10.1080/01635581.2020.1836241

Tao, Y., Liu, J., Li, M., Wang, H., Fan, G., Xie, X., et al. (2024). Abelmoschus manihot (L.) medik. Seeds alleviate rheumatoid arthritis by modulating JAK2/STAT3 signaling pathway. *J. Ethnopharmacol.* 325, 117641. doi:10.1016/j.jep.2023.117641

Tsuji, A., Yoshikawa, S., Ikeda, Y., Taniguchi, K., Sawamura, H., Morikawa, S., et al. (2023). Tactics with prebiotics for the treatment of metabolic dysfunction-associated fatty liver disease *via* the improvement of mitophagy. *Int. J. Mol. Sci.* 24 (6), 5465. doi:10.3390/ijms24065465

Tung, Y. T., Zeng, J. L., Ho, S. T., Xu, J. W., Li, S., and Wu, J. H. (2021). Anti-NAFLD effect of djulis hull and its major compound, rutin, in mice with high-fat diet (HFD)-induced obesity. *Antioxidants (Basel).* 10 (11), 1694. doi:10.3390/antiox10111694

Wang, S., Sheng, F., Zou, L., Xiao, J., and Li, P. (2021). Hyperoside attenuates non-alcoholic fatty liver disease in rats *via* cholesterol metabolism and bile acid metabolism. *J. Adv. Res.* 34, 109–122. doi:10.1016/j.jare.2021.06.001

Wang, W., Liu, M., Fu, X., Qi, M., Zhu, F., Fan, F., et al. (2024). Hydroxysafflor yellow A ameliorates alcohol-induced liver injury through PI3K/Akt and STAT3/NF-κB signaling pathways. *Phytomedicine* 132, 155814. doi:10.1016/j.phymed.2024.155814

Wang, Y. L., Zhou, X., Li, D. L., and Ye, J. M. (2022). Role of the mTOR-autophagy-ER stress pathway in high fructose-induced metabolic-associated fatty liver disease. *Acta Pharmacol. Sin.* 43 (1), 10–14. doi:10.1038/s41401-021-00629-0

Wei, Y., Zhang, Y., Zhan, B., Wang, Y., Cheng, J., Yu, H., et al. (2025). Asiaticoside alleviated NAFLD by activating Nrf2 and inhibiting the NF-κB pathway. *Phytomedicine* 136, 156317. doi:10.1016/j.phymed.2024.156317

Wu, Y., Yang, M., Wu, S. B., Luo, P. Q., Zhang, C., Ruan, C. S., et al. (2024). Zinc finger BED-Type containing 3 promotes hepatic steatosis by interacting with polypyrimidine tract-binding protein 1. *Diabetologia* 67 (10), 2346–2366. doi:10.1007/s00125-024-06224-2

Xu, X., Poulsen, K. L., Wu, L., Liu, S., Miyata, T., Song, Q., et al. (2022). Targeted therapeutics and novel signaling pathways in non-alcohol-associated fatty liver/steatohepatitis (NAFL/NASH). *Signal Transduct. Target Ther.* 7 (1), 287. doi:10.1038/s41392-022-01119-3

Xue, C., Zhang, X., Ge, H., Tang, Q., Jeon, J., Zhao, F., et al. (2023). Total flavone of flowers of abelmoschus manihot (L.) medic inhibits the expression of adhesion molecules in primary mesenteric arterial endothelial cells and ameliorates dextran sodium sulphate-induced ulcerative colitis in mice. *Phytomedicine* 112, 154713. doi:10.1016/j.phymed.2023.154713

Yan, C., Zhang, Y., Zhang, X., Aa, J., Wang, G., and Xie, Y. (2018). Curcumin regulates endogenous and exogenous metabolism *via* Nrf2-FXR-LXR pathway in NAFLD mice. *Biomed. Pharmacother.* 105, 274–281. doi:10.1016/j.biopha.2018.05.135

Yan, L. S., Zhang, S. F., Luo, G., Cheng, B. C., Zhang, C., Wang, Y. W., et al. (2022). Schisandrin B mitigates hepatic steatosis and promotes fatty acid oxidation by inducing autophagy through AMPK/mTOR signaling pathway. *Metabolism* 131, 155200. doi:10.1016/j.metabol.2022.155200

Yang, Y., Qiu, W., Xiao, J., Sun, J., Ren, X., and Jiang, L. (2024). Dihydromyricetin ameliorates hepatic steatosis and insulin resistance *via* AMPK/PGC-1α and PPARα-mediated autophagy pathway. *J. Transl. Med.* 22 (1), 309. doi:10.1186/s12967-024-05060-7

Yi, M., Fasina, O. B., Li, Y., Xiang, L., and Qi, J. (2023). Mixture of peanut skin extract, geniposide, and isoquercitrin improves the hepatic lipid accumulation of mice *via* modification of gut microbiota homeostasis and the TLR4 and AMPK signaling pathways. *Int. J. Mol. Sci.* 24 (23), 16684. doi:10.3390/ijms242316684

Younossi, Z. M., Golabi, P., Paik, J. M., Henry, A., Van Dongen, C., and Henry, L. (2023). The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology* 77 (4), 1335–1347. doi:10.1097/HEP.0000000000000004

Yu, W., Yin, G., Chen, S., Zhang, X., Meng, D., Wang, L., et al. (2024). Diosgenin attenuates metabolic-associated fatty liver disease through the hepatic NLRP3 inflammasome-dependent signaling pathway. *Int. Immunopharmacol.* 138, 112581. doi:10.1016/j.intimp.2024.112581

Zhao, J., Hu, Y., and Peng, J. (2021). Targeting programmed cell death in metabolic dysfunction-associated fatty liver disease (MAFLD): a promising new therapy. *Cell. Mol. Biol. Lett.* 26 (1), 17. doi:10.1186/s11658-021-00254-z

Zhao, R., Guo, Z., Lu, K., Chen, Q., Riaz, F., Zhou, Y., et al. (2024). Hepatocyte-specific NR5A2 deficiency induces pyroptosis and exacerbates non-alcoholic steatohepatitis by downregulating ALDH1B1 expression. *Cell Death Dis.* 15 (10), 770. doi:10.1038/s41419-024-07151-1

Zhou, S., Fan, K. K., Gu, L. F., Yu, B. Y., and Chai, C. Z. (2022). Anti-inflammatory effects of abelmoschus manihot (L.) medik. On LPS-Induced cystitis in mice: potential candidate for cystitis treatment based on classic use. *Chin. J. Nat. Med.* 20 (5), 321–331. doi:10.1016/S1875-5364(22)60140-7

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Natural active botanical metabolites: targeting AMPK signaling pathway to treat metabolic dysfunction-associated fatty liver disease

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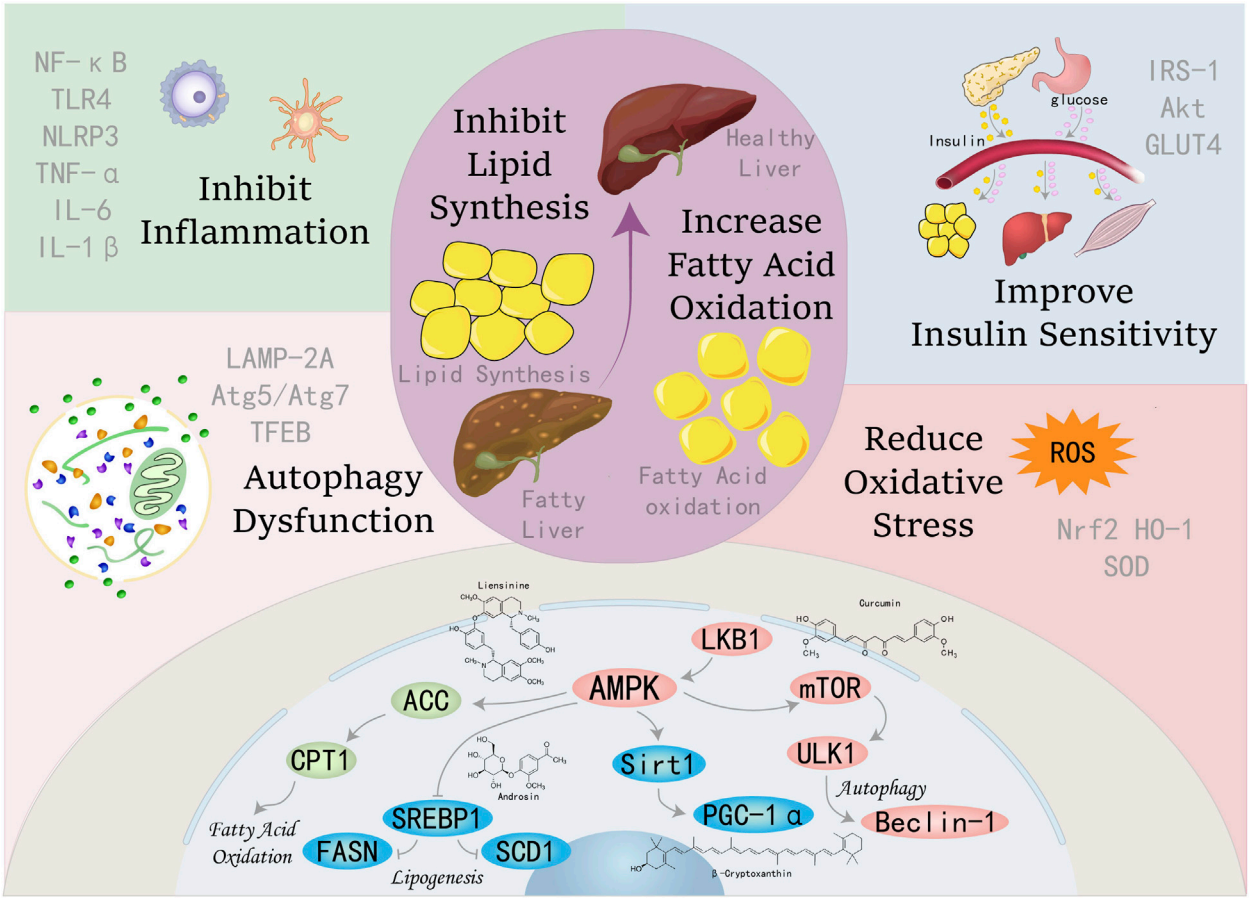
Metabolic dysfunction-associated fatty liver disease (MAFLD), also known as non-alcoholic fatty liver disease (NAFLD), has emerged as one of the most common chronic liver diseases globally, with a tendency to progress gradually. With persistent disease progression, it may subsequently manifest as complications, including non-alcoholic steatohepatitis (NASH), cirrhosis, and liver cancer, and has been clinically established as a primary causative factor for liver failure and clinical scenarios necessitating liver transplantation. AMP-activated protein kinase (AMPK) is the central regulatory hub governing cellular energy homeostasis. It plays a central regulatory role in improving lipid metabolic disorders and represents a key molecular nexus for the management of MAFLD. Currently, the pathogenesis of MAFLD remains unclear, and treatment options are still limited, posing a significant public health challenge. Natural active botanical metabolites, which are important sources of novel therapeutic drugs, are widely available in nature and characterized by strong practicability and low cost. Growing evidence suggests that natural active botanical metabolites have definite therapeutic effects on MAFLD and hold broad application prospects. This study aims to systematically review *in vivo* and *in vitro* experimental evidence on natural active botanical metabolites targeting the AMPK pathway for the treatment of MAFLD. Based on our research findings, it is anticipated that effective natural active botanical metabolites can be incorporated into novel formulations in the future, which are expected to facilitate its bench-to-bedside transformation.

KEYWORDS

metabolic dysfunction-associated fatty liver disease, AMPK, natural active botanical metabolites, lipid metabolism, *de novo* lipogenesis, fatty acid oxidation



GRAPHICAL ABSTRACT

1 Introduction

The nomenclature for non-alcoholic fatty liver disease (NAFLD) has been updated to metabolic dysfunction-associated fatty liver disease (MAFLD), which is the latest terminology for fatty liver disease associated with metabolic syndrome (Chan et al., 2023). This change was first proposed by Eslam et al., in 2020 (Eslam et al., 2020a; Eslam et al., 2020b). The diagnostic criteria for MAFLD have been redefined as follows: based on imaging or histologically confirmed hepatic steatosis, patients must exhibit at least one concurrent metabolic abnormality: ① dysmetabolic weight status; ②confirmed type 2 diabetes mellitus (meeting WHO diagnostic criteria); ③metabolic dysfunction evidenced by two or more cardiometabolic risk factors (abdominal adiposity, atherogenic dyslipidemia, hypertension, or prediabetic states). It is worth noting that, unlike the diagnostic criteria for NAFLD, excluding other liver diseases (including alcoholic, autoimmune, or viral hepatitis) is not a prerequisite for diagnosing MAFLD. This updated definition was introduced to better reflect the metabolic nature of the disease to remove the "non-alcoholic" label, and place greater emphasis on clinical phenotypes (Eslam et al., 2020a). MAFLD is a high-prevalence hepatopathy that causes extensive liver damage. Histologically, it manifests as lobular inflammation and hepatocyte ballooning, among other features (Chan et al., 2023). In severe cases, it can lead to adverse hepatic outcomes such as liver fibrosis, cirrhosis, and hepatocellular carcinoma (Huang and Liu, 2023). Therefore, MAFLD is also considered a significant factor influencing the mortality rate of liver-related diseases. With the gradual prevalence of diabetes, and obesity globally, the overall prevalence of MAFLD has also shown an upward trend (Sangro et al., 2023; Younossi et al., 2023; Liu K. et al., 2024). MAFLD has emerged as a major public health issue, affecting up to 25% of the global adult population (Saiman et al., 2022). Studies estimate that from 1991 to 2019, the prevalence of MAFLD has increased from 21.9% to 37.3%, with a yearly increase of 0.7% ($P < 0.0001$), and it is still increasing, posing a significant economic, health, and social burden globally (Le et al., 2022). The current understanding of the pathogenesis of MAFLD remains unclear. Early researchers proposed the "two-hit" theory to explain the pathomechanism of MAFLD. This theory suggests that hepatic steatosis and insulin resistance (IR) caused by abnormal accumulation of free fatty acids constitute the "first hit". The "second hit" phenomenon is characterized by secondary tissue injury, inflammatory responses, metabolic dysregulation including insulin resistance, fibrotic remodeling, and other pathological alterations, mediated through mechanisms involving elevated oxidative stress, peroxidative damage to lipids, and compromised mitochondrial bioenergetics (Basaranoglu et al., 2013; Friedman et al., 2018; Engin, 2024).

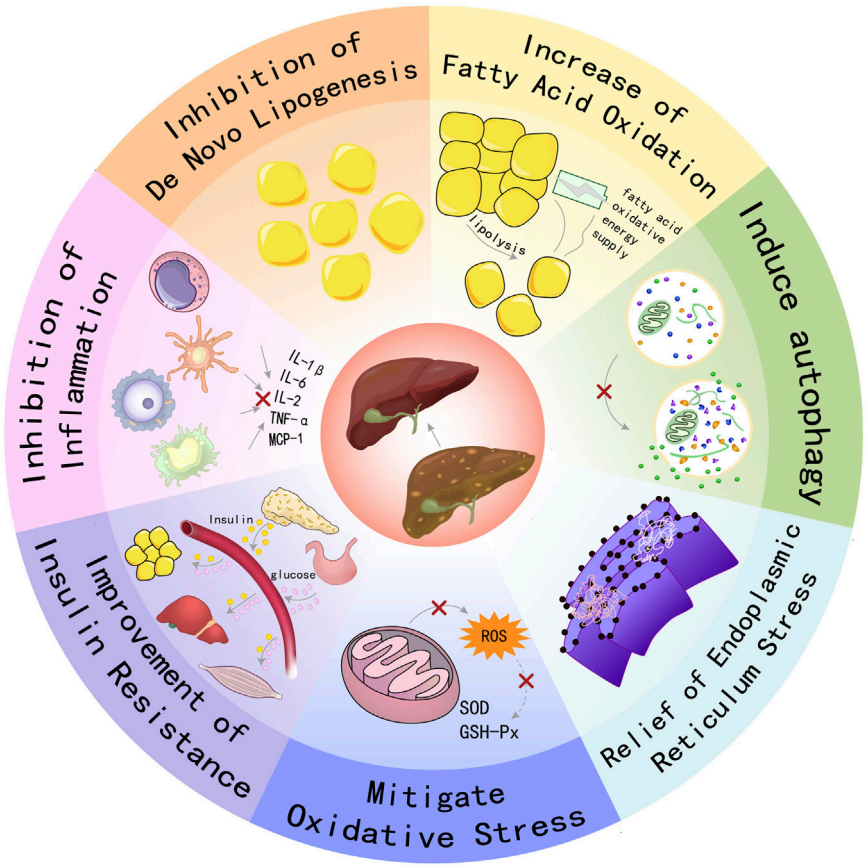


FIGURE 1
Schematic illustration showing partial mechanisms of activated AMPK against MAFLD: (1) Activating AMPK can inhibit key enzymes in the *de novo* lipogenesis pathway, reduce the synthesis and accumulation of fat in the liver, and thereby alleviate the severity of fatty liver; (2) Promoting FAO increases energy supply and reduces the accumulation of fatty acids in the liver, which helps improve hepatic steatosis; (3) Inducing the process of autophagy to clear damaged organelles and protein aggregates, reducing endoplasmic reticulum stress, thereby protecting hepatocytes from damage; (4) Enhancing the cellular antioxidant defense system, reducing the production of ROS, and up-regulating the activities of antioxidant enzymes such as SOD and GSH-Px to protect hepatocytes from oxidative damage; (5) Enhancing insulin signal transduction, increasing insulin sensitivity, promoting glucose uptake and utilization, reducing hepatic gluconeogenesis, thereby improving insulin resistance. (6) Inhibiting inflammatory signaling pathways, reducing the production of inflammatory factors (such as IL-1β, IL-6, TNF-α, etc.), alleviating inflammatory responses in the liver, and preventing the progression of MAFLD to more severe liver diseases.

Recently, it has been generally accepted that the more accurate pathogenesis of MAFLD is the "multiple-hit" hypothesis, which builds upon the "second-hit" theory. It indicates that various harmful factors team up to affect genetically prone individuals, causing MAFLD. These factors include oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, gut microbiota changes, and lipotoxicity (Pafili and Roden, 2021; Guo et al., 2022).

Adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) is a ubiquitously expressed serine/threonine protein kinase in the human body. AMPK consists of a heterotrimeric complex comprising a catalytic α subunit (α1, α2), a structurally and regulatory essential β subunit (β1, β2), and a regulatory γ subunit (γ1, γ2, γ3) (Cui et al., 2023). AMPK can sense the intracellular energy state and is activated when intracellular ATP concentration decreases and AMP or ADP concentration increases (Steinberg and Hardie, 2023). Activated AMPK initiates corresponding biological responses by phosphorylating a series of downstream target proteins to restore cellular energy homeostasis. The specific mechanisms include inhibiting *de novo* lipogenesis (DNL), promoting fatty acid oxidation (FAO) and lipid breakdown, as well as maintaining mitochondrial functional integrity to regulate autophagy and oxidative stress, among others (Smith et al., 2016; Fang et al., 2022). It maintains the steady state of lipid metabolism through the activation of its mediated signaling axes such as the LKB1-AMPK axis, SIRT1-AMPK axis, AMPK-ACC axis, AMPK-SREBP1 axis, and AMPK-mTOR axis. Numerous studies have shown that the AMPK pathway may be a promising target of action for the treatment of MAFLD (Fang et al., 2022).

Unfortunately, the complex pathophysiological characteristics of MAFLD make it difficult to find a single effective treatment method. Clinical options for treating MAFLD are limited, mainly through lifestyle changes such as dietary changes and increased exercise (Romero-Gómez, 2022). The development of pharmacological treatment regimens remains ongoing. Current drugs under clinical investigation include antidiabetic agents, Farnesoid X Receptor (FXR) agonists, Peroxisome Proliferator-Activated Receptor (PPAR) agonists, thyroid hormone receptor

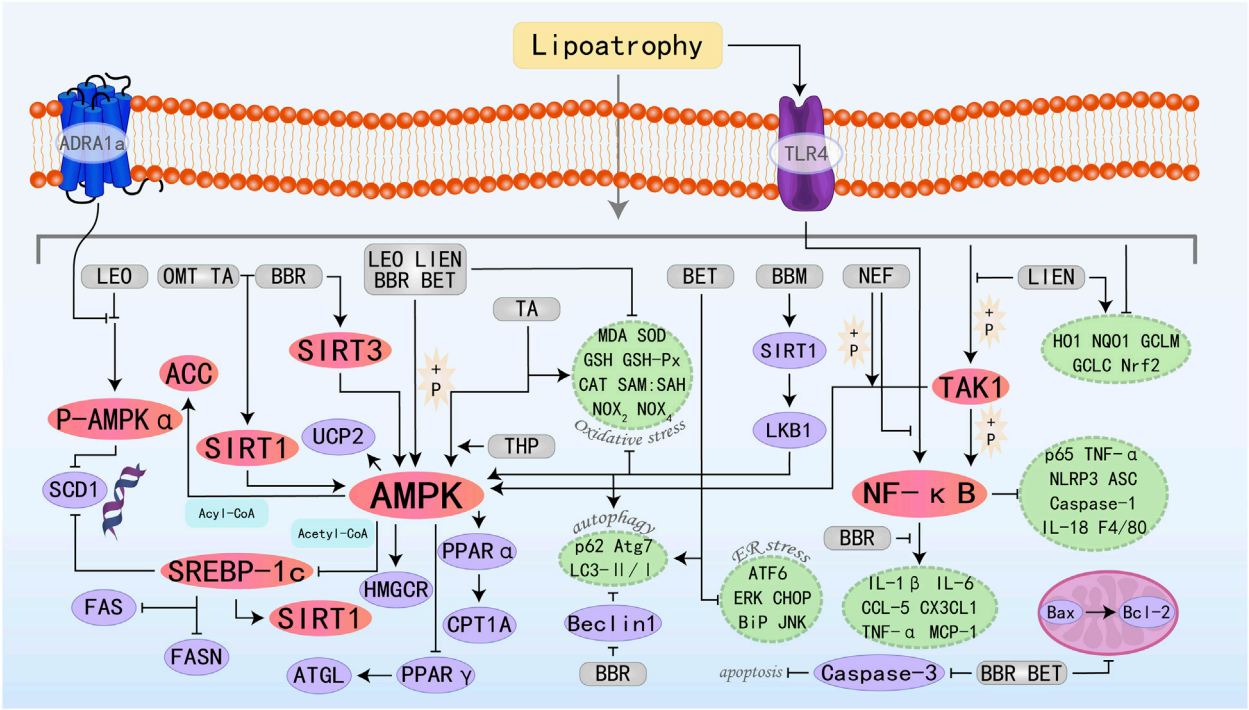


FIGURE 2
Molecular mechanism of alkaloid metabolites from botanical drugs targeting AMPK to treat MAFLD. LEO, Leonurine; LIEN, Liensinine; BBR, Berberine; OMT, Oxymatrine; BET, Betaine; BBM, Berbamine; TA, Tomatidine; NEF, Neferine; THP, Tetrahydropalmatine.

(THR) agonists, etc. However, these therapies face challenges such as toxicity/side effects, undefined optimal dosing regimens, unclear mechanisms of action, and insufficient validation through large-scale clinical trials (Sangro et al., 2023). As of 2024, Resmetirom is the only drug approved by the Food and Drug Administration (FDA). It is an oral, liver-targeted, thyroid hormone beta receptor-selective agonist. Although clinical trials have demonstrated resmetirom's therapeutic potential for non-cirrhotic MASH and moderate to advanced liver fibrosis, gastrointestinal adverse events such as diarrhea and nausea remain significantly higher compared to the placebo group. The potential risks of diseases related to the thyroid, gonads, or bones also need to be monitored. Furthermore, its long-term safety and sustainability have not yet been confirmed through large-scale clinical trials (Bittla et al., 2024; Petta et al., 2024; Suvarna et al., 2024). Consequently, the clinical implementation of resmetirom remains a significant challenge. Compared to Western medicine, traditional Chinese medicine has garnered increasing attention from researchers due to its multi-target and multi-channel mechanisms of action. Natural active botanical metabolites refer to single chemical metabolites isolated, purified, and identified from plants, possessing a well-defined chemical structure (e.g., alkaloids, flavonoids, terpenoids) and exhibiting specific biological activities. They demonstrate a range of biological activities, such as anti-inflammatory and antioxidative effects (Zheng et al., 2023). As isolated bioactive metabolites, they are pivotal in deciphering the molecular mechanisms of traditional herbal therapies and represent a critical source for novel drug discovery. According to a previous study, it was found that many natural active botanical metabolites show great potential in treating MAFLD by inhibiting lipogenesis and promoting FAO, among other effects. Therefore, in this article, we review the research progress on natural active botanical metabolites targeting AMPK-related pathways for the treatment of MAFLD and summarize the associated mechanisms. It is anticipated that effective active metabolites can be incorporated into novel formulations in the future, further advancing the development of clinically effective drugs for MAFLD.

2 Mechanisms by natural active botanical metabolites target AMPK to ameliorate MAFLD

AMPK functions as a pivotal regulator of metabolic homeostasis, employing a repertoire of mechanisms to suppress lipogenesis, enhance FAO, mitigate inflammation and oxidative stress, induce autophagy, and alleviate ER stress and insulin resistance. These pleiotropic effects position AMPK as a promising therapeutic target for the management of MAFLD and other metabolic disorders, see Figure 1.

2.1 Inhibition of *de novo* lipogenesis

De novo lipogenesis (DNL) is the core regulatory axis of energy metabolism homeostasis. The metabolic reprogramming process converts excess carbohydrate substrates (mainly glucose/fructose) into triglycerides (TG) and cholesterol through a series of chemical

reactions, and then these TG and cholesterol lately provide energy through β-oxidation. The DNL pathway is a metabolic process primarily active in the liver (Cross et al., 2023). DNL encompasses a series of coordinated enzymatic reactions. The process initiates with ATP-citrate lyase (ACLY) catalyzing the conversion of citrate into acetyl-CoA. Subsequently, acetyl-CoA carboxylase (ACC) carboxylates the resulting acetyl-CoA to produce malonyl-CoA. Malonyl-CoA then enters the fatty acid synthesis pathway to synthesize fat. Notably, ACC exists in two distinct isoforms - ACC1 and ACC2, which are encoded by separate genes and perform divergent physiological functions (Smith et al., 2016). In detail, ACC1 is key in the DNL process, while ACC2 primarily oversees FAO (Wang et al., 2022b).

Studies have found that AMPK inhibits DNL through multiple pathways. Firstly, AMPK activation phosphorylates ACC, leading to a decrease or inactivation of ACC activity, thereby blocking the first step in fatty acid synthesis. Concurrently, activated AMPK also inhibits the nuclear translocation of SREBP-1c, phosphorylates the precursor of SREBP-1c, and prevents SREBP-1c from converting into its mature form, thus reducing abnormal lipogenesis. In addition, activated AMPK can also inhibit lipogenesis by enhancing the expression of SIRT1. SIRT1, a member of the Sirtuins family, is an NAD^+-dependent class III histone deacetylase. AMPK enhances SIRT1 activity by increasing intracellular NAD^+ levels (Cantó et al., 2009). Activated SIRT1 inhibits SREBP-1c activity in the liver, thereby reducing fat synthesis. For instance, treatment with resveratrol, a potent SIRT1 activator, has been demonstrated to significantly reduce acetylated SREBP-1c levels in obese mouse models (Ponugoti et al., 2010). Interestingly, SIRT1 also promotes the deacetylation of liver kinase B1 (LKB1), an upstream kinase of AMPK, to increase AMPK phosphorylation and activity, and ultimately, through the LKB1-AMPK-SIRT1 signaling axis can attenuate MAFLD through the modulation of DNL process (Lan et al., 2008).

2.2 Increase of fatty acid oxidation

Fatty acid oxidation (FAO) refers to the process by which fatty acids are broken down into CO_2 and H_2O in the presence of oxygen, releasing a significant amount of energy, primarily occurring in mitochondria (Lu and George, 2024). Dysregulation of FAO creates an imbalance between lipid acquisition and processing, resulting in abnormal deposition of lipid droplets in hepatocytes, a pathologic process that is a central driving mechanism of MAFLD (Ipsen et al., 2018). Carnitine palmitoyltransferase 1 (CPT1) is a crucial rate-limiting enzyme in FAO (Schlaepfer and Joshi, 2020), catalyzing the complete oxidation of fatty acids through a series of biochemical reactions. Malonyl-CoA functions as an allosteric inhibitor of CPT1. When AMPK is activated, it phosphorylates and inactivates ACC, which in turn decreases the synthesis of malonyl-CoA. Thereby increasing CPT1 expression and promoting FAO (Tian et al., 2019). Activation of AMPK significantly increases the activity of lipolytic enzymes such as adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), thus promoting lipolysis, increasing the concentration of fatty acids in the cell, and providing substrates for the subsequent oxidation process. The main site of FAO is in the mitochondria, and the activation of AMPK can also regulate the function of mitochondria, which is also conducive to the promotion of FAO. Therefore, multiple regulatory mechanisms interact to form positive feedback, driving the metabolic process of FAO while ensuring precise maintenance of energy homeostasis at the cellular and body levels.

2.3 Inhibition of inflammation

The inflammatory reaction is closely associated with the body's metabolism and often influences each other. For example, obesity and type 2 diabetes, among others, trigger an increase in inflammatory markers in the liver, adipose tissue, and skeletal muscle, while immune dysfunction exacerbates various metabolic disorders (Saltiel and Olefsky, 2017; Fan et al., 2022). Inflammation also plays an important role in MAFLD, and the immune system plays an integral role in the gradual evolution of MAFLD from simple steatosis to NASH and then to the more advanced NASH-associated fibrosis (Friedman et al., 2018; Peiseler et al., 2022). Activated AMPK achieves negative regulation of inflammatory response by inhibiting the expression of pro-inflammatory cytokines (TNF - α, IL-6) and NF - κ B signaling transduction, accompanied by compensatory upregulation of anti-inflammatory proteins. For instance, the activation of AMPK can enhance the activity of SIRT1, which modifies histones and other proteins through deacetylation to suppress the expression of inflammatory genes (Xu C. et al., 2021). AMPK also interacts with other signaling pathways to jointly regulate inflammatory responses. For example, there is an intimate contact between AMPK and the PI3K/Akt/mTOR signaling pathway.

2.4 Induction of autophagy

Autophagy is a lysosome-mediated self-degradative process that eliminates misfolded proteins, aggregated macromolecules, and damaged organelles (e.g., mitochondria and endoplasmic reticulum). By clearing these metabolites, autophagy mitigates oxidative stress and inflammation, thereby maintaining cellular homeostasis. Autophagy is generally regarded as a survival mechanism that breaks down cellular debris to supply recycled metabolites and energy, thereby supporting cellular renewal and homeostasis (Hotamisligil, 2006; Liang L. et al., 2022). The regulatory function of autophagy cannot be overlooked in the pathophysiological progression of multiple disorders, including but not limited to neoplastic transformations, metabolic dysregulations, immune-mediated pathologies, and pathogen-associated conditions. Lipophagy, a subtype of autophagy, can target intracellular lipid droplets and degrade them to regulate fat storage in the liver, and impaired autophagic flux is closely associated with the development of MAFLD (Czaja, 2016; Niture et al., 2021). As an evolutionarily conserved serine/threonine kinase, the mammalian target of rapamycin (mTOR) serves as a central regulatory hub by integrating nutrient signals to coordinate metabolic reprogramming, translational regulation, and programmed cell death, while also dynamically regulating cellular growth and proliferation. Functioning as a critical modulator of cellular energetics, mTOR enhances metabolism and inhibits

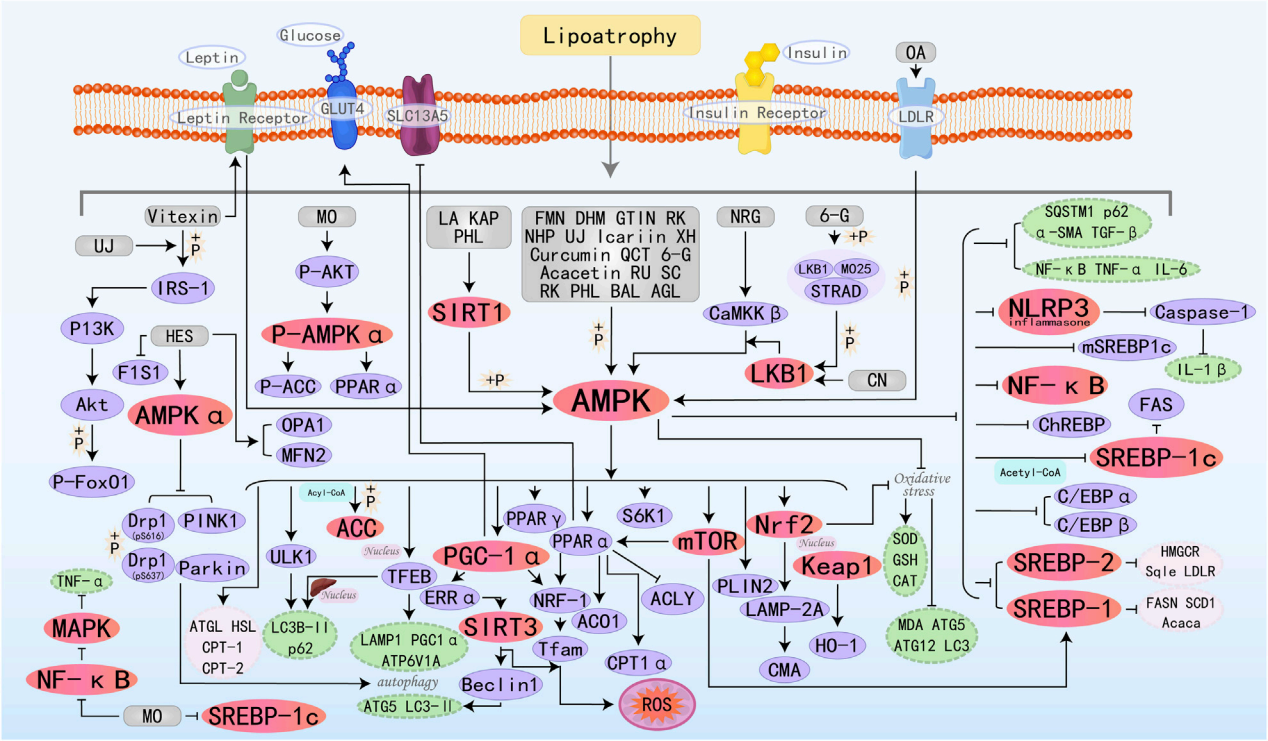


FIGURE 3
Molecular mechanism of flavonoid metabolites from botanical drugs targeting AMPK to treat MAFLD. LA, Licochalcone A; FMN, Formononetin; MO, Morin; DHM, Dihydromyricetin; BAL, Baicalein; NHP, Neohesperidin; UJ, Ugonin J; NRG, Naringenin; XH, Xanthohumol; QCT, Quercetin; CN, Chrysin; BA, Baicalin; KAP, Kaempferol; 6-G, 6-Gingerol; GTIN, Gossypetin; OA, Oroxin A; RU, Rutin; AGL, Apigenin; HES, Hesperitin; SC, Scopoletin; RK, Raspberry ketone; PHL, Phloretin.

autophagy (Xie et al., 2023; Liu X. et al., 2024). mTOR is a key effector in AMPK signaling cascades, and it oppositely regulates autophagy induction with crosstalk in modulating energy balance (Xu et al., 2012). Specifically, mTORC complex 1 (mTORC1) downregulates the autophagy process, whereas AMPK has a direct or indirect positive impact on autophagy induction (Alers et al., 2012). Therefore, the AMPK-mTOR axis plays a crucial role in regulating lipid metabolism. To date, numerous scientific studies have confirmed this notion. Silibinin promotes autophagy in high fructose-exposed HepG2 cells through AMPK activation, mTOR inhibition, and downregulation of autophagy suppressors LC3/beclin-1 (Li et al., 2019). AMPK also alleviates hepatic steatosis by activating autophagy through AMPK-mediated PGC-1α/PPARα pathways (Yang et al., 2024).

2.5 Mitigation of oxidative stress

The fundamental biological nature of oxidative stress (OS) lies in the dynamic imbalance between the intracellular production rate of reactive oxygen species (ROS) and the antioxidant defense capacity, resulting in the accumulation of oxidative damage (Hajam et al., 2022; Sadasivam et al., 2022). Excessive ROS generation depletes endogenous antioxidants, rendering the body unable to neutralize the surplus ROS, thereby leading to cellular damage. OS serves as a pivotal pathogenic driver in MAFLD progression, inducing hepatic inflammatory cascades, compromising mitochondrial bioenergetics, and triggering extracellular matrix deposition within hepatocytes. More severely, excessive OS leading to progressive hepatocyte death can facilitate the development of liver cirrhosis or hepatocellular carcinoma (HCC) (Takaki et al., 2013; Spahis et al., 2017; Clare et al., 2022). Studies have confirmed that berbamine enhances the antioxidant defense system by activating the antioxidant transcription factor nucleus factor erythroid 2-related factor 2 (Nrf2), upregulating the expression of HO-1, Nqo-1, SOD2, and catalase. This effect is primarily achieved through the activation of AMPK, which stimulates the Nrf2/ARE signaling pathway (Sharma et al., 2020). AMPK also inhibits oxidative stress in liver tissue by stimulating SIRT1 expression, which increases GSH-Px, GSH, SOD, and CAT content and significantly decreases MDA levels (Chen et al., 2024).

2.6 Other mechanisms of action

In addition to the major mechanisms mentioned above, endoplasmic reticulum stress (ER stress) is also one of the key pathological mechanisms. As a cellular protective stress response, ER stress functions to alleviate the disruption of cellular homeostasis caused by protein misfolding, thereby triggering the unfolded protein response (UPR) (Lebeaupin et al., 2018). ER stress can initiate and accelerate the progression of diverse liver ailments,

including metabolic-associated fatty liver disease (MAFLD), viral hepatitis, liver injury caused by medications, as well as hepatocellular carcinoma. It has been found that AMPK activation reduces ER stress markers Bip, ATF, and CHOP as well as phosphorylated ERK/JNK, thereby alleviating ER stress. While AMPK-stimulated autophagy is also directly related to the reduction of ER stress (Seo et al., 2024). In addition, insulin resistance is a central driver of MAFLD, promoting disease progression through systemic metabolic disturbances (adipose tissue, skeletal muscle, intestinal tract) and local intrahepatic signaling dysregulation (selective resistance, ER stress) (Sakurai et al., 2021). Activated AMPK improves metabolic status by regulating signaling pathways and metabolic pathways associated with insulin resistance to enhance insulin sensitivity to treat MAFLD (Fouqueray et al., 2021).

To determine the mechanisms by which natural active botanical metabolites ameliorate MAFLD through targeting the AMPK signaling pathway, Non-alcoholic Fatty Liver Disease, Metabolic dysfunction-associated fatty liver disease, NAFLD, MAFLD, AMP-Activated Protein Kinases, AMPK, Chinese herbs, Chinese medicine, Herbal medicine, Plant medicine, Natural medicine, Botanical drug, Phytomedicine were used as keywords or subject headings to search for relevant articles in the Web of Science and PubMed databases from 2019 to 2024. After identifying 1,111 potentially relevant articles, the screening protocol sequentially discarded 342 duplicate records and disqualified 85 review papers, ultimately retaining 684 articles for in-depth analysis. Following initial screening, publications were systematically filtered to eliminate entries conforming to pre-defined exclusion criteria: (1) non-medical articles; (2) articles that study combinations composed of various botanical drugs (e.g., decoctions); (3) articles where the study target was not natural active botanical metabolites; (4) articles classified as commentaries; (5) articles on natural active botanical metabolites unrelated to the AMPK pathway; (6) articles with severely missing experimental data; (7) articles lacking animal experiments; and (8) articles with unavailable full texts. Finally, 120 articles were included, involving 101 natural active botanical metabolites, and summarized information on plant sources, classifications, and other details were summarized (Supplementary Table 1).

3 Natural active botanical metabolites improving MAFLD by targeting AMPK

3.1 Alkaloids

Plant alkaloids are metabolites with complex structures found in natural plants. Due to the different arrangements and combinations of functional groups, various alkaloids can be formed, possessing multifaceted pharmacological profiles, such as anticancer, inflammatory cascade attenuation, pathogen eradication, oxidative damage mitigation, hypertensive state amelioration, and immune homeostasis maintenance (Bhambhani et al., 2021). Therefore, they are regarded as a potentially important source of drugs for the treatment of related diseases in the future. Many alkaloid metabolites have demonstrated positive protective effects against MAFLD. The specific molecular pathways by which nine alkaloid plant metabolites target the AMPK pathway to effectively suppress the onset and advancement of MAFLD are illustrated in Figure 2.

Leonurine (LEO) is a natural alkaloid extract derived from *Herba leonuri*. Studies have shown that LEO exhibits anti-MAFLD effects both *in vivo* and *in vitro* experiments. It regulates lipid homeostasis through modulation of the AMPK/SREBP1 signaling axis, suppressing hepatic fat accumulation while concurrently attenuating oxidative damage and inflammatory responses in liver parenchyma (Zhang et al., 2019a). LEO exerts its regulatory function by upregulating ADRA1a expression—a G protein-coupled receptor—which sequentially induces phosphorylation and activates the downstream AMPK signaling cascade. This regulates the expression of its downstream protein stearoyl-CoA desaturase 1 (SCD1), reducing fatty acid levels (Fan et al., 2024). Liensinine (LIEN) is also a plant-derived isoquinoline alkaloid with various pharmacological effects, including anti-inflammatory, antioxidant, anti-apoptotic, and autophagy-modulating properties (Zhang W. et al., 2023). *In vitro* analyses reveal that LIEN can stimulate the AMPK/ACC signaling axis, which in turn rescues the expression of key FAO-regulatory factors including PPARα, CPT-1α, and uncoupling protein 2 (UCP2). This hierarchical modulation ultimately enhances FAO capacity. Furthermore, it suppresses oxidative stress and inflammation via activation of the transforming growth factor-β-activated kinase 1 (TAK1)-dependent AMPK pathway, culminating in MAFLD improvement (Liang L. et al., 2022). Berberine (BBR) is the main active component in *Coptis chinensis Franch.* In traditional medicine and is regarded as one of the most promising natural product-derived drugs for the treatment of cardiovascular and metabolic diseases (Feng et al., 2019). *In vivo* studies have shown that BBR can inhibit lipogenesis and promote FAO by activating the SIRT3/AMPK/ACC pathway in the liver, thereby improving hepatic steatosis (Zhang et al., 2019a). Further studies have also demonstrated that BBR inhibits the transcriptional activity of the SRE motif (a potential SREBP-1c binding site) within the SCD1 promoter through the AMPK-SREBP-1c pathway, thereby reducing hepatic lipogenesis (Zhu et al., 2019). BBR can also stimulate AMPK/SIRT1 signaling to regulate downstream effector molecules controlling lipid synthesis, transport, and catabolism—specifically SREBP-1c and PPARα. Additionally, it modulates FOXO transcription factors, NF-κB, Bcl-2/Bax, and cleaved caspase 3, thereby controlling oxidative stress, inflammation, and apoptosis, ultimately achieving the goal of treating MAFLD (Chen et al., 2024). Oxymatrine (OMT) belongs to the class of matrine-type alkaloids, which are primarily isolated from *Sophora flavescens Aiton*. Studies have shown that OMT, similar to BBR, primarily regulates hepatic lipid metabolism by activating the SIRT1/AMPK pathway and modulating downstream relevant molecules (Xu et al., 2020). Betaine (BET), a compound also termed trimethylglycine, occurs naturally in various life forms such as animals, plants, and microorganisms. It is a non-essential amino acid that plays a crucial role in metabolic diseases through various effects such as exhibiting anti-inflammatory properties, restoring mitochondrial function, and improving insulin resistance (Chen et al., 2022b). Fibroblast growth factor 10 (FGF10), a key component of the FGF family, is indispensable for the proper development of

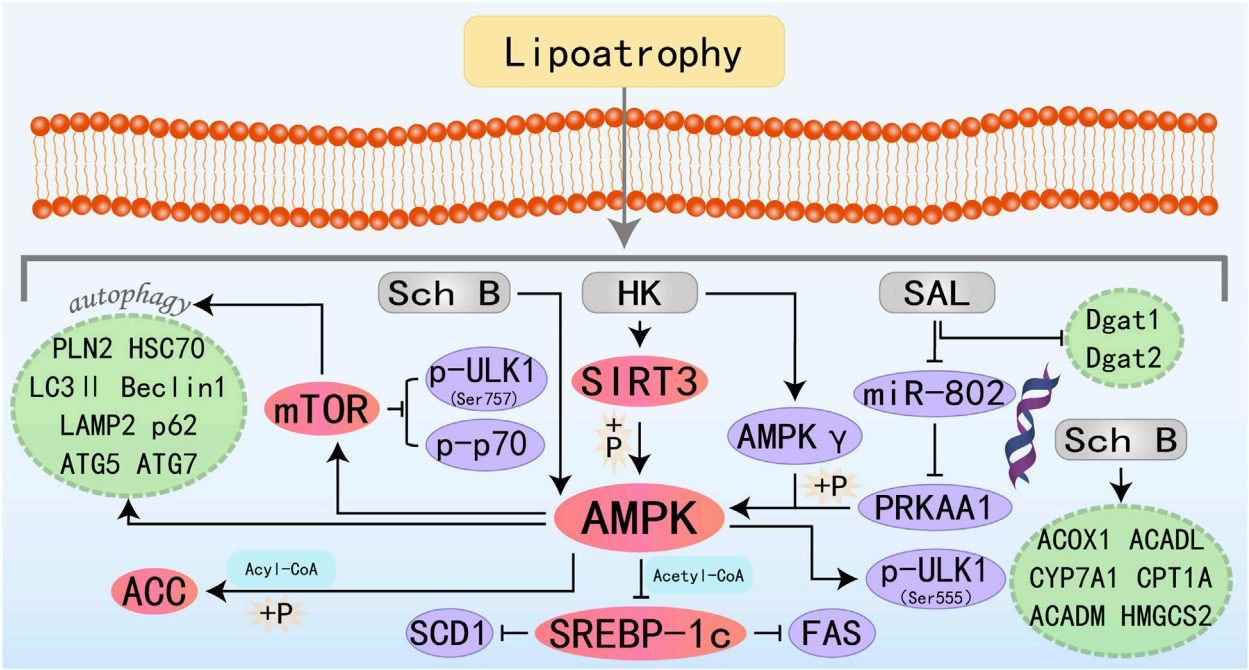


FIGURE 4
Molecular mechanism of lignans metabolites from botanical drugs targeting AMPK to treat MAFLD. HK, Honokiol; Sch B, Schisandrin B; SAL, Schisanhenol.

multiple organs. A previous study suggested that the miR-327-FGF10-FGFR2 signaling axis represents a therapeutic target for the treatment of obesity and metabolic diseases (Fischer et al., 2017). Building upon this foundation, Chen W et al. conducted research and confirmed that BET treatment induces FGF10 secretion, which stimulates AMPK activation and inhibits lipogenesis, increases FAO, and ultimately reduces hepatic lipid accumulation, identifying a potential new therapeutic target for MAFLD (Chen et al., 2021). Additionally, studies have found that BET can also activate autophagy (LC3II/I and p62), thereby reducing downstream signals such as ER stress (BiP, ATF6, and CHOP), apoptosis (Bax, cleaved caspase 3), and steatosis (Seo et al., 2024). Berbamine (BBM) is a dibenzylisoquinoline alkaloid extracted from the Chinese herbal medicine genus Berberis, which possesses anti-inflammatory, leukocytosis-inducing, and anti-tumor effects. For decades, it has maintained its clinical relevance in addressing a broad spectrum of pathological conditions. Among these studies, Sharma A et al. found that BBM activates SIRT1-mediated LKB1 deacetylation, upregulates AMPK signaling, and ultimately regulates the expression of downstream lipid metabolism factors, improves mitochondrial physiological structure and function, restores autophagy, and alleviates oxidative stress, playing a therapeutic role in MAFLD (Sharma et al., 2021). Tomatidine (TA) is a natural steroidal alkaloid and is the aglycone of α-tomatine (αTM). Previous studies have found that TA can act as a vitamin D agonist, activating AMPK through calmodulin-dependent protein kinase β (CaMKKβ) in response to increased intracellular Ca2+ concentrations, inhibiting lipid accumulation (Kusu et al., 2019). Neferine (NEF) exerts its therapeutic potential in MAFLD management by stimulating AMPK-dependent cellular energy reprogramming, demonstrating efficacy *in vitro* and *in vivo*. Additionally, it prevents MAFLD-related fibrosis by inhibiting the TGFβ-Smad2/3 pathway (Wang et al., 2023). Tetrahydropalmatine (THP) is known for its anti-inflammatory and analgesic effects with promising applications and potential development value (Chen X. et al., 2023). Yin X et al. have demonstrated that the AMPKα protein is a direct target of THP, which can promote FAO, improve lipid metabolism, and alleviate lipotoxicity through the AMPK/SREBP-1c/SIRT1 pathway (Yin et al., 2023).

3.2 Flavonoids

Flavonoids represent a class of bioactive phytochemicals renowned for their pleiotropic health-promoting activities, with widespread occurrence in edible horticultural produce and medicinal botany. Flavonoids are characterized by a bipartite aromatic system comprising A and B benzene rings, which are conjugated through a central three-carbon linker, establishing the fundamental C6-C3-C6 carbon framework. Both synthetically produced and naturally isolated flavonoid metabolites exhibit a variety of biological activities, such as anti-tumor, antiplatelet, antimalarial, anti-inflammatory, and antidepressant effects (Guan and Liu, 2016). It exhibits therapeutic efficacy against cancer, inflammatory disorders (particularly cardiovascular and neurological diseases), and related pathologies (Rauf et al., 2023). Notably, flavonoids also demonstrate a potent role in the prevention of MAFLD. The specific molecular mechanisms by which 26 flavonoid active metabolites target the AMPK pathway to improve MAFLD are illustrated in Figure 3.

Vitexin is a natural flavonoid glycoside compound. Research has found that Vitexin activates AMPK by promoting the binding of leptin receptor (LepR) to AMPK, thereby inhibiting lipogenesis and promoting lipolysis and FAO. Additionally, Vitexin improves insulin signaling by activating insulin receptor substrate-1 (IRS-1) and its downstream target protein kinase B (AKT), ultimately leading to an improvement in MAFLD (Inamdar et al., 2019b). Formononetin (FMN) can promote the nuclear translocation of TFEB, a central regulator of the autophagy/lysosome-nuclear signaling pathway, by activating AMPK, thereby activating autophagy (upregulating LAMP1, ATP6V1A, and PGC1α, as well as promoting hepatic nuclear translocation of LC3B-II and p62) to improve autophagosome-lysosome fusion. Additionally, it can also promote FAO by increasing the expression of PPARα and CPT1α(Wang et al., 2019). Dihydromyricetin (DHM) promotes autophagy by activating AMPK/PGC-1α and PPARα, which leads to an increase in the levels of Beclin 1, ATG 5, and LC3-II (Yang et al., 2024). Furthermore, it can also prevent MAFLD by enhancing mitochondrial function through a SIRT3-dependent mechanism (Zeng et al., 2019). Neohesperidin (NHP) is a flavonoid glycoside extracted from citrus peel and utilized as a natural antioxidant. Studies have shown that NHP activates PGC-1α to mediate mitochondrial biogenesis and upregulates nuclear respiratory factor-1(NRF-1) and mitochondrial transcription factor A (TFAM) to enhance mitochondrial capacity and FAO (Wang S. W. et al., 2020). Ugonin J (UJ) is a flavonoid derived from *Helminthostachys zeylanica*, which exhibits anti-inflammatory and anti-osteoporotic effects (Huang et al., 2017). Chang TC et al. showed that UJ regulates lipid metabolism through the activation of factors related to downstream AMPK regulation, increases insulin secretion, improves insulin resistance, and regulates and lipid metabolism disorders by decreasing the ratio of pIRS-1 (Ser307))/IRS-1, and upregulates Akt activity and FoxO1 phosphorylation for the treatment of MAFLD (Chang et al., 2021). Icariin enhances the biosynthesis and membrane redistribution of GLUT4, the transporter protein responsible for glucose uptake, by activating the AMPK/PGC1α pathway. This process enhances glucose uptake and metabolism, while decreasing insulin resistance, ultimately contributing to the improvement of MAFLD (Lin et al., 2021). Curcumin is an active component derived from *Curcuma longa Linn.* With various biological activities including anti-inflammatory, antioxidant, and anticancer properties. It can be used in the treatment of cancer, and metabolic diseases, and as a neuroprotective agent (Mhillaj et al., 2019; Tomeh et al., 2019; Marton et al., 2021; Sadeghi et al., 2023). SLC13A5 is a citrate-selective transmembrane protein in hepatic citrate homeostasis and serves as an alternative energy source for metabolism. ACLY serves as a critical metabolic checkpoint coupling cytosolic glucose-derived carbon flux to DNL via acetyl-CoA availability. Sun QS et al. found that Curcumin may correct the deregulated expression of SLC13A5/ACLY by activating the AMPK-mTOR signaling pathway, thereby inhibiting DNL and reducing hepatic lipid accumulation (Sun et al., 2021). Chrysin (CN) possesses hepatoprotective potential due to its anti-inflammatory properties. Specific investigations have found that CN can activate the LKB1/AMPK/mTOR/SREBP-1c pathway, thereby inhibiting lipogenesis pathways. Additionally, CN also regulates the increased expression of genes involved in mitochondrial biogenesis, such as PGC-1α, NRF-1, and Tfam (Oriquat et al., 2023). The protective effects of Baicalin (BA) on MAFLD rely on AMPK-mediated downstream pathways. Specifically, inhibiting the AMPK/SREBP1 pathway and the AMPK/NF-κB pathway reduces fat synthesis and inflammatory responses, while activating the AMPK/Nrf2 pathway alleviates oxidative stress (Gao et al., 2023b). Kaempferol (KAP) primarily promotes the SIRT1/AMPK pathway, which leads to reduced hepatic lipogenesis and enhanced FAO (Li et al., 2023). 6-Gingerol (6-G) is one of the most biologically potent metabolites in *Zingiber officinale*, exhibiting anti-inflammatory, antioxidant, and anticancer pharmacological activities. STE20-related adapter (STRAD) and MO25 can form a complex with AMPK, promoting the cytosolic distribution and kinase activity of LKB1. Studies have shown that 6-G can induce the activation of the LKB1/AMPK pathway cascade by regulating the stability of the LKB1/STRAD/MO25 complex and activating LKB1, ultimately alleviating MAFLD (Liu Y. et al., 2023). Apigenin (AGL) is an edible flavonoid derived from plants, possessing various biological activities with significant potential applications in cancer and dermatological conditions (Imran et al., 2020; Yoon et al., 2023). Current research has also highlighted its value in treating MAFLD. Previous studies have demonstrated that phosphorylation of perilipin 2 (PLIN2) is a prerequisite for participating in chaperone-mediated autophagy (CMA), while LAMP-2A serves as a rate-limiting enzyme in the degradation of CMA-regulated substrate proteins. In a recent study by Lu J et al., it was found that AGL enhances CMA activity by activating AMPK, which promotes PLIN2 phosphorylation and Nrf2 nuclear translocation, and upregulates LAMP-2A protein. These effects ultimately facilitate lipid droplet degradation and improve MAFLD (Lu et al., 2024). Hesperitin (HES) alleviates MAFLD by reducing the expression of dynamin-related protein 1 (Drp1) through an AMPKα-dependent mechanism, phosphorylating Drp1 at serine 616 (Drp1-pS616), inducing phosphorylated Drp1 at serine 637 (Drp1-pS637), and enhancing mitochondrial autophagy through the induction of PTEN-induced kinase 1 (PINK1) and E3 ubiquitin protein ligase Parkin (Parkin) (Chen et al., 2025a). Many other types of flavonoids can improve hepatic lipid homeostasis by stimulating AMPK and regulating upstream and downstream molecular targets.

3.3 Lignans

Lignans are secondary metabolites with phytoestrogenic physiological activity that are widely present in plants and human food sources, and have a variety of biological activities, including antibacterial, antiviral, antitumor, antiplatelet, antioxidant, and immunosuppressive activities (Fang and Hu, 2018). Lignans have also been found to have a promising role in the prevention and treatment of MAFLD. The specific molecular mechanisms by which three lignan phytomonomers target the AMPK pathway to improve MAFLD are illustrated in Figure 4.

Honokiol (HK) is an active substance isolated from Magnoliaceae species, existing as a fine brown to white powder. It demonstrates antibacterial, anti-inflammatory, oxidative stress-reducing, and cancer-inhibiting activities (Rauf et al., 2018). Emerging evidence suggests that HK exerts hepatoprotective

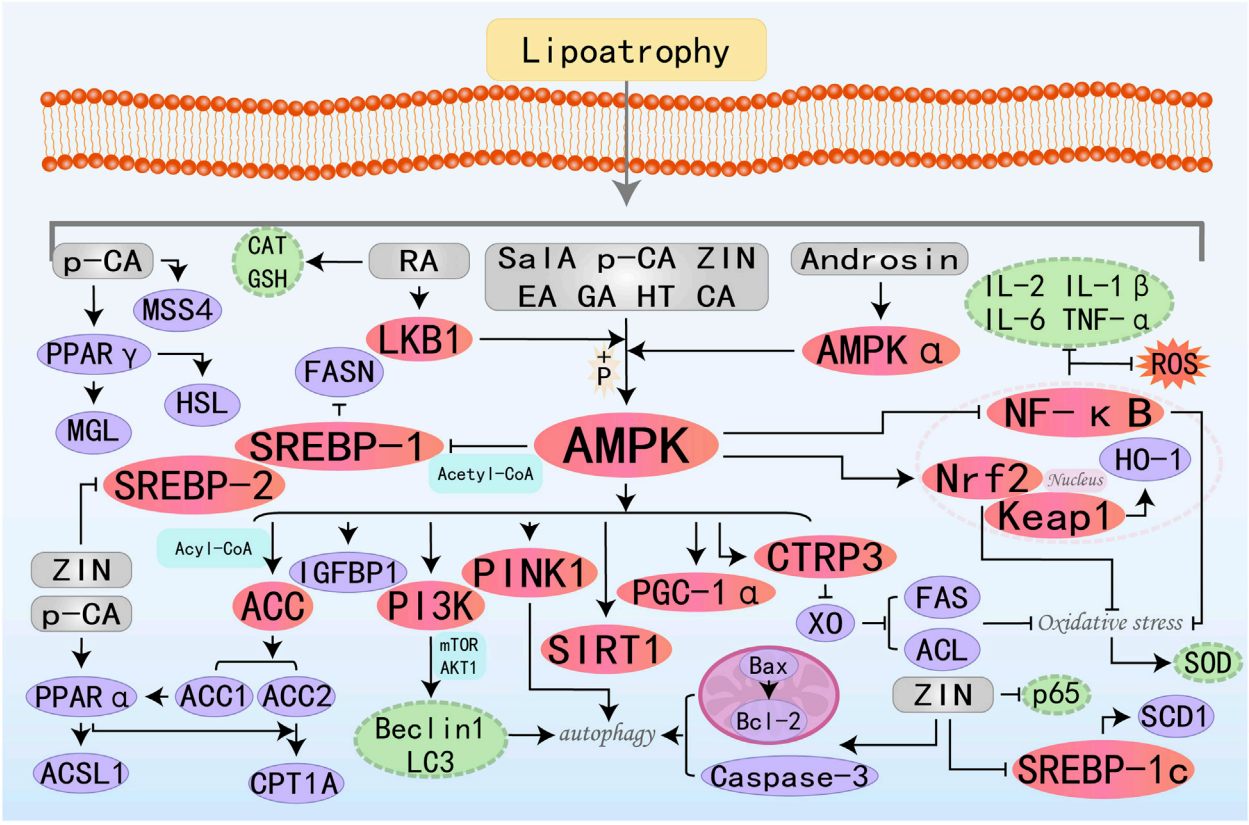


FIGURE 5
Molecular mechanism of phenolic acid metabolites from botanical drugs targeting AMPK to treat MAFLD. Sal A, Salvianolic acid A; HT, Hydroxytyrosol; EA, Ellagic acid; ZIN, Zingerone; GA, Gallic acid; *p*-CA, *p*-Coumaric Acid; RA, Rosmarinic Acid; CA, Chicoric Acid.

effects by modulating lipid metabolism and mitigating oxidative stress associated with hepatic steatosis. Liu J et al. demonstrated that HK confers hepatoprotection against lipotoxic injury by enhancing SIRT3-AMPK-mediated autophagic flux and preserving mitochondrial ultrastructure integrity. These findings establish HK as a promising pharmacological candidate for MAFLD management (Liu et al., 2021b). Interestingly, another study has also found that HK-mediated activation of the AMPK complex does not depend on its classic upstream regulators, but rather directly binds to the AMPKγ1 subunit to act as an agonist of the AMPK complex, thereby modulating downstream molecules and ameliorating hepatic lipid accumulation (Tian et al., 2023). Schisandrin B (Sch B) is one of the most promising bioactive metabolites isolated from *Schisandra chinensis (Turcz.)*. Sch B has a wide range of promising applications in liver diseases and can ameliorate acute liver injury and MAFLD by activating autophagy, anti-inflammation, and direct regulation of adipocyte metabolism (Kwan et al., 2017; Ma et al., 2022; Li X. et al., 2023). The specific molecular mechanism of Sch B treatment of MAFLD may be related to the activation of the autophagy-lysosomal pathway by the AMPK/mTOR signaling axis and the promotion of FAO (Yan et al., 2022). Schisanhenol (SAL) is another lignan with antioxidant and anti-apoptotic properties (Han et al., 2019; Zhang et al., 2025b). MicroRNAs are short non-coding RNA molecules that regulate various biological pathways. Abnormal expression of mRNAs is strongly associated with disorders of glucose-lipid metabolism and contributes to many metabolic diseases, including obesity and MAFLD (Rottiers and Näär, 2012; Agbu and Carthew, 2021; Sun and Kemper, 2023). Research has demonstrated that SAL may activate the AMPK signaling pathway by inhibiting miR-802-mediated PRKAA1 repression, showing a promising therapeutic intervention for MAFLD (Li et al., 2024).

3.4 Phenolic acids

Phenolic acids represent a subclass of plant-derived metabolites defined by their aromatic systems bearing multiple hydroxyl substituents on a shared benzene ring, with C1-C6 and C3-C6 carbon frameworks constituting their primary structural variants. They are abundant in the seeds and peels of fruits as well as in the leaves of vegetables. Phenolic acids are extensively utilized in pharmaceutical formulations, dermatological products, and other fields, offering benefits such as oxidative stress mitigation, inflammation modulation, and carcinogenesis inhibition effects. Research findings indicate various phenolic acids can improve MAFLD. The specific molecular mechanisms by which nine phenolic acids treat MAFLD through the AMPK pathway are illustrated in Figure 5.

Salvianolic acid A (Sal A) exhibits anticancer, anti-inflammatory, and cardioprotection effects and is clinically used

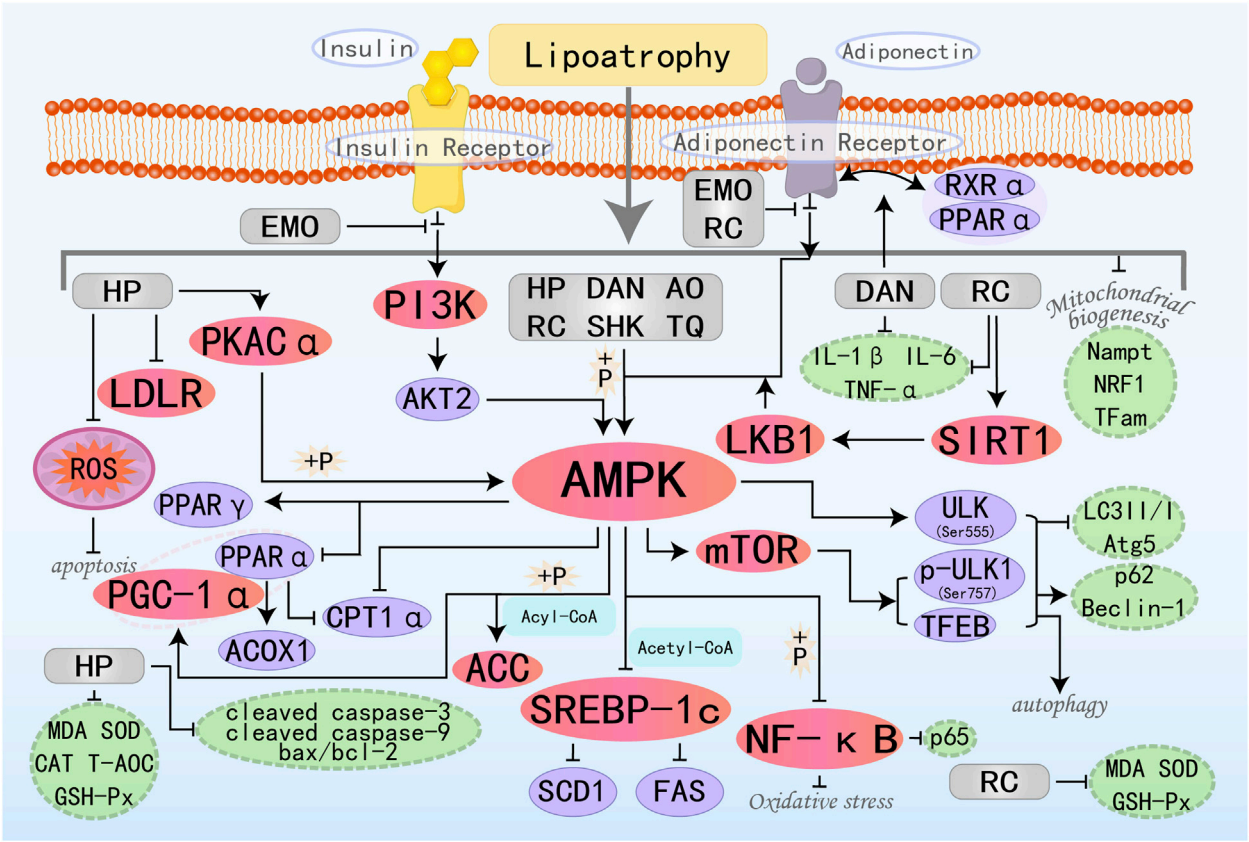


FIGURE 6
Molecular mechanism of quinone metabolites from botanical drugs targeting AMPK to treat MAFLD. HP, Hypericin; EMO, Emodin; DAN, Danthron; AO, Aurantio-Obtusin; RC, Rhinacanthin C; TQ, Thymoquinone; SHK, Shikonin.

to treat cancer and various metabolic diseases such as atherosclerosis and diabetes (Qin et al., 2019; Zhu et al., 2022). Notably, research suggested that Sal A exerts hepatoprotective effects through modulation of the AMPK-SIRT1 signaling axis, thereby counteracting hepatic lipid toxicity (Li et al., 2020). Insulin-like growth factors 1(IGF1) and 2 (IGF2) and IGF-binding proteins (IGFBPs) are produced by the liver to regulate metabolism through insulin. This carrier protein IGFBP-1 plays a pivotal role in governing the metabolic fate of IGF1, with its regulatory action directly impacting glycemic control mechanisms and the development of insulin resistance. Studies have shown that Sal A can improve hepatic fatty acid metabolism by activating the AMPK and IGFBP1 pathways. Additionally, activated AMPK can ameliorate inflammation, fibrosis, and mitochondrial dysfunction (Zhu et al., 2024). Hydroxytyrosol (HT) is the primary polyphenol contained in olive oil and leaves and can produce advantageous effects on MAFLD by regulating mitochondrial function. PINK1, as a protein kinase anchored to the mitochondrial membrane, plays a critical role in autophagy and activates parkin ubiquitin ligase activity to facilitate mitophagy. Studies have shown that HT can activate the AMPK/PINK1 pathway to promote mitophagy, thereby enhancing lipid metabolism, reducing oxidative stress, and mitigating mitochondrial dysfunction (Dong et al., 2022). Ellagic acid (EA) is a polyphenolic compound naturally abundant in dicotyledonous plant species, known for its potent anti-inflammatory and antioxidant capacities. C1q/tumor necrosis factor-related protein-3 (CTRP3) is a widely distributed and functionally diverse adipokine that plays a crucial role in endocrine and metabolic diseases such as inflammatory responses, obesity, and type 2 diabetes. Research has found that EA has the potential to treat MAFLD by improving insulin resistance and reducing liver damage through the activation of the AMPK/CTRP3 pathway (Elseweidy et al., 2022). Zingerone (ZIN), isolated from ginger, is a highly effective compound. Studies have found that it can prevent liver deposition and steatosis induced by high-fat feeding in rats by activating the AMPK/Nrf2 axis to counteract oxidative stress and increasing cleaved caspase-3 and Bax/Bcl2 ratios to promote autophagy (Mohammed, 2022). *p*-Coumaric Acid (*p* -CA) is a phenolic acid abundantly present in various edible plants including vegetables, fruits, and fungi, where it exhibits significant antineoplastic activity and oxidative stress-modulating capabilities. Research has found that p-CA reduces serum and liver lipids by activating PPARα/γ and upregulating HSL, HTGL, MGL, CPT1A, and ACSL1 through AMPK activation. Additionally, it inhibits lipid droplet fusion and growth by increasing MSS4 expression levels, thereby

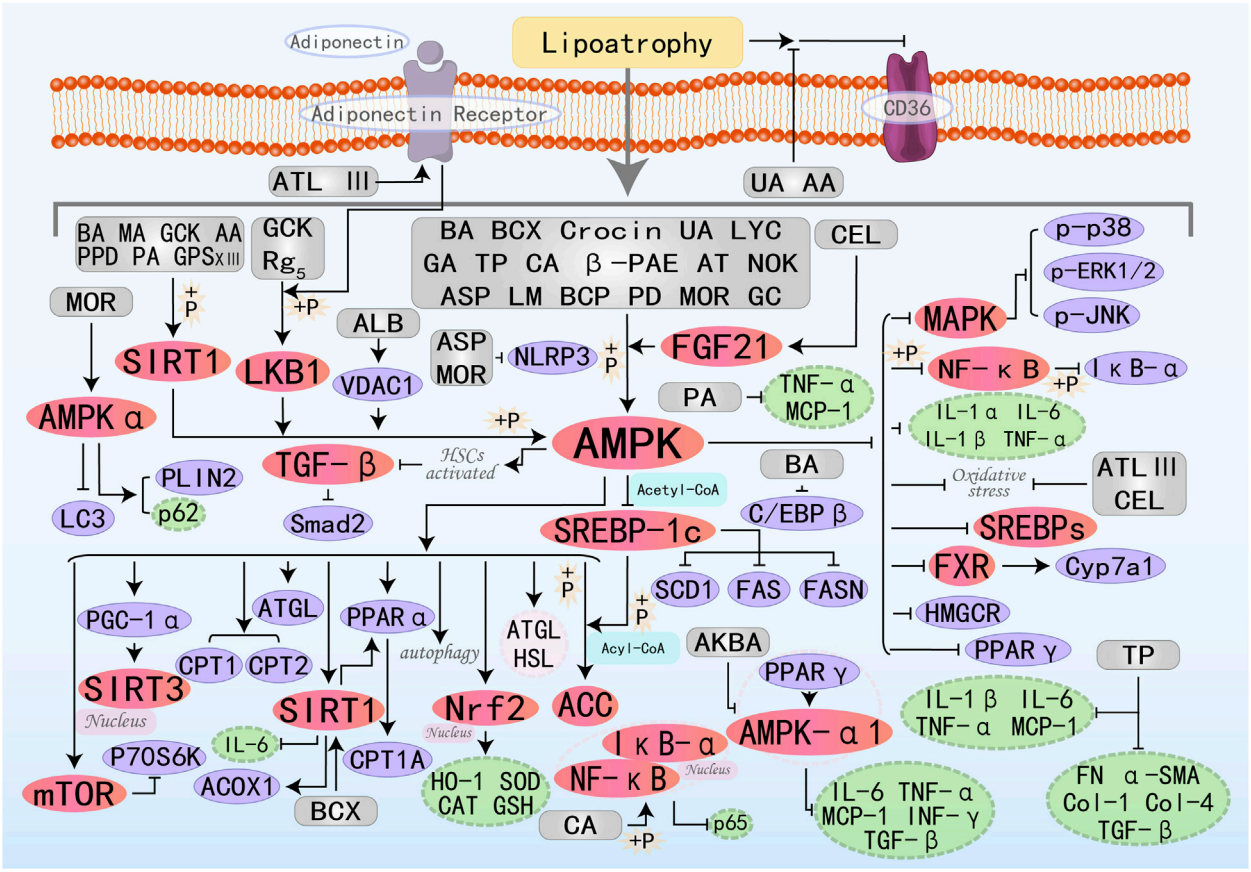


FIGURE 7
Molecular mechanism of terpenoid metabolites from botanical drugs targeting AMPK to treat MAFLD. BA, Betulinic acid; BCX, β-Cryptoxanthin; MA, Maslinic acid; UA, Ursolic acid; GA, Ganoderic acid A; LYC, Lycopene; TP, Triptolide; CA, Corosolic acid; PA, Patchouli alcohol; β-PAE, β-patchoulene; AA, Arjunolic acid; CEL, Celastrol; ATL III, Atractylenolide III; AT, Astaxanthin; LM, Limonin; NOK, Nootkatone; GCK, Ginsenoside CK; AKBA, Acetyl-11-Keto-Beta-Boswellic Acid; PPD, Protopanaxadiol; ASP, Asperuloside; GPS XIII, Gypenoside XIII; ALB, Alisol B; BCP, β-Caryophyllene; Rg_5, Ginsenoside Rg_5; PD, Platycodon D; MOR, Morroniside; GC, Ginkgolide C.

treating MAFLD (Yuan et al., 2023a). Studies have found that Androsin alleviates metabolic-associated fatty liver disease (MAFLD) by stimulating AMPKα, which in turn activates the SREBP-1c/FASN pathway to inhibit DNL and the AMPKα/PI3K/Beclin1/LC3 pathway to activate autophagy (Singh et al., 2024). Rosmarinic Acid (RA) and Chicoric Acid (CA) primarily treat metabolic-associated fatty liver disease (MAFLD) by activating AMPK, which inhibits lipogenesis, promotes fatty acid β-oxidation, and improves oxidative stress and inflammation (Ding et al., 2020a; Kim M. et al., 2020).

3.5 Quinones

Quinones are natural products widely distributed in nature, collectively referring to a class of organic metabolites containing cyclohexadienedione or cyclohexadienedimethylene structures. Quinones can trigger cytoprotective effects through multiple mechanisms: activation of detoxification enzyme systems, modulation of anti-inflammatory signaling pathways, and remodeling of intracellular redox homeostasis. Studies have found that quinones also have some therapeutic potential for MAFLD (Bolton and Dunlap, 2017). The specific molecular mechanisms by which seven quinone substances treat MAFLD through the AMPK pathway are illustrated in Figure 6.

Emodin (EMO) is an anthraquinone derivative derived from various herbal medicines. EMO possesses a wide range of pharmacological properties, including anticancer, hepatoprotective, anti-inflammatory, and antioxidant activities (Dong et al., 2016). Research by Yu LY et al. has found that EMO is the principal bioactive component in *Radix Polygoni Multiflori Preparata* (RPMP) for the therapy of MAFLD. EMO can reduce hepatic lipogenesis and increase insulin sensitivity to combat insulin resistance (IR) by upregulating phosphatidylinositol 3-kinase (PI3K), AKT2, and AMPKα. Additionally, EMO can promote the binding of adiponectin to AdipoR2, thereby activating AMPK-mediated FAO, ultimately improving hepatic lipid accumulation (Yu et al., 2020). Danthron (DAN) is one of the active metabolites found in the Chinese herbal medicine *Rheum rhaponticum L.* Previous studies have shown that DAN can activate AMPK and regulate lipid and glucose metabolism *in vitro*, making it a potentially effective compound for the treatment of obesity and MAFLD (Zhou et al., 2013). PPARα, as a nuclear receptor, coordinates FAO and maintains mitochondrial homeostasis,

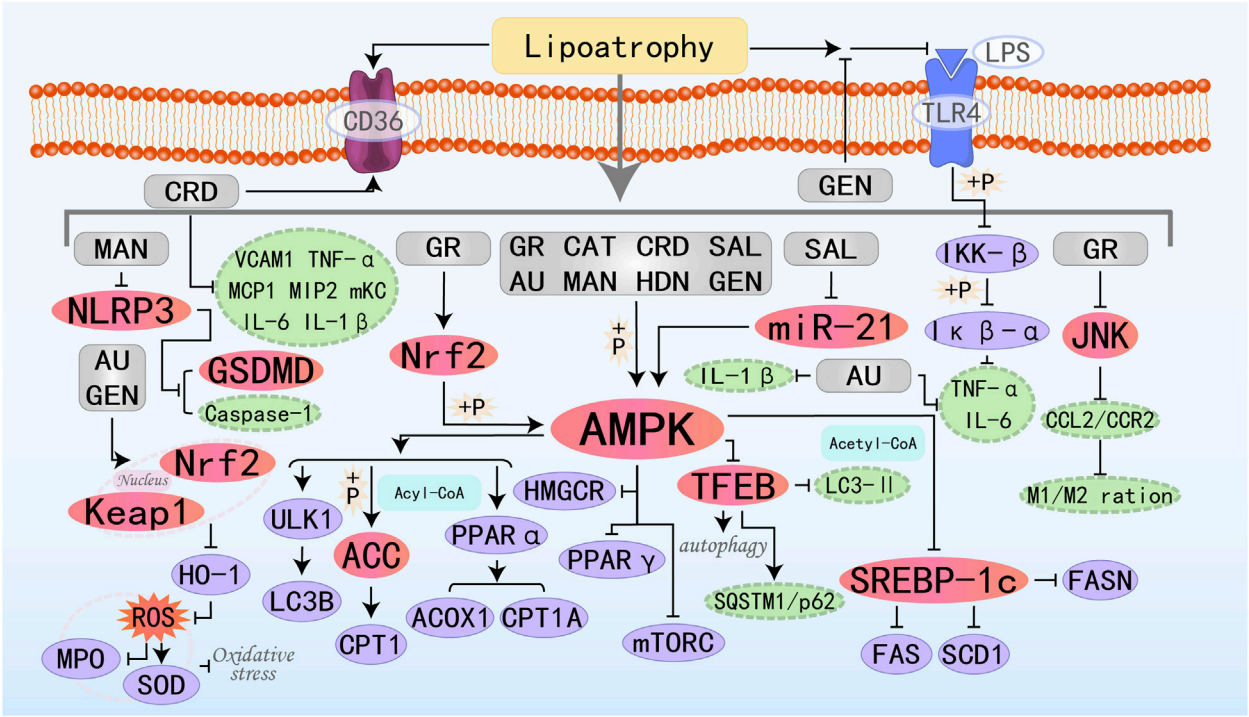


FIGURE 8
Molecular mechanism of glycoside metabolites from botanical drugs targeting AMPK to treat MAFLD. GR, Glucoraphanin; CAT, Catalpol; AU, Aucubin; CRD, Cordycepin; SAL, Salidroside; MAN, Mangiferin; HDN, Hesperidin; GEN, Geniposide.

while the retinoid X receptor (RXR) functions as the indispensable heterodimeric partner for PPARα activity. Recent research by Ma C et al. has found that DAN can enhance nuclear receptor crosstalk between PPARα and RXRα. Additionally, RXRα promotes the upregulation of AdipoR2 by DAN. The ultimately activated AdipoR2 then promotes the expression of AMPKα and PPARα, ultimately restoring mitochondrial biogenesis to enhance FAO (Ma et al., 2021). Aurantio-Obtusin (AO), primarily derived from cassia seed extract, is a major active ingredient within the anthraquinone class. Studies have confirmed that AO activates autophagy and improves lipid accumulation in the liver by upregulating the expression of a series of autophagy-related proteins, including AMPK, mTORC1, ULK, and TFEB (Zhou et al., 2022). Thymoquinone (TQ) is the primary active ingredient isolated from Nigella sativa, and numerous previous *in vivo* and *in vitro* studies have demonstrated its diverse pharmacological activities, including anti-inflammatory, anti-cancer, antioxidant, and neuroprotective effects (Woo et al., 2012; Mahmoud and Abdelrazek, 2019; Isaev et al., 2020). Recent research has found that TQ also holds great potential in the treatment of MAFLD. ULK1 is an autophagy-initiating kinase that can be oppositely regulated by mTOR and AMPK to initiate autophagy (Alers et al., 2012). Studies by Zhang D et al. have shown that TQ triggers autophagy through the activation of the AMPK/ULK1(Ser555) and AMPK/mTOR/ULK1(Ser757) pathway-dependent mechanisms, thereby reducing body weight, alleviating hepatic steatosis, and improving glucose homeostasis (Zhang D. et al., 2023). Shikonin (SHK) is a natural active ingredient with anti-inflammatory and antioxidant properties. Research has found that SHK can act as an AMPK agonist, activating AMPK to inhibit fat synthesis while also promoting the cooperation between PGC-1α and PPARα, inducing mitochondrial FAO. It has certain preventive and therapeutic effects on liver lipid metabolism and MAFLD (Gwon et al., 2020).

3.6 Terpenoids

Terpenoids are one of the most extensive and structural variability classes of both essential and specialized metabolites in nature. All terpenoids are formed by linking multiple isoprene units in a head-to-tail manner and exhibit a range of effects, including anti-inflammatory, antioxidant, antitumor, and immunomodulatory properties (Bergman et al., 2024; Khan et al., 2024). The specific molecular mechanisms by which 28 terpenoids treat MAFLD through the AMPK pathway are illustrated in Figure 7.

β-Cryptoxanthin (BCX) is a provitamin A carotenoid with diverse biological activities, capable of treating numerous diseases, including neoplasm and osteoporosis. Latest research has found its significant potential in treating fatty liver disease (Yamaguchi, 2012; Burri et al., 2016; Clugston, 2023). β-Carotene-15,15′-oxygenase (BCO1) and β-carotene-9′,10′-oxygenase (BCO2) can cleave BCX to generate therapeutic metabolic derivatives, including vitamin A. Experimental evidence elucidates BCX's capacity to enhance hepatic lipid homeostasis via multi-target regulatory effects on the FXR-SIRT1-AMPK signaling axis, demonstrating significant

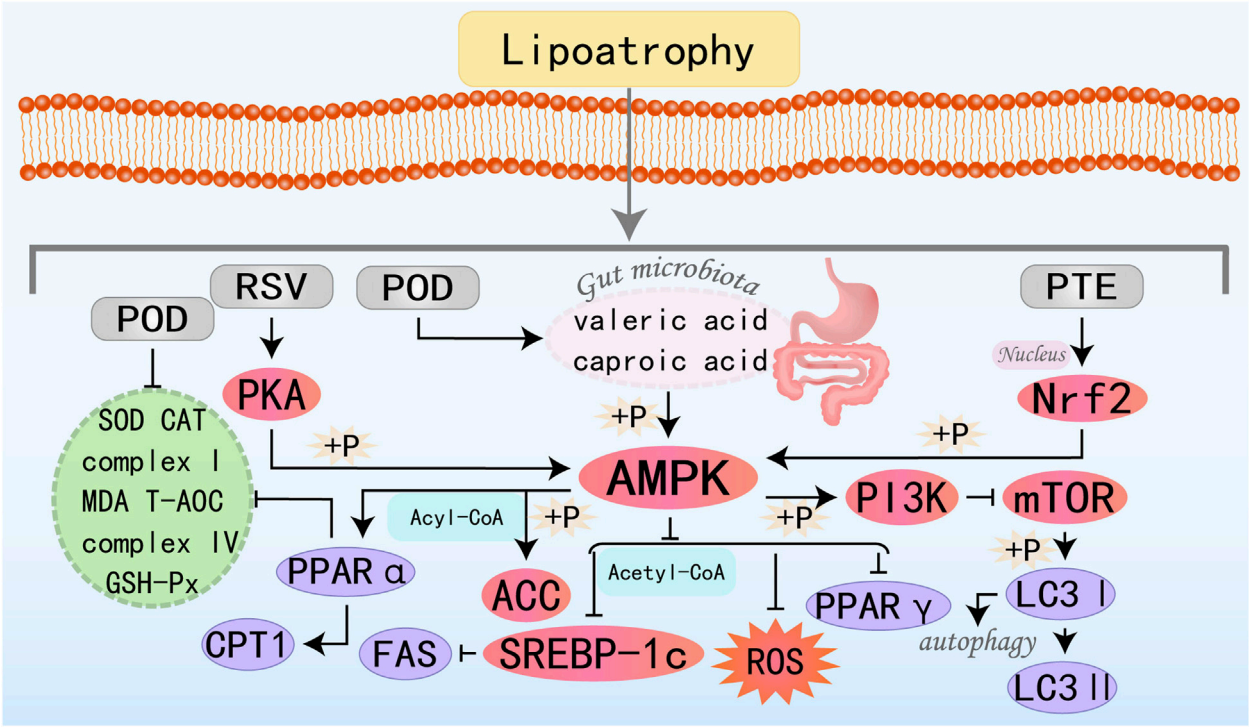


FIGURE 9
Molecular mechanism of stilbenes metabolites from botanical drugs targeting AMPK to treat MAFLD. RSV, Resveratrol; POD, Polydatin; PTE, Pterostilbene.

cholesterol metabolic modulation. Specifically, it depends on the presence or absence of BCO1/BCO2 (Lim et al., 2019). Maslinic acid (MA) (Liou et al., 2019a), Crocin (Luo et al., 2019), and Ursolic acid (UA) (Cheng et al., 2020) all improve hepatic steatosis and treat MAFLD by activating the AMPK pathway, promoting fatty acid oxidation, lipolysis, and inhibiting fat synthesis. Lycopene (LYC) is a lipophilic antioxidant carotenoid derived from tomatoes. Research by Wang J et al. has demonstrated that LYC can reduce lipid synthesis, restore mitochondrial function, and ultimately decrease hepatic lipid accumulation to treat MAFLD by increasing PPARα expression and promoting the activation of the AMPK/SIRT1/PGC1α pathway (Wang J. et al., 2020). Patchouli alcohol (PA) is a characteristic tricyclic terpenoid compound naturally occurring in the *Pogostemon cablin*. Previous studies have shown that it has numerous effects, including anti-inflammatory, anti-cancer, anti-depressant, and anti-viral properties (Lee et al., 2020). Research by Pyun D et al. has found that PA also has potential in hepatic lipid metabolism: it activates the AMPK/SIRT1 pathway to inhibit cellular inflammation (such as TNF-α, MCP-1), improve insulin resistance (IRS-1, HOMA-IR index, IPGTT, and ITT), and positively regulate FAO (Pyun et al., 2021). Arjunolic acid (AA) exhibits antioxidant, anti-inflammatory, and free radical scavenging activities (Hemalatha et al., 2010). Recently, Zheng X et al. discovered that AA indirectly activates SIRT1/AMPK-regulated lipid metabolism by increasing NAMPT-mediated NAD + levels and triggering autophagy, collectively mediating lipid-lowering effects (Zheng et al., 2021). Celastrol (CEL) is a pentacyclic triterpenoid compound separated from the Chinese medicinal plant *Celastrus orbiculatus Thunb.*, possesses various pharmacological activities, including anti-tumor and anti-inflammatory effects. Currently, it is considered to have broad application prospects in metabolic diseases, such as in the treatment of type 2 diabetes, atherosclerosis, cholestasis, and osteoporosis (Xu S. et al., 2021). FGF21 is predominantly secreted by hepatic tissues to coordinate glycemic regulation across both hepatic and adipose metabolic networks, providing protective benefits. Recently, Xue JL et al. explored that CEL treatment can lead to improved mitochondrial morphology, liver lipid accumulation, oxidative stress, and inflammation by activating the FGF21/AMPK/PGC-1α signaling pathway, thereby protecting against MAFLD (Xue et al., 2024a). Atractylenolide III(ATL III) is a natural monomeric herbal bioactive compound with extensive effects in antioxidation and anti-inflammation (Xu et al., 2023). Li Q et al. discovered that ATL III treatment *in vitro* activates the AMPK/SIRT1 signaling pathway downstream of AdipoR1, thereby enhancing oxidative stress resistance (SIRT3, NRF2) and FAO(CPT1A, PGC-1α), ultimately protecting the liver (Li et al., 2022c). Ginsenoside CK(GCK) is the primary intestinal metabolite of protopanaxadiol saponins and exhibits multiple therapeutic effects. Zhang JJ et al. found that GCK can activate LKB1 and AMPK phosphorylation, increase ATGL and SIRT1 expression, and inhibit SREBP-1c activity, thereby promoting lipolysis and FAO while suppressing fat synthesis (Zhang J. J. et al., 2022). Both Protopanaxadiol (PPD) (Li Y. et al., 2023) and Gypenoside XIII(GPS XIII) (Cheng et al., 2024) can reduce lipogenesis, increase lipolysis, and enhance fatty acid β-oxidation by increasing SIRT1 and AMPK phosphorylation, thereby regulating downstream molecules. Alisol B (ALB) is a triterpenoid monomer

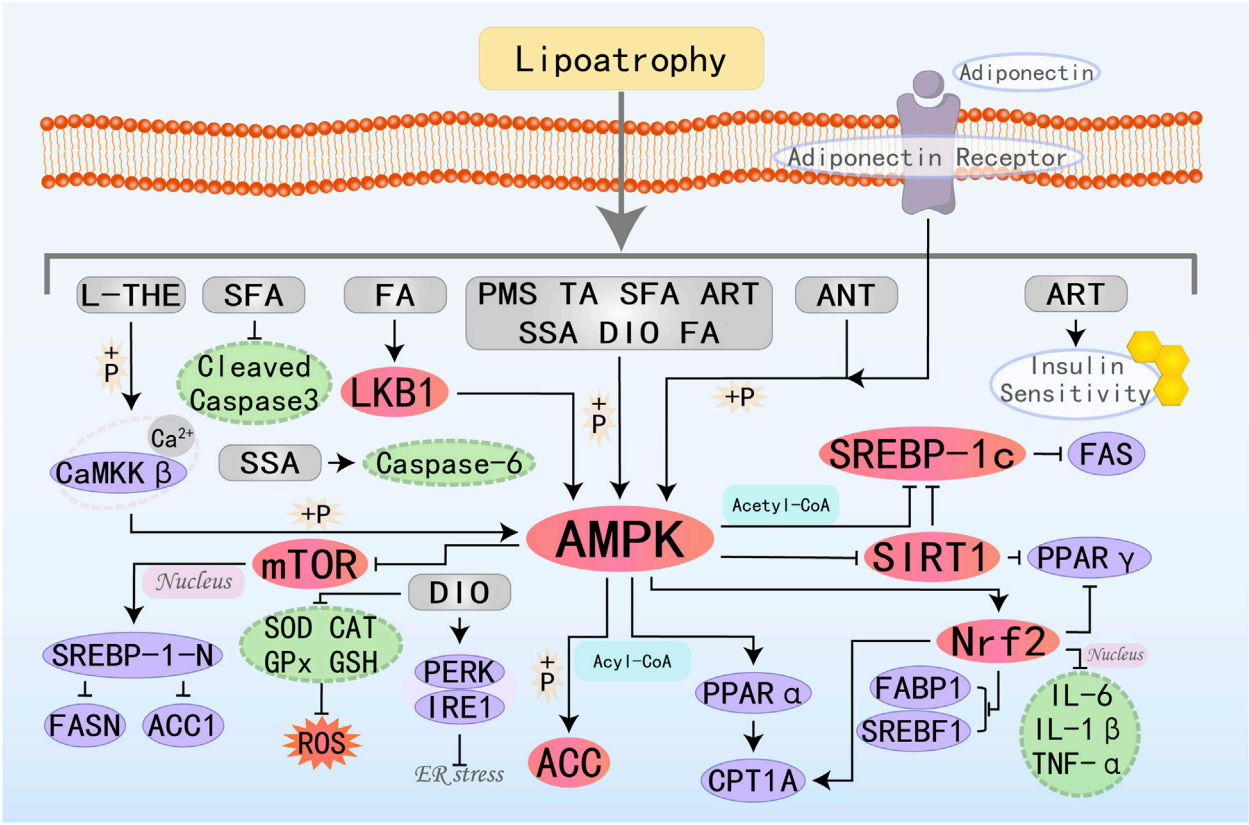


FIGURE 10
Molecular mechanism of other metabolites from botanical drugs targeting AMPK to treat MAFLD. L-THE, L-theanine; PMS, Plantamajoside; TA, Tartaric acid; SFA, Sulforaphane; ART, Atractylodin; ANT, Antrodan; SSA, Salsalate; DIO, Diosgenin; FA, Folic acid.

isolated from classic Chinese medicinal herbs, which plays a role in inhibiting lipogenesis and reducing subcutaneous adipose tissue mass. VDAC1, a voltage-dependent anion channel protein, maintains the balance of the intracellular and extracellular environment by regulating mitochondrial permeability. Gao G et al. found that ALB directly targets VDAC1 to increase the ADP/ATP and AMP/ATP ratios, thereby modulating the AMPK/mTOR/SREBPs pathway to inhibit lipid synthesis (Gao et al., 2024). Ginsenoside Rg5 (Rg5) activates the LKB1/AMPK/mTOR signaling pathway, stimulating energy metabolism and thereby impeding the progression of MAFLD (Shi et al., 2024). Morroniside (MOR) inhibits the progression of NASH by promoting AMPK-dependent lipophagy and inhibiting NLRP3 inflammasome activation (Zhang et al., 2024). β-Caryophyllene (BCP) (Kamikubo et al., 2024) and Ginkgolide C (GC) (Xie et al., 2024) can act as AMPK agonists, regulating downstream molecules involved in lipid synthesis and FAO by activating the AMPK signaling pathway, ultimately improving MAFLD.

3.7 Glycosides

Glycosides, also known as "saponins" or "glycosides," are molecules in which one part is linked to a sugar moiety, while the other part, which is non-sugar, is called the aglycone. Glycosides exhibit extensive application prospects in the medical field, demonstrating favorable pharmacological effects in regulating blood glucose and lipids, as well as possessing antitumor activity, which can be used as adjuvant therapy for cancer. The specific molecular mechanisms by which eight glycosides treat MAFLD through the AMPK pathway are illustrated in Figure 8.

Catalpol (CAT), is a functional substance derived from *Rehmannia glutinosa*. It is commonly used in the treatment of various inflammatory diseases, diabetes, cardiovascular and cerebrovascular diseases, among others (Yan et al., 2018; Bhattamisra et al., 2021; Liu J. et al., 2023; Zhang Z. et al., 2023). Transcription Factor EB (TFEB) is a master regulator whose activation promotes the transcription of genes involved in lysosomal network expansion and regulates autophagy when translocated to the nucleus. CAT can significantly upregulate autophagy-related genes (including Atg7, Atg5, Becn1, Ulk1, and Lamp1) to induce autophagy and regulate hepatic lipid metabolism genes (ACC1α, FAS, PPARα, ACOX1, and CPT1) to improve hepatic lipid accumulation by activating the AMPK/TFEB pathway, and it is a novel therapeutic candidate for MAFLD (Ren et al., 2019; Tian et al., 2020). Aucubin (AU) is an iridoid glycoside derived from natural plants, possessing anti-inflammatory, antioxidant, and anti-fibrotic properties. It has broad application potential in the treatment of atherosclerosis, fatty liver disease, acute hepatitis, diabetes, and other conditions

(Wang H. et al., 2020; Bao et al., 2022; Huang et al., 2022; Liu et al., 2022). Shen B et al. found that the protective effect of AU on MAFLD may be exerted by promoting the escape of Nrf2 from the control of Keap1 and its translocation to the nucleus, which in turn inhibits oxidative stress (reduction of ROS, increase in SOD levels, and decrease in MPO levels) (Shen et al., 2019b). Cordycepin (CRD) is a bioactive compound extracted from Cordyceps sinensis, possessing multiple pharmacological effects. Recent *in vivo* studies have also shown that CRD primarily treats MAFLD by activating AMPK, regulating the expression of key genes related to lipid metabolism (such as SREBP1-c, ACC, SCD-1, LXRα, and CD36), as well as β-oxidation genes (CPT-1 and PPARα), and improving the inflammatory state (Gong et al., 2021). Salidroside (SAL) is also an AMPK agonist that prevents the progression of NASH induced by metabolic stress, inflammation, and other factors by activating AMPK signaling (Hu et al., 2021). Geniposide, an iridoid glycoside extracted from the fruit of Gardenia jasminoides, holds great potential in improving glucose and lipid metabolism (Gao and Feng, 2022). Research by Yi M et al. has demonstrated that GEN can inhibit the inflammatory response induced by LPS directly binding to TLR4 protein and activating NF-κB. Additionally, it regulates lipid metabolism through the AMPK/ACC/CPT1A and AMPK/ULK1/LC3B signaling pathways, thereby preventing and treating MAFLD (Yi et al., 2023).

3.8 Stilbenes

Stilbenoids constitute a group of metabolites defined by their stilbene core structure or polymeric derivatives, functioning as phenolic secondary metabolites in plants. Among these, the most widely recognized is resveratrol, known for its roles as a cardioprotective agent, potent antioxidant, anti-inflammatory agent, and anticancer agent, among others. Current research on the stilbene scaffold continues with the aim of discovering new analogs with higher bioavailability. The specific molecular mechanisms by which three stilbenoid metabolites defend against MAFLD through the AMPK pathway are illustrated in the accompanying Figure 9.

Resveratrol (RSV), a polyphenolic compound of significant research interest, exhibits pleiotropic effects such as quenching oxidative stress, resolving inflammatory cascades, reducing blood pressure and blood sugar levels, combating aging, and exerting anticancer effects. Clinically, it can be utilized in the treatment of metabolic diseases, cardiovascular diseases, and various types of tumors (Ren et al., 2021; Zhang et al., 2021a; Zhou et al., 2021). Research by Huang YJ et al. has shown that RSV modulates lipid metabolism and redox homeostasis by regulating CPT-1, SREBP-1c, and FAS through the PKA/AMPK/PPARα pathway, indicating significant potential for the prevention and treatment of MAFLD (Huang et al., 2020). Polydatin (POD), a glucoside derivative of RSV, boasts higher bioavailability and is primarily involved in modulating homeostatic regulation such as inflammation, oxidative stress, and apoptosis. It plays a significant role in the prevention and treatment of tumors, cardiovascular diseases, and metabolic disorders including diabetes, NASH, and fibrosis (Li et al., 2018; Karami et al., 2022). Research by Zhao G et al. has discovered that POD can significantly elevate the levels of valeric acid and caproic acid in feces by modulating the gut microbiota, thereby activating the AMPK. This activation leads to a reduction in lipid accumulation in the liver and serum, thereby ameliorating MAFLD (Li et al., 2018). Pterostilbene (PTE) is a dimethylated analog of RSV, endowed with physiological activities such as anti-inflammatory, antioxidative stress, and anticancer properties (Estrela et al., 2013; Kim H. et al., 2020; Lin et al., 2020; Gómez-Zorita et al., 2021). Recent research by Shen B et al. has revealed that PTE promotes the nuclear translocation of Nrf2, induces AMPK phosphorylation through Nrf2, and subsequently promotes ACC phosphorylation while inhibiting mTORC, among others. Ultimately, it enhances autophagy, suppresses oxidative stress, and promotes the metabolism and breakdown of fatty acids, thereby ameliorating MAFLD (Shen et al., 2023a).

3.9 Others

In addition to the eight bioactive metabolites extracted from phytomedicines mentioned above, other types of natural active monomers have also been found to improve MAFLD. The specific molecular mechanisms by which nine other metabolites defend against MAFLD through the AMPK pathway are illustrated in Figure 10.

L-Theanine (L-THE), a natural component derived from tea, exhibits immune-regulatory and sedative effects and is commonly used in the management of many psychiatric disorders (Chen S. et al., 2023). Research by Liang J et al. has demonstrated that L-theanine ameliorates hepatic steatosis by boosting the Ca^{2+}-CaMKKβ-AMPK signaling pathway, thereby modulating hepatocyte lipid metabolism pathways (Liang J. et al., 2022). Plantamajoside (PMS), the primary active ingredient in *Plantago asiatica L.*, exhibits various biological activities. It can improve immune dysregulation (downregulate IL-6, IL-1β, TNF-α), reduce fatty acid uptake (downregulate FABP1), and ameliorate abnormal liver lipid metabolism (inhibit SREBF1, PPARγ) in MAFLD rats by activating the AMPK/Nrf2 pathway, thereby treating MAFLD (Wu et al., 2023). Antrodan (ANT) has been shown to effectively alleviate MAFLD through the AMPK/SIRT1/SREBP-1c/PPARγ pathway (Chyau et al., 2020). Diosgenin (DIO) is a natural saponin, and research findings consistently highlight that DIO can treat metabolic diseases through various pathways and mechanisms (Zhang S. Z. et al., 2022). DIO inhibits DNL and increases FAO by modulating the AMPK-ACC/SREBP1 pathway. Additionally, it can suppress ER stress by regulating the PERK and IRE1 branches, reduce ROS by increasing levels of SOD, CAT, and GPx, and enhance antioxidant capacity, thereby offering therapeutic benefits for MAFLD (Zhong et al., 2022).

4 Discussion

MAFLD is a term introduced in 2020 as an improvement over the previous nomenclature of NAFLD, aiming to capture the metabolic essence more precisely. In terms of diagnosis, MAFLD is identified based on metabolic abnormalities such as type 2 diabetes, obesity, or metabolic syndrome, and it is compatible with other hepatic disorders (e.g., viral hepatitis), making the diagnosis more comprehensive and practical. From a clinical perspective, the diagnostic criteria for MAFLD are better at

recognizing populations susceptible to liver fibrosis and metabolic complications and are more intuitive, which helps to enhance understanding of the disease among the public and primary care physicians. The pathogenesis of MAFLD is highly complex, involving multiple processes such as DNL, FAO, oxidative stress, inflammatory responses, autophagy, and endoplasmic reticulum stress. These factors interact, ultimately leading to hepatic fat deposition, inflammation, and fibrosis, which may progress to cirrhosis and hepatocellular carcinoma. It is the complexity and diversity of MAFLD pathogenesis and disease progression that makes drug therapy a challenge, including the difficulty of achieving significant efficacy with single-mechanism drugs, the lack of drugs that are effective at all stages of the disease, the difficulty of identifying drug targets, and the side effects and safety issues of drugs. Consequently, future studies should prioritize investigating the synergy of multidrug combinations to optimize treatment outcomes and minimize adverse reactions, as well as continuing the search and development of novel drug targets directing the pathogenesis of MAFLD. AMPK is a crucial regulatory enzyme for glucose and lipid metabolism, capable of modulating energy metabolism, lipid metabolism, and glucose metabolism. Therefore, it holds potential application value in the treatment of MAFLD. Despite progress, pharmacological interventions targeting AMPK for MAFLD management are still in early-phase development. Compared to Western medicine, natural plant medicine has garnered growing interest in the treatment of MAFLD due to its multi-target, multi-pathway mechanisms of action, as well as its minimal side effects and cost-effectiveness. Emerging evidence from recent investigations suggests natural active botanical metabolites functioning as AMPK activators could represent novel strategies for both prophylactic and therapeutic management of MAFLD. With this goal in view, this study summarizes the *in vivo* and *in vitro* experimental literature from the past 5 years on Natural Active Botanical metabolites improving MAFLD by targeting the AMPK pathway. In this review, we conclude that natural active botanical metabolites ameliorate hepatic lipid accumulation and degeneration, ultimately treating MAFLD, by activating AMPK and its related pathways, inhibiting lipogenesis, exerting anti-inflammatory and antioxidant effects, promoting fatty acid oxidation, lipolysis, autophagy, and improving insulin resistance.

Specifically, natural active botanical metabolites can activate AMPK and increase its phosphorylation by modulating upstream molecules such as kinases (e.g., LKB1 and CaMKK2), energy sensors (AMP), and other upstream regulators (e.g., leptin, adiponectin). They can also directly activate AMPK, thereby inhibiting the activity of lipogenic gene targets like ACC, HMGR, SCD1, and SREBP-1c, and promoting the expression of genes involved in FAO such as PPARα, CPT1α, and ACOX1. Additionally, they improve mitochondrial function, restore mitochondrial homeostasis, regulate oxidative stress-related factors (e.g., ROS, MDA, SOD, GSH-Px, CAT), and modulate autophagy factors (e.g., Bax, p62, cleaved caspase 3, Bcl-2, Atg7, LC3II/I, Beclin1). They also reduce inflammatory cytokines like IL-1β, IL-6, TNF-α, and MCP-1, suppress endoplasmic reticulum stress (downregulating BiP, ATF6, CHOP, ERK, JNK, etc.), promote the expression of HSL and ATGL to accelerate lipolysis, and regulate AUC of ITT, GTT, HOMA-IR index, fasting blood glucose, insulin level and so on to improve the body's insulin resistance, and finally play a role in the treatment of MAFLD. In addition, α-SMA, TGF-β, Col-1, and Col-4 can be regulated to prevent liver fibrosis, prevent the risk of complications, and improve the patient's quality of life and prognosis. The pathways involved are diverse, including AMPK/SREBP1, AMPK/ACC, AMPK/PGC-1α, LKB1/AMPK, SIRT1/AMPK, AMPK/mTOR, AMPK/ULK1, AMPK/NF-κB, AMPK/Nrf2, AMPK/PINK1, and TGFβ-Smad2/3. Natural plant medicine, with its multi-pathway, multi-level, and multi-target characteristics, acts on various links within these pathways, forming a complex network that collectively promotes the alleviation of MAFLD.

Our study demonstrates that natural active botanical metabolites currently targeting MAFLD treatment are predominantly clustered in flavonoids and terpenoids. Flavonoids typically exert their therapeutic effects through multi-target mechanisms, with particularly significant roles in modulating oxidative stress (such as baicalein, baicalin, neohesperidin, and hesperetin), regulating lipid metabolism, and combating inflammatory pathways (such as quercetin, chrysin, and 6-Gingerol). However, we do not know which flavonoids are the most effective or which ones are suitable for dietary therapy. Therefore, more clinical trials are needed to validate this. Our study also demonstrates that multiple terpenoids exhibit a certain degree of convergence in their targeted signaling pathways and molecular mechanisms when treating MAFLD. For example, by modulating PPAR-α, PPAR-γ, Nrf2, and SIRT1, they exert antioxidant, anti-inflammatory, and hepatoprotective effects. Furthermore, alkaloids, phenolic acids, and quinones also demonstrate considerable therapeutic potential, while lignans and stilbenes remain relatively understudied, warranting focused exploration as promising yet underexplored candidates.

Furthermore, it is noteworthy that natural active botanical metabolites demonstrate promising therapeutic potential in the treatment of MAFLD, their clinical application faces multiple challenges: (1) physicochemical limitations: natural monomers often suffer from poor aqueous solubility and strong lipophilicity, leading to restricted oral bioavailability (primarily due to significant hepatic first-pass effects); (2) targeted delivery barriers: inefficient hepatic accumulation and nonspecific systemic distribution may result in off-target effects; (3) pharmacodynamic shortcomings: the lack of tissue-specific targeting capability hampers precise modulation of metabolic dysregulation pathways in the liver. These factors collectively impede their clinical translation. Emerging strategies such as hepatic-targeted drug delivery systems (HTDDS), derivatization, and structural modifications are progressively addressing these issues. These approaches not only enhance drug delivery efficiency and stability but also improve therapeutic efficacy and safety profiles (Tang et al., 2021). In typical situations, the solubility, absorption, and metabolism rates of dietary flavonoids are low, while flavonoid nanoparticles and flavonoid-metal ion complexes not only enhance their effects but also reduce the systemic toxicity side effects of the drugs, demonstrating great potential in the treatment of MAFLD (Selvaraj et al., 2014; Dobrzynska et al., 2020). For example, quercetin-iron complex nanoparticles can significantly improve the stability and solubility of quercetin while also enhancing its antioxidant capacity (Prestianni et al., 2023). Silybin is treated with nanotechnologies such as nanoparticles, liposomes, and nano-suspensions, as well as CD44 receptors, folic acid, vitamin A, and other liver-targeting

methods to treat various liver diseases more effectively (Wu et al., 2024). Based on these significant research findings, we are confident in the development prospects of individual metabolites of natural plant medicine in the field of MAFLD treatment.

Natural active botanical metabolites, leveraging their unique advantages of multi-pathway and multi-target actions, have demonstrated remarkable efficacy in improving MAFLD. These metabolites are not only economical and efficient but also exhibit relatively few side effects. In numerous experimental studies focused on weight control, liver tissue repair, and improvements in TC, TG, LDL-C, and HDL-C levels, natural active botanical metabolites have shown a broad and effective therapeutic dose range. However, there are still some pressing issues in current research in this field. Firstly, in developing approaches for the intervention and therapy of MAFLD, existing experimental studies primarily focus on therapeutic effects and their mechanisms, while assessments of the safety and toxicological characteristics of these metabolites are relatively inadequate. Therefore, there is an urgent need to strengthen systematic safety evaluations of individual metabolites from natural plant medicine and conduct standardized toxicity studies. Secondly, some individual metabolites from natural plant medicine that have been proven to have good therapeutic effects face technical challenges such as poor water solubility, low oral absorption efficiency, and unclear pharmacokinetic profiles, which severely limit their clinical translation and application. Although the development of novel drug delivery systems brings hope for improved liver-targeted drug delivery and enhanced bioavailability, current related research is still mainly confined to animal experiments and *in vitro* cellular studies. The absorption, distribution, metabolism, and excretion processes of these metabolites in humans still require further in-depth research and elucidation. It is particularly important to note that the study is primarily based on animal and cellular research, but lacks clinical trial data, which fails to validate the efficacy, safety, and optimal dosage of these components in humans. Thirdly, Currently, common MAFLD mouse models whether diet-induced (high-fat diet, high-cholesterol diet, methionine, and choline-deficient diet, high-fat, high-cholesterol diets and high-fat, high-fructose diets), genetic (db/db mice, ob/ob mice, and $ApoE^{-/-}$ mice), or chemically induced (CCl4 administration), have certain limitations and fail to meet the characteristics of an "ideal" MAFLD animal model. Furthermore, while some animal models can accurately replicate specific stages of MAFLD, they do not fully reproduce the entire human pathophysiological process. Finally, although natural active metabolites exert preventive and therapeutic effects on MAFLD by acting on the AMPK target, this process is regulated by multiple intertwined and closely related signaling pathways. All conclusions drawn in this study are solely based on the AMPK pathway, overlooking the complexity of the integrated regulatory network.

Given these research gaps, future studies should focus on: (1) advancing clinical translation to validate the efficacy and safety of natural metabolites in humans; (2) systematically investigating long-term toxicity, drug interactions, and effects in special populations; (3) developing nanocarriers and liver-targeting technologies to enhance bioavailability and minimize systemic side effects; and (4) integrating multi-omics approaches to comprehensively map mechanistic networks and clarify multi-target synergistic effects.

Taking all factors into consideration, this article summarizes the significant therapeutic effects of natural active botanical metabolites on MAFLD through targeted regulation of AMPK and its various specific pathway mechanisms. Additionally, we have also pointed out the deficiencies in current research regarding experimental design and subsequent development and application, as well as our future expectations. We believe that if these widely available, low-cost, and complex natural active botanical metabolites can be better utilized, they could provide new and reliable means for the treatment of MAFLD and even various metabolic diseases.

Author contributions

HW: Conceptualization, Data curation, Writing – original draft. XL: Conceptualization, Data curation, Writing – original draft. CW: Conceptualization, Writing – original draft. SY: Writing – review and editing. XY: Writing – review and editing. XC: Writing – review and editing. ML: Writing – review and editing. SL: Supervision, Writing – review and editing. CZ: Funding acquisition, Supervision, Writing – review and editing.

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Conflict of interest

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

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2025.1611400/full#supplementary-material

References

Agbu, P., and Carthew, R. W. (2021). MicroRNA-mediated regulation of glucose and lipid metabolism. *Nat. Rev. Mol. Cell Biol.* 22 (6), 425–438. doi:10.1038/s41580-021-00354-w

Alers, S., Löffler, A. S., Wesselborg, S., and Stork, B. (2012). Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: cross talk, shortcuts, and feedbacks. *Mol. Cell Biol.* 32 (1), 2–11. doi:10.1128/mcb.06159-11

Bao, X., Li, J., Ren, C., Wei, J., Lu, X., Wang, X., et al. (2022). Aucubin ameliorates liver fibrosis and hepatic stellate cells activation in diabetic mice *via* inhibiting ER stress-mediated IRE1α/TXNIP/NLRP3 inflammasome through NOX4/ROS pathway. *Chem. Biol. Interact.* 365, 110074. doi:10.1016/j.cbi.2022.110074

Basaranoglu, M., Basaranoglu, G., and Sentürk, H. (2013). From fatty liver to fibrosis: a tale of second hit. *World J. Gastroenterol.* 19 (8), 1158–1165. doi:10.3748/wjg.v19.i8.1158

Bergman, M. E., Kortbeek, R. W. J., Gutensohn, M., and Dudareva, N. (2024). Plant terpenoid biosynthetic network and its multiple layers of regulation. *Prog. Lipid Res.* 95, 101287. doi:10.1016/j.plipres.2024.101287

Bhambhani, S., Kondhare, K. R., and Giri, A. P. (2021). Diversity in chemical structures and biological properties of plant alkaloids. *Molecules* 26 (11), 3374. doi:10.3390/molecules26113374

Bhattamisra, S. K., Koh, H. M., Lim, S. Y., Choudhury, H., and Pandey, M. (2021). Molecular and biochemical pathways of catalpol in alleviating diabetes mellitus and its complications. *Biomolecules* 11 (2), 323. doi:10.3390/biom11020323

Bittla, P., Paidimarri, S. P., Ayuthu, S., Chauhan, Y. D., Saad, M. Z., Mirza, A. A., et al. (2024). Resmetirom: a systematic review of the revolutionizing approach to non-alcoholic steatohepatitis treatment focusing on efficacy, safety, cost-effectiveness, and impact on quality of life. *Cureus* 16 (9), e69919. doi:10.7759/cureus.69919

Bolton, J. L., and Dunlap, T. (2017). Formation and biological targets of quinones: cytotoxic *versus* cytoprotective effects. *Chem. Res. Toxicol.* 30 (1), 13–37. doi:10.1021/acs.chemrestox.6b00256

Burri, B. J., La Frano, M. R., and Zhu, C. (2016). Absorption, metabolism, and functions of β-cryptoxanthin. *Nutr. Rev.* 74 (2), 69–82. doi:10.1093/nutrit/nuv064

Cantó, C., Gerhart-Hines, Z., Feige, J. N., Lagouge, M., Noriega, L., Milne, J. C., et al. (2009). AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* 458 (7241), 1056–1060. doi:10.1038/nature07813

Chan, W. K., Chuah, K. H., Rajaram, R. B., Lim, L. L., Ratnasingam, J., and Vethakkan, S. R. (2023). Metabolic dysfunction-associated steatotic liver disease (MASLD): a state-of-the-art review. *J. Obes. Metab. Syndr.* 32 (3), 197–213. doi:10.7570/jomes23052

Chang, T. C., Chiou, W. C., Lai, W. H., Huang, H. C., Huang, Y. L., Liu, H. K., et al. (2021). Ugonin J improves metabolic disorder and ameliorates nonalcoholic fatty liver disease by regulating the AMPK/AKT signaling pathway. *Pharmacol. Res.* 163, 105298. doi:10.1016/j.phrs.2020.105298

Chen, C., Liu, X. C., and Deng, B. (2024). Protective effects of berberine on nonalcoholic fatty liver disease in Db/Db mice *via* AMPK/SIRT1 pathway activation. *Curr. Med. Sci.* 44 (5), 902–911. doi:10.1007/s11596-024-2914-y

Chen, S., Kang, J., Zhu, H., Wang, K., Han, Z., Wang, L., et al. (2023a). L-Theanine and immunity: a review. *Molecules* 28 (9), 3846. doi:10.3390/molecules28093846

Chen, S., Lu, H., Yin, G., Zhang, X., Meng, D., Yu, W., et al. (2025a). Hesperitin prevents non-alcoholic steatohepatitis by modulating mitochondrial dynamics and mitophagy *via* the AMPKα-Drp1/PINK1-Parkin signaling pathway. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1870 (1), 159570. doi:10.1016/j.bbalip.2024.159570

Chen, W., Xu, M., Xu, M., Wang, Y., Zou, Q., Xie, S., et al. (2022b). Effects of betaine on non-alcoholic liver disease. *Nutr. Res. Rev.* 35 (1), 28–38. doi:10.1017/s0954422421000056

Chen, W., Zhang, X., Xu, M., Jiang, L., Zhou, M., Liu, W., et al. (2021). Betaine prevented high-fat diet-induced NAFLD by regulating the FGF10/AMPK signaling pathway in ApoE(-/-) mice. *Eur. J. Nutr.* 60 (3), 1655–1668. doi:10.1007/s00394-020-02362-6

Chen, X., Fu, K., Lai, Y., Dong, C., Chen, Z., Huang, Y., et al. (2023b). Tetrahydropalmatine: orchestrating survival - regulating autophagy and apoptosis *via* the PI3K/AKT/mTOR pathway in perforator flaps. *Biomed. Pharmacother.* 169, 115887. doi:10.1016/j.biopha.2023.115887

Cheng, J., Liu, Y., Liu, Y. J., Liu, D., Liu, Y., Guo, Y. T., et al. (2020). Ursolic acid alleviates lipid accumulation by activating the AMPK signaling pathway *in vivo* and *in vitro*. *J. Food Sci.* 85 (11), 3998–4008. doi:10.1111/1750-3841.15475

Cheng, S. C., Liou, C. J., Wu, Y. X., Yeh, K. W., Chen, L. C., and Huang, W. C. (2024). Gypenoside XIII regulates lipid metabolism in HepG2 hepatocytes and ameliorates nonalcoholic steatohepatitis in mice. *Kaohsiung J. Med. Sci.* 40 (3), 280–290. doi:10.1002/kjm2.12795

Chyau, C. C., Wang, H. F., Zhang, W. J., Chen, C. C., Huang, S. H., Chang, C. C., et al. (2020). Antrodan alleviates high-fat and high-fructose diet-induced fatty liver disease in C57BL/6 mice model *via* AMPK/Sirt1/SREBP-1c/PPARγ pathway. *Int. J. Mol. Sci.* 21 (1), 360. doi:10.3390/ijms21010360

Clare, K., Dillon, J. F., and Brennan, P. N. (2022). Reactive oxygen species and oxidative stress in the pathogenesis of MAFLD. *J. Clin. Transl. Hepatol.* 10 (5), 939–946. doi:10.14218/jcth.2022.00067

Clugston, R. D. (2023). β-cryptoxanthin and fatty liver disease: new insights. *Hepatobiliary Surg. Nutr.* 12 (3), 450–452. doi:10.21037/hbsn-23-201

Cross, E., Dearlove, D. J., and Hodson, L. (2023). Nutritional regulation of hepatic *de novo* lipogenesis in humans. *Curr. Opin. Clin. Nutr. Metab. Care* 26 (2), 65–71. doi:10.1097/mco.0000000000000914

Cui, Y., Chen, J., Zhang, Z., Shi, H., Sun, W., and Yi, Q. (2023). The role of AMPK in macrophage metabolism, function and polarisation. *J. Transl. Med.* 21 (1), 892. doi:10.1186/s12967-023-04772-6

Czaja, M. J. (2016). Function of autophagy in nonalcoholic fatty liver disease. *Dig. Dis. Sci.* 61 (5), 1304–1313. doi:10.1007/s10620-015-4025-x

Ding, X., Jian, T., Li, J., Lv, H., Tong, B., Li, J., et al. (2020a). Chicoric acid ameliorates nonalcoholic fatty liver disease *via* the AMPK/Nrf2/NFκB signaling pathway and restores gut microbiota in high-fat-diet-fed mice. *Oxid. Med. Cell Longev.* 2020, 9734560. doi:10.1155/2020/9734560

Dobrzynska, M., Napierala, M., and Florek, E. (2020). Flavonoid nanoparticles: a promising approach for cancer therapy. *Biomolecules* 10 (9), 1268. doi:10.3390/biom10091268

Dong, X., Fu, J., Yin, X., Cao, S., Li, X., Lin, L., et al. (2016). Emodin: a review of its pharmacology, toxicity and pharmacokinetics. *Phytother. Res.* 30 (8), 1207–1218. doi:10.1002/ptr.5631

Dong, Y., Yu, M., Wu, Y., Xia, T., Wang, L., Song, K., et al. (2022). Hydroxytyrosol promotes the mitochondrial function through activating mitophagy. *Antioxidants (Basel)* 11 (5), 893. doi:10.3390/antiox11050893

Elseweidy, M. M., Elesawy, A. E., Sobh, M. S., and Elnagar, G. M. (2022). Ellagic acid ameliorates high fructose-induced hyperuricemia and non-alcoholic fatty liver in wistar rats: focusing on the role of C1q/tumor necrosis factor-related protein-3 and ATP citrate lyase. *Life Sci.* 305, 120751. doi:10.1016/j.lfs.2022.120751

Engin, A. (2024). Nonalcoholic fatty liver disease and staging of hepatic fibrosis. *Adv. Exp. Med. Biol.* 1460, 539–574. doi:10.1007/978-3-031-63657-8_18

Eslam, M., Newsome, P. N., Sarin, S. K., Anstee, Q. M., Targher, G., Romero-Gomez, M., et al. (2020a). A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J. Hepatol.* 73 (1), 202–209. doi:10.1016/j.jhep.2020.03.039

Eslam, M., Sanyal, A. J., George, J., and International Consensus Panel (2020b). MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* 158 (7), 1999–2014.e1. doi:10.1053/j.gastro.2019.11.312

Estrela, J. M., Ortega, A., Mena, S., Rodriguez, M. L., and Asensi, M. (2013). Pterostilbene: biomedical applications. *Crit. Rev. Clin. Lab. Sci.* 50 (3), 65–78. doi:10.3109/10408363.2013.805182

Fan, N., Zhao, J., Zhao, W., Zhang, X., Song, Q., Shen, Y., et al. (2022). Celastrol-loaded lactosylated albumin nanoparticles attenuate hepatic steatosis in non-alcoholic fatty liver disease. *J. Control Release* 347, 44–54. doi:10.1016/j.jconrel.2022.04.034

Fan, W., Pan, M., Zheng, C., Shen, H., Pi, D., Song, Q., et al. (2024). Leonurine inhibits hepatic lipid synthesis to ameliorate NAFLD *via* the ADRA1a/AMPK/SCD1 axis. *Int. J. Mol. Sci.* 25 (19), 10855. doi:10.3390/ijms251910855

Fang, C., Pan, J., Qu, N., Lei, Y., Han, J., Zhang, J., et al. (2022). The AMPK pathway in fatty liver disease. *Front. Physiol.* 13, 970292. doi:10.3389/fphys.2022.970292

Fang, X., and Hu, X. (2018). Advances in the synthesis of lignan natural products. *Molecules* 23 (12), 3385. doi:10.3390/molecules23123385

Feng, X., Sureda, A., Jafari, S., Memariani, Z., Tewari, D., Annunziata, G., et al. (2019). Berberine in cardiovascular and metabolic diseases: from mechanisms to therapeutics. *Theranostics* 9 (7), 1923–1951. doi:10.7150/thno.30787

Fischer, C., Seki, T., Lim, S., Nakamura, M., Andersson, P., Yang, Y., et al. (2017). A miR-327-FGF10-FGFR2-mediated autocrine signaling mechanism controls white fat browning. *Nat. Commun.* 8 (1), 2079. doi:10.1038/s41467-017-02158-z

Fouqueray, P., Bolze, S., Dubourg, J., Hallakou-Bozec, S., Theurey, P., Grouin, J. M., et al. (2021). Pharmacodynamic effects of direct AMP kinase activation in humans with insulin resistance and non-alcoholic fatty liver disease: a phase 1b study. *Cell Rep. Med.* 2 (12), 100474. doi:10.1016/j.xcrm.2021.100474

Friedman, S. L., Neuschwander-Tetri, B. A., Rinella, M., and Sanyal, A. J. (2018). Mechanisms of NAFLD development and therapeutic strategies. *Nat. Med.* 24 (7), 908–922. doi:10.1038/s41591-018-0104-9

Gao, G., Zhao, J., Ding, J., Liu, S., Shen, Y., Liu, C., et al. (2024). Alisol B regulates AMPK/mTOR/SREBPs *via* directly targeting VDAC1 to alleviate hyperlipidemia. *Phytomedicine* 128, 155313. doi:10.1016/j.phymed.2023.155313

Gao, S., and Feng, Q. (2022). The beneficial effects of geniposide on glucose and lipid metabolism: a review. *Drug Des. Devel Ther.* 16, 3365–3383. doi:10.2147/dddt.S378976

Gao, Y., Liu, J., Hao, Z., Sun, N., Guo, J., Zheng, X., et al. (2023b). Baicalin ameliorates high fat diet-induced nonalcoholic fatty liver disease in mice *via* adenosine monophosphate-activated protein kinase-mediated regulation of SREBP1/Nrf2/NF-κB signaling pathways. *Phytother. Res.* 37 (6), 2405–2418. doi:10.1002/ptr.7762

Gómez-Zorita, S., Milton-Laskíbar, I., Aguirre, L., Fernández-Quintela, A., Xiao, J., and Portillo, M. P. (2021). Effects of pterostilbene on diabetes, liver steatosis and serum lipids. *Curr. Med. Chem.* 28 (2), 238–252. doi:10.2174/0929867326666191029112626

Gong, X., Li, T., Wan, R., and Sha, L. (2021). Cordycepin attenuates high-fat diet-induced non-alcoholic fatty liver disease *via* down-regulation of lipid metabolism and inflammatory responses. *Int. Immunopharmacol.* 91, 107173. doi:10.1016/j.intimp.2020.107173

Guan, L. P., and Liu, B. Y. (2016). Antidepressant-like effects and mechanisms of flavonoids and related analogues. *Eur. J. Med. Chem.* 121, 47–57. doi:10.1016/j.ejmech.2016.05.026

Guo, X., Yin, X., Liu, Z., and Wang, J. (2022). Non-alcoholic fatty liver disease (NAFLD) pathogenesis and natural products for prevention and treatment. *Int. J. Mol. Sci.* 23 (24), 15489. doi:10.3390/ijms232415489

Gwon, S. Y., Ahn, J., Jung, C. H., Moon, B., and Ha, T. Y. (2020). Shikonin attenuates hepatic steatosis by enhancing beta oxidation and energy expenditure *via* AMPK activation. *Nutrients* 12 (4), 1133. doi:10.3390/nu12041133

Hajam, Y. A., Rani, R., Ganie, S. Y., Sheikh, T. A., Javaid, D., Qadri, S. S., et al. (2022). Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. *Cells* 11 (3), 552. doi:10.3390/cells11030552

Han, Y., Yang, H., Li, L., Du, X., and Sun, C. (2019). Schisanhenol improves learning and memory in scopolamine-treated mice by reducing acetylcholinesterase activity and attenuating oxidative damage through SIRT1-PGC-1α-Tau signaling pathway. *Int. J. Neurosci.* 129 (2), 110–118. doi:10.1080/00207454.2018.1503183

Hemalatha, T., Pulavendran, S., Balachandran, C., Manohar, B. M., and Puvanakrishnan, R. (2010). Arjunolic acid: a novel phytomedicine with multifunctional therapeutic applications. *Indian J. Exp. Biol.* 48 (3), 238–247.

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature* 444 (7121), 860–867. doi:10.1038/nature05485

Hu, M., Zhang, D., Xu, H., Zhang, Y., Shi, H., Huang, X., et al. (2021). Salidroside activates the AMP-activated protein kinase pathway to suppress nonalcoholic steatohepatitis in mice. *Hepatology* 74 (6), 3056–3073. doi:10.1002/hep.32066

Huang, H., Chang, Y. H., Xu, J., Ni, H. Y., Zhao, H., Zhai, B. W., et al. (2022). Aucubin as a natural potential anti-acute hepatitis candidate: inhibitory potency and hepatoprotective mechanism. *Phytomedicine* 102, 154170. doi:10.1016/j.phymed.2022.154170

Huang, S. C., and Liu, C. J. (2023). Chronic hepatitis B with concurrent metabolic dysfunction-associated fatty liver disease: challenges and perspectives. *Clin. Mol. Hepatol.* 29 (2), 320–331. doi:10.3350/cmh.2022.0422

Huang, Y., Lang, H., Chen, K., Zhang, Y., Gao, Y., Ran, L., et al. (2020). Resveratrol protects against nonalcoholic fatty liver disease by improving lipid metabolism and redox homeostasis *via* the PPARα pathway. *Appl. Physiol. Nutr. Metab.* 45 (3), 227–239. doi:10.1139/apnm-2019-0057

Huang, Y. L., Shen, C. C., Shen, Y. C., Chiou, W. F., and Chen, C. C. (2017). Anti-inflammatory and antiosteoporosis flavonoids from the rhizomes of Helminthostachys zeylanica. *J. Nat. Prod.* 80 (2), 246–253. doi:10.1021/acs.jnatprod.5b01164

Imran, M., Aslam Gondal, T., Atif, M., Shahbaz, M., Batool Qaisarani, T., Hanif Mughal, M., et al. (2020). Apigenin as an anticancer agent. *Phytother. Res.* 34 (8), 1812–1828. doi:10.1002/ptr.6647

Inamdar, S., Joshi, A., Malik, S., Boppana, R., and Ghaskadbi, S. (2019b). Vitexin alleviates non-alcoholic fatty liver disease by activating AMPK in high fat diet fed mice. *Biochem. Biophysical Res. Commun.* 519 (1), 106–112. doi:10.1016/j.bbrc.2019.08.139

Ipsen, D. H., Lykkesfeldt, J., and Tveden-Nyborg, P. (2018). Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell Mol. Life Sci.* 75 (18), 3313–3327. doi:10.1007/s00018-018-2860-6

Isaev, N. K., Chetverikov, N. S., Stelmashook, E. V., Genrikhs, E. E., Khaspekov, L. G., and Illarioshkin, S. N. (2020). Thymoquinone as a potential neuroprotector in acute and chronic forms of cerebral pathology. *Biochem. (Mosc)* 85 (2), 167–176. doi:10.1134/s0006297920020042

Kamikubo, R., Yoshida, H., Fushimi, T., Kamei, Y., and Akagawa, M. (2024). β-Caryophyllene, a dietary phytocannabinoid, alleviates high-fat diet-induced hepatic steatosis in mice *via* AMPK activation. *Biosci. Biotechnol. Biochem.* 88 (12), 1465–1471. doi:10.1093/bbb/zbae129

Karami, A., Fakhri, S., Kooshki, L., and Khan, H. (2022). Polydatin: pharmacological mechanisms, therapeutic targets, biological activities, and health benefits. *Molecules* 27 (19), 6474. doi:10.3390/molecules27196474

Khan, F., Pandey, P., Verma, M., and Upadhyay, T. K. (2024). Terpenoid-mediated targeting of STAT3 signaling in cancer: an overview of preclinical studies. *Biomolecules* 14 (2), 200. doi:10.3390/biom14020200

Kim, H., Seo, K. H., and Yokoyama, W. (2020a). Chemistry of pterostilbene and its metabolic effects. *J. Agric. Food Chem.* 68 (46), 12836–12841. doi:10.1021/acs.jafc.0c00070

Kim, M., Yoo, G., Randy, A., Son, Y. J., Hong, C. R., Kim, S. M., et al. (2020b). Lemon balm and its constituent, rosmarinic acid, alleviate liver damage in an animal model of nonalcoholic steatohepatitis. *Nutrients* 12 (4), 1166. doi:10.3390/nu12041166

Kusu, H., Yoshida, H., Kudo, M., Okuyama, M., Harada, N., Tsuji-Naito, K., et al. (2019). Tomatidine reduces palmitate-induced lipid accumulation by activating AMPK *via* vitamin D receptor-mediated signaling in human HepG2 hepatocytes. *Mol. Nutr. Food Res.* 63 (22), e1801377. doi:10.1002/mnfr.201801377

Kwan, H. Y., Wu, J., Su, T., Chao, X. J., Yu, H., Liu, B., et al. (2017). Schisandrin B regulates lipid metabolism in subcutaneous adipocytes. *Sci. Rep.* 7 (1), 10266. doi:10.1038/s41598-017-10385-z

Lan, F., Cacicedo, J. M., Ruderman, N., and Ido, Y. (2008). SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-Activated protein kinase activation. *J. Biol. Chem.* 283 (41), 27628–27635. doi:10.1074/jbc.M805711200

Le, M. H., Yeo, Y. H., Li, X., Li, J., Zou, B., Wu, Y., et al. (2022). 2019 global NAFLD prevalence: a systematic review and meta-analysis. *Clin. Gastroenterol. Hepatol.* 20 (12), 2809–2817.e28. doi:10.1016/j.cgh.2021.12.002

Lebeaupin, C., Vallée, D., Hazari, Y., Hetz, C., Chevet, E., and Bailly-Maitre, B. (2018). Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. *J. Hepatol.* 69 (4), 927–947. doi:10.1016/j.jhep.2018.06.008

Lee, H. S., Lee, J., Smolensky, D., and Lee, S. H. (2020). Potential benefits of patchouli alcohol in prevention of human diseases: a mechanistic review. *Int. Immunopharmacol.* 89 (Pt A), 107056. doi:10.1016/j.intimp.2020.107056

Li, B., Xiao, Q., Zhao, H., Zhang, J., Yang, C., Zou, Y., et al. (2024). Schisanhenol ameliorates non-alcoholic fatty liver disease *via* inhibiting miR-802 activation of AMPK-Mediated modulation of hepatic lipid metabolism. *Acta Pharm. Sin. B* 14 (9), 3949–3963. doi:10.1016/j.apsb.2024.05.014

Li, N., Yin, L., Shang, J., Liang, M., Liu, Z., Yang, H., et al. (2023). Kaempferol attenuates nonalcoholic fatty liver disease in type 2 diabetic mice *via* the Sirt1/AMPK signaling pathway. *Biomed. Pharmacother.* 165, 115113. doi:10.1016/j.biopha.2023.115113

Li, Q., Tan, J. X., He, Y., Bai, F., Li, S. W., Hou, Y. W., et al. (2022c). Atractylenolide III ameliorates non-alcoholic fatty liver disease by activating hepatic adiponectin receptor 1-Mediated AMPK pathway. *Int. J. Biol. Sci.* 18 (4), 1594–1611. doi:10.7150/ijbs.68873

Li, R., Li, J., Huang, Y., Li, H., Yan, S., Lin, J., et al. (2018). Polydatin attenuates diet-induced nonalcoholic steatohepatitis and fibrosis in mice. *Int. J. Biol. Sci.* 14 (11), 1411–1425. doi:10.7150/ijbs.26086

Li, S., Qian, Q., Ying, N., Lai, J., Feng, L., Zheng, S., et al. (2020). Activation of the AMPK-SIRT1 pathway contributes to protective effects of salvianolic acid A against lipotoxicity in hepatocytes and NAFLD in mice. *Front. Pharmacol.* 11, 560905. doi:10.3389/fphar.2020.560905

Li, X., Zhao, Y., Gong, S., Song, T., Ge, J., Li, J., et al. (2023c). Schisandrin B ameliorates acute liver injury by regulating EGFR-Mediated activation of autophagy. *Bioorg Chem.* 130, 106272. doi:10.1016/j.bioorg.2022.106272

Li, Y., Liu, Y., Chen, Z., Tang, K., Yang, L., Jiang, Y., et al. (2023d). Protopanaxadiol ameliorates NAFLD by regulating hepatocyte lipid metabolism through AMPK/SIRT1 signaling pathway. *Biomed. Pharmacother.* 160, 114319. doi:10.1016/j.biopha.2023.114319

Li, Y., Ren, L., Song, G., Zhang, P., Yang, L., Chen, X., et al. (2019). Silibinin ameliorates fructose-induced lipid accumulation and activates autophagy in HepG2 cells. *Endocr. Metab. Immune Disord. Drug Targets* 19 (5), 632–642. doi:10.2174/1871530319666190207163325

Liang, J., Gu, L., Liu, X., Yan, X., Bi, X., Fan, X., et al. (2022a). L-theanine prevents progression of nonalcoholic hepatic steatosis by regulating hepatocyte lipid metabolic pathways *via* the CaMKKβ-AMPK signaling pathway. *Nutr. Metab. (Lond)* 19 (1), 29. doi:10.1186/s12986-022-00664-6

Liang, L., Ye, S., Jiang, R., Zhou, X., Zhou, J., and Meng, S. (2022b). Liensinine alleviates high fat diet (HFD)-Induced non-alcoholic fatty liver disease (NAFLD) through suppressing oxidative stress and inflammation *via* regulating TAK1/AMPK signaling. *Int. Immunopharmacol.* 104, 108306. doi:10.1016/j.intimp.2021.108306

Lim, J. Y., Liu, C., Hu, K. Q., Smith, D. E., Wu, D. Y., Lamon-Fava, S., et al. (2019). Dietary β-Cryptoxanthin inhibits high-refined carbohydrate diet-induced fatty liver *via* differential protective mechanisms depending on carotenoid cleavage enzymes in Male mice. *J. Nutr.* 149 (9), 1553–1564. doi:10.1093/jn/nxz106

Lin, W., Jin, Y., Hu, X., Huang, E., and Zhu, Q. (2021). AMPK/PGC-1α/GLUT4-Mediated effect of icariin on hyperlipidemia-induced non-alcoholic fatty liver disease and lipid metabolism disorder in mice. *Biochem. (Mosc)* 86 (11), 1407–1417. doi:10.1134/s0006297921110055

Lin, W. S., Leland, J. V., Ho, C. T., and Pan, M. H. (2020). Occurrence, bioavailability, anti-inflammatory, and anticancer effects of pterostilbene. *J. Agric. Food Chem.* 68 (46), 12788–12799. doi:10.1021/acs.jafc.9b07860

Liou, C. J., Dai, Y. W., Wang, C. L., Fang, L. W., and Huang, W. C. (2019a). Maslinic acid protects against obesity-induced nonalcoholic fatty liver disease in mice through regulation of the Sirt1/AMPK signaling pathway. *Faseb J.* 33 (11), 11791–11803. doi:10.1096/fj.201900413RRR

Liu, J., Liu, S., Yu, M., Li, J., Xie, Z., Gao, B., et al. (2023a). Anti-inflammatory effect and mechanism of catalpol in various inflammatory diseases. *Drug Dev. Res.* 84 (7), 1376–1394. doi:10.1002/ddr.22096

Liu, J., Zhang, T., Zhu, J., Ruan, S., Li, R., Guo, B., et al. (2021b). Honokiol attenuates lipotoxicity in hepatocytes *via* activating SIRT3-AMPK mediated lipophagy. *Chin. Med.* 16 (1), 115. doi:10.1186/s13020-021-00528-w

Liu, K., Tang, S., Liu, C., Ma, J., Cao, X., Yang, X., et al. (2024a). Systemic immune-inflammatory biomarkers (SII, NLR, PLR and LMR) linked to non-alcoholic fatty liver disease risk. *Front. Immunol.* 15, 1337241. doi:10.3389/fimmu.2024.1337241

Liu, X., Guo, B., Li, Q., and Nie, J. (2024b). mTOR in metabolic homeostasis and disease. *Exp. Cell Res.* 441 (2), 114173. doi:10.1016/j.yexcr.2024.114173

Liu, Y., Li, D., Wang, S., Peng, Z., Tan, Q., He, Q., et al. (2023b). 6-Gingerol ameliorates hepatic steatosis, inflammation and oxidative stress in high-fat diet-fed mice through activating LKB1/AMPK signaling. *Int. J. Mol. Sci.* 24 (7), 6285. doi:10.3390/ijms24076285

Liu, Y., Zhang, Y., Zhu, H., Shen, W., Chen, Z., Bai, J., et al. (2022). Aucubin administration suppresses STING signaling and mitigated high-fat diet-induced atherosclerosis and steatohepatosis in LDL receptor deficient mice. *Food Chem. Toxicol.* 169, 113422. doi:10.1016/j.fct.2022.113422

Lu, J., Zhou, H., Hu, J., Zhang, R., Meng, Z., and Guan, S. (2024). Apigenin reduces lipid droplet accumulation in hepatocytes by enhancing chaperone-mediated autophagy *via* AMPK. *J. Agric. Food Chem.* 72 (50), 27965–27977. doi:10.1021/acs.jafc.4c08430

Lu, Y., and George, J. (2024). Interaction between fatty acid oxidation and ethanol metabolism in liver. *Am. J. Physiol. Gastrointest. Liver Physiol.* 326 (5), G483–g494. doi:10.1152/ajpgi.00281.2023

Luo, L., Fang, K., Dan, X., and Gu, M. (2019). Crocin ameliorates hepatic steatosis through activation of AMPK signaling in Db/Db mice. *Lipids Health Dis.* 18 (1), 11. doi:10.1186/s12944-018-0955-6

Ma, C., Wang, Z., Xia, R., Wei, L., Zhang, C., Zhang, J., et al. (2021). Danthron ameliorates obesity and MAFLD through activating the interplay between PPARα/RXRα heterodimer and adiponectin receptor 2. *Biomed. Pharmacother.* 137, 111344. doi:10.1016/j.biopha.2021.111344

Ma, R., Zhan, Y., Zhang, Y., Wu, L., Wang, X., and Guo, M. (2022). Schisandrin B ameliorates non-alcoholic liver disease through anti-inflammation activation in diabetic mice. *Drug Dev. Res.* 83 (3), 735–744. doi:10.1002/ddr.21905

Mahmoud, Y. K., and Abdelrazek, H. M. A. (2019). Cancer: thymoquinone antioxidant/pro-oxidant effect as potential anticancer remedy. *Biomed. Pharmacother.* 115, 108783. doi:10.1016/j.biopha.2019.108783

Marton, L. T., Pescinini, E. S. L. M., Camargo, M. E. C., Barbalho, S. M., Haber, J., Sinatora, R. V., et al. (2021). The effects of curcumin on diabetes mellitus: a systematic review. *Front. Endocrinol. (Lausanne)* 12, 669448. doi:10.3389/fendo.2021.669448

Mhillaj, E., Tarozzi, A., Pruccoli, L., Cuomo, V., Trabace, L., and Mancuso, C. (2019). Curcumin and heme oxygenase: neuroprotection and beyond. *Int. J. Mol. Sci.* 20 (10), 2419. doi:10.3390/ijms20102419

Mohammed, H. M. (2022). Zingerone ameliorates non-alcoholic fatty liver disease in rats by activating AMPK. *J. Food Biochem.* 46 (7), e14149. doi:10.1111/jfbc.14149

Niture, S., Lin, M., Rios-Colon, L., Qi, Q., Moore, J. T., and Kumar, D. (2021). Emerging roles of impaired autophagy in fatty liver disease and hepatocellular carcinoma. *Int. J. Hepatol.* 2021, 6675762. doi:10.1155/2021/6675762

Oriquat, G., Masoud, I. M., Kamel, M. A., Aboudeya, H. M., Bakir, M. B., and Shaker, S. A. (2023). The anti-obesity and anti-steatotic effects of chrysin in a rat model of obesity mediated through modulating the hepatic AMPK/mTOR/lipogenesis pathways. *Molecules* 28 (4), 1734. doi:10.3390/molecules28041734

Pafili, K., and Roden, M. (2021). Nonalcoholic fatty liver disease (NAFLD) from pathogenesis to treatment concepts in humans. *Mol. Metab.* 50, 101122. doi:10.1016/j.molmet.2020.101122

Peiseler, M., Schwabe, R., Hampe, J., Kubes, P., Heikenwälder, M., and Tacke, F. (2022). Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease - novel insights into cellular communication circuits. *J. Hepatol.* 77 (4), 1136–1160. doi:10.1016/j.jhep.2022.06.012

Petta, S., Targher, G., Romeo, S., Pajvani, U. B., Zheng, M. H., Aghemo, A., et al. (2024). The first MASH drug therapy on the horizon: current perspectives of resmetirom. *Liver Int.* 44 (7), 1526–1536. doi:10.1111/liv.15930

Ponugoti, B., Kim, D. H., Xiao, Z., Smith, Z., Miao, J., Zang, M., et al. (2010). SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. *J. Biol. Chem.* 285 (44), 33959–33970. doi:10.1074/jbc.M110.122978

Prestianni, L., Espinal, E. R., Hathcock, S. F., Vollmuth, N., Wang, P., Holler, R. A., et al. (2023). Synthesis and characterization of quercetin-iron complex nanoparticles for overcoming drug resistance. *Pharmaceutics* 15 (4), 1041. doi:10.3390/pharmaceutics15041041

Pyun, D., Kim, T. J., Park, S. Y., Lee, H. J., Abd El-Aty, A. M., Jeong, J. H., et al. (2021). Patchouli alcohol ameliorates skeletal muscle insulin resistance and NAFLD *via* AMPK/SIRT1-mediated suppression of inflammation. *Mol. Cell. Endocrinol.* 538, 111464. doi:10.1016/j.mce.2021.111464

Qin, T., Rasul, A., Sarfraz, A., Sarfraz, I., Hussain, G., Anwar, H., et al. (2019). Salvianolic acid A and B: potential cytotoxic polyphenols in battle against cancer *via* targeting multiple signaling pathways. *Int. J. Biol. Sci.* 15 (10), 2256–2264. doi:10.7150/ijbs.37467

Rauf, A., Abu-Izneid, T., Imran, M., Hemeg, H. A., Bashir, K., Aljohani, A. S. M., et al. (2023). Therapeutic potential and molecular mechanisms of the multitargeted flavonoid fisetin. *Curr. Top. Med. Chem.* 23 (21), 2075–2096. doi:10.2174/1568026623666230710162217

Rauf, A., Patel, S., Imran, M., Maalik, A., Arshad, M. U., Saeed, F., et al. (2018). Honokiol: an anticancer lignan. *Biomed. Pharmacother.* 107, 555–562. doi:10.1016/j.biopha.2018.08.054

Ren, B., Kwah, M. X., Liu, C., Ma, Z., Shanmugam, M. K., Ding, L., et al. (2021). Resveratrol for cancer therapy: challenges and future perspectives. *Cancer Lett.* 515, 63–72. doi:10.1016/j.canlet.2021.05.001

Ren, H., Wang, D., Zhang, L., Kang, X., Li, Y., Zhou, X., et al. (2019). Catalpol induces autophagy and attenuates liver steatosis in Ob/Ob and high-fat diet-induced Obese mice. *Aging (Albany NY)* 11 (21), 9461–9477. doi:10.18632/aging.102396

Romero-Gómez, M. (2022). Non-alcoholic steatohepatitis. *Med. Clin. Barc.* 159 (8), 388–395. doi:10.1016/j.medcli.2022.06.017

Rottiers, V., and Näär, A. M. (2012). MicroRNAs in metabolism and metabolic disorders. *Nat. Rev. Mol. Cell Biol.* 13 (4), 239–250. doi:10.1038/nrm3313

Sadasivam, N., Kim, Y. J., Radhakrishnan, K., and Kim, D. K. (2022). Oxidative stress, genomic integrity, and liver diseases. *Molecules* 27 (10), 3159. doi:10.3390/molecules27103159

Sadeghi, M., Dehnavi, S., Asadirad, A., Xu, S., Majeed, M., Jamialahmadi, T., et al. (2023). Curcumin and chemokines: mechanism of action and therapeutic potential in inflammatory diseases. *Inflammopharmacology* 31 (3), 1069–1093. doi:10.1007/s10787-023-01136-w

Saiman, Y., Duarte-Rojo, A., and Rinella, M. E. (2022). Fatty liver disease: diagnosis and stratification. *Annu. Rev. Med.* 73, 529–544. doi:10.1146/annurev-med-042220-020407

Sakurai, Y., Kubota, N., Yamauchi, T., and Kadowaki, T. (2021). Role of insulin resistance in MAFLD. *Int. J. Mol. Sci.* 22 (8), 4156. doi:10.3390/ijms22084156

Saltiel, A. R., and Olefsky, J. M. (2017). Inflammatory mechanisms linking obesity and metabolic disease. *J. Clin. Invest* 127 (1), 1–4. doi:10.1172/jci92035

Sangro, P., de la Torre Aláez, M., Sangro, B., and D'Avola, D. (2023). Metabolic dysfunction-associated fatty liver disease (MAFLD): an update of the recent advances in pharmacological treatment. *J. Physiol. Biochem.* 79 (4), 869–879. doi:10.1007/s13105-023-00954-4

Schlaepfer, I. R., and Joshi, M. (2020). CPT1A-mediated fat oxidation, mechanisms, and therapeutic potential. *Endocrinology* 161 (2), bqz046. doi:10.1210/endocr/bqz046

Selvaraj, S., Krishnaswamy, S., Devashya, V., Sethuraman, S., and Krishnan, U. M. (2014). Flavonoid-metal ion complexes: a novel class of therapeutic agents. *Med. Res. Rev.* 34 (4), 677–702. doi:10.1002/med.21301

Seo, J., Kwon, D., Kim, S. H., Byun, M. R., Lee, Y. H., and Jung, Y. S. (2024). Role of autophagy in betaine-promoted hepatoprotection against non-alcoholic fatty liver disease in mice. *Curr. Res. Food Sci.* 8, 100663. doi:10.1016/j.crfs.2023.100663

Sharma, A., Anand, S. K., Singh, N., Dwarkanath, A., Dwivedi, U. N., and Kakkar, P. (2021). Berbamine induced activation of the SIRT1/LKB1/AMPK signaling axis attenuates the development of hepatic steatosis in high-fat diet-induced NAFLD rats. *Food Funct.* 12 (2), 892–909. doi:10.1039/d0fo02501a

Sharma, A., Anand, S. K., Singh, N., Dwivedi, U. N., and Kakkar, P. (2020). Berbamine induced AMPK activation regulates mTOR/SREBP-1c axis and Nrf2/ARE pathway to allay lipid accumulation and oxidative stress in steatotic HepG2 cells. *Eur. J. Pharmacol.* 882, 173244. doi:10.1016/j.ejphar.2020.173244

Shen, B., Wang, Y., Cheng, J., Peng, Y., Zhang, Q., Li, Z., et al. (2023a). Pterostilbene alleviated NAFLD *via* AMPK/mTOR signaling pathways and autophagy by promoting Nrf2. *Phytomedicine* 109, 154561. doi:10.1016/j.phymed.2022.154561

Shen, B. Y., Zhao, C. X., Wang, Y., Peng, Y., Cheng, J. Q., Li, Z., et al. (2019b). Aucubin inhibited lipid accumulation and oxidative stress *via* Nrf2/HO-1 and AMPK signalling pathways. *J. Cell. Mol. Med.* 23 (6), 4063–4075. doi:10.1111/jcmm.14293

Shi, Y., Chen, J., Qu, D., Sun, Q., Yu, Y., Zhang, H., et al. (2024). Ginsenoside Rg(5) activates the LKB1/AMPK/mTOR signaling pathway and modifies the gut microbiota to alleviate nonalcoholic fatty liver disease induced by a high-fat diet. *Nutrients* 16 (6), 842. doi:10.3390/nu16060842

Singh, A., Ansari, A., Gupta, J., Singh, H., Jagavelu, K., and Sashidhara, K. V. (2024). Androsin alleviates non-alcoholic fatty liver disease by activating autophagy and attenuating *de novo* lipogenesis. *Phytomedicine* 129, 155702. doi:10.1016/j.phymed.2024.155702

Smith, B. K., Marcinko, K., Desjardins, E. M., Lally, J. S., Ford, R. J., and Steinberg, G. R. (2016). Treatment of nonalcoholic fatty liver disease: role of AMPK. *Am. J. Physiology-Endocrinology Metabolism* 311 (4), E730–E740. doi:10.1152/ajpendo.00225.2016

Spahis, S., Delvin, E., Borys, J. M., and Levy, E. (2017). Oxidative stress as a critical factor in nonalcoholic fatty liver disease pathogenesis. *Antioxid. Redox Signal* 26 (10), 519–541. doi:10.1089/ars.2016.6776

Steinberg, G. R., and Hardie, D. G. (2023). New insights into activation and function of the AMPK. *Nat. Rev. Mol. Cell Biol.* 24 (4), 255–272. doi:10.1038/s41580-022-00547-x

Sun, H., and Kemper, J. K. (2023). MicroRNA regulation of AMPK in nonalcoholic fatty liver disease. *Exp. Mol. Med.* 55 (9), 1974–1981. doi:10.1038/s12276-023-01072-3

Sun, Q. S., Niu, Q., Guo, Y. T., Zhuang, Y., Li, X. N., Liu, J., et al. (2021). Regulation on citrate influx and metabolism through inhibiting SLC13A5 and ACLY: a novel mechanism mediating the therapeutic effects of curcumin on NAFLD. *J. Agric. Food Chem.* 69 (31), 8714–8725. doi:10.1021/acs.jafc.1c03105

Suvarna, R., Shetty, S., and Pappachan, J. M. (2024). Efficacy and safety of resmetirom, a selective thyroid hormone receptor-β agonist, in the treatment of metabolic dysfunction-associated steatotic liver disease (MASLD): a systematic review and meta-analysis. *Sci. Rep.* 14 (1), 19790. doi:10.1038/s41598-024-70242-8

Takaki, A., Kawai, D., and Yamamoto, K. (2013). Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). *Int. J. Mol. Sci.* 14 (10), 20704–20728. doi:10.3390/ijms141020704

Tang, R., Li, R., Li, H., Ma, X. L., Du, P., Yu, X. Y., et al. (2021). Design of hepatic targeted drug delivery systems for natural products: insights into nomenclature revision of nonalcoholic fatty liver disease. *ACS Nano* 15 (11), 17016–17046. doi:10.1021/acsnano.1c02158

Tian, L., Cao, W., Yue, R., Yuan, Y., Guo, X., Qin, D., et al. (2019). Pretreatment with tilianin improves mitochondrial energy metabolism and oxidative stress in rats with myocardial ischemia/reperfusion injury *via* AMPK/SIRT1/PGC-1 alpha signaling pathway. *J. Pharmacol. Sci.* 139 (4), 352–360. doi:10.1016/j.jphs.2019.02.008

Tian, R., Yang, J., Wang, X., Liu, S., Dong, R., Wang, Z., et al. (2023). Honokiol acts as an AMPK complex agonist therapeutic in non-alcoholic fatty liver disease and metabolic syndrome. *Chin. Med.* 18 (1), 30. doi:10.1186/s13020-023-00729-5

Tian, X., Ru, Q., Xiong, Q., Wen, R., and Chen, Y. (2020). Catalpol attenuates hepatic steatosis by regulating lipid metabolism *via* AMP-activated protein kinase activation. *Biomed. Res. Int.* 2020, 6708061. doi:10.1155/2020/6708061

Tomeh, M. A., Hadianamrei, R., and Zhao, X. (2019). A review of curcumin and its derivatives as anticancer agents. *Int. J. Mol. Sci.* 20 (5), 1033. doi:10.3390/ijms20051033

Wang, H., Zhou, X. M., Wu, L. Y., Liu, G. J., Xu, W. D., Zhang, X. S., et al. (2020a). Aucubin alleviates oxidative stress and inflammation *via* Nrf2-mediated signaling activity in experimental traumatic brain injury. *J. Neuroinflammation* 17 (1), 188. doi:10.1186/s12974-020-01863-9

Wang, J., Geng, T. H., Zou, Q. H., Yang, N. R., Zhao, W. Y., Li, Y. T., et al. (2020b). Lycopene prevents lipid accumulation in hepatocytes by stimulating PPARα and improving mitochondrial function. *J. Funct. Foods* 67, 103857. doi:10.1016/j.jff.2020.103857

Wang, M. Y., Zhang, S. S., An, M. F., Xia, Y. F., Fan, M. S., Sun, Z. R., et al. (2023). Neferine ameliorates nonalcoholic steatohepatitis through regulating AMPK pathway. *Phytomedicine* 114, 154798. doi:10.1016/j.phymed.2023.154798

Wang, S. W., Sheng, H., Bai, Y. F., Weng, Y. Y., Fan, X. Y., Lou, L. J., et al. (2020c). Neohesperidin enhances PGC-1α-mediated mitochondrial biogenesis and alleviates hepatic steatosis in high fat diet fed mice. *Nutr. and Diabetes* 10 (1), 27. doi:10.1038/s41387-020-00130-3

Wang, Y., Yu, W., Li, S., Guo, D., He, J., and Wang, Y. (2022b). Acetyl-CoA carboxylases and diseases. *Front. Oncol.* 12, 836058. doi:10.3389/fonc.2022.836058

Wang, Y., Zhao, H. L., Li, X., Wang, Q., Yan, M. H., Zhang, H. J., et al. (2019). Formononetin alleviates hepatic steatosis by facilitating TFEB-Mediated lysosome biogenesis and lipophagy. *J. Nutr. Biochem.* 73, 108214. doi:10.1016/j.jnutbio.2019.07.005

Woo, C. C., Kumar, A. P., Sethi, G., and Tan, K. H. (2012). Thymoquinone: potential cure for inflammatory disorders and cancer. *Biochem. Pharmacol.* 83 (4), 443–451. doi:10.1016/j.bcp.2011.09.029

Wu, J., Wen, L., Liu, X., Li, Q., Sun, Z., Liang, C., et al. (2024). Silybin: a review of its targeted and novel agents for treating liver diseases based on pathogenesis. *Phytother. Res.* 38 (12), 5713–5740. doi:10.1002/ptr.8347

Wu, J. M., Zhaori, G., Mei, L., Ren, X. M., Laga, A. T., and Deligen, B. (2023). Plantamajoside modulates immune dysregulation and hepatic lipid metabolism in rats with nonalcoholic fatty liver disease *via* AMPK/Nrf2 elevation. *Kaohsiung J. Med. Sci.* 39 (8), 801–810. doi:10.1002/kjm2.12712

Xie, Y., Lei, X., Zhao, G., Guo, R., and Cui, N. (2023). mTOR in programmed cell death and its therapeutic implications. *Cytokine Growth Factor Rev.* 71-72, 66–81. doi:10.1016/j.cytogfr.2023.06.002

Xie, Y., Wei, L., Guo, J., Jiang, Q., Xiang, Y., Lin, Y., et al. (2024). Ginkgolide C attenuated Western diet-induced non-alcoholic fatty liver disease *via* increasing AMPK activation. *Inflammation* 48, 770–782. doi:10.1007/s10753-024-02086-3

Xu, C., Song, Y., Wang, Z., Jiang, J., Piao, Y., Li, L., et al. (2021a). Pterostilbene suppresses oxidative stress and allergic airway inflammation through AMPK/Sirt1 and Nrf2/HO-1 pathways. *Immun. Inflamm. Dis.* 9 (4), 1406–1417. doi:10.1002/iid3.490

Xu, H., Chen, G. F., Ma, Y. S., Zhang, H. W., Zhou, Y., Liu, G. H., et al. (2020). Hepatic proteomic changes and Sirt1/AMPK signaling activation by oxymatrine treatment in rats with non-alcoholic steatosis. *Front. Pharmacol.* 11, 216. doi:10.3389/fphar.2020.00216

Xu, J., Ji, J., and Yan, X. H. (2012). Cross-talk between AMPK and mTOR in regulating energy balance. *Crit. Rev. Food Sci. Nutr.* 52 (5), 373–381. doi:10.1080/10408398.2010.500245

Xu, S., Feng, Y., He, W., Xu, W., Xu, W., Yang, H., et al. (2021c). Celastrol in metabolic diseases: progress and application prospects. *Pharmacol. Res.* 167, 105572. doi:10.1016/j.phrs.2021.105572

Xu, Y., Hu, X., Cai, J., Li, Y., Zou, Y., Wang, Y., et al. (2023). Atractylenolide-III alleviates osteoarthritis and chondrocyte senescence by targeting NF-κB signaling. *Phytother. Res.* 37 (10), 4607–4620. doi:10.1002/ptr.7929

Xue, J., Liu, Y., Liu, B., Jia, X., Fang, X., Qin, S., et al. (2024a). Celastrus orbiculatus thunb. Extracts and celastrol alleviate NAFLD by preserving mitochondrial function through activating the FGF21/AMPK/PGC-1α pathway. *Front. Pharmacol.* 15, 1444117. doi:10.3389/fphar.2024.1444117

Yamaguchi, M. (2012). Role of carotenoid β-cryptoxanthin in bone homeostasis. *J. Biomed. Sci.* 19 (1), 36. doi:10.1186/1423-0127-19-36

Yan, J., Wang, C., Jin, Y., Meng, Q., Liu, Q., Liu, Z., et al. (2018). Catalpol ameliorates hepatic insulin resistance in type 2 diabetes through acting on AMPK/NOX4/PI3K/AKT pathway. *Pharmacol. Res.* 130, 466–480. doi:10.1016/j.phrs.2017.12.026

Yan, L. S., Zhang, S. F., Luo, G., Cheng, B. C., Zhang, C., Wang, Y. W., et al. (2022). Schisandrin B mitigates hepatic steatosis and promotes fatty acid oxidation by inducing autophagy through AMPK/mTOR signaling pathway. *Metabolism* 131, 155200. doi:10.1016/j.metabol.2022.155200

Yang, Y., Qiu, W., Xiao, J. Y., Sun, J., Ren, X., and Jiang, L. X. (2024). Dihydromyricetin ameliorates hepatic steatosis and insulin resistance *via* AMPK/PGC-1α and PPARα-mediated autophagy pathway. *J. Transl. Med.* 22 (1), 309. doi:10.1186/s12967-024-05060-7

Yi, M., Fasina, O. B., Li, Y., Xiang, L., and Qi, J. (2023). Mixture of peanut skin extract, geniposide, and isoquercitrin improves the hepatic lipid accumulation of mice *via* modification of gut microbiota homeostasis and the TLR4 and AMPK signaling pathways. *Int. J. Mol. Sci.* 24 (23), 16684. doi:10.3390/ijms242316684

Yin, X., Liu, Z., and Wang, J. (2023). Tetrahydropalmatine ameliorates hepatic steatosis in nonalcoholic fatty liver disease by switching lipid metabolism *via* AMPK-SREBP-1c-Sirt1 signaling axis. *Phytomedicine* 119, 155005. doi:10.1016/j.phymed.2023.155005

Yoon, J. H., Kim, M. Y., and Cho, J. Y. (2023). Apigenin: a therapeutic agent for treatment of skin inflammatory diseases and cancer. *Int. J. Mol. Sci.* 24 (2), 1498. doi:10.3390/ijms24021498

Younossi, Z. M., Golabi, P., Paik, J. M., Henry, A., Van Dongen, C., and Henry, L. (2023). The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology* 77 (4), 1335–1347. doi:10.1097/hep.0000000000000004

Yu, L., Gong, L., Wang, C., Hu, N., Tang, Y., Zheng, L., et al. (2020). Radix polygoni multiflori and its main component emodin attenuate non-alcoholic fatty liver disease in zebrafish by regulation of AMPK signaling pathway. *Drug Des. Devel Ther.* 14, 1493–1506. doi:10.2147/dddt.S243893

Yuan, Z., Lu, X., Lei, F., Sun, H., Jiang, J., Xing, D., et al. (2023a). Novel effect of p-Coumaric acid on hepatic lipolysis: inhibition of hepatic lipid-droplets. *Molecules* 28 (12), 4641. doi:10.3390/molecules28124641

Zeng, X. L., Yang, J. N., Hu, O., Huang, J., Ran, L., Chen, M. T., et al. (2019). Dihydromyricetin ameliorates nonalcoholic fatty liver disease by improving mitochondrial respiratory capacity and redox homeostasis through modulation of SIRT3 signaling. *Antioxidants and Redox Signal.* 30 (2), 163–183. doi:10.1089/ars.2017.7172

Zhang, C., Tong, Q., Liu, K., Mao, T., Song, Y., Qu, Y., et al. (2024). Morroniside delays the progression of non-alcoholic steatohepatitis by promoting AMPK-Mediated lipophagy. *Phytomedicine* 129, 155703. doi:10.1016/j.phymed.2024.155703

Zhang, D., Zhang, Y. H., Wang, Z. L., and Lei, L. (2023a). Thymoquinone attenuates hepatic lipid accumulation by inducing autophagy *via* AMPK/mTOR/ULK1-dependent pathway in nonalcoholic fatty liver disease. *Phytotherapy Res.* 37 (3), 781–797. doi:10.1002/ptr.7662

Zhang, J. J., Ma, X. X., and Fan, D. D. (2022a). Ginsenoside CK ameliorates hepatic lipid accumulation *via* activating the LKB1/AMPK pathway *in vitro* and *in vivo*. *Food and Funct.* 13 (3), 1153–1167. doi:10.1039/d1fo03026d

Zhang, L., Li, H. X., Pan, W. S., Khan, F. U., Qian, C., Qi-Li, F. R., et al. (2019a). Novel hepatoprotective role of leonurine hydrochloride against experimental non-alcoholic steatohepatitis mediated *via* AMPK/SREBP1 signaling pathway. *Biomed. Pharmacother.* 110, 571–581. doi:10.1016/j.biopha.2018.12.003

Zhang, L. X., Li, C. X., Kakar, M. U., Khan, M. S., Wu, P. F., Amir, R. M., et al. (2021a). Resveratrol (RV): a pharmacological review and call for further research. *Biomed. Pharmacother.* 143, 112164. doi:10.1016/j.biopha.2021.112164

Zhang, S. Z., Liang, P. P., Feng, Y. N., Yin, G. L., Sun, F. C., Ma, C. Q., et al. (2022b). Therapeutic potential and research progress of diosgenin for lipid metabolism diseases. *Drug Dev. Res.* 83 (8), 1725–1738. doi:10.1002/ddr.21991

Zhang, W., Chen, H., Xu, Z., Zhang, X., Tan, X., He, N., et al. (2023c). Liensinine pretreatment reduces inflammation, oxidative stress, apoptosis, and autophagy to alleviate sepsis acute kidney injury. *Int. Immunopharmacol.* 122, 110563. doi:10.1016/j.intimp.2023.110563

Zhang, Z., Dai, Y., Xiao, Y., and Liu, Q. (2023d). Protective effects of catalpol on cardio-cerebrovascular diseases: a comprehensive review. *J. Pharm. Anal.* 13 (10), 1089–1101. doi:10.1016/j.jpha.2023.06.010

Zhang, Z., Zhong, Y., Han, X., Hu, X., Wang, Y., Huang, L., et al. (2025b). Schisanhenol inhibits the proliferation of hepatocellular carcinoma cells by targeting programmed cell death-ligand 1 *via* the STAT3 pathways. *Anticancer Agents Med. Chem.* 25, 697–710. doi:10.2174/0118715206349131241121091834

Zheng, T., Jiang, T., Huang, Z., Ma, H., and Wang, M. (2023). Role of traditional Chinese medicine monomers in cerebral ischemia/reperfusion injury:a review of the mechanism. *Front. Pharmacol.* 14, 1220862. doi:10.3389/fphar.2023.1220862

Zheng, X., Zhang, X. G., Liu, Y., Zhu, L. P., Liang, X. S., Jiang, H., et al. (2021). Arjunolic acid from Cyclocarya paliurus ameliorates nonalcoholic fatty liver disease in mice *via* activating Sirt1/AMPK, triggering autophagy and improving gut barrier function. *J. Funct. Foods* 86, 104686. doi:10.1016/j.jff.2021.104686

Zhong, Y., Li, Z., Jin, R., Yao, Y., He, S., Lei, M., et al. (2022). Diosgenin Ameliorated Type II Diabetes-Associated Nonalcoholic Fatty Liver Disease through Inhibiting *de novo* Lipogenesis and Improving Fatty Acid Oxidation and Mitochondrial Function in Rats. *Nutrients* 14 (23), 4994. doi:10.3390/nu14234994

Zhou, D. D., Luo, M., Huang, S. Y., Saimaiti, A., Shang, A., Gan, R. Y., et al. (2021). Effects and mechanisms of resveratrol on aging and age-related diseases. *Oxid. Med. Cell Longev.* 2021, 9932218. doi:10.1155/2021/9932218

Zhou, F., Ding, M. N., Gu, Y. Q., Fan, G. F., Liu, C. Y., Li, Y. J., et al. (2022). Aurantio-obtusin attenuates non-alcoholic fatty liver disease through AMPK-mediated autophagy and fatty acid oxidation pathways. *Front. Pharmacol.* 12, 826628. doi:10.3389/fphar.2021.826628

Zhou, R., Wang, L., Xu, X., Chen, J., Hu, L. H., Chen, L. L., et al. (2013). Danthron activates AMP-Activated protein kinase and regulates lipid and glucose metabolism *in vitro*. *Acta Pharmacol. Sin.* 34 (8), 1061–1069. doi:10.1038/aps.2013.39

Zhu, J., Chen, H., Le, Y., Guo, J., Liu, Z., Dou, X., et al. (2022). Salvianolic acid A regulates pyroptosis of endothelial cells *via* directly targeting PKM2 and ameliorates diabetic atherosclerosis. *Front. Pharmacol.* 13, 1009229. doi:10.3389/fphar.2022.1009229

Zhu, J., Guo, J., Liu, Z., Liu, J., Yuan, A., Chen, H., et al. (2024). Salvianolic acid A attenuates non-alcoholic fatty liver disease by regulating the AMPK-IGFBP1 pathway. *Chem. Biol. Interact.* 400, 111162. doi:10.1016/j.cbi.2024.111162

Zhu, X., Bian, H., Wang, L., Sun, X., Xu, X., Yan, H., et al. (2019). Berberine attenuates nonalcoholic hepatic steatosis through the AMPK-SREBP-1c-SCD1 pathway. *Free Radic. Biol. Med.* 141, 192–204. doi:10.1016/j.freeradbiomed.2019.06.019

Glossary

MAFLD	metabolic dysfunction-associated fatty liver disease
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
IR	insulin resistance
AMPK	AMP-activated protein kinase
DNL	*de novo* lipogenesis
FAO	fatty acid oxidation
FXR	farnesoid x receptor
PPAR	peroxisome proliferator-activated receptor
THR	thyroid hormone receptor
TG	triglycerides
ACLY	ATP-citrate lyase
ACC	acetyl-CoA carboxylase
LKB1	liver kinase B1
CPT1	Carnitine palmitoyltransferase 1
ATGL	adipose triglyceride lipase
HSL	hormone-sensitive lipase
mTOR	mammalian target of rapamycin
LC3	light chain 3
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPARα	peroxisome proliferator-activated receptor alpha
OS	oxidative stress
ROS	reactive oxygen species
HCC	hepatocellular carcinoma
Nrf2	nucleus factor erythroid 2-related factor 2
ER	endoplasmic reticulum
UPR	unfolded protein response
SCD1	stearoyl-CoA desaturase 1
UCP2	uncoupling protein 2
TAK1	transforming growth factor-β-activated kinase 1
CaMKKβ	calmodulin-dependent protein kinase β
LepR	leptin receptor
IRS-1	insulin receptor substrate-1
AKT	protein kinase B
NRF-1	nuclear respiratory factor-1
STRAD	STE20-related adapter
PLIN2	perilipin 2
CMA	chaperone-mediated autophagy
Drp1	dynamin-related protein 1
PINK1	PTEN-induced kinase 1
IGF1	insulin-like growth factors 1
IGF2	insulin-like growth factors 2
IGFBPs	IGF-binding proteins
CTRP3	C1q/tumor necrosis factor-related protein-3
PI3K	phosphatidylinositol 3-kinase
RXR	retinoid X receptor
BCO1	β-Carotene-15,15′-oxygenase
BCO2	β-carotene-9′,10′-oxygenase
TFEB	transcription Factor EB

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Potential mechanisms of natural metabolites and botanical drugs foumulae for the treatment of non-alcoholic fatty liver disease: targeting the gut microbiota to modulate the immune system

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Non-alcoholic fatty liver disease (NAFLD) has become the most prevalent liver disorder worldwide and is also a significant risk factor for triggering non-alcoholic steatohepatitis (NASH), hepatic fibrosis, and liver cirrhosis. Disorders in the hepatic immune system constitute one of the key drivers of NAFLD progression; thus, targeting immune dysregulation may represent an effective strategy to delay or reverse NAFLD advancement. Meanwhile, gut microbiota (GM) and its metabolites directly influence liver immune responses throughthe "Gut-Liver Axis." Dysbiosis of the GM triggers damage to the intestinal mucosal barrier. Subsequently, substantial bacterial metabolites derived from GM can induce overactivation of the hepatic immune response, thereby driving NAFLD progression. Thus, targeted intervention in the GM-immune response axis represents an effective therapeutic approach against NAFLD advancement. Numerous current studies indicate that botanical drugs and their metabolites can counteract NAFLD progression by intervening in GM and its metabolites to regulate hepatic immune imbalance. This article reviews the roles of immune cells, GM, and their metabolites in NAFLD development, while exploring the targets and/or pathways through which botanical drugs and their metabolites modulate GM and hepatic immune responses. This aims to provide a foundation for utilizing botanical drugs as natural adjuvants to address immune dysregulation during NAFLD treatment.

KEYWORDS

gut microbiota, metabolite, immune, NAFLD, NASH, botanical drug

1 Introduction

NAFLD is a highly prevalent chronic progressive liver disease (Diehl and Day, 2017). It typically begins as simple hepatic steatosis, which can progress to NASH, liver fibrosis, cirrhosis, and eventually hepatocellular carcinoma (HCC). Immune responses not only determine the progression from NAFLD to NASH/liver fibrosis (Deng et al., 2022), but the retention and recruitment of immune cells within the liver also activate hepatic stellate cells (HSCs), thereby driving the development of cirrhosis and even HCC (Loomba et al., 2021). Therefore, modulating immune responses represents a promising strategy for mitigating NAFLD progression (Martínez-Chantar et al., 2021). On the other hand, there exists extensive crosstalk between the gut and the liver, which is referred to as the "gut–liver axis" (Tilg et al., 2022). The anatomical and functional connections between the gut and the liver make this axis a crucial pathway for bidirectional communication between the GM and the liver (Wang R. et al., 2021). As shown in Figure 1. A balanced GM and intestinal barrier integrity are essential not only for maintaining the homeostasis of the gut–liver axis (Schnabl, 2013), but also for ensuring hepatic immune stability in the host (Wang J. et al., 2023). Disruption of intestinal homeostasis can alter the immune status of the liver. Dysbiosis of the GM can compromise the intestinal mucosal barrier. Microbial components may enter the systemic circulation via a impaired intestinal barrier in the form of extracellular vesicles (EVs), and bind to pathogen recognition receptors (PRRs) in the liver as pathogen-associated molecular patterns (PAMPs) (Wang R. et al., 2021). This interaction leads to overactivation of immune cells, exacerbation of hepatic inflammation, and stimulation of HSCs, thereby promoting the development of liver fibrosis (Vajro et al., 2013). Additionally, metabolites derived from GM can act as damage-associated molecular patterns (DAMPs) by binding to PRRs on the surface of liver cells such as Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs), and cholangiocytes, thereby inducing hepatic immune responses and aggravating inflammatory liver injury (Wang R. et al., 2021). Thus, the GM can modulate liver immunity via the gut–liver axis, thereby influencing the progression of NAFLD.

The pathological mechanism of NAFLD is highly complex, making single-target therapies largely ineffective. To date, only

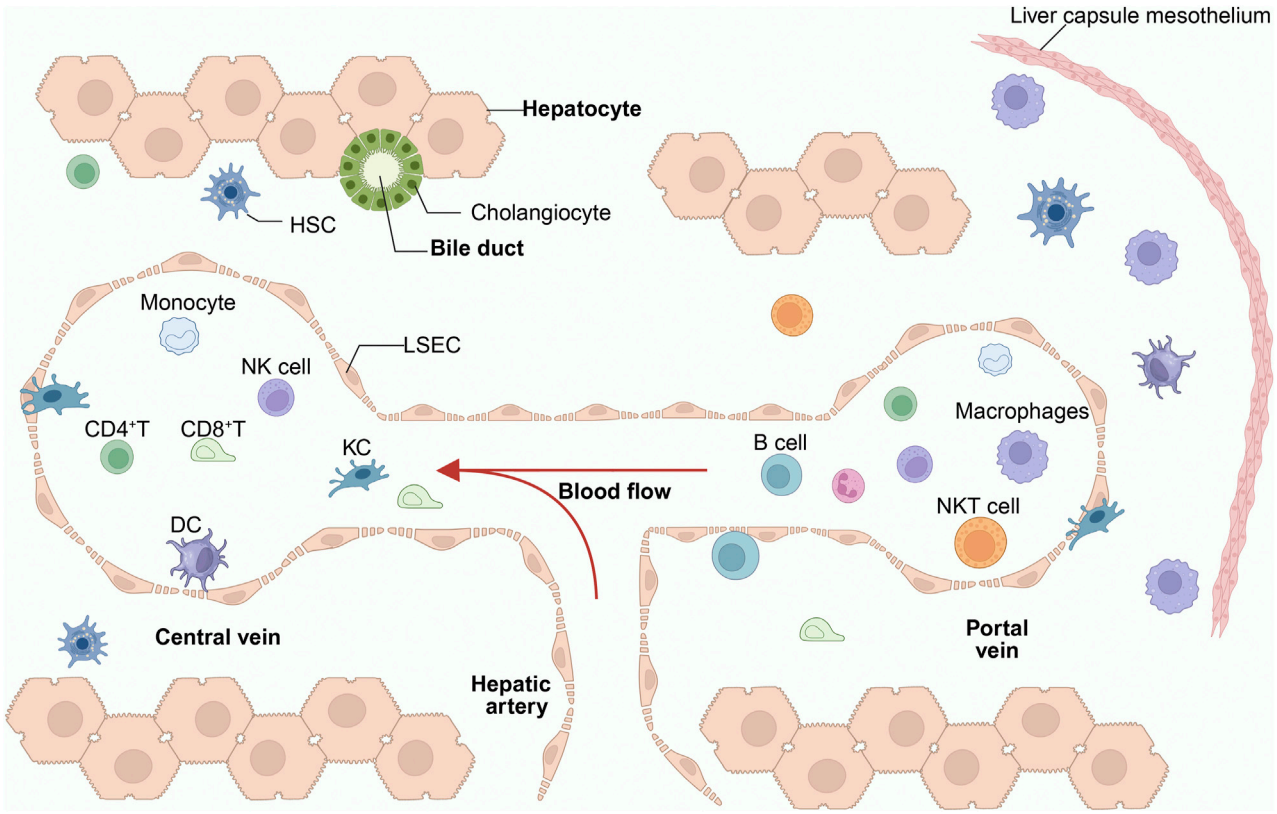

FIGURE 1
Immune status of the liver in a healthy condition. The liver lobules, composed of hepatocytes, are the primary structural units of the liver. The central vein connects to the hepatic artery and the portal vein. Bile ducts transport bile secreted by hepatocytes into the intestines. The liver contains a large number of immune cells, with the hepatic sinusoids being the primary region for their distribution. These include NK cells, LSEC, KCs, $CD4^+$ T cells, DCs, and iNKT cells. Among these, KCs, iNKT cells, and $CD8^+$ T cells are highly enriched in the portal vein region. KCs serve as the cornerstone of liver immunity and are closely connected to LSEC. HSCs primarily reside in the narrow Disse spaces between LSEC and hepatocytes. LCMs are a recently discovered population of macrophages in the liver, primarily distributed in the liver capsule (Glisson's capsule). Under physiological conditions, immune cells maintain a highly organized dynamic equilibrium, simultaneously sustaining basal surveillance against pathogens while avoiding excessive inflammatory responses to harmless antigens from the intestine. KCs, Kupffer Cells; LSECs, Liver Sinusoidal Endothelial Cells; DCs, Dendritic Cells; HSCs, Hepatic Stellate Cells; NK cell, Natural Killer Cell; $CD4^+$ T, CD4-Positive T Lymphocyte; $CD8^+$ T, CD8-Positive T Lymphocyte; LCMs, Liver Capsule Macrophages.

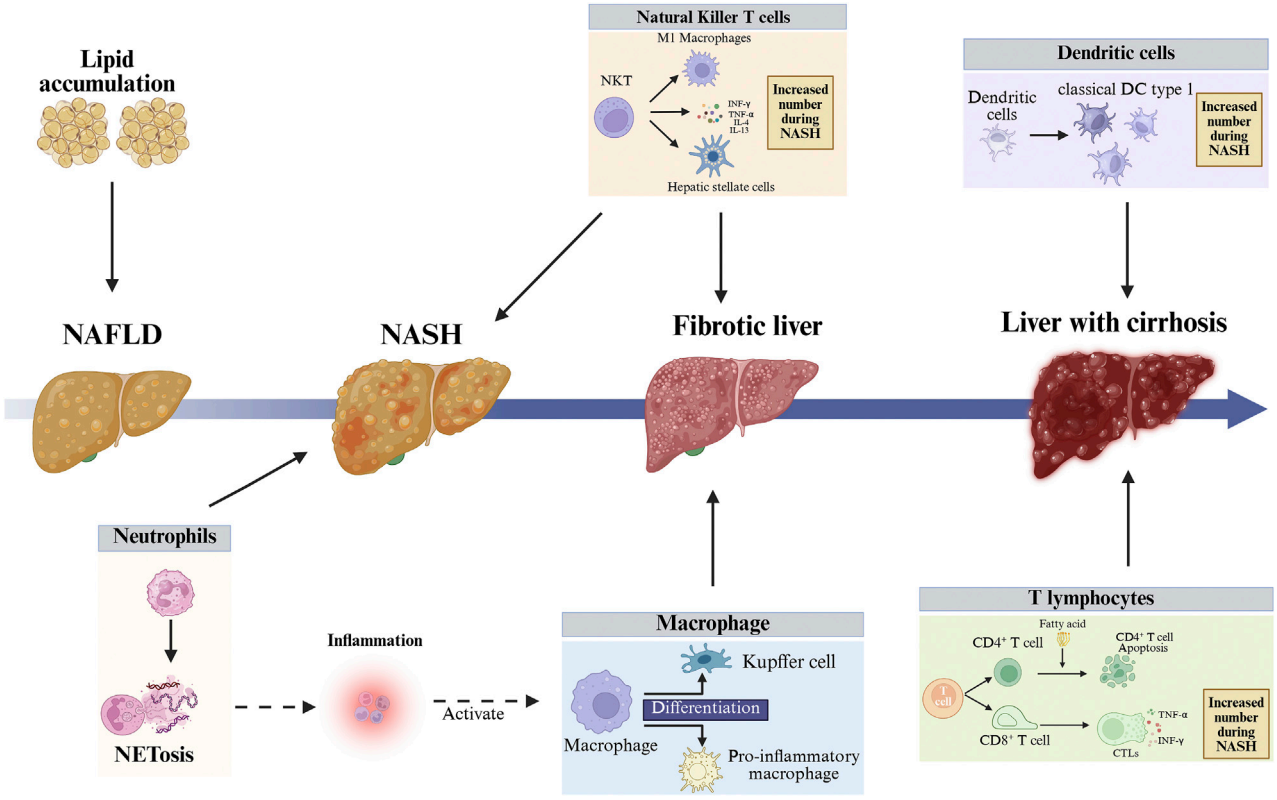

FIGURE 2
Imbalance in the liver's immune environment can lead to liver cell death and liver fibrosis, further exacerbating liver damage. In the early stages of NASH, neutrophils become enriched, and neutrophils exacerbate liver inflammation and liver damage by secreting NETs or through NETosis. cDC1s are an important functional subpopulation of DCs, and their numbers significantly increase in the NASH stage, thereby exacerbating liver immune responses and liver damage. During the progression from NAFLD to NASH, the function of macrophages shifts from "protective" (lipid clearance) to "destructive" (inflammation-driving), with embryonic-derived macrophages differentiating into KCs and recruited monocytes differentiating into pro-inflammatory macrophages. T lymphocytes can be divided into $CD4^+$ T and $CD8^+$ T cells. High levels of free fatty acids in the liver and blood of NAFLD patients induce endoplasmic reticulum stress and oxidative stress in $CD4^+$ T cells, ultimately leading to $CD4^+$ T cell autophagy and downregulation of immune suppressive capacity. Fatty acids activate quiescent $CD8^+$ T cells and drive their differentiation into CTLs. CTLs damage hepatocytes by releasing perforin, Fas ligand, and pro-inflammatory cytokines, exacerbating liver inflammation. NKT cells are activated by free fatty acids, releasing pro-fibrotic cytokines and driving macrophage M1 polarization, thereby activating HSCs. The activation of numerous immune cells disrupts liver immune balance, exacerbating liver inflammation and damage. NASH, Non-alcoholic Steatohepatitis; NAFLD, Non-alcoholic Fatty Liver Disease; NETs, Neutrophil Extracellular Traps; cDC1s, Conventional Dendritic Cells Type 1; KCs, Kupffer Cells; $CD4^+$ T, CD4-Positive T Lymphocyte; $CD8^+$ T, CD8-Positive T Lymphocyte; CTLs, Cytotoxic T Lymphocytes; NKT, Natural Killer T cell; HSCs, Hepatic Stellate Cells.

Resmetirom has been approved for treating NASH patients with moderate hepatic fibrosis (F2~F3 stages). Botanical drugs—natural resources containing multiple bioactive compounds—possess broad pharmacological actions, low toxicity, and high safety profiles, demonstrating excellent potential for treating chronic progressive diseases. Currently, botanical drugs have emerged as significant clinical agents (Hu et al., 2023), attracting considerable research attention. A growing body of studies (Che et al., 2022; Zhu et al., 2023) confirms that botanical drugs can repair damaged intestinal barriers, promote structural remodeling of GM beneficial to host health, alter the production of GM metabolites, and consequently regulate hepatic immune responses. Furthermore, botanical drugs modulate autophagy to promote apoptosis (Niazpour and Meshkani, 2025), inhibit HSC activation and hepatocyte apoptosis to counteract hepatic fibrosis (Wang et al., 2024b), and activate SIRT1 to reduce lipid accumulation and ferroptosis (Liu Y. et al., 2025), and other mechanisms to inhibit NAFLD progression. This also reveals a novel therapeutic strategy for NAFLD. Building upon this foundation, this review synthesizes current research on botanical drugs intervening in NAFLD development through the GM-liver immune axis, thereby providing theoretical support and clinical references for future botanical drug-based NAFLD interventions.

2 Immune cells and NAFLD

As one of the core organs for immune regulation, the liver harbors abundant immune cells that participate in immune responses (Peiseler et al., 2022). Under immune homeostasis, immune cells disperse throughout the liver to expel toxic substances while phagocytosing and eliminating pathogens (Bogdanos et al., 2013). Translocation of gut-derived immune signals to extraintestinal sites triggers hyperactivation of the immune system (Powell et al., 2021), promoting KCs activation in the liver and recruitment of circulatory macrophages to hepatic

tissue (Golabi et al., 2019), thereby intensifying the accumulation and infiltration of inflammatory factors within hepatocytes (Nati et al., 2016). Persistent hepatic inflammation not only induces hepatocyte injury and necrosis, driving NAFLD progression to NASH, but also activates HSCs to accelerate hepatic fibrosis, cirrhosis, and HCC (Kechagias et al., 2020). Research (Huby and Gautier, 2022) has established that NAFLD progression is closely associated with macrophages, neutrophils, dendritic cells (DCs), and natural killer T lymphocytes (NKTs). As shown in Figure 2.

These immune cells synergistically interact through complex networks of cytokines and chemokines, forming an inflammatory cascade. During the progression of NAFLD from early simple fat accumulation to inflammation, fibrosis, and ultimately cirrhosis, the roles of various immune cells exhibit significant stage-dependent changes. In the early stage of fatty liver, excessive lipid deposition in hepatocytes triggers macrophages to initiate inflammation. During the hepatitis phase, macrophages serve as inflammation amplifiers, neutrophils act as executors of early liver injury, while T cells, DCs, and NKT cells participate in amplifying the inflammatory cascade. During the fibrotic stage, macrophages and NKT cells serve as pivotal pro-fibrotic drivers. In the cirrhosis phase, DCs and T cells sustain chronic immune activation while neutrophils persistently promote inflammation, collectively maintaining chronic low-grade inflammatory responses (Huby and Gautier, 2022).

2.1 Macrophages

Hepatic macrophages can be categorized into resident KCs (Res-KCs) and monocyte-derived macrophages (MDMs) based on their cellular origins (Guilliams and Scott, 2022). Lipotoxicity suppresses self-renewal of Res-KCs and induces their apoptosis (Tran et al., 2020; Daemen et al., 2021), thereby inducing monocyte recruitment to the liver and their differentiation into MDMs to replenish the macrophage pool (Guilliams and Scott, 2022). Res-KCs promote liver regeneration by clearing cellular debris and extracellular matrix (Crespo et al., 2023); whereas MDMs typically exhibit high expression of inflammation-related genes, exacerbating liver injury (Tran et al., 2020). Notably, TREM2 expressed in MDMs can reverse their pro-fibrotic function, exerting protective effects through facilitating the clearance of apoptotic hepatocytes and reducing inflammatory factor production (Wang X. et al., 2023). Based on inflammatory phenotypes, macrophages are classified into M1 and M2 subtypes. M1 macrophages drive fibrosis progression by secreting IL-6, TNF-α, various interleukins, and chemokines (CXCL9–11, CCL15/20) (Trinchieri, 2003; Mantovani et al., 2004; Martinez et al., 2006). Conversely, M2 macrophages exert anti-inflammatory effects by expressing TGF-β and IL-10, thereby inhibiting NAFLD progression (Hesse et al., 2001). Studies demonstrate that depleting KCs and MDMs alleviates hepatocyte steatosis, inflammation, and fibrosis, indicating their critical role in NASH pathogenesis (Bartneck et al., 2015; Cynthia and Frank, 2016). Macrophages exhibit dual regulatory functions in NAFLD (Jd and Bl, 2021), offering novel insights for targeted therapies, though challenges persist (Ginhoux et al., 2022). First, macrophage phenotype classification lacks standardization. Second, specific subtypes demonstrate functional complexity and pleiotropy. For instance, while M1 macrophages promote inflammation, they also exert anti-fibrotic effects by phagocytizing debris and secreting MMP-9 to degrade ECM (Heymann et al., 2009). Therefore, clarifying macrophage subtypes and their functions is crucial for developing precise therapeutic strategies.

2.2 Neutrophils

Neutrophils are among the earliest responding immune cells in hepatic inflammation (Rawat and Shrivastava, 2022). In NASH, significant neutrophil infiltration around hepatocytes not only characterizes the disease but also correlates closely with disease progression (Nati et al., 2016). The neutrophil-to-lymphocyte ratio (NLR), as a non-invasive indicator, shows positive correlation with both the NAFLD Activity Score (NAS) and fibrosis staging (Peng et al., 2018). Neutrophils drive hepatic inflammation and directly promote fibrosis through the release of Reactive Oxygen Species (ROS), proteases, Neutrophil Extracellular Traps (NETs), and inflammatory factors (Shrestha and Hong, 2023), among which NETs play a particularly prominent role (Fa et al., 2023). Neutrophils release NETs via a death mechanism known as NETosis (Brinkmann et al., 2004). NETs not only directly cause cellular damage but also induce autoantibody production through immune complex formation, triggering secondary tissue injury (Branzk et al., 2014). Studies have demonstrated that inhibiting NET formation alleviates hepatic inflammation and fibrosis in NASH mouse models while delaying their progression to liver cancer (Zhao et al., 2020). Mechanistically, NETs activate HSCs by triggering the cyclooxygenase-2/prostaglandin E2 pathway through TLR3 signaling, thereby promoting fibrogenesis (Xia et al., 2025). Neutrophil depletion also effectively ameliorates hepatic inflammation and injury in NASH mouse models (Zang et al., 2015). Furthermore, neutrophils indirectly drive NET formation via Notch signaling, exacerbating hepatocyte senescence and lipotoxicity (Xu et al., 2025). NETs can induce intrahepatic microthrombus formation, accelerating the progression from NASH to hepatic fibrosis (Tripodi et al., 2011; Du et al., 2022). This process is associated with their promotion of thrombin and fibrin generation, upregulation of tissue factor expression, and activation of coagulation factor XII (Folco et al., 2018; Shi et al., 2021). Currently, whether NETs-mediated coagulation abnormalities can serve as therapeutic targets for alleviating NASH fibrosis remains understudied and warrants further investigation.

2.3 Dendritic cells

DCs are specialized antigen-presenting cells that sense immune microenvironment changes, recognize pathogens, and detect inflammatory signals (Jenne and Kubes, 2013; Arrese et al., 2016). By transporting phagocytosed antigens to lymphoid organs and activating naive T cells, they bridge innate and adaptive immune responses (Wang H. et al., 2021). In the liver, DCs not only participate in inducing immune tolerance and regulating T cell responses (Bernsmeier and Albano, 2017; Tong

et al., 2023), but also modulate intrahepatic homeostasis and the fibrotic process (Connolly et al., 2009).

In healthy livers, DCs are relatively sparse and exhibit limited capabilities in antigen phagocytosis and T cell stimulation. They primarily maintain tolerance to self-antigens by secreting IL-10 and IL-27 to promote regulatory T cell differentiation (Bernsmeier and Albano, 2017; Méndez-Sánchez et al., 2020). During NASH development, the number of hepatic DCs increases significantly, accompanied by an expansion of classical DC (cDC) progenitor cells in the bone marrow and bloodstream (Deczkowska et al., 2021). Notably, patients exhibit a substantial accumulation of XCR1-expressing cDC1s, whose abundance positively correlates with NASH severity (Deczkowska et al., 2021). Depletion of cDC1s alleviates hepatic inflammation in murine NASH models (Deczkowska et al., 2021). Activated DCs exhibit pro-inflammatory characteristics, releasing inflammatory factors and activating antigen-specific T cells, thereby exacerbating hepatic inflammation (Henning et al., 2013; Deczkowska et al., 2021). Furthermore, lipid accumulation within DCs triggers autoimmune responses, shifting DCs from a tolerogenic state to an immunogenic phenotype (Nati et al., 2016). Accumulated lipids provide precursors for eicosanoid synthesis (e.g., prostaglandins and leukotrienes) (Saka and Valdivia, 2012), while enhancing antigen-presenting function (Anderson and Roche, 2015).

Conversely, some studies report that DCs may also ameliorate NASH-related hepatic inflammation and fibrosis (Henning et al., 2013). For instance, depletion of DCs instead accelerated the progression of hepatic fibrosis (Lukacs-Kornek and Schuppan, 2013), with research indicating that DCs can reverse chemically-induced liver fibrosis through MMP-9 secretion. Specific clearance of DCs delayed fibrosis resolution (Jiao et al., 2012). Therefore, the role of DCs in NASH pathogenesis remains controversial, as DC depletion exhibits opposing effects across different studies. The precise mechanisms by which DCs intervene in NASH require further in-depth investigation.

2.4 T lymphocytes

T lymphocytes originate from hematopoietic multipotent stem cells in the bone marrow and can be classified into two subsets: $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes. Upon binding to MHC-II, $CD4^+$ T lymphocytes differentiate into multiple subsets including helper T cell 1 (Th1), Th2, Th17, and regulatory T cells (Tregs); while $CD8^+$ T lymphocytes are also termed cytotoxic T cells (CTLs) (Nati et al., 2016). Dysregulation of $CD4^+$ T lymphocytes represents one characteristic feature in the progression of chronic liver diseases (Ficht and Iannacone, 2020). Th17 and Tregs constitute crucial $CD4^+$ T lymphocyte subsets involved in NASH pathogenesis regulation. Under physiological conditions, Th17 and Tregs maintain a balanced state (Świderska et al., 2017). Th17/Treg imbalance leads to deterioration of NAFLD/NASH (Josefowicz et al., 2012; Chackelevicius et al., 2016). Inflammatory $CXCR3^+$ Th17 cells accumulate in the liver, driving NAFLD progression to NASH through cytokine release and macrophage activation (Moreno-Fernandez et al., 2021). Studies indicate that the intrahepatic and peripheral Th17/Treg ratio in NAFLD patients reflects disease severity and progression risk (He et al., 2017). However, the role of Treg in NASH remains controversial, with traditional views emphasizing its anti-inflammatory function (Wachtendorf et al., 2024), yet research (Dywicki et al., 2022) conversely demonstrates that Treg supplementation exacerbates hepatic inflammation in murine NASH models and activates HSCs via the amphiregulin (Areg)-epidermal growth factor receptor (EGFR) pathway (Savage et al., 2024).

$CD8^+$ T cells predominantly exert pro-inflammatory effects in NASH by secreting cytotoxic molecules such as IFN-γ, TNF-α, and perforin to induce target cell apoptosis (Wong and Pamer, 2003). NASH patients exhibit increased numbers of activated $CD8^+$ T cells in both hepatic tissues and systemic circulation (Bhattacharjee et al., 2017). Notably, $CXCR6^+$ $CD8^+$ T cells exacerbate hepatic inflammation through an acetate-driven gut-liver axis (Dudek et al., 2021). Depletion of $CD8^+$ T cells reduces the risk of NASH-associated HCC development (Pfister et al., 2021). However, some studies indicate that specific $CD8^+$ tissue-resident memory T cells ($CD8^+$ Trm) can alleviate fibrosis by inducing apoptosis in activated HSCs (Koda et al., 2021). Furthermore, $CD8^+$ T cell function is regulated by the metabolic environment: it activates HSCs in high-fat diet (HFD) models but does not significantly alter liver injury or fibrosis levels in choline-deficient high-fat diet (CD-HFD) models (Breuer et al., 2020). Evidently, T cells and their subsets play diverse and sometimes opposite roles in NASH, which involves a complex immunoregulatory network. Developing strategies targeting specific T cell subsets to inhibit NAFLD may offer novel therapeutic approaches for NASH.

2.5 Natural killer T lymphocytes

Natural killer T (NKT) cells are a specialized T cell subpopulation that co-express T cell receptors (TCR) and NK cell receptors (Zhang and Zhang, 2020), capable of recognizing lipid antigens (such as endogenous sphingolipids or exogenous α-galactosylceramide) presented by CD1d molecules through semi-invariant TCRs (Hung et al., 2017). NKT cells can be categorized into two main subtypes: invariant (iNKT) and diverse (dNKT), with the former typically dominating pro-inflammatory responses while the latter exhibits anti-inflammatory regulatory functions (Tang et al., 2022).

During NAFLD progression, NKT cells demonstrate distinct stage-specific and context-dependent characteristics. During the simple fatty liver stage, iNKT cells suppress macrophage M1 polarization by producing anti-inflammatory factors such as IL-4 and IL-10. When the disease progresses to NASH, the lipotoxic microenvironment (e.g., free fatty acids and oxidized lipids) induces upregulation of CD1d expression in hepatocytes, thereby activating NKT cells (Tajiri and Shimizu, 2012). Activated NKT cells explosively secrete pro-inflammatory factors including IFN-γ, TNF-α, and IL-17, while recruiting neutrophils and monocytes via chemokine pathways, thereby exacerbating hepatic inflammation. (Arrenberg et al., 2011; Maricic et al., 2015; Mathews et al., 2016). Moreover, iNKT cells can directly activate HSCs through the Hedgehog signaling pathway (Nimmerjahn et al., 2005), and promote fibrogenesis via mediators such as osteopontin (OPN) (Marrero et al., 2015). Notably, IL-4 derived from NKT cells induces GARP protein expression on HSCs, thereby activating TGF-

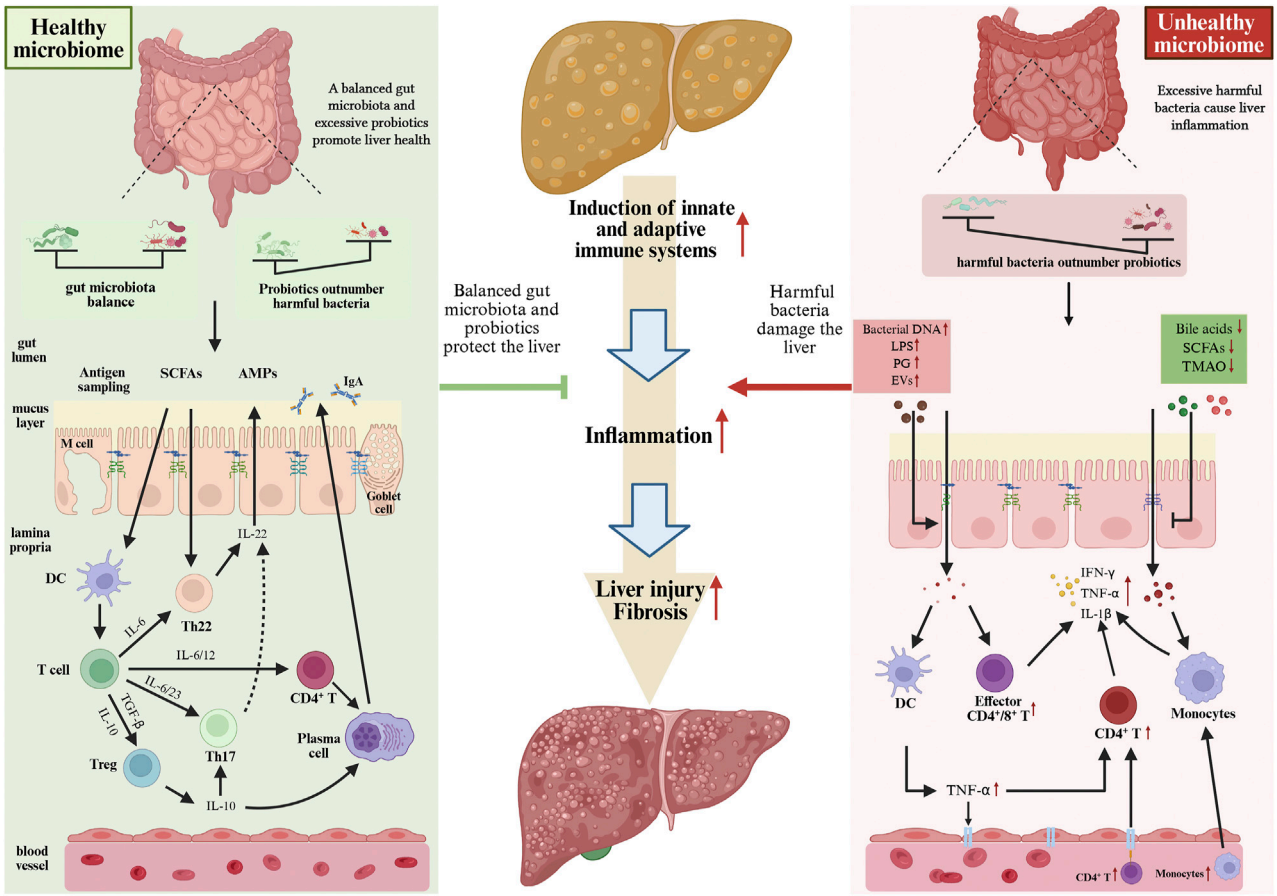

FIGURE 3
Different states of the GM can influence liver immune balance, thereby interfering with the progression of NAFLD to NASH. When the GM is in a balanced state or when the number of probiotics exceeds that of harmful bacteria, it releases various beneficial metabolites, which are crucial for maintaining liver immune balance. However, disruptions in the GM and its metabolites (where harmful bacteria outnumber beneficial bacteria) can induce liver immune imbalance, ultimately driving the progression of NAFLD to NASH. GM, gut microbiota; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

β signaling and driving hepatic fibrosis. Traj18 gene knockout in mice leads to NKT cell deficiency, consequently suppressing GARP expression on HSCs and ultimately delaying NASH progression (Zhang et al., 2023).

NKT cell functionality is also modulated by GM: Bacteroides-derived lipid antigens regulate their activation state (Wieland Brown et al., 2013; An et al., 2014). Fecal microbiota transplantation experiments demonstrate that GM from alcoholic hepatitis patients can induce liver injury in mice, while restructuring the GM prevents alcohol-induced liver injury (Llopis et al., 2016). However, the specific mechanisms underlying microbiota–NKT cell interactions remain to be elucidated (Marrero et al., 2018). Notably, NKT cells constitute 20%–30% of lymphocytes in mouse livers, but account for less than 5% in human livers (Exley and Koziel, 2004). Therefore, extrapolating pathological significance from mouse models to humans requires particular caution. Future research should prioritize validation with human-derived samples and conduct in-depth analyses of regulatory mechanisms among different NKT cell subsets within disease microenvironments.

3 Crosstalk between gut microbiota and immunity in NAFLD

GM participates in the onset and progression of NAFLD through the Gut-Liver Axis (Wu et al., 2021). GM dysbiosis and intestinal barrier damage facilitate the entry of microbial metabolites into systemic circulation, including LPS, peptidoglycan, bacterial DNA, EVs, and trimethylamine N-oxide (TMAO), which activate hepatic immunity and promote inflammation (Song and Zhang, 2022). Certain metabolites, including SCFAs, bile acids (BAs), and tryptophan metabolites, exhibit anti-inflammatory and hepatoprotective effects (Stiglund et al., 2019). Intervention targeting GM has become a crucial strategy for NAFLD treatment (Agus et al.,

2021), and their interaction mechanisms with immunity offer new directions for future therapies. As shown in Figure 3.

3.1 Alterations in gut microbiota composition and abundance in non-alcoholic fatty liver disease

GM exhibits significant inter-individual variations, which are closely associated with geographical regions, dietary patterns, and other factors (Sarfraz et al., 2022; Procházková et al., 2024). For example, a study of populations across different regions in China revealed (Zhang J. et al., 2024) that northern residents exhibited higher abundance of *Bifidobacterium* in their GM, while southern residents showed enrichment of *Blautia* and *Lachnospiracea incertae sedis*, potentially associated with differences in dietary habits. GM directly influences the efficacy of botanical drugs, and its biotransformation capabilities are crucial for the metabolites derived from botanical drugs to exert therapeutic effects. Variations in GM composition and metabolic functions among individuals may lead to inconsistent therapeutic outcomes from identical botanical drug interventions. For instance, ginsenoside Rb1 requires transformation by specific bacterial strains into the highly active Compound K to exert anticancer effects, the absence of these specific bacteria compromises the efficacy of ginsenoside intake (Wan et al., 2017). Significant differences exist in GM composition between NAFLD patients and healthy individuals (Quesada-Vázquez et al., 2022). The pathogenesis of NAFLD is negatively correlated with the alpha-diversity of GM (Alferink et al., 2021). Multiple studies indicate that NAFLD development is associated with reduced GM diversity and alterations in specific bacterial genera: *Ruminococcaceae* and *Veillonellaceae* show positive correlation with hepatic fibrosis severity and demonstrate pro-NAFLD effects in mouse models (Lee et al., 2020).

GM structure undergoes dynamic changes across different pathological stages of NAFLD. During NAFLD progression, Gram-negative bacteria (particularly LPS-producing genera such as *Escherichia*, *Prevotella*) increase, while SCFA-producing Gram-positive bacteria (e.g., *Ruminococcaceae*) decrease (Li F. et al., 2021). Notably, through comparative analysis of GM between NAFLD patients and non-NAFLD individuals, researchers proposed *Phascolarctobacterium*, *Slackia*, and *D. formicigenerans* as biological signatures of NAFLD patients (Leung et al., 2022). However, the small sample size necessitates further validation of these conclusions. During the NASH stage, the abundance of *Clostridium coccoides* significantly increases, and hepatic fibrosis progression exhibits a positive correlation with *Ruminococcus* abundance (Boursier et al., 2016). Patients with hepatic fibrosis show reduced abundance of *Enterococcus faecalis* and *Faecalibacterium prausnitzii*. Butyrate produced by these bacteria plays a crucial role in maintaining intestinal barrier integrity (Kwan et al., 2022). Studies indicate that compositional characteristics of GM can differentiate between early and late stages of hepatic fibrosis (Loomba et al., 2017). Stable GM helps maintain hepatic immune tolerance and suppresses excessive inflammation, whereas GM dysbiosis can induce chronic hepatitis and promote the formation of a microenvironment conducive to NAFLD progression. Targeting GM to modulate the immune system has become a novel strategy for NAFLD treatment. For instance, *Bifidobacterium* exerts immunomodulatory effects by upregulating regulatory T cells, enhancing intestinal barrier function, and suppressing the activity of macrophages and dendritic cells (Gavzy et al., 2023). GM also provides new therapeutic targets for NAFLD-associated HCC. Modulating GM can influence immune regulatory molecules on T cell surfaces, thereby enhancing the efficacy of immune checkpoint inhibitors (e.g., anti-PD-1/PD-L1 and anti-CTLA-4 therapies) in HCC treatment (Huang M. et al., 2025). Although the mechanisms linking GM with NAFLD remain incompletely elucidated, GM-targeted intervention strategies undoubtedly hold broad application prospects.

3.2 GM-derived metabolites

3.2.1 LPS

LPS plays a critical role in the onset and progression of NAFLD (Carpino et al., 2020; Ji et al., 2020). Following intestinal barrier damage, LPS translocates into the portal circulation. By binding to Toll-like receptor 4 (TLR4) on HSCs and KCs, it activates the NF-κB signaling pathway, promoting the expression of inflammatory factors and fibrogenic factors, thereby exacerbating hepatic inflammation and fibrosis (Reid et al., 2016). Beyond TLR4, LPS can also bind to lipopolysaccharide-binding protein (LBP), facilitating the formation of the CD14-TLR4 complex and further enhancing inflammatory responses (Csak et al., 2011). Studies demonstrate that blocking the LPS-TLR4 signaling pathway or reducing plasma LPS levels with polymyxin B significantly alleviates liver injury and steatosis in mice (Pappo et al., 1992; Xu et al., 2023). LPS also accelerates hepatic fibrosis progression by upregulating TGF-β expression, activating the small mother against decapentaplegic (Smad) pathway, and promoting transcription of type I and III collagen (Zhong et al., 2022). For instance, *Escherichia* coli-derived LPS activates macrophages via the TLR4 pathway, exacerbating liver injury, whereas inhibition of this pathway markedly mitigates hepatic lesions (Carpino et al., 2020). On the other hand, LPS also participates in immunomodulatory processes and can induce endotoxin tolerance. Through the LPS/TLR4 pathway, it promotes the expansion of monocyte-derived myeloid-derived suppressor cells (mMDSCs) in the liver and downregulates T cell populations, thereby modulating local immune responses (Schneider et al., 2022). LPS promotes $CD14^+$ $CD8^+$ T cells to secrete protective cytokines such as IL-6 and IL-33, and influences immune cell chemotaxis (Pallett et al., 2023). These effects are closely associated with decreased TLR4 and IRAK expression, along with altered p65/p50 ratios in NF-κB (Fan and Cook, 2004).

3.2.2 Peptidoglycan

Peptidoglycan (PG), derived from gut bacteria in the host intestinal tract, constitutes the core structural component of bacterial cell walls (Meroueh et al., 2006). The diversity of GM gives rise to various types of PG (Krueger et al., 1982). As microbe-associated molecular patterns (MAMPs), PG can activate pattern recognition receptors TLR2, NOD1, and NOD2, thereby eliciting immune responses (Wagner and Cresswell, 2012; Juárez-Verdayes

et al., 2013). NOD1 recognizes iE-DAP fragments while NOD2 identifies MDP fragments; both receptors activate NF-κB/MAPK signaling pathways, promote hepatic inflammation, and contribute to NAFLD progression (Travassos et al., 2004; Al Nabhani et al., 2017; Keestra-Gounder and Tsolis, 2017). In immune regulation, NOD1 perceives nutritional signals and drives neutrophil migration toward the liver, exacerbating inflammatory reactions (Dharancy et al., 2010; Meli et al., 2014). Notably, during advanced NAFLD stages, NOD1 promotes OX40L expression through metabolic reprogramming, upregulates $CD8^+$ T cell activity, and thereby enhances immune responses to combat HCC (Zhang F. et al., 2024). In contrast, NOD2 primarily maintains GM balance and epithelial barrier homeostasis (Balasubramanian and Gao, 2017). Its activation stimulates Paneth cells to produce antimicrobial peptides (e.g., α-defensins) and enhances mucus secretion by goblet cells, thereby restricting bacterial translocation (Tan et al., 2015). Furthermore, NOD2 participates in host defense by modulating the MDP–NF-κB axis while moderately suppressing excessive TLR2 activation, thus alleviating intestinal inflammation (De Bruyn and Vermeire, 2017), this mechanism has been explored for therapeutic application in Crohn's disease (Al Nabhani et al., 2020). Metabolically, NOD1 activation promotes metabolic inflammation and insulin resistance, whereas NOD2 exhibits anti-inflammatory and metabolic protective effects. This process requires the involvement of Receptor-Interacting Serine/Threonine-Protein Kinase 2 (RIPK2) (Cavallari et al., 2020). Therefore, targeting the PG–NOD1/NOD2 signaling pathways may offer novel therapeutic strategies for NAFLD.

3.2.3 Bacterial DNA

As PAMPs, bacterial DNA enters host endosomes through various endocytic pathways. Within endosomes, TLR9 recognizes bacterial DNA-derived CpG oligonucleotides, initiating the MAPK/NF-κB pathway and triggering inflammatory factor secretion (Gomes et al., 2016; Mridha et al., 2017). Moreover, TLR9 signaling can interfere with IRF7 phosphorylation through the IKKα–LC3 pathway, thereby inducing the production of Type I interferon (Hayashi et al., 2018). Additionally, the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway can also recognize bacterial DNA (Zhang and Zhang, 2025). Bacterial DNA triggers dsDNA formation, which then activates cGAS by forming a complex. This activation generates the second messenger cGAMP, which binds to STING to activate TBK1. TBK1 phosphorylates both STING and the IRF3 transcription factor. Phosphorylated IRF3 induces Type I interferon synthesis, while STING accelerates NF-κB activation through IκBα phosphorylation (Zhang and Zhang, 2025). This collectively indicates that bacterial DNA can activate innate immune responses through multiple signaling pathways, playing a significant driving role in the onset and progression of NAFLD.

3.2.4 Extracellular vesicles

GM-derived EVs are bilayer membrane-structured nanoparticles released by bacteria, carrying substantial amounts of toxic microbial molecules. These particles can traverse the compromised intestinal barrier into circulation, target the liver, and participate in NAFLD pathogenesis by activating immune-inflammatory responses (Yáñez-Mó et al., 2015). Components such as LPS carried by EVs can enter the cytoplasm via the TLR4–TRIF–GBP3 pathway, activate Caspase-11, and induce inflammation through receptors including TLR2 and NOD1/2 (Bielig et al., 2011; Gu et al., 2019). Furthermore, EVs can promote hepatic inflammation and fibrosis through the TLR4 and NLRP3–GSDMD signaling pathways (Dorner et al., 2024). EVs derived from feces of NASH patients (NASH-fEVs) disrupt intestinal barrier integrity, increase permeability, and activate HSCs via the TLR/LPS pathway, thereby upregulating fibrosis-related protein expression (Fizanne et al., 2023). Notably, the impact of EVs on the liver is context-dependent: for instance, miRNA (miR-129-2-3p) derived from *Fusobacterium nucleatum* can exacerbate intestinal inflammation by promoting cellular senescence (Wei et al., 2023). Conversely, remodeling GM using the PPARα inhibitor GW6471—which increases the abundance of probiotics (e.g., *Bacteroides*) while reducing harmful bacterial populations—alleviates hepatic lipid accumulation, ferroptosis, and oxidative stress, thereby improving NAFLD (Yang X. et al., 2025). In summary, EVs exhibit dual roles in NAFLD, where their specific effects are contingent upon both their microbial origin and the host microenvironment. Further investigation into the immune-regulatory mechanisms of active components within EVs will help elucidate their pathological significance and therapeutic potential in NAFLD.

3.2.5 Indole and its derivatives

Intestinal tract commensal bacteria metabolize tryptophan (Trp) into various indole derivatives, including indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), indole-3-aldehyde (IAld), indole-3-lactic acid (ILA), and tryptamine (Su et al., 2022). Different bacteria possess distinct tryptophan enzymes that produce specific indole derivatives (Dodd et al., 2017). Indole derivatives accumulate in the intestinal tract and activate the Aryl Hydrocarbon Receptor (AhR) on innate lymphoid cells, thereby promoting goblet cell differentiation and mucus secretion (Powell et al., 2020), and induce tight junction protein expression (Shimada et al., 2013), enhancing the integrity and functionality of the intestinal barrier. On the other hand, AhR is widely expressed in various immune cells, such as DCs, T cells, and lymphocytes (Nguyen et al., 2010; Rothhammer and Quintana, 2019), mediating the regulation of the immune system by indole derivatives, including promoting Treg differentiation (Goettel et al., 2016), inducing T cell apoptosis (Landfried et al., 2011), and suppressing the inflammatory activity of Th17 cells (Rothhammer and Quintana, 2019), thereby alleviating hepatic inflammation. Furthermore, indole derivatives (e.g., IAA and IPA) produced by specific strains (e.g., probiotics) inhibit the NF-κB pathway, reduce levels of pro-inflammatory factors (IL-8), and promote the release of anti-inflammatory factors (IL-10) (Bansal et al., 2010). Clinical studies have revealed decreased levels of IPA and IAA in the feces of NAFLD patients. Increasing the abundance of *Bifidobacterium bifidum*—the primary source of these metabolites—significantly ameliorates hepatic steatosis and inflammation in mice (Min et al., 2024). Furthermore, indole derivatives promote the proliferation of intestinal crypt epithelial-tubular cells in mice, which plays a critical role in maintaining intestinal immune homeostasis (Powell et al., 2020).

3.2.6 Bile acids

BAs are important signaling molecules generated through enzymatic conversion of cholesterol in the liver. By activating specific receptors, they regulate hepatic lipid metabolism and suppress the transcription of inflammatory factors, thereby influencing NAFLD progression. Among these, Farnesoid X Receptor (FXR) and Takeda G-protein Receptor 5 (TGR5) represent two critical BAs receptors (Chávez-Talavera et al., 2017). FXR plays a pivotal role in anti-inflammatory and immunomodulatory processes, BAs activate FXR to suppress NF-κB signaling, thereby reducing its induction of inflammatory mediators such as IFNγ and COX-2 (Wang et al., 2008). This downregulates monocyte chemoattractant protein-1 (MCP-1) expression, diminishing macrophage infiltration into the liver (Li et al., 2015). Simultaneously, FXR activation restores the intestinal vascular barrier by triggering the endothelial Wnt/β-catenin pathway, which blocks bacterial translocation and alleviates hepatic inflammation (Mouries et al., 2019). FXR signaling also acts on macrophages, NK cells, and DCs, restricting the production of pro-inflammatory factors and suppressing inflammasome activation (Sun et al., 2021; Fiorucci et al., 2022). Since TGR5 is widely distributed in cell types including HSCs, LSECs, and macrophages (Keitel et al., 2007). TGR5 activated by BAs promotes macrophage M2 polarization (Shao et al., 2022), inhibits the TLR4–NF-κB pathway (Biagioli et al., 2017; Hosseinkhani et al., 2021), suppresses NLRP3 inflammasome activation and IL-1β secretion through the cAMP–PKA signaling axis (Pols et al., 2011; Guo C. et al., 2016), and induces endothelial nitric oxide synthase (eNOS) expression to exert anti-inflammatory effects (Keitel et al., 2007). Additionally, TGR5 promotes the release of glucagon-like peptide-1 (GLP-1) in the intestinal tract, thereby improving insulin sensitivity and lipid metabolism (Thomas et al., 2009). Notably, BAs with different structures exhibit distinct effects, for example, 12α-hydroxylated BAs (12α-OH BAs) paradoxically promote HSCs proliferation by binding to TGR5. Simultaneously, they upregulate hepatic fibrosis-related proteins (α-SMA, TGF-ß, COL I, PDGF) and exacerbate fibrosis progression through activation of ERK1/2 and p38 MAPK signaling pathways (Xie et al., 2021).

3.2.7 Short-Chain fatty acids

SCFAs modulate immune and metabolic responses through multiple pathways, thereby influencing the progression of NAFLD. SCFAs promote proliferation of intestinal epithelial cells, enhance expression of tight junction proteins (e.g., ZO-1, occludin, claudin-1, and claudin-2), and activate hypoxia-inducible factor (HIF) to maintain intestinal barrier integrity (Nicolas and Chang, 2019). Butyrate also induces expression of the antimicrobial peptide β-defensin-1, reducing levels of LPS-carrying bacteria and LPS (Beisner et al., 2021). Furthermore, it inhibits the increase in intestinal permeability mediated through the TLR4/myeloid differentiation factor (MyD88) signaling pathway (Nighot et al., 2017). SCFAs deficiency impairs barrier function by causing inadequate energy supply to intestinal epithelium and disrupting mucosal immune homeostasis (Matsumoto et al., 2017).

Secondly, SCFAs regulate immune cell function through G protein-coupled receptors (GPRs) and Toll-like receptors (TLRs) (Canfora et al., 2015). For instance, butyrate promotes anti-inflammatory factor IL-10 secretion and Treg differentiation via GPR109a, while suppressing release of inflammatory factors such as IL-1β, IL-6, and TNF-α through TLR4 (Feingold et al., 2014; Sam et al., 2021). SCFAs also promote Treg differentiation via epigenetic mechanisms including HDAC inhibition and enhanced histone H3 acetylation in the Foxp3 promoter region, thereby ameliorating hepatic inflammation (Furusawa et al., 2013; Park et al., 2015). Supplementation of butyrate-producing *Clostridium* butyricum B1 (CB) in the NASH mouse model reversed HFD-induced hepatic steatosis, suppressed hepatic MCP-1 and TNF-α expression, reduced pro-inflammatory factors (IFN-γ and IL-17) in both liver and intestinal tract, and increased anti-inflammatory factors ($FOXP3^+$, IL-4, and IL-22). These findings were corroborated by *in vitro* experiments (Zhou et al., 2017).

Additionally, SCFAs intervene in NAFLD through energy metabolism regulation. SCFA supplementation ameliorated hepatic steatosis in mice (Shimizu et al., 2019). Acetate and propionate stimulate peptide YY (PYY) and insulin-like growth factor-1 (IGF-1) release via GPR41/43, thereby suppressing appetite and energy intake. Concurrently activates AMP-activated protein kinase (AMPK) to reduce lipid accumulation (Deng et al., 2020). Sodium butyrate regulates hepatic lipid metabolism by promoting GLP-1 secretion from intestinal L cells (Zhou et al., 2018). *Clostridium* butyricum capsules combined with rosuvastatin demonstrate superior efficacy over monotherapy in lipid regulation, anti-fibrotic effects, and liver function improvement (Zhu et al., 2022). Notably, acetate promotes liver regeneration by inducing SCD1 expression (Yin et al., 2023), providing novel directions for NAFLD treatment.

3.2.8 TMAO

Circulating TMAO levels exhibit positive correlations with NAFLD incidence risk, disease severity, and all-cause mortality (Flores-Guerrero et al., 2021). TMAO promotes NAFLD progression through multiple mechanisms. TMAO elevates mitochondrial ROS levels, activates the NF-κB signaling pathway, thereby promoting NLRP3 inflammasome assembly and the release of inflammatory factors such as IL-1β. It also disrupts calcium homeostasis in pancreatic β-cells, leading to dysfunction (Kong et al., 2024). Secondly, TMAO impairs intestinal barrier function by suppressing the Wnt/β-catenin pathway and activating TLR4/MyD88/NF-κB signaling. Simultaneously, it induces LSEC dysfunction and capillarization, while promoting macrophage M1 polarization (Nian et al., 2024). Research reveals that TMAO activates the PERK signaling pathway in zebrafish liver and HepG2 cells, inducing pathological alterations including lipid accumulation, inflammation, and fibrosis (Yang et al., 2024). TMAO triggers endoplasmic reticulum stress (ERS), activates macrophages via the TLR pathway, and exacerbates inflammatory responses (Hakhamaneshi et al., 2021). Concurrently, TMAO suppresses BAs synthesis, disrupts cholesterol metabolism, aggravates intrahepatic lipid accumulation, promotes foam cell formation, and inhibits reverse cholesterol transport (RCT), thereby further compromising hepatic lipid homeostasis (Janeiro et al., 2018). Reduced TMAO synthesis can lower the risk of NAFLD onset and progression (Corbin and Zeisel, 2012).

TABLE 1 The mechanism of botanical drugs in treating NAFLD by targeting GM-immune response.

Metabolites	Botanical drug	Experimental model	Regulation of GM and its metabolism	Targeting immune	Refs
Berberine (BBR)	*Coptis chinensis* Franch	C57BL/6J mice	*Bifidobacterium* ↑, *Bacteroidetes/Firmicutes* ↑	IL-1 ↓, IL-6 ↓, TNF-α ↓, CD14 ↓	Cao et al. (2016)
		Six-week-old SD male rats	*Faecalibacterium prausnitzii* ↓; *Bacteroides* ↑	BBR increases the expression level of occludin, improves intestinal mucosal damage, and reduces the level of inflammatory factors in serum	Li et al. (2017)
		SD male rats	Atopobiaceae ↓, Rikenellaceae ↓, Christensenellaceae ↑; Coriobacteriales ↓; Brevibacterium ↑, Papillibacter↓; gut microbiota diversity ↑	Reduce damage to the intestinal barrier, decrease the translocation of LPS from the intestines to the liver, thereby alleviating liver inflammation	Chen et al. (2023a)
		C57BL/6J mice	No specific mechanism	Inhibits JNK1 signaling and downregulates NF-KB pathway activity	Guo et al. (2016b)
Resveratrol, (RSV)	*Polygonum cuspidatum Sieb. et Zucc*	C57BL/6J mice	No specific mechanism	Activate the AMPKα-SIRT1 signaling pathway to inhibit the NF-κB inflammatory pathway	Tian et al. (2016)
		SD male rats	*Ruminococcaceae*↑, *Lachnospiraceae*↑, *Desulfovibrio*↓	Enhance the expression occludin, ZO1, and claudin-1 to improve intestinal barrier function and reduce liver inflammation	Chen et al. (2020)
		SD male rats	*Desulfovibrio*↓,*Lachnospiraceae_NK4A316_group*↓, *Alistipes*↓, *Allobaculum*↑, *Bacteroides*↑, *Blautia*↑; SCFAs ↑	Improved intestinal barrier integrity, inhibited the migration of LPS from the intestine to the liver, and alleviated low-grade inflammation in the liver	Wang et al. (2020b)
Curcumin (Cur)	*Curcuma longa* L	Male C57BL/6J mice	*Firmicutes/Bacteroidetes* ↓, *Akkermansia* ↑	Increasing the expression levels of occludin and ZO1, inhibiting the activation of the TLR4/NF-κB signaling pathway in the liver, and reducing the suppression of LPS-induced immune responses in the liver	Hong et al. (2022)
		Male C57BL/6J mice	*Firmicutes/Bacteroidetes* ↓, *Desulfovibrio* ↓ *Akkermansia* ↑, *Bacteroides* ↑, *Parabacteroides* ↑, *Alistipes* ↑, *Alloprevotella* ↑	Reduce HFD-induced hepatic steatosis and serum LPS concentration in mice, and alleviate LPS-induced hepatic inflammation	Li et al. (2021b)
		Male C57BL/6J mice	No specific mechanism	Cur effectively inhibits lipopolysaccharide and IFN-γ-induced M1 macrophage activation and reduces IL-1β and TNF-α	Tong et al. (2021)
Quercetin (QUE)	*Scutellaria baicalensis* Georgi	Male C57BL/6J mice	*Firmicutes/Bacteroidetes* ↓, *Helicobacter* ↓	Inhibits LPS synthesis, suppresses activation of the TLR4/NF-κB signaling pathway, and inhibits inflammasome activation	Porras et al. (2017)
		C57BL/6J mice	No specific mechanism	Increase the expression of SOD and GPX1 to enhance antioxidant capacity, and	Jiang et al. (2025)

(Continued on following page)

TABLE 1 (*Continued*) The mechanism of botanical drugs in treating NAFLD by targeting GM-immune response.

Metabolites	Botanical drug	Experimental model	Regulation of GM and its metabolism	Targeting immune	Refs
				block the phosphorylation of IκBα and NF-κB p65 to inhibit excessive activation of the immune response	
Lycium barbarum polysaccharides (LBPs)	*Lycium chinense* Mill	SD male rats	*Verrucomicrobia* ↓, *Enterococcaceae* ↓	Reduce intestinal LPS synthesis and LPS migration to the liver, and block LPS activation of KCs in the liver	Hu et al. (2020)
		SD male rats	*Butyricicoccus* ↑, *Butyricimonas* ↑	Promote butyrate secretion, thereby increasing intestinal mucus and the expression of ZO-1 and occludin, and inhibiting LPS-induced liver inflammation	Gao et al. (2021)
		Human	*Bacteroides* ↑, *Bifidobacterium* ↑, *Phascollarctobacterium* ↑, *Prevotella* ↑, *Collinsella* ↑, SCFAs ↑	No specific mechanism	Ding et al. (2019)
Poria cocos Polysaccharide (PCP)	*Wolfiporia cocos* (F. A. Wolf) Ryvarden & Gilb	C57BL/6J mice	*Faecalibaculum* ↑, gut microbiota diversity ↑, LPS ↓	Inhibiting the NF-κB/CCL3/CCR1 signaling pathway to reduce immune responses in the liver	Tan et al. (2022)
		C57BL/6J mice	No specific mechanism	Improving liver cell apoptosis and repairing the intestinal barrier by inhibiting the CYP2E1/ROS/MAPKs signaling pathway to reduce liver immune response	Jiang et al. (2022)
Ginsenoside	*Panax ginseng* C. A. Mey	C57BL/6J mice	*Akkermansia* ↑,*Oscillospira* ↑,*Phascolarctobacterium* ↑, *Bacteroides* ↑, *Dehalobacterium*↑, *Allobaculum* ↓, *Olsenla* ↓	Increase the expression of ZO-1, Occludin, and Claudin-1 to maintain intestinal barrier function and reduce the risk of liver inflammation driven by enteric LPS.	Shi et al. (2024)
		C57BL/6J mice	No specific mechanism	Inhibiting SIRT1 and FOXO1 in the liver to interfere with NF-κB signaling and oxidative stress, thereby alleviating liver inflammation	Wu et al. (2025)
pachymic acid (Pac)	*Wolfiporia cocos* (F. A. Wolf) Ryvarden & Gilb	C57BL/6J mice	*Firmicutes*/*Bacteroidetes* ↓, *Akkermansia* ↑, *Desulfovibrio* ↓, *Streptococcus* ↓, gut microbiota diversity ↑	Inhibiting the LPS/TLR4/MYD88/NFκB signaling pathway to reduce liver inflammation. Pac downregulates the expression of FASN, SREBP1c, and SCD1 to reduce lipid synthesis, while promoting the expression of PPARα and CPT1α to enhance fatty acid oxidation, ultimately reducing liver inflammation induced by lipid accumulation	Ren et al. (2025a)

TABLE 2 Researches on the treatment of NAFLD by targeting the GM-immune response with botanical drugs formulae.

Botanical drugs formulae	Botanical drugs	Model	Regulation of GM and its metabolism	Targeting immune and inflammatory responses	Refs
Yinzhihuang granule (YZHG)	*Artemisia capillaris* Thunb *Gardenia jasminoides* J.Ellis;*Scutellaria baicalensis* Georgi; *Lonicera japonica* Thunb	C57BL/6J mice	*Firmicutes*↓, *Proteobacteria*↓, *Patescibacteria*↑, *Tenericutes*↑ *RuminococcaceAE_UCG-014*↑, *Lactobacillus*↑, *Desulfovibrio*↑	ZO1 ↑, Occludin ↑, Claudin 1 ↑; ACC1↓, FASN ↓, CD36 ↓	Tan et al. (2023)
Xie Zhuo Tiao Zhi formula (XZTZ)	*Alisma plantago-aquatica* L; *Atractylodes macrocephala* Koidz *Wolfiporia cocos* (F. A. Wolf) Ryvarden & Gilb *Citrus* × *aurantium* Siebold & Zucc. ex Engl.; *Crataegus pinnatifida* Bunge *Nelumbo nucifera* Gaertn	C57BL/6J mice	*Ileibacterium valens*↑, *Bifidobacterium pseudolongum* ↑	Inosine inhibits the expression of focal death-associated proteins NLPR3, GSDMD, Nek7, Caspase 1 and ASC, and reduces the levels of inflammatory factors IL-1, IL-6 and TNFα	Qiu et al. (2023)
Yinchen-Gancao decoction (YG)	*Artemisia capillaris* Thunb;*Glycyrrhiza uralensis* Fisch	C57BL/6J mice	No special changes	Reduces fatty acid synthesis and uptake, increases fatty acid oxidation, and reduces inflammatory factors and chemokines to suppress hepatic inflammation and endoplasmic reticulum stress	(J et al., 2025)
Si-Wu-Tang (SWT)	*Rehmannia glutinosa* (Gaertn.) *Libosch.* ex Fisch. & C. A. Mey; *Paeonia lactiflora* Pall; *Angelica sinensis* (Oliv.) Diels *Ligusticum* chuanxiong Hort	C57BL/6J mice	*Bacteroides*↑, *Lachnoclostridium*↑; *Alistipes*↓, *Rikenellaceae*↓	Regulation of bile acid metabolism to treat liver fibrosis	Xue et al. (2021)
Lingguizhugan decoction (LGZG)	*Wolfiporia cocos* (F. A. Wolf) Ryvarden & Gilb;*Cinnamomum cassia* (L.) D. Don; *Atractylodes macrocephala* Koidz.; *Glycyrrhiza uralensis* Fisch	C57BL/6J mice	*Bacteroides*↑, *Lachnoclostridium*↑; *Alistipes*↓, *Rikenellaceae*↓	Reduction of hepatic mitochondrial damage and oxidative stress and inhibition of inflammatory factor release via STING-TBK1-NF-κB signaling pathway	Cao et al. (2022)
Yindanxinnaotong formula (YDX)	*Ginkgo biloba* Leaf; *Salvia miltiorrhiza* Bunge; *Asarum heterotropoides* F. Schmidt; *Panax notoginseng* (Burkill) F. H. Chen ex C. H. Chow; *Crataegus pinnatifida* Bunge; *Gynostemma pentaphyllum* (Thunb.) Makino; *Borneolum Syntheticum; Allium sativum* Bulb	C57BL/6J mice	*Firmicutes*/*Bacteroidetes* ↑; *Odoribacter* ↑, *Alistipes*↑, *Flavonifractor*↑, *Oscillibacter*↑, *Pseudoflavonifractor* ↑, *Desulfovibrio* ↑, *Mucispirillum*↑, *Acetatifactor* ↑, *Clostridium cluster* XIVa ↓, *Barmesiella*↓; SCFA ↑	Protecting the intestinal barrier as well as lowering LPS levels, which can help reduce the risk of liver exposure	Huang et al. (2024)

4 Botanical drugs and their metabolites alleviate the progression of non-alcoholic fatty liver disease by modulating the gut microbiota-immune responses axis

Currently, botanical drugs and their metabolites are receiving increasing attention as adjuvant therapies. Through synergistic effects, they regulate multiple interconnected targets and pathways within the disease network, promoting the restoration of immune system balance and thereby reducing hepatic inflammation (Zhi et al., 2025). Numerous studies indicate they can intervene in NAFLD progression via the GM-immune responses axis.As shown in Tables 1, 2.

4.1 Metabolites originating from botanical drugs

4.1.1 Berberine

Berberine (BBR), a natural metabolite derived from *Coptis chinensis* Franch., alleviates hepatic inflammation by targeting GM. Administration of BBR (40 mg/kg) to NAFLD mouse models increased the relative abundance of *Akkermansia* and *Bacteroides* at the genus level, while decreasing the relative abundance of *Lactobacillus* and *Romboutsia* (Yang et al., 2022). BBR supplementation elevated intestinal *Bifidobacterium* abundance and *Bacteroidetes*/*Firmicutes* ratio in NASH mouse models, concurrently reducing serum concentrations of inflammatory factors including IL-1, IL-6, TNF-α, and CD14

(Cao et al., 2016). The study also found that BBR supplementation reduced the abundance of *F. prausnitzii* (Li et al., 2017). Regarding intestinal barrier function, BBR promotes the expression of tight junction proteins (ZO-1 and Occludin), increases the number of colonic glands and mucus secretion by goblet cells, reduces translocation of Gut-Derived LPS to the liver, and alleviates hepatic inflammation (Chen D. et al., 2023; Chen et al., 2023 Y.). Beyond regulating GM, BBR exerts effects through direct anti-inflammatory and anti-fibrotic mechanisms, including inhibition of JNK1 signal transduction (Guo T. et al., 2016) and downregulation of TLR4/MyD88/NF-κB pathway activity (Wang L. et al., 2020), reducing the expression and activity of neutrophil elastase (NE), upregulating α1-antitrypsin (α1-AT), and inhibiting the CXCR4/CXCL12 axis (Yang et al., 2017), as well as inducing apoptosis of HSCs and suppressing their proliferation (Eissa et al., 2018). Animal studies demonstrate that BBR supplementation (200 mg/kg/d) significantly alleviates hepatic inflammation and steatosis in HFD-induced NASH mouse models, with these effects closely linked to GM modulation and intestinal barrier repair. (Cao et al., 2016). A clinical trial involving 184 NAFLD patients further confirmed that oral BBR administration (1.5 g/d for 16 weeks) significantly reduced hepatic fat content, lipid parameters (TG, TC), and liver enzyme levels (ALT, AST), while exhibiting a favorable safety profile (Yan et al., 2015). BBR demonstrates promising therapeutic potential for NAFLD/NASH prevention and treatment through multi-target modulation of GM, enhancement of barrier function, and suppression of inflammatory signaling pathways and fibrotic progression.

4.1.2 Resveratrol

Resveratrol (RSV), a natural polyphenolic metabolite primarily extracted from *Reynoutria japonica* Houtt., exhibits antioxidant, anti-apoptotic, and anti-inflammatory properties (Tian and Liu, 2020). As a natural agonist of silent information regulator 1 (SIRT1), RSV ameliorates hepatic lesions by exerting anti-inflammatory and anti-fibrotic effects through multiple pathways. In hepatocytes, RSV significantly alleviates hepatic inflammation by activating the AMPKα–SIRT1 signaling pathway to inhibit the NF-κB inflammatory pathway (Tian et al., 2016). Simultaneously, RSV induces apoptosis of activated HSCs and suppresses their activation in a dose-dependent manner via the SIRT1 and JNK signaling pathways, thereby reversing hepatic fibrosis (Zhang et al., 2022). On the other hand, RSV also exerts significant regulatory effects on immune cells. It interferes with interferon-gamma (IFN-γ)-mediated macrophage activation by inhibiting the JAK/STAT-1 pathway, consequently reducing the production of inflammatory mediators such as nitric oxide (NO), IP-10, and MIG, while downregulating the expression of inducible nitric oxide synthase (Chung et al., 2011). Additionally, RSV promotes macrophage polarization toward the M2 anti-inflammatory phenotype and upregulates IL-10 synthesis, thereby alleviating fibrosis (Yu et al., 2019; Jin et al., 2025). Furthermore, RSV inhibits NF-κB nuclear translocation and reduces inflammatory factor release by disrupting crosstalk between the TLR2/MyD88/ERK and NF-κB/NLRP3 inflammasome pathways, thereby delaying the progression of hepatic fibrosis (Lei et al., 2025). Regarding intestinal effects, RSV not only remodels GM and enhances microbial diversity but also strengthens intestinal mucosal barrier function by upregulating tight junction proteins (Occludin, ZO-1, and Claudin1). It concurrently suppresses mRNA expression of cannabinoid receptor type 1 (CB1), further consolidating its protective effects along the gut-liver axis (Chen et al., 2020).

Supplementation with RSV (50/75/100 mg/kg/d) significantly reduced hepatic steatosis and fibrosis in HFD-induced NASH rats, with the ameliorative effect demonstrating dose dependency. RSV restructured the GM composition in NASH rats. At the species level, the abundance of *Akkermansia muciniphila* increased; at the genus level, *Bacteroides* abundance increased while *Desulfovibrio* abundance decreased; at the family level, *Ruminococcaceae* and *Lachnospiraceae* abundances increased. However, these GM alterations were not observed in the low-dose RSV group (50 mg/kg/d) (Campbell et al., 2019). The study also found that RSV supplementation reduced the abundance of harmful bacteria *Desulfovibrio*, *Lachnospiraceae_NK4A316_group*, and *Alistipes* in the intestinal tract, while increasing the abundance of SCFA-producing bacteria *Allobaculum*, *Bacteroides*, and *Blautia* (Wang P. et al., 2020). An RCT involving 60 NAFLD patients demonstrated that 3 months of RSV supplementation (300 mg/d) significantly reduced serum levels of ALT, AST, TC, and TNF-α (Chen et al., 2015).

4.1.3 Curcumin

Curcumin (Cur), a natural metabolite extracted from *Curcuma longa* L., exhibits multiple biological activities including antioxidant, anti-inflammatory, and antitumor effects. Cur intervenes in NAFLD progression through multiple mechanisms, including anti-inflammatory effects, regulation of lipid metabolism, improvement of insulin resistance, and modulation of fibrotic processes (Li X. et al., 2024). In recent years, its role in modulating GM has garnered increasing attention. Studies demonstrate that Cur significantly increases *Bacteroides* abundance and ameliorates hepatic lipid accumulation through microbiota-dependent BAs metabolism (He et al., 2024). In NAFLD models, Cur reverses the elevated *Firmicutes*/*Bacteroidetes* ratio and reduced *Akkermansia* abundance (Hong et al., 2022), while concurrently upregulating expression of tight junction proteins Occludin and ZO-1, suppressing TLR4/NF-κB pathway activation, and reducing LPS exposure, thereby alleviating hepatic inflammation (Hong et al., 2022). Further studies (Li S. et al., 2021; Hong et al., 2022) confirmed that Cur effectively ameliorated hepatic steatosis and reduced serum LPS levels in HFD-fed mice. This protective mechanism correlates with modulation of GM composition, specifically manifested through: decreased *Firmicutes*/*Bacteroidetes* ratio and reduced *Desulfovibrio* abundance, alongside elevated abundance of *Akkermansia* and multiple SCFA-producing genera including *Bacteroides*, *Parabacteroides*, *Alistipes*, and *Alloprevotella*. Cur supplementation (200 mg/kg/d for 16 weeks) significantly alleviated hepatic steatosis and oxidative stress in HFD-fed mice, mediated through multiple pathways (Yang J. et al., 2025). Cur enhanced the alpha-diversity of the GM. At the family taxonomic level, a significant increase in the abundance of *Coriobacteriaceae* was observed. At the genus level, the abundance of *Mailhella* and *Parabacteroides* increased, while that of *Alistipes* decreased. At the species level, the abundance of *Phocaeicola vulgatus* and *Bacteroides intestinalis* rose, whereas *Acutalibacter muris* abundance declined,

thereby reducing the synthesis of enterogenic toxins. Simultaneously, Cur inhibits the JNK2/FOXO1/Bcl6 signaling axis to alleviate lipid accumulation (Yang J. et al., 2025). Moreover, Cur suppresses M1 polarization of macrophages and reduces the secretion of IL-1β and TNF-α (Tong et al., 2021). By promoting PPARα mRNA m6A methylation through inhibition of FTO protein, it activates the PPARα/CPT1α pathway to enhance fatty acid β-oxidation (Fan et al., 2025b), regulates AMPK, ChREBP, and SREBP1-c expression to ameliorate lipid metabolism (Guariglia et al., 2023), downregulates CYP2E1 and C/EBPβ, reduces ROS generation, and alleviates oxidative stress (Afrin et al., 2017). A meta-analysis encompassing 1,028 NAFLD patients also demonstrated that curcumin effectively ameliorates hepatic steatosis (Ngu et al., 2022).

4.1.4 Quercetin

Quercetin (QUE), a metabolite isolated from *Scutellaria baicalensis* Georgi, intervenes in NAFLD through multiple pathways. QUE reverses HFD-induced GM dysbiosis, significantly lowering the *Firmicutes*/*Bacteroidetes* ratio and reducing the abundance of Gram-negative bacteria such as *Helicobacter*. This diminishes endotoxemia occurrence, ultimately suppressing TLR4/NF-κB signaling pathway activation and inflammasome initiation, thereby ameliorating hepatic inflammation (Porras et al., 2017). QUE supplementation also inhibits oxidative stress in hepatocytes by regulating cytochrome P450 2E1 (CYP2E1), thereby conferring hepatoprotective effects against NAFLD (Porras et al., 2017). Conversely, QUE inhibits the NF-κB p65/iNOS signaling pathway in a concentration-dependent manner and reduces serum TNF-α levels (Ying et al., 2013). It blocks phosphorylation of IκBα and NF-κB p65 while upregulating expression of superoxide dismutase (SOD) and glutathione peroxidase 1 (GPX1), thus systematically alleviating hepatic oxidative stress and excessive immune activation (Jiang et al., 2025). Furthermore, QUE can activate the antioxidant transcription factor Nrf2 and alleviate hepatic lipid accumulation, mitochondrial dysfunction, and oxidative stress through the AMPK-dependent autophagy pathway (Panchal et al., 2012; Cao et al., 2023).

The effect of QUE on ameliorating lipid metabolism is closely associated with its promotion of beneficial *A. muciniphila* proliferation. Indole-3-lactic acid (ILA), produced by this bacterium's metabolism, upregulates CYP8B1 via the FTO/m6A/YTHDF2 pathway, driving cholesterol conversion into cholic acid (CA). The latter suppresses lipid accumulation through FXR receptor activation (Liu J. et al., 2025). A randomized controlled trial (n = 41) demonstrated the clinical value of QUE. NAFLD patients supplemented with 500 mg QUE daily for 12 weeks exhibited significant reductions in hepatic lipid content, body weight, and body mass index (BMI), with favorable safety profiles (Li N. et al., 2024).

4.1.5 Lycium barbarum polysaccharides

Lycium barbarum polysaccharides (LBPs) constitute a key active metabolite in *Lycium chinense* Mill. LBPs delay NAFLD progression by modulating GM and enhancing barrier function. In NAFLD mouse models, LBPs reduce the relative abundance of *Verrucomicrobia* and *Enterococcaceae* (Gao et al., 2021), and the latter serves as the primary source of gut-derived LPS. This helps reduce the risk of LPS translocation and hepatic inflammation (Hu et al., 2020), while simultaneously increasing the abundance of *Deferribacteres* and butyrate-producing bacteria (such as *Butyricicoccus* and *Butyricimonas*) (Gao et al., 2021). Butyrate enhances the expression of intestinal mucus and tight junction proteins (ZO-1, occludin), thereby improving intestinal barrier function (Peng et al., 2007). The study (Ding et al., 2019) further confirmed that LBPs promote the proliferation of *Bacteroides*, *Bifidobacterium*, and SCFA-producing bacteria such as *Phascolarctobacterium*, *Prevotella*, and *Collinsella*. LBPs play a crucial role in inhibiting the progression of NAFLD.

Notably, the effects of LBPs on modulating GM are not solely determined by their metabolites; the structural domains of LBPs also exert influence. This structure-function relationship is particularly evident in *Bacteroidetes* and *Parabacteroides*. RG-I and its neutral sugar side chains increase the abundance of beneficial bacterial families such as *Comamonadaceae*; whereas linear homogalacturonan (HG) promotes the proliferation of potentially harmful bacterial families including *Pseudomonadaceae*, *Xanthomonadaceae*, *Caulobacteraceae*, and *Oxalobacteraceae* (Wei et al., 2025).

LBPs also stimulate putrescine secretion by murine GM, which subsequently inhibits the JAK2-STAT3 pathway via TRAF6-mediated suppression, thereby reducing Th17 cell differentiation and inflammatory factor release (Wang et al., 2025). Moreover, Th17 differentiation drives the progression from NASH to HCC, rendering the inhibition of this differentiation a crucial intervention strategy for NASH (Huang Y. et al., 2025). Supplementation with LBPs (100 mg/kg, 10 weeks) significantly alleviated liver injury, dyslipidemia, and inflammation in NASH rats. This effect was achieved by activating the AMPK/PPARα/PGC-1α pathway to promote hepatic lipid consumption (Li et al., 2022). LBP inhibited caspase-9/3 activity and TNF-α levels in CCl4-induced hepatic fibrosis mice, thereby mitigating hepatic inflammation and fibrosis (Chiang and Chao, 2018). A pre-post study also demonstrated that LBP supplementation (300 mg/day, 12 weeks) improved both GM diversity and liver function in NAFLD patients (Fan et al., 2025a).

4.1.6 Poria cocos polysaccharide

Poria cocos polysaccharide (PCP) is a botanical drug-derived metabolite (Zhao et al., 2023). PCP increases the relative abundance of *Faecalibaculum*, reduces gut-derived LPS levels, thereby inhibiting the NF-κB/CCL3/CCR1 signaling pathway, alleviates hepatic inflammation, and delays NASH progression (Tan et al., 2022). Furthermore, PCP reduces hepatocyte apoptosis by suppressing the CYP2E1/ROS/MAPKs signaling pathway, and decreases the liver's exposure risk in inflammatory environments through restoration of intestinal barrier function (Jiang et al., 2022). In both zebrafish and mouse NAFLD models, PCP demonstrated efficacy in counteracting hepatic steatosis (Ye et al., 2022). This beneficial effect stems from reducing translocation of gut-derived LPS to the liver and suppressing PARP-1-mediated pyroptosis in intestinal cells. Furthermore, PCP inhibited disease progression in methionine-choline-deficient (MCD)-induced NASH mice by downregulating expression of F4/80, CD68, IL-1β, CD11b, and CCL5 genes (Gao et al., 2023). Simultaneously, PCP alleviated

hepatic steatosis in NAFLD mice by modulating glucose and lipid metabolism through upregulation of lipid transport proteins and suppression of lipid synthesis-associated proteins (Wang et al., 2022). PCP is even considered a prebiotic. Supplementation with PCP (50 mg/kg/d, for 8 weeks) can alleviate insulin resistance, lipid metabolism imbalance, and inflammation in the NASH mouse model. This effect stems from PCP enhancing the intestinal barrier, improving GM diversity, and increasing the relative abundance of probiotics including *Lactobacillus*, *Allobaculum*, and *Phascolarctobacterium*. These probiotics synthesize SCFAs, which subsequently ameliorate insulin resistance through the FGF21-PI3K/AKT signaling pathway (Liu et al., 2025b).

4.1.7 Ginsenoside

Ginsenoside (Rg) is a metabolite extracted from *Panax ginseng* C. A. Mey. Rg supplementation ameliorates hepatic steatosis and reduces hepatic inflammation in HFD-induced NAFLD mouse models, with its therapeutic effects on NAFLD being closely related to modulation of the GM-immune axis (Shi et al., 2024). Studies demonstrate that Rg can restructure GM composition, and this alteration proves beneficial to host health. At the phylum level, *Bacteroidota* abundance increases while the *Firmicutes*/*Bacteroidetes* ratio decreases; at the family level, *Muribaculaceae* abundance elevates; At the genus level, the relative abundances of *Akkermansia*, *Parabacteroides*, *Lachnospiraceae_NK4A136_group*, *Oscillospira*, *Phascolarctobacterium*, *Bacteroides*, and *Dehalobacterium* increased, while the relative abundances of *Allobaculum* and *Olsenella* decreased (Liang et al., 2021; Shi et al., 2024). Concurrently, Rg enhances intestinal barrier integrity by upregulating the expression of tight junction proteins (including ZO-1, Occludin, and Claudin-1), thereby reducing the translocation of Gut-Derived LPS. Furthermore, Rg reduces LPS levels and inhibits the TLR4/NF-κB signaling pathway, consequently decreasing the production of pro-inflammatory factors and suppressing macrophage activation and inflammatory cell infiltration in the liver, ultimately alleviating hepatic inflammation (Liang et al., 2021).

Beyond the intestinal tract, Rg delays NAFLD progression by multi-target regulation of lipid metabolism. Regarding lipid metabolism, Rg reduces lipid uptake through suppression of CD36 expression (Wu et al., 2025) while activating the hepatic LKB1/AMPK/mTOR signaling pathway (Shi et al., 2024). This dual action cooperatively downregulates key lipid synthesis genes (e.g., SREBP-1c, FAS, ACC) and upregulates the fatty acid oxidation gene CPT-1a, thereby significantly ameliorating hepatic lipid accumulation (Liang et al., 2021; Shi et al., 2024). Furthermore, activated AMPK exerts hepatoprotective effects through metabolic and anti-inflammatory mechanisms: it inhibits mTORC1 and SREBP-1c to reduce lipid synthesis, while suppressing NF-κB signaling and oxidative stress via the sirtuin 1/FOXO1 pathway (Wu et al., 2025). Notably, lipid overload can induce ferroptosis, thereby driving the progression of NAFLD (Chen et al., 2022). Rg effectively enhances antioxidant capacity by activating the Keap1/Nrf2 signaling pathway and preserving mitochondrial structural and functional integrity, consequently inhibiting ferroptosis (Liu et al., 2025c). Significantly, the anti-ferroptosis effect of Rg markedly diminished following antibiotic intervention, indicating its dependence on GM involvement. This demonstrates that Rg primarily suppresses NAFLD development through multiple pathways by modulating the "GM–immune inflammation" axis while synergistically improving lipid metabolism and antioxidant pathways.

4.1.8 Pachymic acid

Pachymic acid (Pac) is a metabolite derived from *Wolfiporia cocos* (F. A. Wolf) Ryvarden & Gilb. Pac supplementation alleviated hepatic inflammation in HFD-induced NAFLD mouse models (Ren et al., 2025a). Pac reshaped the GM structure in NAFLD mice, reversing HFD-induced intestinal dysbiosis. At the phylum level, the *Firmicutes*/*Bacteroidetes* ratio decreased. At the genus level, the abundance of *Akkermansia* increased, while *Desulfovibrio* and *Streptococcus* decreased. Furthermore, Pac reduced the expression of hepatic inflammatory factors by suppressing the LPS/TLR4/MYD88/NFκB pathway, thereby mitigating liver inflammation. Pac inhibits the expression of lipid synthesis-related proteins including FASN, SREBP1c, and SCD1, while promoting the expression of fatty acid oxidation-related proteins such as PPARα and CPT1α, thereby reducing hepatic inflammation induced by lipid accumulation. Studies demonstrate that Pac ameliorates HFD-induced NAFLD through synergistic multi-pathway effects. In animal models, Pac supplementation (20/40 mg/kg for 4 weeks) significantly alleviates hepatic steatosis, reduces serum lipid levels, and improves liver function (Ren et al., 2025b). On one hand, Pac upregulates the expression and activity of PPARα, thereby promoting hepatic fatty acid oxidation to accelerate lipid consumption. Additionally, activated PPARα upregulates GPX4 protein expression, thus inhibiting ferroptosis. On the other hand, Pac downregulates TFR1 protein expression by inhibiting the MAPKs signaling pathway, consequently reducing Fe^{3+} uptake and intracellular Fe^{2+} accumulation in hepatocytes. This suppresses the Fenton reaction and further alleviates hepatocellular ferroptosis. Through multiple mechanisms including promoting lipid metabolism, inhibiting inflammation, and suppressing ferroptosis, Pac collectively delays the progression of NAFLD.

4.2 Botanical drugs formulae

Botanical drugs formulae comprise complex systems of multiple botanical drugs, corresponding to diverse metabolites. Their core therapeutic mechanism lies in the synergistic effects of metabolites through multi-target and multi-system interventions, achieving holistic regulation of diseases.

Zexie Tang contains two botanical drugs: *Alisma plantago-aquatica* L. and *Atractylodes macrocephala* Koidz. Researchers discovered that Zexie Tang contains a metabolite composed of fructose and glucose, designated as Zexie Tang Polysaccharides (ZXTPs) (Zhang et al., 2025). ZXTPs ameliorate hepatic steatosis in NAFLD mouse models via the gut-liver axis. ZXTPs remodeled the GM, increasing the abundance of beneficial bacteria including *Akkermansia*, *Lachnospiraceae_NK4A136*, and *Bacteroides*, while reducing pathogenic bacteria such as *Prevotella_9* and *Phascolarctobacterium*. This alteration promoted the secretion of tryptophan metabolites (including indole-3-acetic acid and serotonin) and SCFAs. Tryptophan metabolites play a key role in alleviating hepatic inflammation and modulating immunity by

activating AhR. Concurrently, ZXTPs upregulate the expression of tight junction proteins (ZO-1 and Occludin), repairing the intestinal mucosal barrier and thereby inhibiting gut-derived LPS from entering systemic circulation. Meanwhile, tryptophan metabolites exert crucial effects in mitigating hepatic inflammation and modulating immunity through activation of the AhR. Regarding lipid metabolism, SCFAs and ZXTPs enhance hepatic AMPK phosphorylation through regulating LKB1/AMPK and PI3K/AKT/mTOR signaling pathways along with the autophagy pathway. This subsequently suppresses expression of the lipogenesis key enzyme SREBP1 while upregulating PPARα expression, ultimately leading to significant amelioration of hepatic lipid metabolism.

Yinzhihuang granule (YZHG) contains four types of botanical drugs, including *Artemisia capillaris* Thunb, *Gardenia jasminoides* J.Ellis, *S. baicalensis* Georgi, *Lonicera japonica* Thunb. The study (Tan et al., 2023) revealed that YZHG contains 42 blood-absorbed metabolites, primarily flavonoids, phenolic acids, iridoids. YZHG could reduce blood lipid levels in NAFLD mouse models and decrease concentrations of LPS, TNF-α, IL-1β, and IL-6 in liver tissues. YZHG modulated the GM structure in mice: at the phylum level, the abundance of *Firmicutes* and *Proteobacteria* decreased, while *Patescibacteria* and *Tenericutes* increased; At the genus level, the abundance of *Ruminococcaceae_UCG-014*, *Lactobacillus*, and *Desulfovibrio* elevated. 16S rRNA sequencing and metabolomics demonstrated that this therapeutic effect stems from YZHG metabolites influencing intestinal tract and lipid metabolism-related proteins, particularly chrysin, baicalein, wogonin, hispidulin, and negletein A.

Xie Zhuo Tiao Zhi formula (XZTZ) contains *Crataegus pinnatifida* Bunge, *Nelumbo nucifera* Gaertn., *Citrus* × *aurantium* Siebold & Zucc. ex Engl., *A. macrocephala* Koidz., *W. cocos* (F. A. Wolf) Ryvarden & Gilb, *A. plantago-aquatica* L. These botanical drug-derived metabolites (including naringin, neohesperidin, atractylenolide III, 23-acetyl alisol B, pachymic acid, and ursolic acid) elevate circulating and hepatic inosine levels by increasing the abundance of *A. muciniphila*, *Bifidobacterium pseudolongum*, and *Ileibacterium valens* in the intestinal tract of NAFLD mouse models. This subsequently inhibits hepatocyte pyroptosis, as evidenced by downregulation of NLRP3, GSDMD, Nek7, Caspase-1, and ASC protein expression, while reducing inflammatory factors such as IL-1β, IL-6, and TNF-α. Concurrently, other metabolites (such as Inosine) effectively alleviate hepatic lipid accumulation by regulating the expression of proteins involved in lipid synthesis, transport, and oxidation (Qiu et al., 2023).

Yinchen-Gancao decoction (YG) consists of two botanical drugs: *A. capillaris* Thunb. and *Glycyrrhiza uralensis* Fisch., which carry multiple metabolites, including Chlorogenic Acid (CGA), Glycyrrhizic Acid (GZA), Isochlorogenic Acid (ICGA), and glycyrrhetinic acid (GTA). YG significantly ameliorates hepatic lipid accumulation and inflammation in the NASH mouse model. This effect stems from CGA suppressing the fatty acid synthesis pathway (SREBP1c-ACC/FASN) via an FXR-dependent mechanism, downregulating the expression of the uptake protein CD36, and enhancing lipid oxidation through the PPARα-CPT1α pathway (Jing et al., 2025).

Si-Wu-Tang (SWT) treats CCL4-induced hepatic fibrosis by remodeling the composition of GM and regulating BAs metabolism (Xue et al., 2021). Twenty-two major metabolites derived from botanical drugs in SWT, particularly paeoniflorin, ferulic acid, verbascoside, and senkyunolide A, play crucial roles. They regulate BAs metabolism by activating the FXR-fibroblast growth factor 15 (FGF15) and FXR-SHP pathways, which facilitates hepatic lipid excretion and reduces lipotoxicity-induced inflammation. Conversely, paeoniflorin and ferulic acid synergistically improve the intestinal microenvironment, at the phylum level, the abundance of *Bacteroides* and *Lachnoclostridium* increases; at the genus level, the abundance of *Alistipes* decreases. At the family level, reduced abundance of *Rikenellaceae* suppresses gut-derived endotoxin translocation, thereby alleviating hepatic inflammation burden.

Lingguizhugan decoction (LGZG) can alleviate hepatic inflammation in HFD-fed mice. This effect is closely associated with metabolites derived from botanical drugs, including Paclitaxel (Pac), Cinnamaldehyde, Atractylenolide II, and Glycyrrhizic acid (GZA). These metabolites inhibit TNFα and IFNβ release in a dose-dependent manner; Notably, both Cinnamaldehyde and GZA further block activation of the STING–TBK1–NF-κB signaling pathway by suppressing TBK1 and NF-κB phosphorylation in macrophages. Notably, the effects of mixed metabolites surpassed those of single metabolites, indicating synergistic interactions among metabolites (Cao et al., 2022). Yindanxinnaotong (YDX) comprises eight botanical drugs containing a total of 124 metabolites. Studies demonstrate that YDX reduces gut-derived LPS production by remodeling the GM. At the genus level, it significantly increases the abundance of *Odoribacter*, *Alistipes*, and *Flavonifractor* while decreasing *Clostridium cluster XIVa* and *Barmesiella*. Furthermore, YDX downregulates hepatic expression of lipid synthesis-related proteins (including SREBP-1c, SCD-1, and CD36) and pro-inflammatory cytokines (IL-6, TNF-α), while enhancing expression of key fatty acid β-oxidation proteins (AMPKα, CPT-1). This effectively suppresses hepatic lipid accumulation and inflammatory responses (Huang et al., 2024). Although studies have observed alterations in GM alongside improvements in hepatic lipid metabolism and inflammatory status, the direct link between these two pathways, the primary metabolites involved, and the specific mechanisms remain to be elucidated, this warrants further investigation.

Shugan Xiaozhi (SG), composed of 15 botanical drugs, is commonly employed in the treatment of NAFLD. SG ameliorates hepatic inflammation and fibrosis in HFD-induced mice, suppresses intrahepatic ROS generation, elevates levels of SOD, GSH, and CAT, reduces MDA levels in murine liver in a dose-dependent manner, and preserves the integrity of hepatic mitochondrial function and structure. These effects depend on SG's regulation of BNIP3/BNIP3L-mediated mitophagy. Metabolites derived from SG—including naringin, hesperetin 7-O-rutinoside, frangulin A, and 3″-p-Coumaroylprunin—exhibit close interactions with key targets regulating mitophagy, suggesting their pivotal roles in this process (Chen M. et al., 2023).

Shugan Xiaozhi (SG), composed of 15 botanical drugs, is commonly used to treat NAFLD. Studies demonstrate that SG alleviates HFD-induced hepatic inflammation and fibrosis in mice, suppresses ROS generation, enhances SOD, GSH, and CAT activities, and reduces MDA levels in a dose-dependent manner, thereby protecting mitochondrial structural and functional integrity in hepatocytes. The therapeutic effect of SG on NASH is closely associated with its regulation of BNIP3/BNIP3L-mediated mitophagy. Metabolites in SG—including naringin, hesperetin 7-

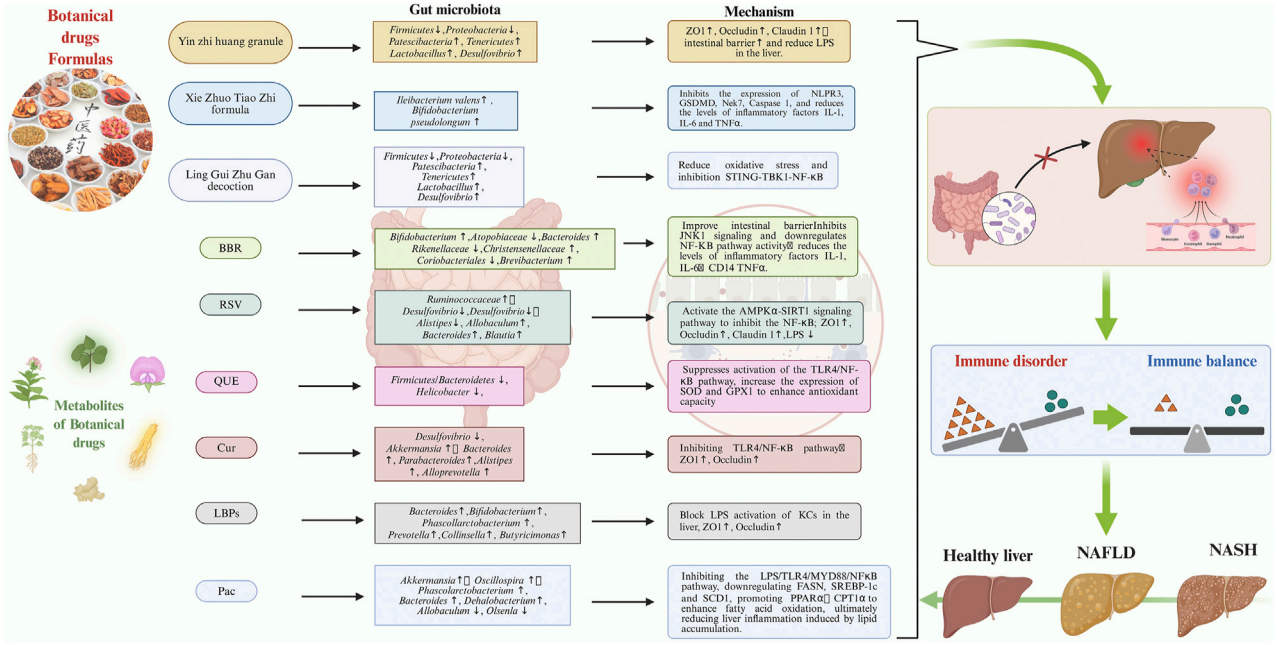

FIGURE 4
The specific targets and mechanisms of action of botanical drugs formulae and metabolites from botanical drugs, in treating NAFLD by targeting the GM-immune response. GM, gut microbiota; NAFLD, Non-alcoholic Fatty Liver Disease.

O-rutinoside, frangulin A, and 3″-p-Coumaroylprunin—exhibit significant interactions with key mitophagy targets, thereby playing a central role in this mechanism (Chen M. et al., 2023).

5 Discussion

Immune dysregulation serves as a critical driver of NAFLD progression, making the maintenance of immune homeostasis an essential intervention strategy. GM regulates host immune balance through the gut-liver axis and participates in hepatic inflammation processes. Therefore, utilizing metabolites derived from botanical drugs to treat NAFLD through the GM-Immune Axis represents a promising strategy. As shown in Figure 4. However, its mechanisms remain incompletely elucidated and face multifaceted challenges.

GM is highly susceptible to environmental influences, resulting in significant research heterogeneity. This necessitates integrating multi-center large-sample cohorts with multi-omics data to enhance conclusion reliability. Organoid co-culture systems (Wang et al., 2024a) and CRISPR-based microbiota editing (Jin et al., 2022) provide novel approaches to overcome mechanistic bottlenecks. The former can simulate gut-liver axis immune interactions for target screening, while the latter enables precise identification of functional genes in GM and drug-action pathways.

The clinical efficacy evidence and translation of botanical drugs still face dilemmas. Current research predominantly focuses on basic mechanisms, with limited and low-quality clinical studies. These manifest experimental design flaws (e.g., inadequate sample size, suboptimal blinding, insufficient endpoint indicators, significant regional variations, and short follow-up periods), lax quality control, and lack of collaborative mechanisms, substantially diminishing the evidence level. Future efforts should initiate pilot experiments and observational studies to preliminarily evaluate efficacy, identify benefiting subpopulations, and define treatment endpoints, thereby providing foundations for subsequent large-scale RCTs. Studies must strictly adhere to the PICOTS framework, establish intelligent data centers, and follow international reporting standards (e.g., CONSORT, STRICTA) to advance the generation of high-quality clinical evidence.

The translation from basic research to clinical applications of botanical drugs also faces challenges. The multi-target characteristics result in unclear onset of action metabolites and dose-response relationships, while the lack of standardized preparation processes leads to inconsistent drug quality and irreproducible efficacy (Hu et al., 2019). Multidisciplinary platforms should be integrated to establish artificial intelligence-based metabolites screening and quality control systems, evaluate pharmacological effects using organoids/organs-on-chips, and ultimately develop a research paradigm featuring well-defined mechanisms, rigorous quality control, and quantifiable efficacy.

The safety of metabolites derived from botanical drugs also requires significant attention. Toxic side effects may be associated with exogenous contaminants (pesticides, heavy metals, mycotoxins), endogenous factors (origin, dosage, treatment duration, preparation processes), and individual variations (Gao et al., 2019). Current toxicological research remains limited, with unclear toxicity mechanisms and a lack of clinical risk warnings. Regulatory oversight of drug quality should be enhanced, toxicity evaluation systems improved, toxicity metabolites pre-screened using chemical structure warning databases, pharmacokinetic-toxicokinetic (PK-TK) models established through integrated *in vivo* and *in vitro* experiments, and novel technologies such as

microfluidic chips, high-throughput screening, and systems toxicology introduced to develop more comprehensive safety evaluation standards.

In summary, elucidating the interaction targets among botanical drugs, GM, and immune dysregulation provides novel insights for NAFLD treatment, deepening our understanding of the gut-liver axis mechanism. Integrating mechanistic studies, clinical validation, and safety assessment holds promise for advancing systematic, standardized, and internationally recognized applications of botanical drugs in NAFLD treatment.

Author contributions

YZ: Writing – original draft, Writing – review and editing. LaL: Writing – original draft, Writing – review and editing. RS: Writing – original draft. ZQ: Writing – original draft. TW: Visualization, Writing – original draft. LeL: Writing – original draft, Visualization. SW: Project administration, Methodology, Validation, Conceptualization, Writing – original draft. SZ: Writing – original draft, Visualization. HL: Writing – review and editing, Supervision, Methodology, Writing – original draft. HW: Writing – review and editing, Funding acquisition, Supervision.

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Conflict of interest

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

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References

Afrin, R., Arumugam, S., Rahman, A., Wahed, M. I. I., Karuppagounder, V., Harima, M., et al. (2017). Curcumin ameliorates liver damage and progression of NASH in NASH-HCC mouse model possibly by modulating HMGB1-NF-κB translocation. *Int. Immunopharmacol.* 44, 174–182. doi:10.1016/j.intimp.2017.01.016

Agus, A., Clément, K., and Sokol, H. (2021). Gut microbiota-derived metabolites as central regulators in metabolic disorders. *Gut* 70, 1174–1182. doi:10.1136/gutjnl-2020-323071

Al Nabhani, Z., Dietrich, G., Hugot, J.-P., and Barreau, F. (2017). Nod2: the intestinal gate keeper. *PLoS Pathog.* 13, e1006177. doi:10.1371/journal.ppat.1006177

Al Nabhani, Z., Berrebi, D., Martinez-Vinson, C., Montcuquet, N., Madre, C., Roy, M., et al. (2020). Nod2 protects the gut from experimental colitis spreading to small intestine. *J. Crohns Colitis* 14, 669–679. doi:10.1093/ecco-jcc/jjz196

Alferink, L. J. M., Radjabzadeh, D., Erler, N. S., Vojinovic, D., Medina-Gomez, C., Uitterlinden, A. G., et al. (2021). Microbiomics, metabolomics, predicted metagenomics, and hepatic steatosis in a population-based study of 1,355 adults. *Hepatol. Balt. Md* 73, 968–982. doi:10.1002/hep.31417

An, D., Oh, S. F., Olszak, T., Neves, J. F., Avci, F. Y., Erturk-Hasdemir, D., et al. (2014). Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. *Cell.* 156, 123–133. doi:10.1016/j.cell.2013.11.042

Anderson, H. A., and Roche, P. A. (2015). MHC class II association with lipid rafts on the antigen presenting cell surface. *Biochim. Biophys. Acta* 1853, 775–780. doi:10.1016/j.bbamcr.2014.09.019

Arrenberg, P., Maricic, I., and Kumar, V. (2011). Sulfatide-mediated activation of type II natural killer T cells prevents hepatic ischemic reperfusion injury in mice. *Gastroenterology* 140, 646–655. doi:10.1053/j.gastro.2010.10.003

Arrese, M., Cabrera, D., Kalergis, A. M., and Feldstein, A. E. (2016). Innate immunity and inflammation in NAFLD/NASH. *Dig. Dis. Sci.* 61, 1294–1303. doi:10.1007/s10620-016-4049-x

Balasubramanian, I., and Gao, N. (2017). From sensing to shaping microbiota: insights into the role of NOD2 in intestinal homeostasis and progression of Crohn's disease. *Am. J. Physiol. Gastrointest. Liver Physiol.* 313, G7–G13. doi:10.1152/ajpgi.00330.2016

Bansal, T., Alaniz, R. C., Wood, T. K., and Jayaraman, A. (2010). The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proc. Natl. Acad. Sci. U. S. A.* 107, 228–233. doi:10.1073/pnas.0906112107

Bartneck, M., Scheyda, K. M., Warzecha, K. T., Rizzo, L. Y., Hittatiya, K., Luedde, T., et al. (2015). Fluorescent cell-traceable dexamethasone-loaded liposomes for the treatment of inflammatory liver diseases. *Biomaterials* 37, 367–382. doi:10.1016/j.biomaterials.2014.10.030

Beisner, J., Filipe Rosa, L., Kaden-Volynets, V., Stolzer, I., Günther, C., and Bischoff, S. C. (2021). Prebiotic inulin and sodium butyrate attenuate obesity-induced intestinal barrier dysfunction by induction of antimicrobial peptides. *Front. Immunol.* 12, 678360. doi:10.3389/fimmu.2021.678360

Bernsmeier, C., and Albano, E. (2017). Liver dendritic cells and NAFLD evolution: a remaining open issue. *J. Hepatol.* 66, 1120–1122. doi:10.1016/j.jhep.2017.02.018

Bhattacharjee, J., Kirby, M., Softic, S., Miles, L., Salazar-Gonzalez, R.-M., Shivakumar, P., et al. (2017). Hepatic natural killer T-cell and CD8+ T-cell signatures in mice with Nonalcoholic steatohepatitis. *Hepatol. Commun.* 1, 299–310. doi:10.1002/hep4.1041

Biagioli, M., Carino, A., Cipriani, S., Francisci, D., Marchianò, S., Scarpelli, P., et al. (2017). The bile acid receptor GPBAR1 regulates the M1/M2 phenotype of intestinal macrophages and activation of GPBAR1 rescues mice from Murine Colitis. *J. Immunol. Balt. Md 1950* 199, 718–733. doi:10.4049/jimmunol.1700183

Bielig, H., Rompikuntal, P. K., Dongre, M., Zurek, B., Lindmark, B., Ramstedt, M., et al. (2011). NOD-like receptor activation by outer membrane vesicles from Vibrio cholerae non-O1 non-O139 strains is modulated by the quorum-sensing regulator HapR. *Infect. Immun.* 79, 1418–1427. doi:10.1128/IAI.00754-10

Bogdanos, D. P., Gao, B., and Gershwin, M. E. (2013). Liver immunology. *Compr. Physiol.* 3, 567–598. doi:10.1002/cphy.c120011

Boursier, J., Mueller, O., Barret, M., Machado, M., Fizanne, L., Araujo-Perez, F., et al. (2016). The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatol. Balt. Md* 63, 764–775. doi:10.1002/hep.28356

Branzk, N., Lubojemska, A., Hardison, S. E., Wang, Q., Gutierrez, M. G., Brown, G. D., et al. (2014). Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. *Nat. Immunol.* 15, 1017–1025. doi:10.1038/ni.2987

Breuer, D. A., Pacheco, M. C., Washington, M. K., Montgomery, S. A., Hasty, A. H., and Kennedy, A. J. (2020). CD8+ T cells regulate liver injury in obesity-related nonalcoholic fatty liver disease. *Am. J. Physiol. Gastrointest. Liver Physiol.* 318, G211–G224. doi:10.1152/ajpgi.00040.2019

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D. S., et al. (2004). Neutrophil extracellular traps kill bacteria. *Science* 303, 1532–1535. doi:10.1126/science.1092385

Campbell, C. L., Yu, R., Li, F., Zhou, Q., Chen, D., Qi, C., et al. (2019). Modulation of fat metabolism and gut microbiota by resveratrol on high-fat diet-induced obese mice. *Diabetes Metab. Syndr. Obes. Targets Ther.* 12, 97–107. doi:10.2147/DMSO.S192228

Canfora, E. E., Jocken, J. W., and Blaak, E. E. (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat. Rev. Endocrinol.* 11, 577–591. doi:10.1038/nrendo.2015.128

Cao, Y., Pan, Q., Cai, W., Shen, F., Chen, G.-Y., Xu, L.-M., et al. (2016). Modulation of Gut Microbiota by berberine improves steatohepatitis in high-fat diet-fed BALB/C mice. *Arch. Iran. Med.* 19, 197–203.

Cao, L., Xu, E., Zheng, R., Zhangchen, Z., Zhong, R., Huang, F., et al. (2022). Traditional Chinese medicine Lingguizhugan decoction ameliorate HFD-induced hepatic-lipid deposition in mice by inhibiting STING-mediated inflammation in macrophages. *Chin. Med.* 17, 7. doi:10.1186/s13020-021-00559-3

Cao, P., Wang, Y., Zhang, C., Sullivan, M. A., Chen, W., Jing, X., et al. (2023). Quercetin ameliorates nonalcoholic fatty liver disease (NAFLD) *via* the promotion of AMPK-mediated hepatic mitophagy. *J. Nutr. Biochem.* 120, 109414. doi:10.1016/j.jnutbio.2023.109414

Carpino, G., Del Ben, M., Pastori, D., Carnevale, R., Baratta, F., Overi, D., et al. (2020). Increased liver localization of lipopolysaccharides in human and experimental NAFLD. *Hepatol. Balt. Md* 72, 470–485. doi:10.1002/hep.31056

Cavallari, J. F., Barra, N. G., Foley, K. P., Lee, A., Duggan, B. M., Henriksbo, B. D., et al. (2020). Postbiotics for NOD2 require nonhematopoietic RIPK2 to improve blood glucose and metabolic inflammation in mice. *Am. J. Physiol. Endocrinol. Metab.* 318, E579–E585. doi:10.1152/ajpendo.00033.2020

Chackelevicius, C. M., Gambaro, S. E., Tiribelli, C., and Rosso, N. (2016). Th17 involvement in nonalcoholic fatty liver disease progression to non-alcoholic steatohepatitis. *World J. Gastroenterol.* 22, 9096–9103. doi:10.3748/wjg.v22.i41.9096

Chávez-Talavera, O., Tailleux, A., Lefebvre, P., and Staels, B. (2017). Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic Fatty liver disease. *Gastroenterology* 152, 1679–1694.e3. doi:10.1053/j.gastro.2017.01.055

Che, Q., Luo, T., Shi, J., He, Y., and Xu, D.-L. (2022). Mechanisms by which traditional Chinese medicines influence the intestinal flora and intestinal barrier. *Front. Cell. Infect. Microbiol.* 12, 863779. doi:10.3389/fcimb.2022.863779

Chen, S., Zhao, X., Ran, L., Wan, J., Wang, X., Qin, Y., et al. (2015). Resveratrol improves insulin resistance, glucose and lipid metabolism in patients with non-alcoholic fatty liver disease: a randomized controlled trial. *Dig. Liver Dis. Off. J. Ital. Soc. Gastroenterol. Ital. Assoc. Study Liver* 47, 226–232. doi:10.1016/j.dld.2014.11.015

Chen, M., Hou, P., Zhou, M., Ren, Q., Wang, X., Huang, L., et al. (2020). Resveratrol attenuates high-fat diet-induced non-alcoholic steatohepatitis by maintaining gut barrier integrity and inhibiting gut inflammation through regulation of the endocannabinoid system. *Clin. Nutr. Edinb. Scotl.* 39, 1264–1275. doi:10.1016/j.clnu.2019.05.020

Chen, J., Li, X., Ge, C., Min, J., and Wang, F. (2022). The multifaceted role of ferroptosis in liver disease. *Cell. Death Differ.* 29, 467–480. doi:10.1038/s41418-022-00941-0

Chen, D., Xiong, J., Chen, G., Zhang, Z., Liu, Y., Xu, J., et al. (2023a). Comparing the influences of metformin and berberine on the intestinal microbiota of rats with Nonalcoholic steatohepatitis. *Vivo Athens Greece* 37, 2105–2127. doi:10.21873/invivo.13308

Chen, Y., Hao, Z., Zhao, H., Duan, X., Jia, D., Li, K., et al. (2023b). Berberine alleviates intestinal barrier dysfunction in glucolipid metabolism disorder hamsters by modulating gut microbiota and gut-microbiota-related tryptophan metabolites. *J. Sci. Food Agric.* 103, 1464–1473. doi:10.1002/jsfa.12242

Chen, M., Huang, F., Chen, B., Kang, J., Yao, Y., Liua, M., et al. (2023c). A classical herbal formula alleviates high-fat diet induced nonalcoholic steatohepatitis (NASH) *via* targeting mitophagy to rehabilitate dysfunctional mitochondria, validated by UPLC-HRMS identification combined with *in vivo* experiment. *Biomed. Pharmacother. Biomedecine Pharmacother.* 168, 115831. doi:10.1016/j.biopha.2023.115831

Chiang, Y.-Y., and Chao, J. C.-J. (2018). Olive oil combined with Lycium barbarum polysaccharides attenuates liver apoptosis and inflammation induced by carbon tetrachloride in rats. *J. Funct. Foods* 48, 329–336. doi:10.1016/j.jff.2018.07.029

Chung, E. Y., Kim, B. H., Hong, J.-T., Lee, C.-K., Ahn, B., Nam, S.-Y., et al. (2011). Resveratrol down-regulates interferon-γ-inducible inflammatory genes in macrophages: molecular mechanism *via* decreased STAT-1 activation. *J. Nutr. Biochem.* 22, 902–909. doi:10.1016/j.jnutbio.2010.07.012

Connolly, M. K., Bedrosian, A. S., Mallen-St Clair, J., Mitchell, A. P., Ibrahim, J., Stroud, A., et al. (2009). In liver fibrosis, dendritic cells govern hepatic inflammation in mice *via* TNF-alpha. *J. Clin. Investig.* 119, 3213–3225. doi:10.1172/JCI37581

Corbin, K. D., and Zeisel, S. H. (2012). Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. *Curr. Opin. Gastroenterol.* 28, 159–165. doi:10.1097/MOG.0b013e32834e7b4b

Crespo, M., Nikolic, I., Mora, A., Rodríguez, E., Leiva-Vega, L., Pintor-Chocano, A., et al. (2023). Myeloid p38 activation maintains macrophage-liver crosstalk and BAT thermogenesis through IL-12-FGF21 axis. *Hepatol. Balt. Md* 77, 874–887. doi:10.1002/hep.32581

Csak, T., Velayudham, A., Hritz, I., Petrasek, J., Levin, I., Lippai, D., et al. (2011). Deficiency in myeloid differentiation factor-2 and toll-like receptor 4 expression attenuates nonalcoholic steatohepatitis and fibrosis in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 300, G433–G441. doi:10.1152/ajpgi.00163.2009

Cynthia, J., and Frank, T. (2016). Hepatic macrophages in homeostasis and liver diseases: from pathogenesis to novel therapeutic strategies. *Cell. Mol. Immunol.* 13, 316–327. doi:10.1038/cmi.2015.104

Daemen, S., Gainullina, A., Kalugotla, G., He, L., Chan, M. M., Beals, J. W., et al. (2021). Dynamic shifts in the composition of resident and recruited macrophages influence tissue remodeling in NASH. *Cell. Rep.* 34, 108626. doi:10.1016/j.celrep.2020.108626

De Bruyn, M., and Vermeire, S. (2017). NOD2 and bacterial recognition as therapeutic targets for Crohn's disease. *Expert Opin. Ther. Targets* 21, 1123–1139. doi:10.1080/14728222.2017.1397627

Deczkowska, A., David, E., Ramadori, P., Pfister, D., Safran, M., Li, B., et al. (2021). XCR1+ type 1 conventional dendritic cells drive liver pathology in non-alcoholic steatohepatitis. *Nat. Med.* 27, 1043–1054. doi:10.1038/s41591-021-01344-3

Deng, M., Qu, F., Chen, L., Liu, C., Zhang, M., Ren, F., et al. (2020). SCFAs alleviated steatosis and inflammation in mice with NASH induced by MCD. *J. Endocrinol.* 245, 425–437. doi:10.1530/JOE-20-0018

Deng, C.-J., Lo, T.-H., Chan, K.-Y., Li, X., Wu, M.-Y., Xiang, Z., et al. (2022). Role of B Lymphocytes in the Pathogenesis of NAFLD: a 2022 update. *Int. J. Mol. Sci.* 23, 12376. doi:10.3390/ijms232012376

Dharancy, S., Body-Malapel, M., Louvet, A., Berrebi, D., Gantier, E., Gosset, P., et al. (2010). Neutrophil migration during liver injury is under nucleotide-binding oligomerization domain 1 control. *Gastroenterology* 138, 1546–1556.e15565. doi:10.1053/j.gastro.2009.12.008

Diehl, A. M., and Day, C. (2017). Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis. *N. Engl. J. Med.* 377, 2063–2072. doi:10.1056/NEJMra1503519

Ding, Y., Yan, Y., Peng, Y., Chen, D., Mi, J., Lu, L., et al. (2019). *In vitro* digestion under simulated saliva, gastric and small intestinal conditions and fermentation by human gut microbiota of polysaccharides from the fruits of Lycium barbarum. *Int. J. Biol. Macromol.* 125, 751–760. doi:10.1016/j.ijbiomac.2018.12.081

Dodd, D., Spitzer, M. H., Van Treuren, W., Merrill, B. D., Hryckowian, A. J., Higginbottom, S. K., et al. (2017). A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. *Nature* 551, 648–652. doi:10.1038/nature24661

Dorner, H., Stolzer, I., Mattner, J., Kaminski, S., Leistl, S., Edrich, L.-M., et al. (2024). Gut pathobiont-derived outer membrane vesicles drive liver inflammation and fibrosis in primary sclerosing cholangitis-associated inflammatory bowel disease. *Gastroenterology* 167, 1183–1197.e16. doi:10.1053/j.gastro.2024.06.032

Du, J., Zhang, J., Chen, X., Zhang, S., Zhang, C., Liu, H., et al. (2022). Neutrophil extracellular traps induced by pro-inflammatory cytokines enhance procoagulant activity in NASH patients. *Clin. Res. Hepatol. Gastroenterol.* 46, 101697. doi:10.1016/j.clinre.2021.101697

Dudek, M., Pfister, D., Donakonda, S., Filpe, P., Schneider, A., Laschinger, M., et al. (2021). Auto-aggressive CXCR6+ CD8 T cells cause liver immune pathology in NASH. *Nature* 592, 444–449. doi:10.1038/s41586-021-03233-8

Dywicki, J., Buitrago-Molina, L. E., Noyan, F., Davalos-Misslitz, A. C., Hupa-Breier, K. L., Lieber, M., et al. (2022). The detrimental role of regulatory T cells in nonalcoholic steatohepatitis. *Hepatol. Commun.* 6, 320–333. doi:10.1002/hep4.1807

Eissa, L. A., Kenawy, H. I., El-Karef, A., Elsherbiny, N. M., and El-Mihi, K. A. (2018). Antioxidant and anti-inflammatory activities of berberine attenuate hepatic fibrosis induced by thioacetamide injection in rats. *Chem. Biol. Interact.* 294, 91–100. doi:10.1016/j.cbi.2018.08.016

Exley, M. A., and Koziel, M. J. (2004). To be or not to be NKT: natural killer T cells in the liver. *Hepatol. Balt. Md* 40, 1033–1040. doi:10.1002/hep.20433

Fa, P., Ke, B. G., Dupre, A., Tsung, A., and Zhang, H. (2023). The implication of neutrophil extracellular traps in nonalcoholic fatty liver disease. *Front. Immunol.* 14, 1292679. doi:10.3389/fimmu.2023.1292679

Fan, H., and Cook, J. A. (2004). Molecular mechanisms of endotoxin tolerance. *J. Endotoxin Res.* 10, 71–84. doi:10.1179/096805104225003997

Fan, Y., Pu, Y., Xu, X., and Yang, J. (2025a). Benefit of Lycium barbarum polysaccharide supplementation in patients with nonalcoholic fatty liver disease: a pre-post study. *Nutr. Health*, 02601060251357979. doi:10.1177/02601060251357979

Fan, Y., Yang, C., Pan, J., Ye, W., Zhang, J., Zeng, R., et al. (2025b). A new target for curcumin in the treatment of non-alcoholic fatty liver disease: the FTO protein. *Int. J. Biol. Macromol.* 319, 145642. doi:10.1016/j.ijbiomac.2025.145642

Feingold, K. R., Moser, A., Shigenaga, J. K., and Grunfeld, C. (2014). Inflammation stimulates niacin receptor (GPR109A/HCA2) expression in adipose tissue and macrophages. *J. Lipid Res.* 55, 2501–2508. doi:10.1194/jlr.M050955

Ficht, X., and Iannacone, M. (2020). Immune surveillance of the liver by T cells. *Sci. Immunol.* 5, eaba2351. doi:10.1126/sciimmunol.aba2351

Fiorucci, S., Zampella, A., Ricci, P., Distrutti, E., and Biagioli, M. (2022). Immunomodulatory functions of FXR. *Mol. Cell. Endocrinol.* 551, 111650. doi:10.1016/j.mce.2022.111650

Fizanne, L., Villard, A., Benabbou, N., Recoquillon, S., Soleti, R., Delage, E., et al. (2023). Faeces-derived extracellular vesicles participate in the onset of barrier dysfunction leading to liver diseases. *J. Extracell. Vesicles* 12, e12303. doi:10.1002/jev2.12303

Flores-Guerrero, J. L., Post, A., van Dijk, P. R., Connelly, M. A., Garcia, E., Navis, G., et al. (2021). Circulating trimethylamine-N-oxide is associated with all-cause mortality in subjects with nonalcoholic fatty liver disease. *Liver Int. Off. J. Int. Assoc. Study Liver* 41, 2371–2382. doi:10.1111/liv.14963

Folco, E. J., Mawson, T. L., Vromman, A., Bernardes-Souza, B., Franck, G., Persson, O., et al. (2018). Neutrophil extracellular traps induce endothelial cell activation and tissue factor production through Interleukin-1α and Cathepsin G. *Arterioscler. Thromb. Vasc. Biol.* 38, 1901–1912. doi:10.1161/ATVBAHA.118.311150

Furusawa, Y., Obata, Y., Fukuda, S., Endo, T. A., Nakato, G., Takahashi, D., et al. (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504, 446–450. doi:10.1038/nature12721

Gao, Y., Liang, A., Fan, X., Hu, L., Hao, F., and Li, Y. (2019). Safety research in traditional Chinese medicine: methods, applications, and outlook. *Engineering* 5, 76–82. doi:10.1016/j.eng.2018.11.019

Gao, L.-L., Ma, J.-M., Fan, Y.-N., Zhang, Y.-N., Ge, R., Tao, X.-J., et al. (2021). Lycium barbarum polysaccharide combined with aerobic exercise ameliorated nonalcoholic fatty liver disease through restoring gut microbiota, intestinal barrier and inhibiting hepatic inflammation. *Int. J. Biol. Macromol.* 183, 1379–1392. doi:10.1016/j.ijbiomac.2021.05.066

Gao, W., Zhang, L., Wu, J., Xu, Y., Qi, S., Liu, W., et al. (2023). Extraction, characterization, and anti-nonalcoholic steatohepatitis activity of a (1,3) (1,6)-β-D-glucan from the Polyporus umbellatus (Pers.) fries. *Int. J. Biol. Macromol.* 230, 123252. doi:10.1016/j.ijbiomac.2023.123252

Gavzy, S. J., Kensiski, A., Lee, Z. L., Mongodin, E. F., Ma, B., and Bromberg, J. S. (2023). Bifidobacterium mechanisms of immune modulation and tolerance. *Gut Microbes* 15, 2291164. doi:10.1080/19490976.2023.2291164

Ginhoux, F., Yalin, A., Dutertre, C. A., and Amit, I. (2022). Single-cell immunology: past, present, and future. *Immunity* 55, 393–404. doi:10.1016/j.immuni.2022.02.006

Goettel, J. A., Gandhi, R., Kenison, J. E., Yeste, A., Murugaiyan, G., Sambanthamoorthy, S., et al. (2016). AHR activation is protective against colitis driven by T cells in humanized mice. *Cell. Rep.* 17, 1318–1329. doi:10.1016/j.celrep.2016.09.082

Golabi, P., Paik, J., Reddy, R., Bugianesi, E., Trimble, G., and Younossi, Z. M. (2019). Prevalence and long-term outcomes of non-alcoholic fatty liver disease among elderly individuals from the United States. *BMC Gastroenterol.* 19, 56. doi:10.1186/s12876-019-0972-6

Gomes, M. T., Campos, P. C., Pereira, G. de S., Bartholomeu, D. C., Splitter, G., and Oliveira, S. C. (2016). TLR9 is required for MAPK/NF-κB activation but does not cooperate with TLR2 or TLR6 to induce host resistance to Brucella abortus. *J. Leukoc. Biol.* 99, 771–780. doi:10.1189/jlb.4A0815-346R

Gu, L., Meng, R., Tang, Y., Zhao, K., Liang, F., Zhang, R., et al. (2019). Toll-Like receptor 4 signaling licenses the cytosolic transport of lipopolysaccharide from bacterial outer membrane vesicles. *Shock Augusta Ga* 51, 256–265. doi:10.1097/SHK.0000000000001129

Guariglia, M., Saba, F., Rosso, C., and Bugianesi, E. (2023). Molecular mechanisms of curcumin in the pathogenesis of Metabolic Dysfunction associated steatotic liver disease. *Nutrients* 15, 5053. doi:10.3390/nu15245053

Guilliams, M., and Scott, C. L. (2022). Liver macrophages in health and disease. *Immunity* 55, 1515–1529. doi:10.1016/j.immuni.2022.08.002

Guo, C., Xie, S., Chi, Z., Zhang, J., Liu, Y., Zhang, L., et al. (2016a). Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. *Immunity* 45, 802–816. doi:10.1016/j.immuni.2016.09.008

Guo, T., Woo, S.-L., Guo, X., Li, H., Zheng, J., Botchlett, R., et al. (2016b). Berberine ameliorates hepatic steatosis and suppresses liver and adipose tissue inflammation in mice with diet-induced obesity. *Sci. Rep.* 6, 22612. doi:10.1038/srep22612

Hakhamaneshi, M. S., Abdolahi, A., Vahabzadeh, Z., Abdi, M., and Andalibi, P. (2021). Toll-Like receptor 4: a macrophage cell surface receptor is activated by Trimethylamine-N-Oxide. *Cell. J.* 23, 516–522. doi:10.22074/cellj.2021.7849

Hayashi, K., Taura, M., and Iwasaki, A. (2018). The interaction between IKKα and LC3 promotes type I interferon production through the TLR9-containing LAPosome. *Sci. Signal.* 11, eaan4144. doi:10.1126/scisignal.aan4144

He, B., Wu, L., Xie, W., Shao, Y., Jiang, J., Zhao, Z., et al. (2017). The imbalance of Th17/Treg cells is involved in the progression of nonalcoholic fatty liver disease in mice. *BMC Immunol.* 18, 33. doi:10.1186/s12865-017-0215-y

He, Y., Chen, X., Li, Y., Liang, Y., Hong, T., Yang, J., et al. (2024). Curcumin supplementation alleviates hepatic fat content associated with modulation of gut microbiota-dependent bile acid metabolism in patients with nonalcoholic simple fatty liver disease: a randomized controlled trial. *Am. J. Clin. Nutr.* 120, 66–79. doi:10.1016/j.ajcnut.2024.05.017

Henning, J. R., Graffeo, C. S., Rehman, A., Fallon, N. C., Zambirinis, C. P., Ochi, A., et al. (2013). Dendritic cells limit fibroinflammatory injury in nonalcoholic steatohepatitis in mice. *Hepatol. Balt. Md* 58, 589–602. doi:10.1002/hep.26267

Hesse, M., Modolell, M., La Flamme, A. C., Schito, M., Fuentes, J. M., Cheever, A. W., et al. (2001). Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines *in vivo:* granulomatous pathology is shaped by the pattern of L-arginine metabolism. *J. Immunol. Balt. Md 1950* 167, 6533–6544. doi:10.4049/jimmunol.167.11.6533

Heymann, F., Trautwein, C., and Tacke, F. (2009). Monocytes and macrophages as cellular targets in liver fibrosis. *Inflamm. Allergy Drug Targets* 8, 307–318. doi:10.2174/187152809789352230

Hong, T., Jiang, X., Zou, J., Yang, J., Zhang, H., Mai, H., et al. (2022). Hepatoprotective effect of curcumin against bisphenol A-induced hepatic steatosis *via* modulating gut microbiota dysbiosis and related gut-liver axis activation in CD-1 mice. *J. Nutr. Biochem.* 109, 109103. doi:10.1016/j.jnutbio.2022.109103

Hosseinkhani, F., Heinken, A., Thiele, I., Lindenburg, P. W., Harms, A. C., and Hankemeier, T. (2021). The contribution of gut bacterial metabolites in the human immune signaling pathway of non-communicable diseases. *Gut Microbes* 13, 1–22. doi:10.1080/19490976.2021.1882927

Hu, Z.-Q., Xian, J.-C., Chu, S.-C., Wang, J.-Y., Shen, L., Zhang, L., et al. (2019). Development status and research strategy of traditional Chinese medicine temporary prescription preparation technology. *Zhongguo Zhong Yao Za Zhi Zhongguo Zhongyao Zazhi China J. Chin. Mat. Medica* 44, 28–33. doi:10.19540/j.cnki.cjcmm.20181107.002

Hu, H., Lin, A., Kong, M., Yao, X., Yin, M., Xia, H., et al. (2020). Intestinal microbiome and NAFLD: molecular insights and therapeutic perspectives. *J. Gastroenterol.* 55, 142–158. doi:10.1007/s00535-019-01649-8

Hu, Y.-L., Ma, Q., Dong, X., Kong, Y., Cai, J., Li, J., et al. (2023). Research progress on the therapeutic effects of polysaccharides on non-alcoholic fatty liver diseases. *Front. Nutr.* 10, 1107551. doi:10.3389/fnut.2023.1107551

Huang, L., Rao, Q., Wang, C., Mou, Y., Zheng, X., Hu, E., et al. (2024). Multi-omics joint analysis reveals that the Miao medicine Yindanxinnaotong formula attenuates non-alcoholic fatty liver disease. *Phytomedicine Int. J. Phytother. Phytopharm.* 135, 156026. doi:10.1016/j.phymed.2024.156026

Huang, M., Ji, Q., Huang, H., Wang, X., and Wang, L. (2025a). Gut microbiota in hepatocellular carcinoma immunotherapy: immune microenvironment remodeling and gut microbiota modification. *Gut Microbes* 17, 2486519. doi:10.1080/19490976.2025.2486519

Huang, Y., Xie, Y., Zhang, Y., Liu, Z., Jiang, W., Ye, Y., et al. (2025b). Single-cell transcriptome reveals the reprogramming of immune microenvironment during the transition from MASH to HCC. *Mol. Cancer* 24, 177. doi:10.1186/s12943-025-02370-2

Huby, T., and Gautier, E. L. (2022). Immune cell-mediated features of non-alcoholic steatohepatitis. *Nat. Rev. Immunol.* 22, 429–443. doi:10.1038/s41577-021-00639-3

Hung, J.-T., Huang, J.-R., and Yu, A. L. (2017). Tailored design of NKT-stimulatory glycolipids for polarization of immune responses. *J. Biomed. Sci.* 24, 22. doi:10.1186/s12929-017-0325-0

Janeiro, M. H., Ramírez, M. J., Milagro, F. I., Martínez, J. A., and Solas, M. (2018). Implication of trimethylamine N-Oxide (TMAO) in disease: potential biomarker or new therapeutic target. *Nutrients* 10, 1398. doi:10.3390/nu10101398

Jd, S., and Bl, C. (2021). Modulation of macrophage phenotype to treat liver fibrosis-current approaches and future possibilities. *Adv. Pharmacol. San. Diego Calif.* 91, 213–228. doi:10.1016/bs.apha.2021.03.001

Jenne, C. N., and Kubes, P. (2013). Immune surveillance by the liver. *Nat. Immunol.* 14, 996–1006. doi:10.1038/ni.2691

Ji, Y., Yin, Y., Sun, L., and Zhang, W. (2020). The molecular and mechanistic insights based on gut-liver axis: nutritional target for non-alcoholic fatty liver disease (NAFLD) improvement. *Int. J. Mol. Sci.* 21, 3066. doi:10.3390/ijms21093066

Jiang, Y.-H., Wang, L., Chen, W.-D., Duan, Y.-T., Sun, M.-J., Huang, J.-J., et al. (2022). Poria cocos polysaccharide prevents alcohol-induced hepatic injury and inflammation

by repressing oxidative stress and gut leakiness. *Front. Nutr.* 9, 963598. doi:10.3389/fnut.2022.963598

Jiang, L., Yi, R., Chen, H., and Wu, S. (2025). Quercetin alleviates metabolic-associated fatty liver disease by tuning hepatic lipid metabolism, oxidative stress and inflammation. *Anim. Biotechnol.* 36, 2442351. doi:10.1080/10495398.2024.2442351

Jiao, J., Sastre, D., Fiel, M. I., Lee, U. E., Ghiassi-Nejad, Z., Ginhoux, F., et al. (2012). Dendritic cell regulation of carbon tetrachloride-induced murine liver fibrosis regression. *Hepatol. Balt. Md* 55, 244–255. doi:10.1002/hep.24621

Jin, W.-B., Li, T.-T., Huo, D., Qu, S., Li, X. V., Arifuzzaman, M., et al. (2022). Genetic manipulation of gut microbes enables single-gene interrogation in a complex microbiome. *Cell.* 185, 547–562.e22. doi:10.1016/j.cell.2021.12.035

Jin, R., Neufeld, L., and McGaha, T. L. (2025). Linking macrophage metabolism to function in the tumor microenvironment. *Nat. Cancer* 6, 239–252. doi:10.1038/s43018-025-00909-2

Jing, Z., Jingyi, J., Yue, L., Zhang, S., Li, F., Cui, J., et al. (2025). Yinchen-Gancao decoction ameliorated NASH in mice by reducing hepatic lipid accumulation, inhibiting hepatic inflammatory and endoplasmic reticulum stress. *J. Ethnopharmacol.* 341, 119318. doi:10.1016/j.jep.2025.119318

Josefowicz, S. Z., Lu, L.-F., and Rudensky, A. Y. (2012). Regulatory T cells: mechanisms of differentiation and function. *Annu. Rev. Immunol.* 30, 531–564. doi:10.1146/annurev.immunol.25.022106.141623

Juárez-Verdayes, M. A., Rodríguez-Martínez, S., Cancino-Diaz, M. E., and Cancino-Diaz, J. C. (2013). Peptidoglycan and muramyl dipeptide from Staphylococcus aureus induce the expression of VEGF-A in human limbal fibroblasts with the participation of TLR2-NFκB and NOD2-EGFR. *Graefes Arch. Clin. Exp. Ophthalmol. Albr. Von. Graefes Arch. Klin. Exp. Ophthalmol.* 251, 53–62. doi:10.1007/s00417-012-2130-5

Kechagias, S., Nasr, P., Blomdahl, J., and Ekstedt, M. (2020). Established and emerging factors affecting the progression of nonalcoholic fatty liver disease. *Metabolism* 111S, 154183. doi:10.1016/j.metabol.2020.154183

Keestra-Gounder, A. M., and Tsolis, R. M. (2017). NOD1 and NOD2: beyond peptidoglycan sensing. *Trends Immunol.* 38, 758–767. doi:10.1016/j.it.2017.07.004

Keitel, V., Reinehr, R., Gatsios, P., Rupprecht, C., Görg, B., Selbach, O., et al. (2007). The G-protein coupled bile salt receptor TGR5 is expressed in liver sinusoidal endothelial cells. *Hepatol. Balt. Md* 45, 695–704. doi:10.1002/hep.21458

Koda, Y., Teratani, T., Chu, P.-S., Hagihara, Y., Mikami, Y., Harada, Y., et al. (2021). CD8+ tissue-resident memory T cells promote liver fibrosis resolution by inducing apoptosis of hepatic stellate cells. *Nat. Commun.* 12, 4474. doi:10.1038/s41467-021-24734-0

Kong, L., Zhao, Q., Jiang, X., Hu, J., Jiang, Q., Sheng, L., et al. (2024). Trimethylamine N-oxide impairs β-cell function and glucose tolerance. *Nat. Commun.* 15, 2526. doi:10.1038/s41467-024-46829-0

Krueger, J. M., Pappenheimer, J. R., and Karnovsky, M. L. (1982). The composition of sleep-promoting factor isolated from human urine. *J. Biol. Chem.* 257, 1664–1669. doi:10.1016/s0021-9258(19)68088-9

Kwan, S.-Y., Jiao, J., Joon, A., Wei, P., Petty, L. E., Below, J. E., et al. (2022). Gut microbiome features associated with liver fibrosis in Hispanics, a population at high risk for fatty liver disease. *Hepatol. Balt. Md* 75, 955–967. doi:10.1002/hep.32197

Landfried, K., Zhu, W., Waldhier, M. C., Schulz, U., Ammer, J., Holler, B., et al. (2011). Tryptophan catabolism is associated with acute GVHD after human allogeneic stem cell transplantation and indicates activation of indoleamine 2,3-dioxygenase. *Blood* 118, 6971–6974. doi:10.1182/blood-2011-06-357814

Lee, G., You, H. J., Bajaj, J. S., Joo, S. K., Yu, J., Park, S., et al. (2020). Distinct signatures of gut microbiome and metabolites associated with significant fibrosis in Non-obese NAFLD. *Nat. Commun.* 11, 4982. doi:10.1038/s41467-020-18754-5

Lei, J.-Q., Xie, Q.-L., Li, Q.-P., Qin, S.-Y., Wu, J.-H., Jiang, H.-X., et al. (2025). Resveratrol alleviates liver fibrosis by targeting cross-talk between TLR2/MyD88/ERK and NF-κB/NLRP3 inflammasome pathways in macrophages. *J. Biochem. Mol. Toxicol.* 39, e70208. doi:10.1002/jbt.70208

Leung, H., Long, X., Ni, Y., Qian, L., Nychas, E., Siliceo, S. L., et al. (2022). Risk assessment with gut microbiome and metabolite markers in NAFLD development. *Sci. Transl. Med.* 14, eabk0855. doi:10.1126/scitranslmed.abk0855

Li, L., Zhang, Q., Peng, J., Jiang, C., Zhang, Y., Shen, L., et al. (2015). Activation of farnesoid X receptor downregulates monocyte chemoattractant protein-1 in murine macrophage. *Biochem. Biophys. Res. Commun.* 467, 841–846. doi:10.1016/j.bbrc.2015.10.056

Li, D., Zheng, J., Hu, Y., Hou, H., Hao, S., Liu, N., et al. (2017). Amelioration of intestinal barrier dysfunction by berberine in the treatment of nonalcoholic Fatty liver disease in rats. *Pharmacogn. Mag.* 13, 677–682. doi:10.4103/pm.pm_584_16

Li, F., Ye, J., Shao, C., and Zhong, B. (2021a). Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and meta-analysis. *Lipids Health Dis.* 20, 22. doi:10.1186/s12944-021-01440-w

Li, S., You, J., Wang, Z., Liu, Y., Wang, B., Du, M., et al. (2021b). Curcumin alleviates high-fat diet-induced hepatic steatosis and obesity in association with modulation of gut microbiota in mice. *Food Res. Int. ott. Ont.* 143, 110270. doi:10.1016/j.foodres.2021.110270

Li, D.-D., Ma, J.-M., Li, M.-J., Gao, L.-L., Fan, Y.-N., Zhang, Y.-N., et al. (2022). Supplementation of Lycium barbarum polysaccharide combined with aerobic exercise ameliorates high-fat-induced nonalcoholic steatohepatitis *via* AMPK/PPARα/PGC-1α pathway. *Nutrients* 14, 3247. doi:10.3390/nu14153247

Li, N., Cui, C., Xu, J., Mi, M., Wang, J., and Qin, Y. (2024a). Quercetin intervention reduced hepatic fat deposition in patients with nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled crossover clinical trial. *Am. J. Clin. Nutr.* 120, 507–517. doi:10.1016/j.ajcnut.2024.07.013

Li, X., Chen, W., Ren, J., Gao, X., Zhao, Y., Song, T., et al. (2024b). Effects of curcumin on non-alcoholic fatty liver disease: a scientific metrogy study. *Phytomedicine Int. J. Phytother. Phytopharm.* 123, 155241. doi:10.1016/j.phymed.2023.155241

Liang, W., Zhou, K., Jian, P., Chang, Z., Zhang, Q., Liu, Y., et al. (2021). Ginsenosides improve nonalcoholic Fatty liver disease *via* integrated regulation of Gut Microbiota, inflammation and energy homeostasis. *Front. Pharmacol.* 12, 622841. doi:10.3389/fphar.2021.622841

Liu, J., Liu, Y., Huang, C., He, C., Yang, T., Ren, R., et al. (2025a). Quercetin-Driven Akkermansia Muciniphila alleviates obesity by modulating bile acid metabolism *via* an ILA/m6A/CYP8B1 signaling. *Adv. Sci. Weinh. Baden-Wurtt. Ger.* 12, e2412865. doi:10.1002/advs.202412865

Liu, W., Yu, L., Chen, Q., Zhang, C., Wang, L., Yu, N., et al. (2025b). Poria cocos polysaccharides alleviate obesity-related adipose tissue insulin resistance *via* gut microbiota-derived short-chain fatty acids activation of FGF21/PI3K/AKT signaling. *Food Res. Int. ott. Ont.* 215, 116671. doi:10.1016/j.foodres.2025.116671

Liu, W., Zhou, X., Xiao, L., Huang, X., Chang, D., Zhong, X., et al. (2025c). The gut microbiota-mediated ferroptosis pathway: a key mechanism of ginsenoside Rd against metabolism-associated fatty liver disease. *Chin. Med.* 20, 83. doi:10.1186/s13020-025-01121-1

Liu, Y., Zhou, F., Zhao, H., Song, J., Song, M., Zhu, J., et al. (2025d). Dimeric guaianolide sesquiterpenoids from the flowers of Chrysanthemum indicum ameliorate hepatic steatosis through mitigating SIRT1-mediated lipid accumulation and ferroptosis. *J. Adv. Res.* S2090-1232 (24), 00625–00628. doi:10.1016/j.jare.2024.12.047

Llopis, M., Cassard, A. M., Wrzosek, L., Boschat, L., Bruneau, A., Ferrere, G., et al. (2016). Intestinal microbiota contributes to individual susceptibility to alcoholic liver disease. *Gut* 65, 830–839. doi:10.1136/gutjnl-2015-310585

Loomba, R., Seguritan, V., Li, W., Long, T., Klitgord, N., Bhatt, A., et al. (2017). Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic Fatty liver disease. *Cell. Metab.* 25, 1054–1062.e5. doi:10.1016/j.cmet.2017.04.001

Loomba, R., Friedman, S. L., and Shulman, G. I. (2021). Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell.* 184, 2537–2564. doi:10.1016/j.cell.2021.04.015

Lukacs-Kornek, V., and Schuppan, D. (2013). Dendritic cells in liver injury and fibrosis: shortcomings and promises. *J. Hepatol.* 59, 1124–1126. doi:10.1016/j.jhep.2013.05.033

Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., and Locati, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol.* 25, 677–686. doi:10.1016/j.it.2004.09.015

Maricic, I., Sheng, H., Marrero, I., Seki, E., Kisseleva, T., Chaturvedi, S., et al. (2015). Inhibition of type I natural killer T cells by retinoids or following sulfatide-mediated activation of type II natural killer T cells attenuates alcoholic liver disease in mice. *Hepatol. Balt. Md* 61, 1357–1369. doi:10.1002/hep.27632

Marrero, I., Ware, R., and Kumar, V. (2015). Type II NKT cells in inflammation, autoimmunity, microbial immunity, and cancer. *Front. Immunol.* 6, 316. doi:10.3389/fimmu.2015.00316

Marrero, I., Maricic, I., Feldstein, A. E., Loomba, R., Schnabl, B., Rivera-Nieves, J., et al. (2018). Complex network of NKT cell subsets controls immune homeostasis in liver and gut. *Front. Immunol.* 9, 2082. doi:10.3389/fimmu.2018.02082

Martinez, F. O., Gordon, S., Locati, M., and Mantovani, A. (2006). Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J. Immunol. Balt. Md 1950* 177, 7303–7311. doi:10.4049/jimmunol.177.10.7303

Martínez-Chantar, M. L., Delgado, T. C., and Beraza, N. (2021). Revisiting the role of natural killer cells in non-alcoholic fatty liver disease. *Front. Immunol.* 12, 640869. doi:10.3389/fimmu.2021.640869

Mathews, S., Feng, D., Maricic, I., Ju, C., Kumar, V., and Gao, B. (2016). Invariant natural killer T cells contribute to chronic-plus-binge ethanol-mediated liver injury by promoting hepatic neutrophil infiltration. *Cell. Mol. Immunol.* 13, 206–216. doi:10.1038/cmi.2015.06

Matsumoto, K., Ichimura, M., Tsuneyama, K., Moritoki, Y., Tsunashima, H., Omagari, K., et al. (2017). Fructo-oligosaccharides and intestinal barrier function in a methionine-choline-deficient mouse model of nonalcoholic steatohepatitis. *PloS One* 12, e0175406. doi:10.1371/journal.pone.0175406

Meli, R., Mattace Raso, G., and Calignano, A. (2014). Role of innate immune response in non-alcoholic Fatty liver disease: metabolic complications and therapeutic tools. *Front. Immunol.* 5, 177. doi:10.3389/fimmu.2014.00177

Méndez-Sánchez, N., Valencia-Rodríguez, A., Coronel-Castillo, C., Vera-Barajas, A., Contreras-Carmona, J., Ponciano-Rodríguez, G., et al. (2020). The cellular pathways of liver fibrosis in non-alcoholic steatohepatitis. *Ann. Transl. Med.* 8, 400. doi:10.21037/atm.2020.02.184

Meroueh, S. O., Bencze, K. Z., Hesek, D., Lee, M., Fisher, J. F., Stemmler, T. L., et al. (2006). Three-dimensional structure of the bacterial cell wall peptidoglycan. *Proc. Natl. Acad. Sci. U. S. A.* 103, 4404–4409. doi:10.1073/pnas.0510182103

Min, B. H., Devi, S., Kwon, G. H., Gupta, H., Jeong, J.-J., Sharma, S. P., et al. (2024). Gut microbiota-derived indole compounds attenuate metabolic dysfunction-associated steatotic liver disease by improving fat metabolism and inflammation. *Gut Microbes* 16, 2307568. doi:10.1080/19490976.2024.2307568

Moreno-Fernandez, M. E., Giles, D. A., Oates, J. R., Chan, C. C., Damen, M. S. M. A., Doll, J. R., et al. (2021). PKM2-dependent metabolic skewing of hepatic Th17 cells regulates pathogenesis of non-alcoholic fatty liver disease. *Cell. Metab.* 33, 1187–1204.e9. doi:10.1016/j.cmet.2021.04.018

Mouries, J., Brescia, P., Silvestri, A., Spadoni, I., Sorribas, M., Wiest, R., et al. (2019). Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. *J. Hepatol.* 71, 1216–1228. doi:10.1016/j.jhep.2019.08.005

Mridha, A. R., Haczeyni, F., Yeh, M. M., Haigh, W. G., Ioannou, G. N., Barn, V., et al. (2017). TLR9 is up-regulated in human and murine NASH: pivotal role in inflammatory recruitment and cell survival. *Clin. Sci. Lond. Engl. 1979* 131, 2145–2159. doi:10.1042/CS20160838

Nati, M., Haddad, D., Birkenfeld, A. L., Koch, C. A., Chavakis, T., and Chatzigeorgiou, A. (2016). The role of immune cells in metabolism-related liver inflammation and development of non-alcoholic steatohepatitis (NASH). *Rev. Endocr. Metab. Disord.* 17, 29–39. doi:10.1007/s11154-016-9339-2

Ngu, M. H., Norhayati, M. N., Rosnani, Z., and Zulkifli, M. M. (2022). Curcumin as adjuvant treatment in patients with non-alcoholic fatty liver (NAFLD) disease: a systematic review and meta-analysis. *Complement. Ther. Med.* 68, 102843. doi:10.1016/j.ctim.2022.102843

Nguyen, N. T., Kimura, A., Nakahama, T., Chinen, I., Masuda, K., Nohara, K., et al. (2010). Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity *via* a kynurenine-dependent mechanism. *Proc. Natl. Acad. Sci. U. S. A.* 107, 19961–19966. doi:10.1073/pnas.1014465107

Nian, F., Chen, Y., Xia, Q., Zhu, C., Wu, L., and Lu, X. (2024). Gut microbiota metabolite trimethylamine N-oxide promoted NAFLD progression by exacerbating intestinal barrier disruption and intrahepatic cellular imbalance. *Int. Immunopharmacol.* 142, 113173. doi:10.1016/j.intimp.2024.113173

Niazpour, F., and Meshkani, R. (2025). Unlocking the therapeutic potential of autophagy modulation by natural products in tackling non-alcoholic fatty liver disease. *Phytother. Res.* PTR, 2357–2373. doi:10.1002/ptr.8463

Nicolas, G. R., and Chang, P. V. (2019). Deciphering the chemical lexicon of host-gut microbiota interactions. *Trends Pharmacol. Sci.* 40, 430–445. doi:10.1016/j.tips.2019.04.006

Nighot, M., Al-Sadi, R., Guo, S., Rawat, M., Nighot, P., Watterson, M. D., et al. (2017). Lipopolysaccharide-Induced increase in intestinal epithelial tight permeability is mediated by toll-like receptor 4/Myeloid differentiation primary response 88 (MyD88) activation of Myosin Light chain kinase expression. *Am. J. Pathol.* 187, 2698–2710. doi:10.1016/j.ajpath.2017.08.005

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318. doi:10.1126/science.1110647

Pallett, L. J., Swadling, L., Diniz, M., Maini, A. A., Schwabenland, M., Gasull, A. D., et al. (2023). Tissue CD14+CD8+ T cells reprogrammed by myeloid cells and modulated by LPS. *Nature* 614, 334–342. doi:10.1038/s41586-022-05645-6

Panchal, S. K., Poudyal, H., and Brown, L. (2012). Quercetin ameliorates cardiovascular, hepatic, and metabolic changes in diet-induced metabolic syndrome in rats. *J. Nutr.* 142, 1026–1032. doi:10.3945/jn.111.157263

Pappo, I., Bercovier, H., Berry, E. M., Haviv, Y., Gallily, R., and Freund, H. R. (1992). Polymyxin B reduces total parenteral nutrition-associated hepatic steatosis by its antibacterial activity and by blocking deleterious effects of lipopolysaccharide. *JPEN J. Parenter. Enter. Nutr.* 16, 529–532. doi:10.1177/0148607192016006529

Park, J., Kim, M., Kang, S. G., Jannasch, A. H., Cooper, B., Patterson, J., et al. (2015). Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol.* 8, 80–93. doi:10.1038/mi.2014.44

Peiseler, M., Schwabe, R., Hampe, J., Kubes, P., Heikenwälder, M., and Tacke, F. (2022). Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease - novel insights into cellular communication circuits. *J. Hepatol.* 77, 1136–1160. doi:10.1016/j.jhep.2022.06.012

Peng, L., He, Z., Chen, W., Holzman, I. R., and Lin, J. (2007). Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. *Pediatr. Res.* 61, 37–41. doi:10.1203/01.pdr.0000250014.92242.f3

Peng, Y., Li, Y., He, Y., Wei, Q., Xie, Q., Zhang, L., et al. (2018). The role of neutrophil to lymphocyte ratio for the assessment of liver fibrosis and cirrhosis: a systematic review. *Expert Rev. Gastroenterol. Hepatol.* 12, 503–513. doi:10.1080/17474124.2018.1463158

Pfister, D., Núñez, N. G., Pinyol, R., Govaere, O., Pinter, M., Szydlowska, M., et al. (2021). NASH limits anti-tumour surveillance in immunotherapy-treated HCC. *Nature* 592, 450–456. doi:10.1038/s41586-021-03362-0

Pols, T. W. H., Nomura, M., Harach, T., Lo Sasso, G., Oosterveer, M. H., Thomas, C., et al. (2011). TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. *Cell. Metab.* 14, 747–757. doi:10.1016/j.cmet.2011.11.006

Porras, D., Nistal, E., Martínez-Flórez, S., Pisonero-Vaquero, S., Olcoz, J. L., Jover, R., et al. (2017). Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease in mice is mediated by modulating intestinal microbiota imbalance and related gut-liver axis activation. *Free Radic. Biol. Med.* 102, 188–202. doi:10.1016/j.freeradbiomed.2016.11.037

Powell, D. N., Swimm, A., Sonowal, R., Bretin, A., Gewirtz, A. T., Jones, R. M., et al. (2020). Indoles from the commensal microbiota act *via* the AHR and IL-10 to tune the cellular composition of the colonic epithelium during aging. *Proc. Natl. Acad. Sci. U. S. A.* 117, 21519–21526. doi:10.1073/pnas.2003004117

Powell, E. E., Wong, V. W.-S., and Rinella, M. (2021). Non-alcoholic fatty liver disease. *Lancet lond. Engl.* 397, 2212–2224. doi:10.1016/S0140-6736(20)32511-3

Procházková, N., Laursen, M. F., La Barbera, G., Tsekitsidi, E., Jørgensen, M. S., Rasmussen, M. A., et al. (2024). Gut physiology and environment explain variations in human gut microbiome composition and metabolism. *Nat. Microbiol.* 9, 3210–3225. doi:10.1038/s41564-024-01856-x

Qiu, J., Chen, L., Zhang, L., Xu, F., Zhang, C., Ren, G., et al. (2023). Xie Zhuo Tiao Zhi formula modulates intestinal microbiota and liver purine metabolism to suppress hepatic steatosis and pyroptosis in NAFLD therapy. *Phytomedicine Int. J. Phytother. Phytopharm.* 121, 155111. doi:10.1016/j.phymed.2023.155111

Quesada-Vázquez, S., Bone, C., Saha, S., Triguero, I., Colom-Pellicer, M., Aragonès, G., et al. (2022). Microbiota dysbiosis and gut barrier dysfunction associated with non-alcoholic fatty liver disease are modulated by a specific metabolic cofactors' combination. *Int. J. Mol. Sci.* 23, 13675. doi:10.3390/ijms232213675

Rawat, K., and Shrivastava, A. (2022). Neutrophils as emerging protagonists and targets in chronic inflammatory diseases. *Inflamm. Res. Off. J. Eur. Histamine Res. Soc. Al* 71, 1477–1488. doi:10.1007/s00011-022-01627-6

Reid, D. T., Reyes, J. L., McDonald, B. A., Vo, T., Reimer, R. A., and Eksteen, B. (2016). Kupffer cells undergo fundamental changes during the development of experimental NASH and are critical in initiating liver damage and inflammation. *PloS One* 11, e0159524. doi:10.1371/journal.pone.0159524

Ren, G., Lin, Y., Fu, Y., Liu, F., Wang, R., Zhang, C., et al. (2025a). Multi-omics joint analysis: pachymic acid ameliorated non-alcoholic fatty liver disease by regulating gut microbiota. *Food Res. Int. ott. Ont.* 209, 116178. doi:10.1016/j.foodres.2025.116178

Ren, G., Lin, Y., Qiu, J., Zhang, C., Chen, L., Zhu, L., et al. (2025b). Pachymic acid alleviates metabolic dysfunction-associated steatotic liver disease by inhibiting ferroptosis through PPARα. *Front. Pharmacol.* 16, 1554850. doi:10.3389/fphar.2025.1554850

Rothhammer, V., and Quintana, F. J. (2019). The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nat. Rev. Immunol.* 19, 184–197. doi:10.1038/s41577-019-0125-8

Saka, H. A., and Valdivia, R. (2012). Emerging roles for lipid droplets in immunity and host-pathogen interactions. *Annu. Rev. Cell. Dev. Biol.* 28, 411–437. doi:10.1146/annurev-cellbio-092910-153958

Sam, Q. H., Ling, H., Yew, W. S., Tan, Z., Ravikumar, S., Chang, M. W., et al. (2021). The divergent immunomodulatory effects of short chain fatty acids and medium chain fatty acids. *Int. J. Mol. Sci.* 22, 6453. doi:10.3390/ijms22126453

Sarfraz, M. H., Shahid, A., Asghar, S., Aslam, B., Ashfaq, U. A., Raza, H., et al. (2022). Personalized nutrition, microbiota, and metabolism: a triad for eudaimonia. *Front. Mol. Biosci.* 9, 1038830. doi:10.3389/fmolb.2022.1038830

Savage, T. M., Fortson, K. T., de Los Santos-Alexis, K., Oliveras-Alsina, A., Rouanne, M., Rae, S. S., et al. (2024). Amphiregulin from regulatory T cells promotes liver fibrosis and insulin resistance in non-alcoholic steatohepatitis. *Immunity* 57, 303–318.e6. doi:10.1016/j.immuni.2024.01.009

Schnabl, B. (2013). Linking intestinal homeostasis and liver disease. *Curr. Opin. Gastroenterol.* 29, 264–270. doi:10.1097/MOG.0b013e32835ff948

Schneider, K. M., Mohs, A., Gui, W., Galvez, E. J. C., Candels, L. S., Hoenicke, L., et al. (2022). Imbalanced gut microbiota fuels hepatocellular carcinoma development by shaping the hepatic inflammatory microenvironment. *Nat. Commun.* 13, 3964. doi:10.1038/s41467-022-31312-5

Shao, J., Ge, T., Tang, C., Wang, G., Pang, L., and Chen, Z. (2022). Synergistic anti-inflammatory effect of gut microbiota and lithocholic acid on liver fibrosis. *Inflamm. Res. Off. J. Eur. Histamine Res. Soc. Al* 71, 1389–1401. doi:10.1007/s00011-022-01629-4

Shi, Y., Gauer, J. S., Baker, S. R., Philippou, H., Connell, S. D., and Ariëns, R. a. S. (2021). Neutrophils can promote clotting *via* FXI and impact clot structure *via* neutrophil extracellular traps in a distinctive manner *in vitro*. *Sci. Rep.* 11, 1718. doi:10.1038/s41598-021-81268-7

Shi, Y., Chen, J., Qu, D., Sun, Q., Yu, Y., Zhang, H., et al. (2024). Ginsenoside Rg5 activates the LKB1/AMPK/mTOR signaling pathway and modifies the gut

Microbiota to alleviate nonalcoholic Fatty liver disease induced by a high-fat diet. *Nutrients* 16, 842. doi:10.3390/nu16060842

Shimada, Y., Kinoshita, M., Harada, K., Mizutani, M., Masahata, K., Kayama, H., et al. (2013). Commensal bacteria-dependent indole production enhances epithelial barrier function in the colon. *PloS One* 8, e80604. doi:10.1371/journal.pone.0080604

Shimizu, H., Masujima, Y., Ushiroda, C., Mizushima, R., Taira, S., Ohue-Kitano, R., et al. (2019). Dietary short-chain fatty acid intake improves the hepatic metabolic condition *via* FFAR3. *Sci. Rep.* 9, 16574. doi:10.1038/s41598-019-53242-x

Shrestha, S., and Hong, C.-W. (2023). Extracellular mechanisms of neutrophils in immune cell crosstalk. *Immune Netw.* 23, e38. doi:10.4110/in.2023.23.e38

Song, Q., and Zhang, X. (2022). The role of gut-liver axis in gut Microbiome dysbiosis associated NAFLD and NAFLD-HCC. *Biomedicines* 10, 524. doi:10.3390/biomedicines10030524

Stiglund, N., Strand, K., Cornillet, M., Stål, P., Thorell, A., Zimmer, C. L., et al. (2019). Retained NK cell phenotype and functionality in non-alcoholic fatty liver disease. *Front. Immunol.* 10, 1255. doi:10.3389/fimmu.2019.01255

Su, X., Gao, Y., and Yang, R. (2022). Gut Microbiota-Derived tryptophan metabolites maintain gut and systemic homeostasis. *Cells* 11, 2296. doi:10.3390/cells11152296

Sun, L., Cai, J., and Gonzalez, F. J. (2021). The role of farnesoid X receptor in metabolic diseases, and gastrointestinal and liver cancer. *Nat. Rev. Gastroenterol. Hepatol.* 18, 335–347. doi:10.1038/s41575-020-00404-2

Świderska, M., Jaroszewicz, J., Stawicka, A., Parfieniuk-Kowerda, A., Chabowski, A., and Flisiak, R. (2017). The interplay between Th17 and T-regulatory responses as well as adipokines in the progression of non-alcoholic fatty liver disease. *Clin. Exp. Hepatol.* 3, 127–134. doi:10.5114/ceh.2017.68466

Tajiri, K., and Shimizu, Y. (2012). Role of NKT cells in the pathogenesis of NAFLD. *Int. J. Hepatol.* 2012, 850836. doi:10.1155/2012/850836

Tan, G., Zeng, B., and Zhi, F.-C. (2015). Regulation of human enteric α-defensins by NOD2 in the Paneth cell lineage. *Eur. J. Cell. Biol.* 94, 60–66. doi:10.1016/j.ejcb.2014.10.007

Tan, Y.-Y., Yue, S.-R., Lu, A.-P., Zhang, L., Ji, G., Liu, B.-C., et al. (2022). The improvement of nonalcoholic steatohepatitis by Poria cocos polysaccharides associated with gut microbiota and NF-κB/CCL3/CCR1 axis. *Phytomedicine Int. J. Phytother. Phytopharm.* 103, 154208. doi:10.1016/j.phymed.2022.154208

Tan, Y., Huang, Z., Liu, Y., Li, X., Stalin, A., Fan, X., et al. (2023). Integrated serum pharmacochemistry, 16S rRNA sequencing and metabolomics to reveal the material basis and mechanism of Yinzhihuang granule against non-alcoholic fatty liver disease. *J. Ethnopharmacol.* 310, 116418. doi:10.1016/j.jep.2023.116418

Tang, W., Zhou, J., Yang, W., Feng, Y., Wu, H., Mok, M. T. S., et al. (2022). Aberrant cholesterol metabolic signaling impairs antitumor immunosurveillance through natural killer T cell dysfunction in obese liver. *Cell. Mol. Immunol.* 19, 834–847. doi:10.1038/s41423-022-00872-3

Thomas, C., Gioiello, A., Noriega, L., Strehle, A., Oury, J., Rizzo, G., et al. (2009). TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell. Metab.* 10, 167–177. doi:10.1016/j.cmet.2009.08.001

Tian, B., and Liu, J. (2020). Resveratrol: a review of plant sources, synthesis, stability, modification and food application. *J. Sci. Food Agric.* 100, 1392–1404. doi:10.1002/jsfa.10152

Tian, Y., Ma, J., Wang, W., Zhang, L., Xu, J., Wang, K., et al. (2016). Resveratrol supplement inhibited the NF-κB inflammation pathway through activating AMPKα-SIRT1 pathway in mice with fatty liver. *Mol. Cell. Biochem.* 422, 75–84. doi:10.1007/s11010-016-2807-x

Tilg, H., Adolph, T. E., and Trauner, M. (2022). Gut-liver axis: pathophysiological concepts and clinical implications. *Cell. Metab.* 34, 1700–1718. doi:10.1016/j.cmet.2022.09.017

Tong, C., Wu, H., Gu, D., Li, Y., Fan, Y., Zeng, J., et al. (2021). Effect of curcumin on the non-alcoholic steatohepatitis *via* inhibiting the M1 polarization of macrophages. *Hum. Exp. Toxicol.* 40, S310–S317. doi:10.1177/09603271211038741

Tong, C., Liang, Y., Han, X., Zhang, Z., Zheng, X., Wang, S., et al. (2023). Research progress of dendritic cell surface receptors and targeting. *Biomedicines* 11, 1673. doi:10.3390/biomedicines11061673

Tran, S., Baba, I., Poupel, L., Dussaud, S., Moreau, M., Gélineau, A., et al. (2020). Impaired kupffer cell self-renewal alters the liver response to lipid overload during non-alcoholic steatohepatitis. *Immunity* 53, 627–640.e5. doi:10.1016/j.immuni.2020.06.003

Travassos, L. H., Girardin, S. E., Philpott, D. J., Blanot, D., Nahori, M.-A., Werts, C., et al. (2004). Toll-like receptor 2-dependent bacterial sensing does not occur *via* peptidoglycan recognition. *EMBO Rep.* 5, 1000–1006. doi:10.1038/sj.embor.7400248

Trinchieri, G. (2003). Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat. Rev. Immunol.* 3, 133–146. doi:10.1038/nri1001

Tripodi, A., Anstee, Q. M., Sogaard, K. K., Primignani, M., and Valla, D. C. (2011). Hypercoagulability in cirrhosis: causes and consequences. *J. Thromb. Haemost. JTH* 9, 1713–1723. doi:10.1111/j.1538-7836.2011.04429.x

Vajro, P., Paolella, G., and Fasano, A. (2013). Microbiota and gut-liver axis: their influences on obesity and obesity-related liver disease. *J. Pediatr. Gastroenterol. Nutr.* 56, 461–468. doi:10.1097/MPG.0b013e318284abb5

Wachtendorf, S., Jonin, F., Ochel, A., Heinrich, F., Westendorf, A. M., Tiegs, G., et al. (2024). The ST2+ Treg/amphiregulin axis protects from immune-mediated hepatitis. *Front. Immunol.* 15, 1351405. doi:10.3389/fimmu.2024.1351405

Wagner, C. S., and Cresswell, P. (2012). TLR and nucleotide-binding oligomerization domain-like receptor signals differentially regulate exogenous antigen presentation. *J. Immunol. Balt. Md 1950* 188, 686–693. doi:10.4049/jimmunol.1102214

Wan, J.-Y., Wang, C.-Z., Zhang, Q.-H., Liu, Z., Musch, M. W., Bissonnette, M., et al. (2017). Significant difference in active metabolite levels of ginseng in humans consuming Asian or Western diet: the link with enteric microbiota. *Biomed. Chromatogr. BMC* 31, e3851. doi:10.1002/bmc.3851

Wang, Y.-D., Chen, W.-D., Wang, M., Yu, D., Forman, B. M., and Huang, W. (2008). Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. *Hepatol. Balt. Md* 48, 1632–1643. doi:10.1002/hep.22519

Wang, L., Jia, Z., Wang, B., and Zhang, B. (2020a). Berberine inhibits liver damage in rats with non-alcoholic fatty liver disease by regulating TLR4/MyD88/NF-κB pathway. *Turk. J. Gastroenterol. Off. J. Turk. Soc. Gastroenterol.* 31, 902–909. doi:10.5152/tjg.2020.19568

Wang, P., Wang, J., Li, D., Ke, W., Chen, F., and Hu, X. (2020b). Targeting the gut microbiota with resveratrol: a demonstration of novel evidence for the management of hepatic steatosis. *J. Nutr. Biochem.* 81, 108363. doi:10.1016/j.jnutbio.2020.108363

Wang, H., Mehal, W., Nagy, L. E., and Rotman, Y. (2021a). Immunological mechanisms and therapeutic targets of fatty liver diseases. *Cell. Mol. Immunol.* 18, 73–91. doi:10.1038/s41423-020-00579-3

Wang, R., Tang, R., Li, B., Ma, X., Schnabl, B., and Tilg, H. (2021b). Gut microbiome, liver immunology, and liver diseases. *Cell. Mol. Immunol.* 18, 4–17. doi:10.1038/s41423-020-00592-6

Wang, J., Zheng, D., Huang, F., Zhao, A., Kuang, J., Ren, Z., et al. (2022). Theabrownin and Poria cocos polysaccharide improve lipid metabolism via modulation of bile acid and Fatty acid metabolism. *Front. Pharmacol.* 13, 875549. doi:10.3389/fphar.2022.875549

Wang, J., Zhu, N., Su, X., Gao, Y., and Yang, R. (2023a). Gut-Microbiota-derived metabolites maintain gut and systemic immune homeostasis. *Cells* 12, 793. doi:10.3390/cells12050793

Wang, X., He, Q., Zhou, C., Xu, Y., Liu, D., Fujiwara, N., et al. (2023b). Prolonged hypernutrition impairs TREM2-dependent efferocytosis to license chronic liver inflammation and NASH development. *Immunity* 56, 58–77.e11. doi:10.1016/j.immuni.2022.11.013

Wang, Q., Guo, F., Zhang, Q., Hu, T., Jin, Y., Yang, Y., et al. (2024a). Organoids in gastrointestinal diseases: from bench to clinic. *MedComm* 5, e574. doi:10.1002/mco2.574

Wang, Q., Lu, T., Song, P., Dong, Y., Dai, C., Zhang, W., et al. (2024b). Glycyrrhizic acid ameliorates hepatic fibrosis by inhibiting oxidative stress via AKR7A2. *Phytomedicine Int. J. Phytother. Phytopharm.* 133, 155878. doi:10.1016/j.phymed.2024.155878

Wang, X., Yue, S.-Y., Chen, L., Chen, J., Li, C., Wei, S.-A., et al. (2025). *Lycium barbarum* polysaccharides alleviate experimental autoimmune prostatitis by putrescine/TRAF/JAK/STAT-mediated Th17 differentiation. *Phytomedicine* 147, 157210. doi:10.1016/j.phymed.2025.157210

Wei, S., Wu, X., Chen, M., Xiang, Z., Li, X., Zhang, J., et al. (2023). Exosomal-miR-129-2-3p derived from fusobacterium nucleatum-infected intestinal epithelial cells promotes experimental colitis through regulating TIMELESS-Mediated cellular senescence pathway. *Gut Microbes* 15, 2240035. doi:10.1080/19490976.2023.2240035

Wei, Y., Jiang, Y., Liu, M., Zhang, X., Guo, S., Su, S., et al. (2025). Chemical structures of lycii fructus polysaccharides tailored the gut microbiota composition of aged *Caenorhabditis elegans*. *Food Hydrocoll.* 169, 111608. doi:10.1016/j.foodhyd.2025.111608

Wieland Brown, L. C., Penaranda, C., Kashyap, P. C., Williams, B. B., Clardy, J., Kronenberg, M., et al. (2013). Production of α-galactosylceramide by a prominent member of the human gut microbiota. *PLoS Biol.* 11, e1001610. doi:10.1371/journal.pbio.1001610

Wong, P., and Pamer, E. G. (2003). CD8 T cell responses to infectious pathogens. *Annu. Rev. Immunol.* 21, 29–70. doi:10.1146/annurev.immunol.21.120601.141114

Wu, L., Feng, J., Li, J., Yu, Q., Ji, J., Wu, J., et al. (2021). The gut microbiome-bile acid axis in hepatocarcinogenesis. *Biomed. Pharmacother. Biomedecine Pharmacother.* 133, 111036. doi:10.1016/j.biopha.2020.111036

Wu, Y., Duan, Z., Qu, L., Liu, Y., Ma, X., and Fan, D. (2025). Ginsenoside Rk1 ameliorates non-alcoholic fatty liver disease by targeting CD36 to modulate the AMPK signaling pathway. *Food Res. Int. ott. Ont.* 211, 116426. doi:10.1016/j.foodres.2025.116426

Xia, Y., Wang, Y., Xiong, Q., He, J., Wang, H., Islam, M., et al. (2025). Neutrophil extracellular traps promote MASH fibrosis by metabolic reprogramming of HSC. *Hepatol. Balt. Md* 81, 947–961. doi:10.1097/HEP.0000000000000762

Xie, G., Jiang, R., Wang, X., Liu, P., Zhao, A., Wu, Y., et al. (2021). Conjugated secondary 12α-hydroxylated bile acids promote liver fibrogenesis. *EBioMedicine* 66, 103290. doi:10.1016/j.ebiom.2021.103290

Xu, Y., Huang, X., Huangfu, B., Hu, Y., Xu, J., Gao, R., et al. (2023). Sulforaphane ameliorates nonalcoholic fatty liver disease induced by high-fat and high-fructose diet via LPS/TLR4 in the gut-liver axis. *Nutrients* 15, 743. doi:10.3390/nu15030743

Xu, M., Xu, H., Ling, Y.-W., Liu, J.-J., Song, P., Fang, Z.-Q., et al. (2025). Neutrophil extracellular traps-triggered hepatocellular senescence exacerbates lipotoxicity in non-alcoholic steatohepatitis. *J. Adv. Res.* S2090-1232 (25), 00175–4. doi:10.1016/j.jare.2025.03.015

Xue, X., Wu, J., Ding, M., Gao, F., Zhou, F., Xu, B., et al. (2021). Si-wu-tang ameliorates fibrotic liver injury via modulating intestinal microbiota and bile acid homeostasis. *Chin. Med.* 16, 112. doi:10.1186/s13020-021-00524-0

Yan, H.-M., Xia, M.-F., Wang, Y., Chang, X.-X., Yao, X.-Z., Rao, S.-X., et al. (2015). Efficacy of berberine in patients with non-alcoholic fatty liver disease. *PloS One* 10, e0134172. doi:10.1371/journal.pone.0134172

Yáñez-Mó, M., Siljander, P. R.-M., Andreu, Z., Zavec, A. B., Borràs, F. E., Buzas, E. I., et al. (2015). Biological properties of extracellular vesicles and their physiological functions. *J. Extracell. Vesicles* 4, 27066. doi:10.3402/jev.v4.27066

Yang, J., Ma, X.-J., Li, L., Wang, L., Chen, Y.-G., Liu, J., et al. (2017). Berberine ameliorates non-alcoholic steatohepatitis in ApoE-/- mice. *Exp. Ther. Med.* 14, 4134–4140. doi:10.3892/etm.2017.5051

Yang, S., Cao, S., Li, C., Zhang, J., Liu, C., Qiu, F., et al. (2022). Berberrubine, a main metabolite of berberine, alleviates non-alcoholic fatty liver disease via modulating glucose and lipid metabolism and restoring gut microbiota. *Front. Pharmacol.* 13, 913378. doi:10.3389/fphar.2022.913378

Yang, B., Tang, G., Wang, M., Ni, Y., Tong, J., Hu, C., et al. (2024). Trimethylamine N-oxide induces non-alcoholic fatty liver disease by activating the PERK. *Toxicol. Lett.* 400, 93–103. doi:10.1016/j.toxlet.2024.08.009

Yang, J., Song, B., Zhang, F., Liu, B., Yan, J., Wang, Y., et al. (2025a). Curcumin ameliorates hepatic steatosis and insulin resistance through the JNK2/FOXO1/Bcl6 axis and regulate the intestinal flora structure. *J. Physiol. Biochem.* 81, 771–791. doi:10.1007/s13105-025-01101-x

Yang, X., Wang, J., Qi, X., Hou, M., Liu, M., Xiao, Y., et al. (2025b). HLF and PPARα axis regulates metabolic-associated fatty liver disease through extracellular vesicles derived from the intestinal microbiota. *iMeta* 4, e70022. doi:10.1002/imt2.70022

Ye, H., Ma, S., Qiu, Z., Huang, S., Deng, G., Li, Y., et al. (2022). Poria cocos polysaccharides rescue pyroptosis-driven gut vascular barrier disruption in order to alleviates non-alcoholic steatohepatitis. *J. Ethnopharmacol.* 296, 115457. doi:10.1016/j.jep.2022.115457

Yin, Y., Sichler, A., Ecker, J., Laschinger, M., Liebisch, G., Höring, M., et al. (2023). Gut microbiota promote liver regeneration through hepatic membrane phospholipid biosynthesis. *J. Hepatol.* 78, 820–835. doi:10.1016/j.jhep.2022.12.028

Ying, H.-Z., Liu, Y.-H., Yu, B., Wang, Z.-Y., Zang, J.-N., and Yu, C.-H. (2013). Dietary quercetin ameliorates nonalcoholic steatohepatitis induced by a high-fat diet in gerbils. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* 52, 53–60. doi:10.1016/j.fct.2012.10.030

Yu, B., Qin, S.-Y., Hu, B.-L., Qin, Q.-Y., Jiang, H.-X., and Luo, W. (2019). Resveratrol improves CCL4-induced liver fibrosis in mouse by upregulating endogenous IL-10 to reprogramme macrophages phenotype from M(LPS) to M(IL-4). *Biomed. Pharmacother. Biomedecine Pharmacother.* 117, 109110. doi:10.1016/j.biopha.2019.109110

Zang, S., Wang, L., Ma, X., Zhu, G., Zhuang, Z., Xun, Y., et al. (2015). Neutrophils play a crucial role in the early stage of nonalcoholic steatohepatitis via neutrophil elastase in mice. *Cell. biochem. Biophys.* 73, 479–487. doi:10.1007/s12013-015-0682-9

Zhang, M., and Zhang, S. (2020). T cells in fibrosis and fibrotic diseases. *Front. Immunol.* 11, 1142. doi:10.3389/fimmu.2020.01142

Zhang, Z., and Zhang, C. (2025). Regulation of cGAS–STING signalling and its diversity of cellular outcomes. *Nat. Rev. Immunol.* 25, 425–444. doi:10.1038/s41577-024-01112-7

Zhang, J., Ping, J., Jiang, N., and Xu, L. (2022). Resveratrol inhibits hepatic stellate cell activation by regulating autophagy and apoptosis through the SIRT1 and JNK signaling pathways. *J. Food Biochem.* 46, e14463. doi:10.1111/jfbc.14463

Zhang, X., Sharma, P., Maschmeyer, P., Hu, Y., Lou, M., Kim, J., et al. (2023). GARP on hepatic stellate cells is essential for the development of liver fibrosis. *J. Hepatol.* 79, 1214–1225. doi:10.1016/j.jhep.2023.05.043

Zhang, F., Jiang, Q., Cai, J., Meng, F., Tang, W., Liu, Z., et al. (2024a). Activation of NOD1 on tumor-associated macrophages augments CD8+ T cell-mediated antitumor immunity in hepatocellular carcinoma. *Sci. Adv.* 10, eadp8266. doi:10.1126/sciadv.adp8266

Zhang, J., Qi, H., Li, M., Wang, Z., Jia, X., Sun, T., et al. (2024b). Diet mediate the impact of host habitat on gut microbiome and influence clinical indexes by modulating gut microbes and serum metabolites. *Adv. Sci. Weinh. Baden-Wurtt. Ger.* 11, e2310068. doi:10.1002/advs.202310068

Zhang, Z., Zhi, Y., Wang, S., Zhang, F., Zhang, S., and Wu, J. (2025). Zexie Tang Polysaccharides (ZXTPs) alleviate high-fat diet-induced NAFL in mice through improving intestinal microbiota imbalance and liver metabolic dysfunction. *Carbohydr. Polym.* 367, 123937. doi:10.1016/j.carbpol.2025.123937

Zhao, X., Yang, L., Chang, N., Hou, L., Zhou, X., Yang, L., et al. (2020). Neutrophils undergo switch of apoptosis to NETosis during murine fatty liver injury via S1P receptor 2 signaling. *Cell. Death Dis.* 11, 379. doi:10.1038/s41419-020-2582-1

Zhao, M., Guan, Z., Tang, N., and Cheng, Y. (2023). The differences between the water- and alkaline-soluble Poria cocos polysaccharide: a review. *Int. J. Biol. Macromol.* 235, 123925. doi:10.1016/j.ijbiomac.2023.123925

Zhi, N., Chang, X., Zha, L., Zhang, K., Wang, J., and Gui, S. (2025). Platycodonis radix polysaccharides suppress progression of high-fat-induced obesity through modulation of intestinal microbiota and metabolites. *Phytomedicine Int. J. Phytother. Phytopharm.* 142, 156653. doi:10.1016/j.phymed.2025.156653

Zhong, T., Zhang, W., Guo, H., Pan, X., Chen, X., He, Q., et al. (2022). The regulatory and modulatory roles of TRP family channels in malignant tumors and relevant therapeutic strategies. *Acta Pharm. Sin. B* 12, 1761–1780. doi:10.1016/j.apsb.2021.11.001

Zhou, D., Pan, Q., Liu, X.-L., Yang, R.-X., Chen, Y.-W., Liu, C., et al. (2017). Clostridium butyricum B1 alleviates high-fat diet-induced steatohepatitis in mice via enterohepatic immunoregulation. *J. Gastroenterol. Hepatol.* 32, 1640–1648. doi:10.1111/jgh.13742

Zhou, D., Chen, Y.-W., Zhao, Z.-H., Yang, R.-X., Xin, F.-Z., Liu, X.-L., et al. (2018). Sodium butyrate reduces high-fat diet-induced non-alcoholic steatohepatitis through upregulation of hepatic GLP-1R expression. *Exp. Mol. Med.* 50, 1–12. doi:10.1038/s12276-018-0183-1

Zhu, W., Yan, M., Cao, H., Zhou, J., and Xu, Z. (2022). Effects of Clostridium butyricum capsules combined with Rosuvastatin on intestinal flora, lipid metabolism, liver function and inflammation in NAFLD patients. *Cell. Mol. Biol. noisy–gd. Fr.* 68, 64–69. doi:10.14715/cmb/2022.68.2.10

Zhu, L.-R., Li, S.-S., Zheng, W.-Q., Ni, W.-J., Cai, M., and Liu, H.-P. (2023). Targeted modulation of gut microbiota by traditional Chinese medicine and natural products for liver disease therapy. *Front. Immunol.* 14, 1086078. doi:10.3389/fimmu.2023.1086078

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Flavonoids as modulators of gut-liver axis: emerging therapeutic strategies for MAFLD

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Metabolic dysfunction-associated fatty liver disease (MAFLD) is a significant global health challenge affecting approximately 25% of adults worldwide. Given the limited efficacy of existing therapies, there is an urgent need for novel treatment strategies. Flavonoids, a diverse class of natural polyphenolic compounds, exhibit significant potential in ameliorating MAFLD by modulating hepatic lipid metabolism and immune-inflammatory responses via gut-liver axis. This review systematically explores the interactions between flavonoids and gut microbiota, elucidating their role in MAFLD progression. We highlight how flavonoid structural diversity and microbial biotransformation modulate multiple key pathways, such as PPARα, PPARγ, ERβ, Nrf2, NF-κB, and FXR signalling. These multi-target mechanisms underpin the therapeutic potential of flavonoids in reducing lipid accumulation, oxidative stress, inflammation, and fibrosis in MAFLD. We also discuss innovative strategies, including flavonoid-probiotic synergies, nanotechnology-enhanced delivery systems, and personalized nutrition strategies. By integrating evidence from preclinical models and clinical trials, we highlight the translational potential of flavonoid-based interventions for MAFLD management. Our analysis underscores flavonoids as multi-target, safe and effective solutions for MAFLD management, warranting further clinical studies to translate these findings into routine clinical practice.

KEYWORDS

MAFLD, flavonoids, gut-liver axis, gut microbial modulation, natural herbal products

1 Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a global health challenge affecting an estimated 25% of the adult, with its prevalence rising due to the increasing rates of obesity, diabetes, and metabolic syndrome (Younossi et al., 2023; Man et al., 2023). Its severe form, metabolic dysfunction-associated steatohepatitis (MASH), is a leading cause of liver cirrhosis and hepatocellular carcinoma (HCC) (Huang et al., 2021;

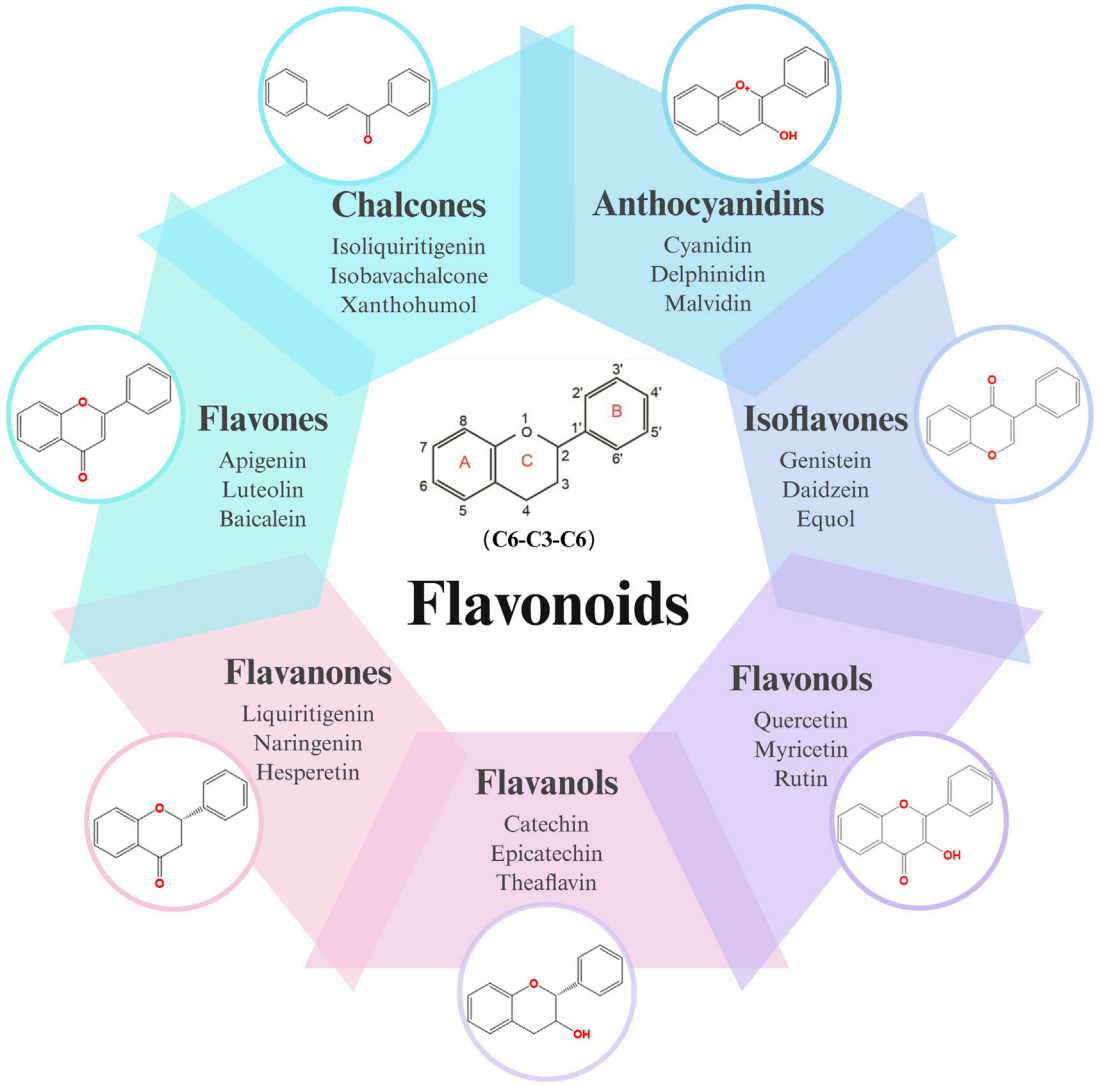
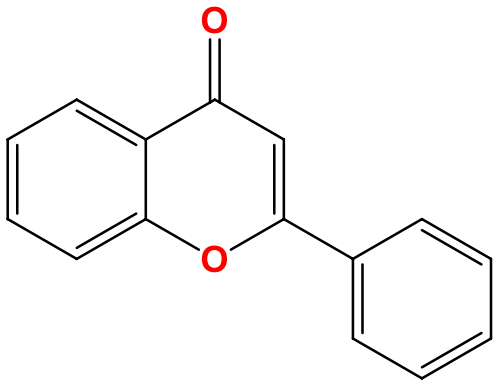
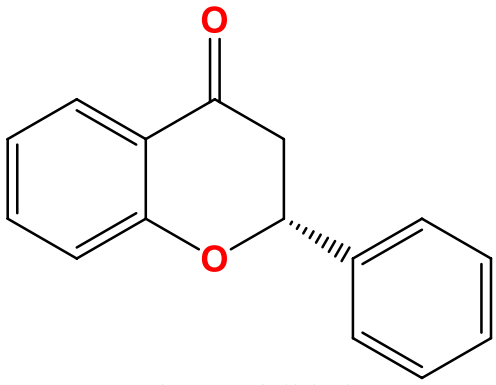
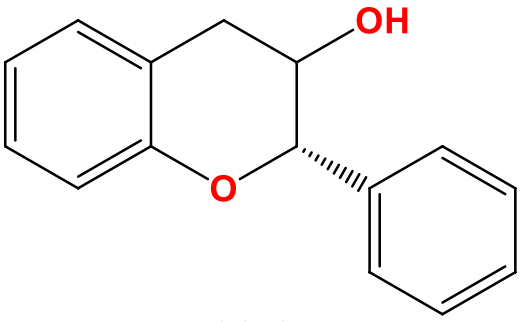
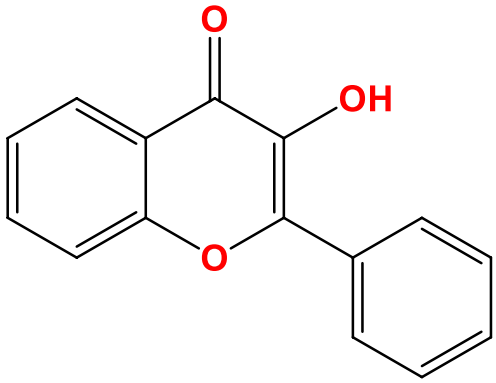


FIGURE 1
Skeletal structure of flavonoids and their seven subclassifications. Original image drawn for this review using Biorender software.

Paik et al., 2022). Despite this significant burden, effective drug therapies for MAFLD are lacking, as many anticipated drugs have failed in clinical trials (Rong et al., 2023; Piero et al., 2024). Diet is a critical factor in MAFLD pathogenesis, with diets rich in refined sugars, unhealthy fats, and low in essential micronutrients exacerbating its progression (Francesca et al., 2022). Of particular interest is the role of flavonoid-rich diets, such as the Mediterranean diet, which has been consistently linked to a reduced risk of developing MAFLD (Ilaria et al., 2020; Riazi et al., 2022). Epidemiological studies have demonstrated that individuals with diets deficient in flavonoids are at an increased risk of developing MAFLD (Iino et al., 2022), further emphasizing the importance of dietary interventions in MAFLD management (Sherouk and Joseph, 2023).

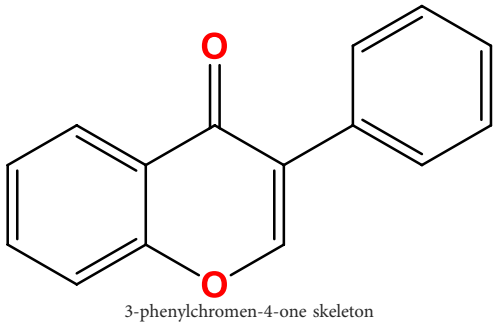
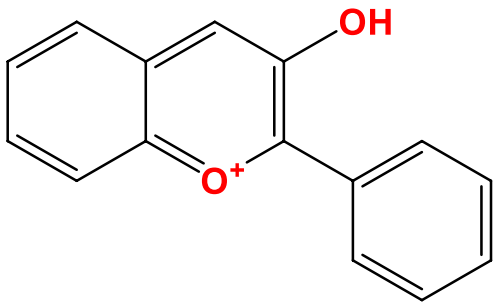
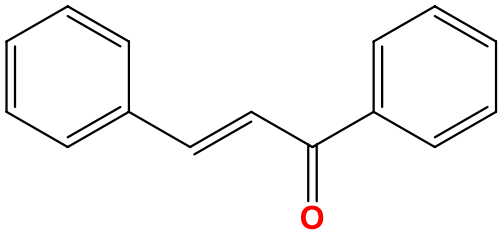
Flavonoids, a diverse group of natural polyphenolic compounds, have garnered attention as "food-derived medicine" therapeutic potential. These compounds exhibit remarkable structural diversity, characterized by their C6-C3-C6 backbone (Figure 1), which allows for a wide range of substitution patterns and bioactive properties (Dias et al., 2021; Kaushal et al., 2022). Flavonoids are abundant in citrus fruits, soy, tea, and medicinal herbals such as snow lotus (*Saussurea eriocephala* Franch), Chinese skullcap (*Scutellaria baicalensis* Georgi), *Dendrobium officinale* Kimura and Migo, Dragon's blood (*Dracaena draco* (L.) L.) and Ficus hirta (*Ficus simplicissima* Lour) (Dias et al., 2021; Shen et al., 2022; Yi et al., 2012; Yi et al., 2009a; Chen et al., 2017; Xue et al., 2017; Yi et al., 2009b) (Table 1). Pharmacologically, flavonoids show potent antioxidant and anti-inflammatory properties by modulating oxidative stress pathways (e.g., Nrf2, CYP2E1, ROS) and inhibiting pro-inflammatory signalling pathways (e.g., NF-κB, TNF-α, TLR4) (Kaushal et al., 2022; Shen et al., 2022). As estrogen analogs, flavonoids may modulate diseases associated with estrogen abnormalities with low toxicity and low side effects (Kiyama, 2023). As food-derived medicine, flavonoids were found to modulate intestinal microbiota, which in turn affect host metabolism and immune function (Kuziel et al., 2025). After

TABLE 1 Structure, characteristics, food sources and representative molecules of the seven subclasses of flavonoids.

Flavonoids subclass	Structural features	Food source	MALFD related compounds	References
Flavones	 The chemical structure consists of 4H-chromen-4-one, which bears a phenyl substituent at position 2	Chamomile, Parsley, Lamiaceae, Bergamot, Tea leaves and Herbs	Apigenin, Luteolin, Baicalein	Jeong et al. (2022), Hostetler et al. (2017)
Flavanones (dihydroflavones)	 The C2 = C3 double bond	Citrus fruits (Grapefruits, Lemons, Oranges), Tomatoes, Cherries	Hesperetin, Naringenin, Liquiritigenin	Barreca et al. (2017), Motallebi et al. (2022)
Flavanols (flavan-3-ols)	 C-3 hydroxyl group	Cereals, Legumes, Forages, Hops, Beers, Red wine, Tea, Cocoa	(+)-Catechin, (−)-Epicatechin, Theaflavin	Luo et al. (2022), Martín and Ramos (2021), Ottaviani et al. (2018)
Flavonols (3-hydroxyflavone)	 There are several specific substitutions in the A and B rings, which are connected by a three-carbon chain	Onions, Apples, Green tea, Berries, Nuts	Quercetin, Myricetin, Rutin	Jazvinšćak et al. (2023), Popiolek-Kalisz and Fornal (2022), Wendlocha et al. (2024)

(Continued on following page)

TABLE 1 (*Continued*) Structure, characteristics, food sources and representative molecules of the seven subclasses of flavonoids.

Flavonoids subclass	Structural features	Food source	MALFD related compounds	References
Isoflavones	 3-phenylchromen-4-one skeleton	Soybean and soybean products, Red clover	Genistein, Daidzein, Equol	Gómez-Zorita et al. (2020), Křížová et al. (2019), Yamagata and Yamori (2021)
Anthocyanidins	 Anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives	Flowers, Fruits, Seeds, Plant leaves	Cyanidin, Delphinidin, Malvidin	de Sousa Moraes et al. (2019), Lee et al. (2017), Wallace and Giusti (2015)
Chalcones (1,3-diaryl-2-propen-1-ones)	 Open-chain flavonoids containing up to three modified or unmodified C5, C10 and C15 olefin molecules in the A and B rings	Fruits, Vegetables, Teas	Isoliquiritigenin, Isobavachalcone, Xanthohumol	Constantinescu and Lungu (2021), WalyEldeen et al. (2023)

being ingested by the body, the metabolic fate of flavonoids is influenced by their structural characteristics and their interactions with the intestinal microbiota (Kuziel et al., 2025; Li C. et al., 2023). A major challenge lies in their typically low bioavailability and extensive first-pass metabolism, which severely limits the flavonoid concentration in systemic circulation and target tissues. Furthermore, the diverse metabolic transformations by gut microbiota and host enzymes can lead to varied, making their precise *in vivo* effects difficult to predict and standardize across individuals. Therefore, how to enhance the bioavailability of flavonoids within the context of gut-liver crosstalk is crucial for alleviating liver diseases such as MAFLD. In summary, flavonoids' structural diversity, rich dietary sources, and broad spectrum of pharmacological activities position them as promising candidates for developing novel therapeutic strategies of MAFLD (Kaushal et al., 2022; Li C. et al., 2023; Xiaopeng et al., 2022).

Recent studies highlight the central role of the gut-liver axis in MAFLD pathogenesis through oxidative stress, inflammation, and lipid dysregulation (De Cól et al., 2024; Fianchi et al., 2021; Hu Y. et al., 2025; Peng et al., 2024). This communication system between the gut microbiota, the gastrointestinal tract, and the liver underpins MAFLD development (Kuziel et al., 2025; Luo et al., 2023; Martín-Mateos and Albillos, 2021). For instance, microbiota dysbiosis can cause translocation of microbial products like lipopolysaccharides (LPS), triggering endotoxemia, systemic inflammation, and liver injury (Luo et al., 2023; Peiseler et al., 2022). Microbial metabolites also have emerged as important modulators in the development of hepatic steatosis. Altered bile acid (BA) profile resulting from microbiome imbalances contributes to hepatic steatosis by affecting both BA signalling and lipid metabolism, reinforcing the gut-liver interaction and advancing the pathogenesis of MAFLD (De Cól et al., 2024; Luo et al., 2023).Short-chain fatty acids (SCFAs), produced by gut microbiota fermentation, enhance the intestinal barrier and modulate liver fatty acid (FA) metabolism. SCFAs decrease hepatic triglyceride accumulation, improve insulin sensitivity, and reduce inflammation, mitigating MAFLD progression (Fianchi et al., 2021; Barber et al., 2023).

Metabolite of dietary flavonoids by gut bacteria, such as S-equol, which improve liver health by modulating oxidative stress and inflammation (Rao et al., 2021; Qi et al., 2025). The interaction between gut metabolism and flavonoid bioavailability also provides an important basis to understands flavonoid's role to intervene

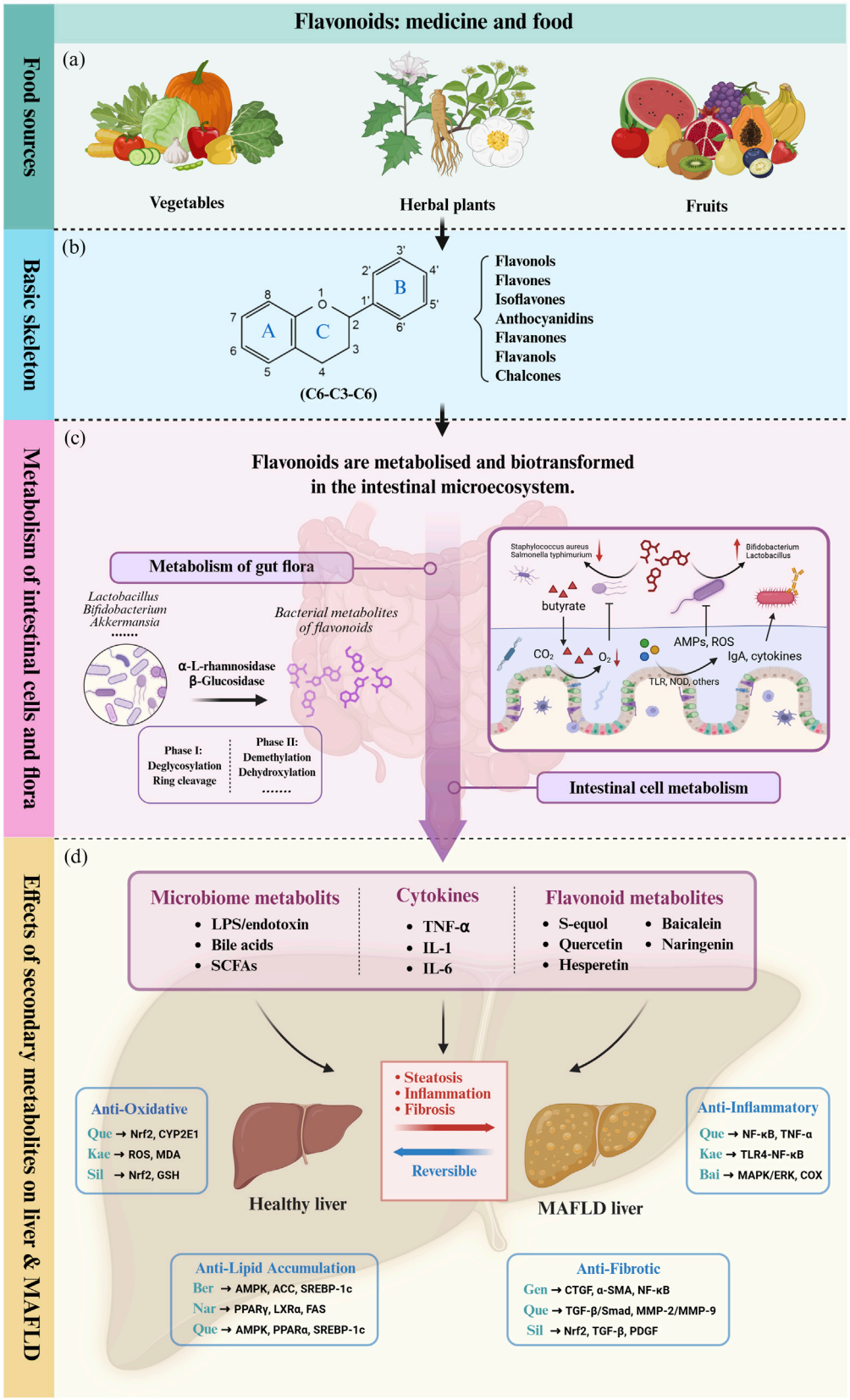


FIGURE 2
Flavonoids influence liver metabolism and MALFD progression directly or indirectly through the gut-liver axis. **(a)** Flavonoids are widely distributed in the natural diet and are abundant in various medicinal plants, vegetables and fruits. **(b)** The basic skeletal structure and seven subclasses of flavonoids. **(c)** The intestinal microenvironment and its role in flavonoid metabolism and biotransformation. **(d)** Flavonoids and intestinal secondary metabolites

(*Continued*)

FIGURE 2 (Continued)
ultimately act on the liver and affect pathophysiological processes of MAFLD such as hepatic inflammatory cascade, oxidative stress and lipid accumulation through complex molecular mechanisms (In the image, upstream and downstream pathways use the same color to distinguish signaling pathways driven by different bioactive molecules). TLR: Toll-like Receptor. TLR4: Toll-like Receptor 4. NOD: Nucleotide-binding Oligomerization Domain-containing protein. ROS: Reactive Oxygen Species. LPS: Lipopolysaccharide. SCFA: Short-Chain Fatty Acid. TNF-α: Tumor Necrosis Factor-alpha. IL-1: Interleukin-1. IL-6: Interleukin-6. Nrf-2: Nuclear Factor Erythroid 2-related Factor 2. CYP2E1: Cytochrome P450 Family 2 Subfamily E Member 1. MDA: Malondialdehyde. GSH: Glutathione. AMPK: AMP-activated Protein Kinase. ACC: Acetyl-CoA Carboxylase. SREBP-1c: Sterol Regulatory Element-binding Protein 1c. PPARα: Peroxisome Proliferator-activated Receptor Alpha. PPARγ: Peroxisome Proliferator-activated Receptor Gamma. LXRα: Liver X Receptor Alpha. FAS: Fatty Acid Synthase. CTGF: Connective Tissue Growth Factor. α-SMA: Alpha-Smooth Muscle Actin. NF-κB: Nuclear Factor-kappa B. TGF-β: Transforming Growth Factor-beta. Smad: Homolog of the *Caenorhabditis elegans* protein SMA and *Drosophila* protein MAD. MMP-2: Matrix Metalloproteinase-2. MMP-9: Matrix Metalloproteinase-9. MAPK: Mitogen-Activated Protein Kinase. COX: Cyclooxygenase. ERK: Extracellular Regulated Protein Kinase. ApoB: Apolipoprotein B. Bid: BH3 interacting-domain death agonist. SIRT1: Sirtuin 1. UQ: Ubiquinone. Que: Quercetin. Kae: Kaempferol. Bai: Baicalein. Sil: Silymarin. Gen: Genistein. Nar: Naringenin. Ber: Berberine. Original image drawn for this review using Biorender software.

MAFLD development (Cheng et al., 2024). Studies have shown that gut dysbiosis, which is often associated with MAFLD, can impair the metabolic conversion of flavonoids, reducing their protective effects on liver health (Long et al., 2024; Fang et al., 2024).

Given the structural diversity of flavonoid and their complex interaction with gut microbial community, this review aims to summarize how flavonoids target the gut-liver axis to mitigate MAFLD. It emphasizes microbiota-dependent and -independent mechanisms to provide novel therapeutic strategies for MAFLD management through medical or dietary interventions.

2 Flavonoid, and their metabolism by gut microbiota

Flavonoids represent the principal bioactive constituents in a wide array of medicinal plants and have been employed in the management of numerous diseases, including MAFLD. Owing to their multi-targeted biological activity, minimal toxicity, and dietary origin, flavonoids have garnered increasing scientific interest for their therapeutic potential in mitigating MAFLD. This growing interest is substantiated by findings from evidence-based medicine derived from population-level data. For instance, analyses of the National Health and Nutrition Examination Survey (NHANES) database have highlighted the hepatoprotective properties of dietary flavonoids in reducing the risk of MAFLD (Tong et al., 2022). Moreover, a meta-analysis of randomised controlled trials has indicated that flavonoids—such as quercetin, epicatechin, naringenin, apigenin, among others—ameliorate MAFLD by enhancing hepatic metabolic function, attenuating inflammatory responses, and modulating gut microbiota composition (Li et al., 2023b).

Actually, the intricate interplay between flavonoids and gut microbiota constitutes a bidirectional relationship that profoundly influences host physiology. Emerging evidence highlights the gut microbiome's role in modulating flavonoid bioavailability and bioactivity, while flavonoids reciprocally reshape microbial ecology and metabolic output (Murota et al., 2018; Shabbir et al., 2021; Pei et al., 2020). This dynamic interaction forms a critical axis for understanding the therapeutic potential of flavonoids in metabolic diseases, including MAFLD (Figure 2), as will be elaborated upon in subsequent sections.

2.1 Introduction of diverse flavonoid structures

Flavonoids are a diverse group of plant-derived polyphenolic compounds, characterized by a common 15-carbon skeleton comprising two aromatic rings (A and B) and a heterocyclic ring (C), forming a C6–C3–C6 structure (Kumar and Pandey, 2013; Latos-Brozio and Masek, 2019). This core structure serves as the foundation of flavonoids subclasses, each distinguished by specific modifications that influence their biological activities and physicochemical properties (Dias et al., 2021; Shen et al., 2022). Through extensive modification, it yields >8,000 species across seven major subclasses (Figure 1): flavones characterized by a 2-phenylchromen-4-one backbone (e.g., luteolin, apigenin), flavonols which are 3-hydroxylated flavones (e.g., quercetin, kaempferol) flavanones that lack the C2 = C3 double bond (e.g., naringenin, hesperetin), flavanols that lack the C2 = C3 double bond and C4 = O carboxyl group, but have C3-hydroxyl group (e.g., catechins, epicatechins), isoflavones with B ring attached to C3 (e.g., genistein, daidzein), anthocyanidins with C1 oxonium ion and C3-hydroxyl group (e.g., cyanidin, delphinidin), chalcones which are open-chain flavonoids serving as precursors in flavonoid biosynthesis (e.g., phloretin, isoliquiritigenin) (Shen et al., 2022; Kumar and Pandey, 2013).

Beyond these core structures, flavonoids undergo various modifications through hydroxylation, methylation (mainly O-methylation), glycosylation, and polymerization, that enhance their structural diversity and functional properties (Wang et al., 2020). Additional hydroxylation increases polarity and antioxidant capacity, while methoxylation enhances lipophilicity and membrane permeability, facilitating absorption and bioavailability. Glycosylation, the attachment of sugar moieties, improves water solubility and stability, and can substantially influence their bioactivity. Polymerization leads to the formation of oligomeric compounds, such as proanthocyanidins, which exhibit unique antioxidant properties (Cao et al., 2015).

These structural features and modifications not only define the chemical nature of flavonoids but also underpin their vast array of biological activities, including antioxidant, anti-inflammatory, and anticancer properties. Understanding the structural diversity of flavonoids is crucial for elucidating their mechanisms of action and potential therapeutic applications.

2.2 Flavonoid metabolism by gut microbiota

2.2.1 Two-stage microbial biotransformation of flavonoids

The gut microbiota mediates the structural modification and metabolism of dietary flavonoids through a complex enzymatic network, including phase I (deglycosylation, ring cleavage) and phase II (demethylation, dehydroxylation) reactions (Loo et al., 2020; Liu C. et al., 2024; Tian et al., 2019). In the first stage, key bacterial enzymes (e.g., β-glucosidase, α-rhamnosidase, and UDP-glucuronosyltransferases) and host intestinal lactase convert β-glycosylated flavonoids to more easily absorbed aglycone compounds (Loo et al., 2020; Liu Z. et al., 2021; Wang et al., 2023). Following absorption, these flavonoid aglycones undergo further biotransformation through phase II reactions. These reactions, predominantly mediated by the gut microbiota, include critical modifications such as glycosylation, hydroxylation, O-methylation and depolymerization. Microbial metabolites derived from flavonoids exhibit different bioactivity compared to their parent compounds. These metabolites often survive hepatic first-pass metabolism, achieving higher systemic concentrations and longer half-lives (Liu C. et al., 2024).

2.2.2 Impact of flavonoid structure on microbial metabolism

The structure of flavonoids not only dictates their biological activities and target interactions but also profoundly influences their metabolic fate within the gut microbiota. We summarised the metabolic transformations of selected flavonoids based on gut flora and their targets of action regarding MAFLD treatment (Table 2).

2.2.2.1 Glycosylation

Glycosylation significantly impacts flavonoid bioavailability and target engagement (Zeng et al., 2020; Shi et al., 2022). C-linked glycosides (e.g., vitexin), resistant to hydrolysis by mammalian digestive enzymes, require microbial glycoside hydrolases (e.g., C-glycoside hydrolases contained in *Enterococcus faecalis*) for deglycosylation (Braune and Blaut, 2011). The resulting aglycones can directly interact with cellular targets, including nuclear receptors such as ER, FXR (Tian et al., 2019; Kiriyama et al., 2024). In addition, O-glycosylated flavonoids like rutin (quercetin-3-O-rutinoside) are hydrolyzed by intestinal α-L-rhamnosidases (e.g., from *Bifidobacterium* spp. and *Lactobacillus*), generating quercetin aglycone (Murota et al., 2018; Liu C. et al., 2024; Liu Z. et al., 2021; Fan et al., 2018). Flavonols with 3-O-glycone are preferentially hydrolyzed by *Lactobacillus* strains, which express β-glucosidases (Wang et al., 2023; Chen-Chen et al., 2024; Li B. et al., 2021; Zhu et al., 2024). Reciprocally, unique glycan constituents of flavonoids also affect microbial ecology. For instance, the rutin glycoside moiety acts as a prebiotic, selectively promoting *Bifidobacterium* growth and enhancing SCFA production, which in turn activates G-protein-coupled receptor 43 (GPR43) to improve hepatic energy metabolism (Shi et al., 2022; Ferreira-Lazarte et al., 2021).

2.2.2.2 Hydroxylation

The number and position of hydroxyl groups on the flavonoid skeleton also dictate substrate specificity for microbial enzymes and target binding affinity. For instance, flavones possessing a 5,7-dihydroxy configuration, such as apigenin, are recognized substrates for gut microbes like *Flavonifractor plautii*. A key initial step in the intestinal catabolism of apigenin is the hydrogenation of its C2-C3 double bond. This reaction is catalysed by a flavone reductase (FLR) from *F. plautii*, which stereospecifically reduces apigenin to naringenin (a dihydroflavone), an essential intermediate for further degradation into phenolic acids (Yang et al., 2021). The activity of FLR is a critical gateway to the breakdown of dietary flavones.

Importantly, the specific hydroxylation pattern of a flavonoid can enable it to selectively modulate inflammatory responses. In the case of kaempferol, the 4′-hydroxyl group on its B-ring is crucial for the inhibition of the mitogen-activated protein kinase (MAPK) pathway, which suppresses the production of inflammatory cytokines by downregulating ERK1/2 phosphorylation (Niziński et al., 2025; Li N. et al., 2023). Furthermore, the 3,5,7-trihydroxy arrangement of kaempferol facilitates its interaction with Toll-like receptor 4 (TLR4), thereby blocking lipopolysaccharide (LPS)-induced NF-κB activation and attenuating liver inflammation (Niziński et al., 2025; Qu et al., 2021; Wu et al., 2024). Therefore, the anti-inflammatory properties of flavonoids can be modified by gut microbiome.

2.2.2.3 O-methylation (methoxylation) and O-demethylation

Structural alkyl modifications of flavonoids, particularly O-methylation (forming methoxyl groups, -OCH_3), critically determine their metabolic fate and bioactivity (Kim et al., 2014). Polymethoxyflavones (PMFs) like nobiletin, confers unique biological activities by enhancing membrane permeability and membrane receptor binding (Xu et al., 2024; Zhang et al., 2020). Increasing evidence highlights the significant impact of gut microbiota on flavonoid methylation status. Although direct microbe-mediated O-methylation of flavonoids has been less extensively studied, specific microbial enzymes, such as DnrK from *Streptomyces peucetius*, have been shown to perform O-methylation *in vitro* on various flavonoids, including apigenin and genistein, typically at the C7 hydroxyl group (Kim et al., 2007). This suggests a potential for similar activities within the complex gut microbiome, even if not yet fully characterized *in situ*.

Conversely, microbial O-demethylation of many methoxylated flavonoids is relatively well documented. For instance, PMFs often undergo extensive O-demethylation by gut bacteria, particularly at positions like C-3′ and C-4′ on the B ring (Cao et al., 2015; Braune and Blaut, 2016). This may exhibit different bioactivities, absorption profiles, and targets in the host compared to their parent compounds. For example, *Aspergillus niger* strains have been shown to regioselectively O-demethylate tangeretin and 3-hydroxytangeretin into their 4′-O-demethylated metabolites, demonstrating a microbial capacity for targeted demethylation similar to some mammalian P450 systems (Murota et al., 2018; Buisson et al., 2007).

2.2.2.4 Depolymerization

Flavonoid polymers such as oligomeric proanthocyanidins, polymeric rutin, and condensation complexes of catechin exhibit significantly delayed microbial catabolism compared to their

TABLE 2 Gut microbiota-mediated flavonoid metabolism and its molecular mechanisms in MAFLD.

Flavonoid substrate	Gut microbiota metabolite	Reaction (enzyme)	Microbial species/Strain	Molecular targets/ Pathways	Effects on liver and MAFLD	References
Rutin	Quercetin	Deglycosylation (α-L-rhamnosidase, β-Glucosidase)	*Bifidobacterium* spp.	PPARα*, SREBP-1c#, NF-κB#	Reduces hepatic lipid accumulation and inflammation via activation of FA β-oxidation, suppression of lipogenesis, and enhancements of mitochondrial biogenesis	Shi et al. (2022), Ferreira-Lazarte et al. (2021), Feng et al. (2021)
			Clostridium orbiscindens, *Lactobacillus* spp.	Nrf2*, HO-1*, NQO1*		
			Bacteroides spp., *Eubacterium* spp.	AMPK*, PPARγ*		
Daidzein	S-Equol	Reductive ring cleavage (Daidzein reductase)	*Slackia isoflavoniconvertens*, *Eggerthella lenta*	ERβ*, PPARα*, CPT1A*, SREBP-1c#	Suppresses hepatic lipogenesis and enhances FA β-oxidation via estrogen receptor signalling	Kiyama (2023), Huang et al. (2019), Kumari et al. (2024), Křížová et al. (2019), Yamagata and Yamori (2021), Zhang et al. (2024b)
Genistein	Equol	Reductive ring cleavage (Daidzein reductase)	*Slackia isoflavoniconvertens*	ERβ*, Wnt/β-catenin#	Inhibits hepatic stellate cell activation and fibrosis by targeting Wnt signalling	Kiyama (2023), Kuziel et al. (2025), Farhat et al. (2023)
Hesperidin	Hesperetin	Deglycosylation (β-glucosidase)	*Bacteroides* spp.	FXR*, SREBP-1c#, PPARγ/ Adiponectin*	Reduces hepatic triglycerides by activating farnesoid X receptor and suppressing lipogenesis, and improves insulin sensitivity	Xu et al. (2024), Barreca et al. (2017)
Apigenin	p-Hydroxyphenylacetic acid	Ring cleavage (Flavone reductase)	*Clostridium orbiscindens*	LXRα*, ABCA1/ ABCG1*	Enhances cholesterol efflux and reverse cholesterol transport in macrophages	Jeong et al. (2022), Hostetler et al. (2017), Li and Somerset (2018)
Luteolin	3-Hydroxyphenylacetic acid	Ring cleavage (Flavone reductase)	*Clostridium orbiscindens*	NF-κB#, NLRP3#	Suppresses hepatic inflammation by inhibiting NLRP3 inflammasome activation	Li et al. (2021a), Yang et al. (2025), Jeong et al. (2022), Hostetler et al. (2017), Liu et al. (2021b)
	Protocatechuic acid		*Lactobacillus* spp., *Bacteroides* spp.	PPARγ*, GLUT4*		
Myricetin	Pyrogallol	Deglycosylation, Ring Cleavage (β-glucosidase, Ring-cleaving dioxygenase)	*Lactobacillus* spp., *Bacteroides* spp.	Nrf2*, HO-1*	Enhances antioxidant defense and reduces oxidative stress in hepatocytes	Dias et al. (2021), Fan et al. (2018), Popiolek-Kalisz and Fornal (2022), Wendlocha et al. (2024), Sun et al. (2021)
Catechin	5-(3,4-Dihydroxyphenyl)-γ-valerolactone	Ring cleavage (Catechin dioxygenase)	*Eubacterium* spp., *Lactobacillus* spp.	AMPK*, SIRT1*	Activates energy-sensing pathways and improves mitochondrial function	Shabbir et al. (2021), Liu et al. (2024a), Talib et al. (2024), Dey et al. (2020), Huang et al. (2020)
Baicalin	Baicalein	Hydrolysis (β-glucuronidase)	*Lactobacillus* spp.	TLR4/NF-κB#, Nrf2*	Reduces hepatic oxidative stress and inflammation via Nrf2 activation	Lin et al. (2022), Peng et al. (2021), Yu et al. (2024), Hu et al. (2021)
Naringin	Naringenin	Hydrolysis (β-glucosidase)	*Clostridium* spp.	FXR/TGR5*, LXRα*	Improves bile acid homeostasis and cholesterol metabolism	Ferreira-Lazarte et al. (2021), Feng et al. (2020)
Quercetin-3-O-glucoside	Quercetin aglycone	Deglycosylation (β-glucosidase)	*Akkermansia muciniphila*	SIRT1*, Nrf2*, NF-κB#	Reduces LPS-induced hepatic inflammation and insulin resistance	Ferreira-Lazarte et al. (2021)

"*" Indicates that the target/pathway is activated by flavonoids. "#" indicates that the target/pathway is inhibited by flavonoids.

monomers (Latos-Brozio and Masek, 2019; Patanè et al., 2023). Specifically, crosslinked rutin shows a 5.6-fold prolonged intestinal retention time relative to its monomeric form due to reduced passive diffusion across enterocytes (Latos-Brozio and Masek, 2019). This kinetic property allows sustained release of bioactive metabolites in the distal colon (Liu C. et al., 2024; Shi et al., 2021). Due to their complex structures and high molecular weights, polymeric flavonoids like proanthocyanidins are poorly absorbed in the small intestine and a substantial portion reaches the colon largely intact (Braune and Blaut, 2016; Niwano et al., 2022). Here, the diverse enzymatic machinery of the gut microbiota plays a vital role in their breakdown. This microbial processing is initiated by depolymerization, a critical prerequisite for their biological activity where gut bacteria cleave the interflavan bonds (C-C and C-O-C linkages) holding the monomers together (Murota et al., 2018; Braune and Blaut, 2016). For instance, glycoside hydrolases from *Lactobacillus* and *Bacteroides* species target the glycosidic bonds in proanthocyanidins. These enzymes are induced by flavonoid exposure and show higher activity toward oligomers (DP 2–4) than high-molecular-weight polymers (Xiong et al., 2023). In addition, proanthocyanidin polymers (DP > 20) show limited depolymerization within the gastrointestinal tract, but their partial degradation by *Bifidobacterium* species produces metabolites (e.g., 5-(hydroxyphenyl)-γ-valerolactone) that modulate hepatic lipid metabolism via the gut-liver axis (Niwano et al., 2022; Shoji et al., 2023; Déprez et al., 2000). These differences in kinetics and bioactivity place the gut microbiota in a unique position in the gut-liver axis regulation of polymeric flavonoids.

However, despite the well-established enzymatic framework we outlined in 2.2, a critical translational gap remains between identifying microbial metabolic capabilities and confirming their physiological relevance in MAFLD. For instance, while bacterial β-glucosidases from *Lactobacillus* spp. are known to hydrolyze rutin to quercetin, and *F. plautii* can hydrogenate apigenin to naringenin, the functional outcomes of these transformations are often inferred rather than definitively proven. Meanwhile, the field suffers from significant quantification deficit, the inadequate characterization and quantification of the terminal active metabolites: despite knowing that microbial metabolites like equol exhibit potent bioactivities *in vitro* (e.g., activating AMPK or FXR), their actual concentrations achieved in human portal circulation or hepatic tissue following dietary flavonoid intake are scarcely measured. It is therefore plausible that many proposed mechanisms operate at pharmacologically irrelevant concentrations. If the intrinsic concentrations fall substantially below these thresholds, the proposed mechanisms and physiological significance of flavonoid-derived metabolites become questionable.

Furthermore, the immense inter-individual variability in gut microbiota composition means that the metabolic pathways detailed herein—such as the production of S-equol from daidzein by *Slackia isoflavoniconvertens*—may be absent or inefficient in a substantial proportion of the MAFLD population. Consequently, the promising effects observed in preclinical models may not consistently translate to human patients. Future research must prioritize absolute quantification of microbial flavonoid metabolites in human biospecimens using advanced techniques—such as targeted metabolomics, and *in vivo* imaging—to accurately quantify and spatially resolve the distribution of these metabolites in target tissues, thereby moving beyond correlative associations to establish causative links, and finally distinguish truly impactful metabolic pathways from mere observational curiosities.

2.3 Modulation of microbiota by flavonoid

As mentioned previously, flavonoids and gut microbiota engage in dynamic, reciprocal interactions that transcend mere metabolism, influencing both microbial composition and host physiology. Many flavonoids exert prebiotic-like effects by selectively enriching beneficial symbionts while suppressing pathobionts (Shabbir et al., 2021; Zhu et al., 2024; Naudhani et al., 2021).

For instance, theabrownin and quercetin increases symbionts *Bifidobacterium* and *Akkermansia muciniphila* abundances, while concurrently decrease the abundance of detrimental bacteria, such as *Proteobacteria*, *Bacteroides*, *Escherichia-Shigella*, and *Escherichia_coli* in murine models (Huang et al., 2019; Yuan et al., 2024). Importantly, these shifts in microbial community structure are often accompanied by significant alterations in overall microecological diversity. Studies have indeed reported that quercetin can modulate both alpha and beta diversity, leading to a more balanced and diverse gut microbiota composition, which is generally associated with improved gut health outcomes (e.g., increased Shannon, Simpson and Chao1 indices for alpha diversity, and PCoA and weighted UniFrac tree analysis for beta diversity) (Mi et al., 2022; Li et al., 2024a). Furthermore, these microecological changes translate into tangible physiological improvements in the host. Quercetin has been shown to reverse gut microbiota dysbiosis and inhibit the endotoxemia-mediated TLR-4 pathway, thereby ameliorating lipid metabolism abnormalities and mitigating systemic inflammation (Yuan et al., 2024; Porras et al., 2017; Cai et al., 2024). Compared to quercetin, isoquercetin exhibits a stronger ability to improve the MAFLD phenotype in mice induced by high-fat diet-fed (HFD). Isoquercetin significantly increases the abundance of *Bifidobacterium*, *Lactobacillus*, and *Akkermansia*, leading to the production of more SCFAs and indole metabolites, which leads to a reduction in hepatic steatosis in HFD mice (Tan et al., 2018). Similarly, luteolin and kaempferol increase the *Firmicutes*/*Bacteroidetes* ratio through upregulation of mucin-degrading *A. muciniphila*, which enhances gut barrier integrity (Li B. et al., 2021; Qu et al., 2021). This effect is partially mediated by flavonoid-induced inhibition of bile salt hydrolases (BSH), which alters intraluminal BA profiles. Specifically, BSH inhibition generally leads to an increase in conjugated BAs and a decrease in deconjugated BAs, thereby creating a microenvironment less favourable for certain pathogenic bacteria and more conducive to the growth of probiotic bacteria (Xu et al., 2024; Collins et al., 2023; Sayin et al., 2013).

Anthocyanins, which derived from black rice and blackcurrant (*Ribes nigrum* L.), was shown to enhance the proportion of SCFA-producing microbiota by promoting the growth of *Lactobacillus*, *Bifidobacterium*, and *A. muciniphila*, while suppressing pro-inflammatory pathogenic taxa such as *Helicobacter* and *Desulfovibrio*. Concurrently, these anthocyanins activate the PPARα, FXR, and AMPK signalling pathways and downregulate the expression of SREBP-1c, thereby contributing to improved hepatic lipid metabolism (Song et al., 2021a; Song et al., 2021b).

2.4 MAFLD evidence: based on the gut-liver axis

Accumulating clinical evidence positions flavonoids as promising therapeutics for MAFLD through microbiota-dependent mechanisms. Notably, fecal microbiota transplantation from flavonoid-treated mice recapitulates these metabolic benefits, confirming the functionality of flavonoid-trained microbial community (Fang et al., 2021). Moreover, flavonoids also mitigate MAFLD through multiple microbial-triggered pathways, including the inhibition of TLR4-NF-κB signalling to dampen hepatic inflammation (Qu et al., 2021; Porras et al., 2017; Huiru et al., 2023; Li et al., 2024b; Ting et al., 2022), activation of AMPK to promote FA β-oxidation (Song et al., 2021b; Li X. et al., 2021), and regulation of SCFA production to improve energy metabolism, among others (Kiriyama et al., 2024). These observations highlight the gut microbiota as a central hub through which flavonoids exert their hepatoprotective effects, underscoring the therapeutic potential of microbiota-targeted flavonoid interventions in MAFLD. Obviously, further exploration of the specific mechanisms is a great temptation for researchers in this field.

3 Mechanism of flavonoids action in MAFLD via the gut-liver axis

In recent years, a growing number of meta-analyses and systematic reviews with high-quality of evidence-based medicine evidence have pointed to flavonoid supplementation as a promising pharmacological option for the management of MAFLD and its associated complications (Li et al., 2023b; Liu H. et al., 2024). Their therapeutic efficacy is largely attributed to their ability to modulate the gut-liver axis: flavonoids can act directly on the liver after modification by the gut microbiota, or they can work synergistically with microbial metabolites to accomplish cooperative signalling (Figure 3). These findings are supported by a large body of animal studies, which have elucidated the diverse mechanisms by which flavonoids exert their therapeutic effects on MAFLD (Table 3). However, these promising results must be interpreted with caution due to significant translational limitations inherent in current animal models. The widely used HFD model effectively recapitulates hepatic steatosis and insulin resistance, but often fails to fully replicate the profound inflammatory component and fibrotic progression characteristic of human MASH. Conversely, while the methionine-choline deficient (MCD) diet model rapidly induces steatohepatitis and fibrosis, its accompanying weight loss paradoxically contradicts the typical obese phenotype observed in most human MASH patients. Furthermore, the pharmacological doses employed in many animal studies (e.g., baicalein at 400 mg/kg/day) vastly exceed achievable human dietary intake levels—when converted to human equivalent doses, these doses fall far beyond reasonable supplementation ranges, and raise legitimate concerns about potential toxicity. Therefore, considerable challenges still remain in translating these findings into clinically relevant, dietary achievable interventions for human MAFLD.

3.1 Direct hepatic effects of microbiota-modified flavonoids

The human gut-liver axis is increasingly recognized as a key regulator of hepatic metabolic health, and in addition to its direct involvement in hepatic physiology (e.g., BA metabolism), it can affect the liver through its ability to alter bioactive compounds. The gut microbiota converts dietary flavonoids into metabolites that directly modulate hepatic signalling. This is expected to address the lipid dysregulation, oxidative stress, and inflammatory response that are core pathological features of MAFLD.

3.1.1 Regulation of lipid metabolism

Flavonoids can inhibit *de novo* lipogenesis via key transcription factors like Sterol Regulatory Element-Binding Protein 1c (SREBP-1c) and lipogenic enzymes. In some cases, they also promote fatty acid oxidation by activating master regulators like PPARα, rate-limiting enzymes such as Carnitine Palmitoyltransferase 1 (CPT1), and enhancing alternative oxidation pathways like ω-oxidation. In brief, flavonoids contribute to systemic remodelling of lipid, improving circulating lipid profiles and influencing BA synthesis and excretion.

3.1.1.1 Inhibition of hepatic lipogenesis

The synthesis process of new fatty acids and triglycerides in the liver is known as *de novo* lipogenesis (DNL), which is a critical contributor to hepatic steatosis in MAFLD (Gnoni et al., 2022; Ponugoti et al., 2010). A primary molecular target in this process is SREBP-1c, a master transcriptional regulator of hepatic lipogenesis (Ponugoti et al., 2010; Yoon et al., 2009). Flavonoids such as quercetin and baicalin have been shown to significantly reduce the expression of SREBP-1c and lipogenic genes (Gnoni et al., 2022; Jiang L. et al., 2025). Acetyl-CoA Carboxylase (ACC) and Fatty Acid Synthase (FASN) are key rate-limiting enzymes in fatty acid synthesis (Yoon et al., 2009; Jiang L. et al., 2025; Mu et al., 2020; Dong et al., 2025). Quercetin exerts its anti-lipogenic effect by phosphorylating Acetyl-CoA Carboxylase Alpha (ACACA), a key player that catalyses the committing step in the DNL pathway (Gnoni et al., 2022; Wan et al., 2025). Furthermore, baicalin suppresses DNL by inhibiting the AMPK/acetyl-CoA carboxylase pathway and downregulating FASN (Dai et al., 2018). Licorice chalcone and luteolin also inhibit adipogenesis by activating the Sirtuin1/AMPK pathway (Tan et al., 2022). The consistent targeting of SREBP-1c, ACC, and FASN by various flavonoids indicates a convergent therapeutic strategy to suppress the core DNL pathway. However, the distinct upstream mechanisms, such as quercetin's action on the ACACA/AMPK/PP2A axis versus licorice chalcone/luteolin's SIRT1/AMPK activation, reveal diverse molecular effect points to achieve this common outcome.

3.1.1.2 Promotion of hepatic fatty acid oxidation

Flavonoids enhance the breakdown of fatty acids for energy, thereby reducing intrahepatic fat levels (Aneta et al., 2024; Ipsen et al., 2018). Fatty acid oxidation (FAO), which is crucial for maintaining lipid homeostasis (Ipsen et al., 2018), is mainly regulated by hepatic Peroxisome Proliferator-Activated Receptor alpha (PPARα), particularly during fasting, that orchestrate the transcription of numerous FAO genes (Ipsen et al., 2018; Silva

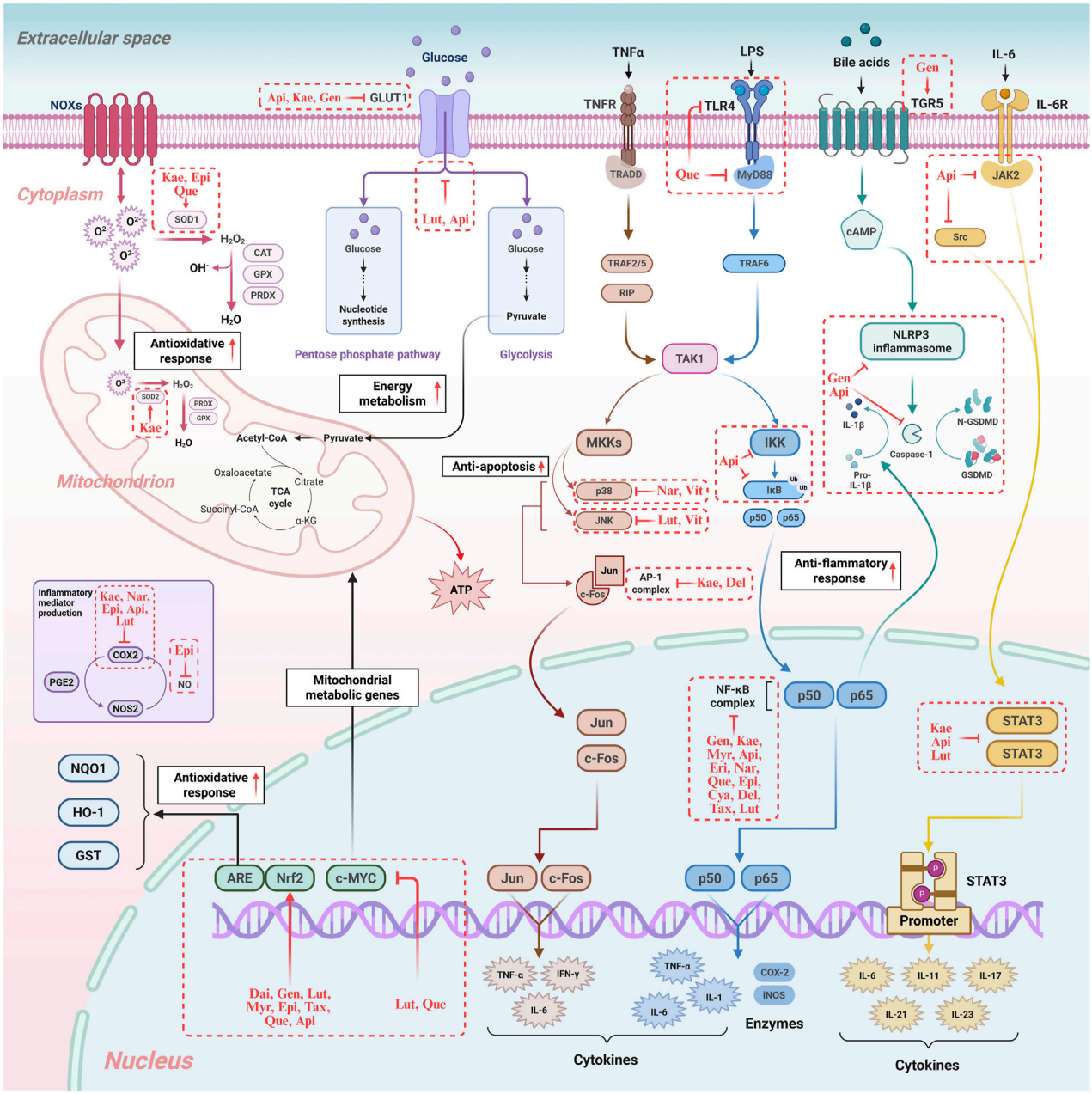


FIGURE 3
Regulatory mechanisms of flavonoids in hepatocyte oxidative stress, inflammation, immunity, and energy metabolism. The diagram illustrates how flavonoids modulate crucial signalling pathways, including those regulating oxidative stress (e.g., Nrf2 activation), inflammation (e.g., NF-κB inhibition), and energy metabolism (e.g., TCA cycle), highlighting their multi-target therapeutic potential. NOXS: NADPH Oxidases. SOD1: Superoxide Dismutase 1. SOD2: Superoxide Dismutase 2. CAT: Catalase. GPX: Glutathione Peroxidase. PRDX: Peroxiredoxin. GLUT1: Glucose Transporter 1. ATP: Adenosine Triphosphate. PGE2: Prostaglandin E2. COX2: Cyclooxygenase-2. iNOS: Inducible Nitric Oxide Synthase. NOS2: Nitric Oxide Synthase 2. NQO1: NAD(P)H Quinone Dehydrogenase 1. IDH1: Isocitrate Dehydrogenase 1. ME1: Malic Enzyme 1. MAF: Musculoaponeurotic Fibrosarcoma Proteins. Nrf2: Nuclear Factor Erythroid 2-related Factor 2. c-MYC: cellular myelocytomatosis viral oncogene homolog. TNFR: Tumor Necrosis Factor Receptor. TRADD: TNF Receptor-Associated Death Domain. TRAF2/5: TNF Receptor-Associated Factor 2/5. TRAF6: TNF Receptor-Associated Factor 6. RIP: Receptor-Interacting Protein. TAK1: TGF-β-Activated Kinase 1. MKKs: Mitogen-Activated Protein Kinase Kinases. p38: p38 Mitogen-Activated Protein Kinase. p50: NF-κB subunit p50. p65: NF-κB subunit p65. JNK: c-Jun N-terminal Kinase. c-Fos: FBJ murine osteosarcoma viral oncogene homolog. Jun: Transcription factor Jun. AP-1: Activator Protein-1. TNF-α: Tumor Necrosis Factor-alpha. IFN-γ: Interferon-gamma. IL-1: Interleukin-1. IL-1β: Interleukin-1 beta. IL-6: Interleukin-6. IL-11: Interleukin-11. IL-17: Interleukin-17. IL-21: Interleukin-21. IL-23: Interleukin-23. LPS: Lipopolysaccharide. TLR4: Toll-like Receptor 4. MyD88: Myeloid Differentiation Primary Response 88. IKK: IκB Kinase. IκB: Inhibitor of κB. NF-κB: Nuclear Factor-kappa B. TGR5: G Protein-coupled Bile Acid Receptor 1. cAMP: Cyclic Adenosine Monophosphate. NLRP3: NLR Family Pyrin Domain Containing 3. GSDMD: Gasdermin D. IL-6R: Interleukin-6 Receptor. JAK2: Janus Kinase 2. Src: Proto-oncogene tyrosine-protein kinase Src. STAT3: Signal Transducer and Activator of Transcription 3. Kae: Kaempferol. Epi: Epigallocatechin. Que: Quercetin. Api: Apigenin. Gen: Genistein. Lut: Luteolin. Nar: Naringenin. Dai: Daidzein. Myr: Myricetin. Tax: Taxifolin. Eri: Eriodictyol. Cya: Cyanidin. Del: Delphinidin. Vit: Vitexin. Original image drawn for this review using Biorender software.

TABLE 3 Animal experiments related to the treatment of MAFLD.

Flavonoid name	Disease treated	Animal model/ Number	Intervention dosage	Key results	Mechanisms	References
Quercetin	NAFLD	C57BL/6J mice (n = 40)	Quercetin 0.05%/day, oral, 16 weeks	Significantly reduced hepatic lipid accumulation, improved metabolic markers and gut microbiota dysbiosis	Quercetin modulates intestinal microbiota imbalance and relates gut-liver axis activation. It reduces the *Firmicutes*/*Bacteroidetes* ratio, inhibits endotoxemia-mediated TLR-4/NF-κB signaling, and upregulates lipid β-oxidation genes like PPAR-α and CPT-1a	Porras et al. (2017)
Silymarin	MASLD	ICR mice (n = 50)	30 or 80 mg/kg/day, oral, 8 weeks	Attenuated liver inflammation and fibrosis, and gut microbiota modulation	Silymarin regulates gut microbiota homeostasis and the TLR4/NF-κB signaling pathway. It specifically targets the FXR protein through the microbial metabolite 7-keto-deoxycholic acid (7-KDCA), which acts as an FXR antagonist	Yi et al. (2024)
Baicalein	Metabolic disorder	C57BL/6J mice (n = 80)	400 mg/kg/day, oral, 29 weeks	Selectively activated $AMPK_{\alpha 2}$, ameliorating insulin resistance and lipid abnormalities	Baicalein selectively activates $AMPK_{\alpha 2}$ which leads to the inhibition of the MAPKs pathway, the blocking of lipid synthesis by inhibiting SERBP-1c and PPARγ, and the enhancement of fatty acid oxidation	Pu et al. (2012)
Baicalein	NAFLD	C57BL/6N mice (n = 50)	100 or 200 mg/kg/day, oral, 5 weeks	Suppressed hepatic steatosis and oxidative stress and altered gut microbiota composition	Baicalein remodels the gut microbiota structure and affects lipid metabolism in the liver by regulating the gut-liver axis	Li et al. (2022)
Quercetin + *Akkermansia*	Obesity and NAFLD	Wistar rats (n = 60)	Quercetin 37.5 mg/kg/day, *Akkermansia* 2×10^8 CFU oral, 10 weeks	Synergistically reshaped gut microbiota and modulated bile acid metabolism	The synbiotic combination reshapes the gut microbiota, modulates bile acid metabolism, modulates liver lipogenesis and increases the plasma levels of unconjugated hydrophilic bile acids	Juárez-Fernández et al. (2021)

and Peixoto, 2018). Flavonoids (including quercetin, naringenin and baicalin) have been shown to ameliorate hepatic fat accumulation by targeting PPARα/γ (Jiang L. et al., 2025; Dong et al., 2025; Dai et al., 2018; Zhao et al., 2023).

Another critical molecular target is CPT1, a rate-limiting enzyme that facilitates lipid influx into mitochondria for FAO (Jiang L. et al., 2025; Dai et al., 2018). Baicalin directly activates hepatic CPT1, accelerating this process (Dai et al., 2018). Quercetin enhances CPT1A expression, and increases hepatic lipid ω-oxidation, leading to lowered circulating lipid levels (Jiang L. et al., 2025; Hoek-van den Hil et al., 2013). The consistent promotion of FAO by flavonoids via PPARα and CPT1 directly addresses the insufficiency of compensatory FAO often observed in MAFLD, which can otherwise lead to oxidative stress and disease progression (Ipsen et al., 2018). The ability of quercetin to increase ω-oxidation provides an additional, distinct pathway for fatty acid disposal, which is particularly important when mitochondrial β-oxidation is overwhelmed or compromised. This suggests that flavonoids may protect against lipotoxicity not merely by reducing fat production, but by enhancing the liver's capacity to safely process excess fatty acids, thereby preventing the "second hit" of oxidative stress and inflammation (Zhang S. et al., 2025).

3.1.2 Regulation of BA Enterohepatic metabolism

Flavonoids act on FXR in the liver and intestine to regulate BA synthesis, excretion and reabsorption. This modulation of BA metabolism is one of the key axes influencing the pathological processes associated with MAFLD. By inhibiting intestinal FXR signalling, compounds like theabrownin from Pu-erh tea increase hepatic BA synthesis and fecal excretion, reducing hepatic cholesterol accumulation (Huang et al., 2019; Liang et al., 2024). Quercetin, a paradigmatic flavonol which alleviates hepatic steatosis in HFD mice, maintains lipid homeostasis and attenuates hepatic fat accumulation mainly by regulating intestinal BA metabolism and activating FXR and TGR5 in the liver (Yuan et al., 2024; Porras et al., 2017; Cai et al., 2024). S-equol, as previously noted, binds estrogen receptors with higher affinity than precursor daidzein, which not only exerts stronger anti-inflammatory and anticancer effects, (Farhat et al., 2023), but also activates hepatic FXR to regulate BA synthesis (Kumari et al., 2024). Moreover, naringenin enhances the production of secondary BAs (e.g., lithocholic acid) by inducing BSH activity in *Bacteroides ovatus*. These BAs activate FXR in the ileum and stimulate fibroblast growth factor 19 (FGF19) secretion (Katafuchi and Makishima, 2022). Hepatic FGF19 receptor (FGFR4) activation inhibits cytochrome P450 7A1 (CYP7A1), the rate-

limiting enzyme in the synthesis of bile acids, and activates c-Jun N-terminal kinase (JNK), which phosphorylates and inhibits carbohydrate-responsive element-binding protein (ChREBP), reducing hepatic gluconeogenesis (Huang et al., 2019). At another metabolic node, flavonoids modulate BA metabolism by inhibiting BSH activity in *Clostridium* and *Bacteroides*, thereby increasing conjugated BA that antagonize intestinal FXR signalling (Zhang et al., 2020; Huang et al., 2019; Lin et al., 2022). Concurrently, altered BA profiles feedback on gut microbiota, suppressing BSH-positive pathogens and promoting beneficial bacteria (Xu et al., 2024). This bidirectional crosstalk targeting dysregulated BA metabolism that contributes to hepatic steatosis and inflammation holds key to the development of novel therapy for MAFLD (Xu et al., 2024; Huang et al., 2019).

3.1.3 Modulation of oxidative stress

Various Flavonoids such as citrus-enriched naringenin exhibited potent anti-oxidative and anti-inflammatory properties. Demethylation of naringin by microorganisms produces naringenin, which undergoes further ring cleavage to produce phenolic acids (e.g., 4-hydroxyphenylacetic acid). These metabolites activate nuclear factor erythroid 2-related factor 2 (Nrf2), promoting its translocation to the nucleus and binding to antioxidant response elements (AREs) in the promoters of HO-1 (Heme Oxygenase-1), NQO1 (NAD(P)H: quinone oxidoreductase 1) and GCLC (glutamate-cysteine ligase catalytic subunit) (Dias et al., 2021). In HFD-induced MAFLD mice, naringenin supplementation increased hepatic glutathione (GSH) levels by 60%, reduced malondialdehyde (MDA) by 40%, and attenuated cytochrome P450 2E1 (CYP2E1)-mediated oxidative damage (Shi et al., 2022).

3.1.4 Regulation of inflammatory via Kupffer cells (KCs)

Kupffer cells (KCs), the liver's resident macrophages, their critical functions include recognizing and clearing foreign materials (such as bacterial products like LPS), and endogenous danger signals (Baffy, 2009). Activated KCs are significant contributors to hepatic inflammation and the progression of MAFLD to steatohepatitis. They release a variety of pro-inflammatory mediators, including cytokines, chemokines, and reactive oxygen species (ROS) (Baffy, 2009).

Flavonoids exert their anti-inflammatory effects on the liver through a dual approach. First, they can directly interact with KCs, as in case of bergamot polyphenols that shown to decrease hepatic inflammation by the expression of pro-inflammatory cytokines like interleukin-6 (IL-6) while increasing the anti-inflammatory cytokine IL-10 (Parafati et al., 2018). This effect correlated with fewer KCs and lower inflammatory foci scores in the liver, suggesting a direct immunomodulatory action (Parafati et al., 2018). Second, flavonoids modulate the inflammatory response via gut-liver axis by influencing the production and translocation of key microbial metabolites. In MAFLD, an impaired gut barrier allows the translocation of bacterial components like LPS from the intestinal lumen to the liver, that activate KCs via the Toll-like receptor 4 (TLR4) signalling pathway and subsequently the MyD88/NF-κB cascade, a central driver of pro-inflammatory gene expression. Flavonoids can directly inhibit this cascade, but they also have an indirect effect by modulating the gut microbiota to increase the production of anti-inflammatory metabolites, such as short-chain fatty acids (SCFAs) like butyrate. These SCFAs can then reach the liver via the portal vein and directly interact with KCs to suppress their inflammatory response. This multi-pronged approach is further facilitated by the fact that specific flavonoids, such as quercetin and luteolin, can modulate broader inflammatory networks. For example, they can affect the production of cytokines such as IL-17, which in turn influences KC activation and the subsequent inflammatory cascade (Jiang L. et al., 2025; Ma et al., 2020; Meng et al., 2012; Kelepouri et al., 2018).

3.1.5 Regulation of inflammatory via hepatic T-cell

Beyond their influence on KCs, flavonoids exert a direct immunomodulatory effect on hepatic T-cells, which is crucial for managing liver inflammation and fibrosis. Specific flavonoids, such as curcumin and quercetin, have been shown to regulate T-cell activity by modulating key signalling pathways. For instance, curcumin suppresses T-cell activation by inhibiting calcium mobilization and the NFAT (Nuclear Factor of Activated T Cells) signalling pathway, leading to a dose-dependent reduction in the expression of pro-inflammatory cytokines like IL-2 and IFN-γ (Kliem et al., 2012). This effect is further supported by evidence that curcumin inhibits the proliferation of $CD4^+$ T-cells (Kim et al., 2013). Similarly, quercetin has been found to modulate the balance between pro-inflammatory Th17 cells and anti-inflammatory regulatory T cells (Tregs), promoting an anti-inflammatory state within the liver (Jiang Z. et al., 2025).

Furthermore, flavonoids can induce apoptosis in activated T-cells, a mechanism essential for resolving inflammation. For example, baicalein selectively promotes apoptosis in activated lymphocytes, which helps to mitigate hepatitis by removing excessive inflammatory cells (Zhang et al., 2013). The anti-inflammatory actions of these compounds are often mediated by their ability to inhibit central signalling pathways such as NF-κB, MAPK, and the NLRP3 inflammasome, all of which are critical for T-cell activation and cytokine production (Jiang Z. et al., 2025; Martinez et al., 2019). These findings provide a cellular and molecular basis for how flavonoids can directly modulate hepatic immune responses, offering a promising therapeutic approach for MAFLD and other liver inflammatory conditions (Li et al., 2018; Wu and Wang, 2025).

3.1.6 Regulation of fibrosis via hepatic stellate cells (HSCs)

Hepatic Stellate Cells (HSCs) play pivotal roles in the development and progression of liver fibrosis in chronic inflammation conditions such as in MAFLD. The activated HSCs are the primary producers of excessive extracellular matrix (ECM) proteins, which leads to the accumulation of fibrotic tissue (Zhang Y. et al., 2024). The activation of HSCs is driven by the dysregulation of multiple signalling pathways, including TGF-β/Smads, MAPK (ERK, JNK, p38), PI3K/AKT, Wnt, NF-κB, and AMPK (Zhang Y. et al., 2024). Transforming growth factor-beta (TGF-β) is a particularly potent activator of HSCs, promoting fibrosis through the Smad2/3 signalling pathway (Zhang Y. et al., 2024).

Flavonoids are recognized as promising natural compounds for alleviating or reversing hepatic fibrosis (Tauil et al., 2024). For example, quercetin, hydrolyzed from rutin by *Lactobacillus*

β-glucosidases, can work with butyrate (a SCFA) to suppress TGF-β/Smad signalling in HSCs (Feng et al., 2021). Quercetin blocks TGF-β type I receptor (ALK5) phosphorylation, preventing Smad2/3 nuclear translocation and reducing COL1A1 and α-SMA transcription, while butyrate enhances this effect by inhibiting histone deacetylases (HDACs), increasing acetylation of the TGF-β promoter and reducing its expression (Wang S. et al., 2022). Genistein, a soy isoflavone, and urolithin A (derived from ellagitannins by *Enterococcus* and *Gordonella spp*) cooperate to inhibit the Wnt/β-catenin pathway, a key driver of HSCs activation (Farhat et al., 2023). Genistein binds to low-density lipoprotein receptor-related protein 5/6 (LRP5/6), blocking Wnt ligand binding and β-catenin stabilization, while urolithin A enhances this effect by promoting β-catenin ubiquitination and proteasomal degradation, reducing nuclear β-catenin levels and downstream fibrosis-related genes (e.g., CTGF, VEGFA) (Liu et al., 2025). Specific flavonoids have demonstrated clear anti-fibrotic effects.

3.1.7 Flavonoids act on MAFLD via liver sinusoidal endothelial cells (LSECs)

Liver sinusoidal endothelial cells (LSECs) play a pivotal role in the development and progression of MAFLD. As a specialized cell type lining the liver sinusoids, LSECs are essential for maintaining liver homeostasis, regulating blood flow, and facilitating the bidirectional exchange of nutrients, hormones, and immune signals between the portal blood and hepatocytes. LSEC dysfunction, which can precede the development of inflammation and fibrosis, is now recognized as an early and critical event in MAFLD pathogenesis (Hammoutene et al., 2020; Velliou et al., 2023; Hammoutene and Rautou, 2019). This dysfunction is mechanistically characterized by several key changes, including the loss of fenestrations (defenestration) and the formation of a continuous basement membrane (capillarization). These structural alterations hinder the metabolic exchange between the bloodstream and hepatocytes, leading to lipotoxicity and a subsequent pro-inflammatory state. At the molecular level, this pathological process is exacerbated by a defect in endothelial autophagy, which has been observed in patients with non-alcoholic steatohepatitis (NASH) and contributes to inflammation and fibrosis by allowing the accumulation of damaged cellular components (Hammoutene et al., 2020). Furthermore, MAFLD-associated inflammation drives the overexpression of adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1), on the surface of LSECs. This promotes the recruitment and adhesion of inflammatory cells, such as macrophages, to the liver, thereby accelerating the inflammatory cascade and the progression of fibrosis (Guo et al., 2022). By targeting these specific pathways—such as by protecting LSEC integrity, enhancing endothelial autophagy, or modulating adhesion molecule expression—flavonoids offer a promising therapeutic avenue for mitigating MAFLD progression.

3.2 Indirect effects of flavonoids on the MALFD via the gut-liver axis

In addition to direct effects on the liver, flavonoids also mediate synergistic signalling via intestinal epithelial cells and immune cells, which can significantly affect liver metabolism and disease progression. Such synergistic signalling networks amplify their effects on liver inflammation, fibrosis and metabolic homeostasis.

3.2.1 Flavonoids act on MAFLD via intestinal epithelial cells (IECs)

Intestinal epithelial cells form a critical component of the gut barrier, regulating nutrient absorption and playing a significant role in metabolic signalling. Flavonoids have a multifaceted effect on these cells, particularly on regulating intestinal barrier function.

Dysregulation of the gut microbiota and subsequent intestinal barrier dysfunction are recognized contributors to the pathogenesis of MAFLD (Sun et al., 2025). Research shows that flavonoids can inhibit the loss of tight junction proteins such as ZO-1 and occludin, thereby improving intestinal barrier function. This protective action is attributed to the flavonoids' ability to modulate the gut microbiota and an increased production of beneficial SCFAs (Dong et al., 2025; Aneta et al., 2024; Zhou et al., 2024). These SCFAs then exert protective effects on IECs, including the upregulation or maintenance of tight junction proteins like ZO-1 and occluding (Vancamelbeke and Vermeire, 2017). This sequence of events results in enhanced intestinal barrier function, a reduction in the translocation of bacterial endotoxins (such as LPS) to the liver, and ultimately, an attenuation of hepatic inflammation and MAFLD progression. This pathway highlights a crucial indirect mechanism by which flavonoids contribute to liver protection. For instance, total flavonoids derived from *Dracocephalum moldavica L.* have been shown to alleviate HFD rats by enhancing the intestinal barrier, alongside their anti-inflammatory and lipid metabolism-regulating effects (Sun et al., 2025).

3.2.2 Flavonoids act on MAFLD via intestinal immune cells

The liver functions as a central immunological organ, and its susceptibility to inflammatory responses is particularly evident in chronic liver diseases such as MAFLD. The balance of intestinal T-cell responses, notably the Th17/Treg axis, further influences liver inflammation (Hammerich et al., 2011). Flavonoids exhibit immunomodulatory effects that can influence this delicate balance of intestinal T cell, impacting hepatic lipid inflammation.

The balance between Th17 cells and T regulatory (Treg) cells is crucial, as Tregs secrete anti-inflammatory cytokines and can mitigate the Th17 response, such as produce pro-inflammatory cytokines like IL-17 and IL-22 (Hammerich et al., 2011; Olveira et al., 2023; Abdelnabi et al., 2024; Pan et al., 2014). Levels of IL-17 have been correlated with the progression from MAFLD to steatohepatitis, cirrhosis, and even hepatocellular carcinoma (Hammerich et al., 2011; Olveira et al., 2023; Chackelevicius et al., 2016). In contrast, IL-22 is a pleiotropic cytokine that abrogates MASH-related inflammation and fibrosis development by inducing antioxidant and anti-apoptotic factors (Abdelnabi et al., 2024; Pan et al., 2014). This differentiation in function suggests that effective therapeutic strategies for MAFLD should aim to suppress the detrimental effects of IL-17 while potentially enhancing the beneficial actions of IL-22.

Previous studies have shown that flavonoids can significantly affect the release of IL-17 and IL-22 by regulating immune cell function and signalling pathways. For example, in the LPS-induced RAW 264.7 macrophage model, luteolin blocked the NF-κB signalling pathway, reduced the p65 binding activity in the promoter region of the IL-17A gene by 40%, led to downregulation of IL-17A mRNA expression, and inhibited the secretion of IL-17 (Gendrisch et al., 2021). In addition, a variety of

TABLE 4 Clinical trial related to the treatment of MAFLD.

Flavonoid name	Participants	Clinical trial ID	Intervention dose	Clinical endpoints		Key results	References
				Primary outcomes	Secondary outcomes		
Quercetin	41 NAFLD patients (Randomized, Double-Blind, Placebo-Controlled)	ChiCTR2100047904	500 mg/day, oral, 12 weeks	Intrahepatic lipid (IHL) content evaluated by MRI	Liver and renal function, blood lipids and glucose metabolism, body composition and anthropometryetc.	Significantly reduced hepatic fat deposition (MRI-PDFF decreased, $p < 0.05$)	Li et al. (2024c)
Silymarin	83 MASLD patients (Randomized, Double-Blind, Placebo-Controlled)	ChiCTR2200059043	103.2 mg/day, 6 months	Liver health (including liver stiffness, hepatic steatosis, and liver function)	Metabolic risk factors (including body composition, blood pressure, glucose and lipid profiles, inflammation, and antioxidant capacity)	Reduced liver stiffness ($p < 0.01$) and correlated with gut microbiota diversity	Jin et al. (2024)
Naringenin	44 NAFLD patients (Randomized, Double-Blind, Placebo-Controlled)	—	200 mg/day, oral, 4 weeks	Improvement of liver steatosis and NAFLD fibrosis score (NFS)	Changes in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lipid profile	Improved lipid profile, hepatic steatosis severity, and fibrosis probability	Namkhah et al. (2021)

flavonoids such as quercetin and naringenin have been shown to regulate the Th17/Treg cell ratio and affect IL-17/IL-22 levels in intestinal tissue (Yang et al., 2018; Ke et al., 2023; Wang et al., 2012; Wang et al., 2018).

In addition to influence key molecular pathways such as NF-κB, MAPK and PPARγ directly, flavonoids can also affect the Th17/Treg balance by improving the composition of intestinal flora (Kelepouri et al., 2018; Fu et al., 2025). A commensal bacterium, *Bacteroides fragilis*, could inhibit IL-17 production and enhance intestinal Treg cell activity by producing polysaccharide A (PSA) with anti-inflammatory effects (Jin et al., 2012; Round et al., 2011). PSA is an immunomodulatory molecule present in the pod membrane of *B. fragilis*, which mediates the conversion of CD_4^+ T cells into Treg cells via toll-like receptor 2 (TLR2) (Round et al., 2011). In addition, PSA is recognized by dendritic cells (DCs) in the intestine and then causes IL-10 production by DC cells, thus promoting Treg production (Chu et al., 2016). Quercetin and luteolin can both decrease the abundance of *B. fragilis*, thereby regulating Th17/Treg balance and cytokine secretion (Yuan et al., 2024; Kelepouri et al., 2018; Fu et al., 2025; Liu et al., 2020; Yang et al., 2025).

Currently, it is still unclear how flavonoids regulate the cytokine profile to make the Th17/Treg axis more balanced in the MAFLD model, such as reducing pro-inflammatory IL-17 and supporting protective IL-22. Future studies should focus on specific flavonoid metabolites and their direct effects on immune cell differentiation and cytokine production within the gut-liver axis to fully characterize these complex interactions. In summary, the synergistic actions of flavonoids with enterocyte and microbial metabolites represent a complex and dynamic regulatory network that can significantly impact hepatic health. By modulating key metabolic pathways and inflammatory responses, these cooperative interactions offer promising therapeutic avenues for the management of MAFLD and its associated complications.

However, while the mechanistic pathways delineated in this section present a compelling framework, the evidence supporting these mechanisms remains largely correlative and derived from imperfect model systems. For instance, the proposed anti-fibrotic effects of quercetin and urolithin A are primarily founded on preclinical models that may not fully recapitulate human disease pathophysiology. A critical, unresolved question is whether the observed microbial shifts (e.g., enrichment of *A. muciniphila* or *Bacteroides* spp.) are related to consequence of improved liver health by flavonoids. This requires causal validation in germ-free or antibiotic depletion animal models to tell whether the absence of gut microbiota abrogates the hepatoprotective effects of flavonoids like naringenin or baicalein. Faecal microbiota transplantation (FMT) studies could also establish whether microbiota from flavonoid-treated donors is sufficient to transfer metabolic benefits. Future research must prioritise these approaches to transcend correlation and establish causality, ensuring that the compelling narrative of flavonoid action via the gut-liver axis is robustly anchored in definitive experimental evidence.

4 Clinical evidence and trials

Flavonoids have been explored as potential modulators of the gut-liver axis in the context of metabolic-associated fatty liver disease. Animal studies have shown that flavonoids can modulate the gut microbiota and its metabolites to alleviate MAFLD. Other than preclinical studies in animal models as discussed in previous sections and summarized in Table 3, several clinical studies have documented the efficacy of flavonoids for MALFD management (Table 4).

A randomised, double-blind, placebo-controlled crossover clinical trial assessed the impact of quercetin supplementation on intrahepatic lipid content in patients with MAFLD. In this trial, 41 patients were randomised to receive either quercetin (500 mg) or placebo capsules for 12 weeks, followed by a 4-week washout period and subsequent intervention crossover. The primary outcome was

intrahepatic lipid content evaluated by magnetic resonance imaging (MRI) estimated proton density fat fraction. Secondary outcomes included liver function measurements and safety assessments. The results showed that quercetin intervention moderately decreased intrahepatic lipid contents from 11.5% ± 6.4%–9.6% ± 5.8%, compared with a minimal decrease of 0.1% ± 2.6% in the placebo group (P = 0.013). Body weight and body mass index (BMI) were also mildly reduced after quercetin intervention (P < 0.05 and adjusted P values of 0.038), while the placebo group experienced much smaller reductions. The reduction in intrahepatic lipid content was positively associated with body weight loss after both interventions. No significant differences were found in other secondary and safety outcomes, and no adverse events were associated with the study intervention. This trial demonstrated that 12 weeks of quercetin treatment could reduce intrahepatic lipid content in MAFLD patients. However, the trial was limited by its relatively small sample size and crossover design, which may have introduced carryover effects despite the washout period. Further trials with larger cohorts and longer intervention durations are needed to confirm these clinical findings and to explore the long-term safety and efficacy of quercetin in MAFLD management (Li N. et al., 2024).

Another randomised, double-blind, placebo-controlled trial registered at the Chinese Clinical Trial Registry (ChiCTR2200059043) investigated the potential efficacy of silymarin in improving MAFLD indicators and the underlying mechanisms related to gut microbiota. In this 24-week trial, 83 patients with MAFLD were randomised to either placebo (n = 41) or silymarin (103.2 mg/d, n = 42). Liver stiffness and hepatic steatosis were assessed using FibroScan at 0, 12, and 24 weeks, while blood samples were collected for biochemical detection and faecal samples were gathered at 0 and 24 weeks for 16S rRNA sequencing. The results showed that silymarin supplementation significantly reduced liver stiffness (LSM, −0.21 ± 0.17 vs. 0.41 ± 0.17, P = 0.015) and serum levels of γ-glutamyl transpeptidase (GGT, −8.21 ± 3.01 vs. 1.23 ± 3.16, P = 0.042), but had no significant effect on other biochemical indicators, physical measurements or fibrosis indices (AST to Platelet Ratio Index and Fibrosis-4 Index). Gut microbiota analysis revealed increased species diversity and enrichment of *Oscillospiraceae* in the silymarin group. These clinical findings suggest that silymarin supplementation could improve liver stiffness in MAFLD patients, possibly by modulating gut microbiota. The trial was limited by its relatively small sample size and the lack of long-term follow-up to assess the sustainability of the observed effects. Further trials are needed to confirm these results and to explore the optimal dosing and duration of silymarin treatment in MAFLD management Meanwhile, the specific mechanism linking gut microbiota changes to liver stiffness was not directly elucidated. Therefore, the findings may have limited generalizability, and future research should focus on confirming these results in larger, more diverse cohorts and exploring the causality of the proposed mechanism (Jin et al., 2024).

Also eligible for randomised, double-blind, placebo-controlled a clinical trial of naringenin included 44 eligible overweight/obese patients with MAFLD. This study assessed the effect of naringenin supplementation on lipid profile, transaminase levels, severity of steatosis and probability of fibrosis. Participants were randomised to receive naringenin capsules (100 mg) or identical placebo capsules twice daily for 4 weeks. The primary outcomes were improvement of liver steatosis and MAFLD fibrosis score (NFS), while secondary outcomes included changes in ALT, AST and lipid profile. The results showed that naringenin consumption significantly reduced the percentages of MAFLD grades (P < 0.001), as well as serum levels of triglyceride (TG) (P < 0.001), total cholesterol (TC) (P = 0.01), and low-density lipoprotein (LDL) (P = 0.02), and increased serum levels of high-density lipoprotein (HDL) (P = 0.02) compared with the control group. However, no significant changes were observed in AST, ALT and NFS. The trial concluded that daily intake of 200 mg of naringenin for 4 weeks had beneficial effects on lipid profile and MAFLD grades as an indicator for the severity of hepatic steatosis (Namkhah et al., 2021).

The aforementioned clinical trials, while providing foundational evidence for the therapeutic potential of flavonoids in MAFLD, are subject to several limitations that warrant careful consideration. A primary and common limitation is the relatively small sample size in all trials (n = 41, n = 83, and n = 44, respectively), with insufficient statistical power to detect smaller, yet clinically meaningful effects, thereby increasing the risk of Type II errors (false negatives), such as failing to identify significant changes in fibrosis scores or other metabolic parameters. Furthermore, the short intervention durations (4, 12, and 24 weeks) are a significant constraint, as meaningful improvements in hepatic fibrosis or metabolic outcomes needs long-term observation, and the reversal of fibrosis typically requires extended periods beyond these timeframes, limiting the ability to capture true therapeutic effects. For instance, the lack of significant change in transaminases and fibrosis scores in the naringenin trial is likely a reflection of its extremely short 4-week duration rather than a true absence of effect, as these markers typically require a longer period to respond to interventions.

Additionally, the reliance on non-invasive surrogate endpoints (e.g., MRI-PDFF for steatosis and FibroScan for stiffness) instead of liver biopsy introduces uncertainty in accurately evaluating the severity of MAFLD and the full extent of histological improvement, as these imaging and biochemical markers may not fully correlate with pathological changes. The crossover design of the quercetin trial, despite its washout period, introduces the potential for carryover effects, where the influence of the initial treatment may persist and confound the results of the subsequent treatment period, a bias that can only be definitively ruled out with a longer washout period or a parallel-group design. The silymarin trial did not establish a direct causal link between the observed changes in *Oscillospiraceae* enrichment and the reduction in liver stiffness. The use of 16S rRNA sequencing also provides only a taxonomic snapshot of the microbiota, lacking the functional insights that could be provided by shotgun metagenomics or metabolomics to track specific flavonoid-derived metabolites. Moreover, these trials did not account for individual variability in flavonoid bioavailability, which is profoundly influenced by gut microbiota composition and genetic background (e.g., polymorphisms in drug-metabolizing enzymes or bile acid receptors); the absence of patient stratification based on enterotypes or genetic markers may obscure subgroup effects and contribute to inconsistent outcomes across studies.

These methodological constraints significantly limit the generalizability of the findings to the broader, heterogeneous

MAFLD population, as the specific patient characteristics and baseline disease severity are likely to influence treatment response. Future research must, therefore, be guided by more robust methodologies. This includes large-scale, multi-center, parallel-group randomized controlled trials with intervention periods of at least 6 months to 1 year to properly evaluate sustained efficacy and long-term safety. The inclusion of more definitive clinical endpoints, such as a histological response (via liver biopsy) or significant and sustained changes in liver stiffness (FibroScan) and metabolic markers, will be essential. Furthermore, future studies should incorporate a multi-omics approach to thoroughly investigate the mechanistic link between flavonoids, gut microbiota, and hepatic pathology, moving beyond basic sequencing to measure microbial metabolites and host-derived signalling molecules in the gut-liver axis. Establishing optimal dosing regimens through dose-ranging studies and exploring the therapeutic efficacy in specific patient subgroups (e.g., by genetic polymorphisms or gut enterotypes) will be critical for translating these promising preclinical and preliminary clinical findings into personalized, effective clinical practice.

5 Beyond monotherapy: nanotechnology and probiotic Co-administration

5.1 Synbiotics: flavonoid-probiotic combinations

As discussed previously, the gut microbiota plays a crucial role in mediating the physiological effects of flavonoids via the gut-liver axis. Probiotics, defined as live microorganisms that confer health benefits when consumed in adequate amounts, can interact synergistically with flavonoids, which are potential prebiotics, to form synbiotics. The combination of dietary flavonoids with specific probiotic strains has emerged as an innovative approach to address gut-liver axis dysregulation in the context of MAFLD (Jazvinšćak et al., 2023). Probiotic enzymatic activity activates flavonoid precursors into bioactive metabolites, while flavonoids selectively modulate the gut microbiota and microbial composition, creating a self-amplifying loop that enhances MAFLD management. This positive feedback loop targets multiple pathological mechanisms and exerts beneficial effects on oxidative stress, inflammation, and the gut microbiome (Zhu et al., 2022). In this section, we review several studies investigating the combined effects of flavonoids and probiotics on MAFLD, elucidating their therapeutic potential.

5.1.1 Quercetin and *Akkermansia muciniphila*

Akkermansia muciniphila is a prominent gut bacterium that has shown potential in improving metabolic diseases, including obesity and MAFLD. In a landmark study involving HFD-induced obese mice, researchers demonstrated that the synbiotic combination of *A. muciniphila* with quercetin was superior in reducing hepatic steatosis and insulin resistance compared to either intervention alone. This combination restored intestinal barrier integrity, as evidenced by upregulation of tight junction proteins, such as Claudin-1 and Occludin, while decreasing LPS translocation (Le Barz et al., 2019). Moreover, the combination reshaped the microbiota by enriching *Roseburia* and *Faecalibacterium*, bacteria that produce butyrate, a SCFA with anti-inflammatory properties. Notably, the quercetin-*A. muciniphila* synergy also modulated BA metabolism with enhanced FXR signalling, which suppressed hepatic lipogenesis by downregulating SREBP-1c and activated mitochondrial β-oxidation by upregulating PGC-1α. Co-administration of quercetin (50 mg/kg/day) with *A. muciniphila* (1×10^9 CFU/day) resulted in a 38% greater reduction in hepatic TG content compared to either treatment alone. This highlights the potential of flavonoid-probiotic combinations to modulate multiple metabolic pathways and improve liver health in MAFLD (Juárez-Fernández et al., 2021).

5.1.2 Grapeseed flour and *Lactobacillus acidophilus*

Grapeseed flour (GSF) is rich in flavonoids, particularly proanthocyanidins, which possess potent antioxidant and anti-inflammatory properties (Cho et al., 2018). In a 24-week clinical trial, a synbiotic combination of GSF and kefir-derived probiotics *L. acidophilus LA-5* was showed to reduced liver fat content, as measured by the controlled attenuation parameter, by 22% compared to the use of probiotics alone. Additionally, this combination downregulated hepatic SREBP-1c (62%) and ACC (55%) expression through AMPK phosphorylation, as validated by CRISPR-Cas9 knockout models. The synbiotic treatment also reversed HFD-induced gut barrier dysfunction, which is crucial for preventing endotoxemia and inflammation in MAFLD. Moreover, *Lactobacillus acidophilus* enhanced the absorption of procyanidins by degrading mucus-bound glycoproteins in the intestine, thereby improving their bioavailability. These clinical findings suggest that combining flavonoid-rich GSF with probiotics can significantly ameliorate hepatic steatosis and related metabolic disturbances (Kwon et al., 2019; Seo et al., 2020).

5.1.3 Green tea EGCG and *Lactobacillus fermentum*

Epigallocatechin gallate (EGCG), a flavonoid extracted from green tea, has been extensively studied for its antioxidant and anti-inflammatory properties, both of which are essential for the management of MAFLD (Carrasco-Pozo et al., 2019; Talib et al., 2024). In a study involving aged C57BL/6 mice with diet-induced MAFLD, a synbiotic combination of EGCG and the probiotic *L. fermentum* was tested for its protective effects against oxidative stress and inflammation (Sharma et al., 2019). The synbiotic was found to increase hepatic glutathione peroxidase activity by 40%, decrease malondialdehyde (a marker of lipid peroxidation) level by 35%, and suppress $CD8^+$ T-cell hepatic infiltration, which is indicative of liver inflammation. Transcriptome analysis revealed upregulation of antioxidant genes (HO-1, NQO1) and downregulation of Th17-related cytokines, suggesting that the synbiotic combination modulated both oxidative stress and immune responses (Ting et al., 2022). The EGCG component inhibited JNK phosphorylation, a key pathway in oxidative stress, while *Lactobacillus fermentum* promoted T-regulatory cell proliferation, further supporting the anti-inflammatory effects (Dey et al., 2020). The strong upregulation of hepatic Nrf2 expression exclusively in the synbiotic-fed animals provides additional evidence of the robust antioxidant defence induced by

EGCG-*L. fermentum* combination (Sharma et al., 2019). These results highlight the potential of flavonoid-probiotic synbiotics as a therapeutic strategy for MAFLD, particularly in populations prone to oxidative stress, such as the elderly (Sharma et al., 2019; Huang et al., 2020).

5.1.4 Flavonoid-probiotic synbiotics: challenges and future

In conclusion, flavonoid-probiotic combinations represent a promising avenue for the treatment of MAFLD. The synergistic effects of these bioactive compounds, targeting multiple pathways such as gut microbiota modulation, bile acid metabolism, and hepatic lipid metabolism, provide a multifaceted cutting-edge strategy to managing this increasingly prevalent liver disease (Axling et al., 2012; Xiong et al., 2023; Thilakarathna and Rupasinghe, 2024; Peng et al., 2020). However, existing studies highlight several challenges, most notably the critical issue of strain-specificity where effects observed with particular bacterial strains (e.g., *Lactobacillus* or *B. spp*.) cannot be extrapolated to the entire species, yet many studies fail to adequately characterize or report the specific strains utilised, rendering results difficult to interpret and replicate. Other challenges include the need for optimal strain selection, biosafety evaluation, and the impact of patient variability, including genetic predisposition, individual microbiome composition, and dietary habits, on therapeutic outcomes (DiStefano, 2023; Oh et al., 2023; Wang M. et al., 2022; Ribeiro et al., 2018). Furthermore, there is a conspicuous lack of standardisation in formulations and dosages across studies, with highly variable flavonoid-to-probiotic ratios, delivery formats, and intervention regimens creating significant obstacles for comparing outcomes and establishing reproducible therapeutic protocols. Future research must focus on strain-specific screening, pharmacokinetic modelling, and long-term safety assessments to ensure the clinical translation of these promising preclinical findings (Palencia-Argel et al., 2024).

Genetic polymorphisms have also been identified as crucial determinants of the therapeutic response to flavonoid-based treatments, such as silibinin. For instance, Lrp6(+/−) mice exhibited less severe liver injury in response to MCD, but a reduced treatment response to silibinin, compared to Lrp6(+/+) mice, suggesting that Lrp6 may serve as a target for silibinin's therapeutic action (Chen et al., 2021). This highlights the need for personalized treatment approaches based on genetic variations, as individual susceptibility to MAFLD may be modulated by specific genetic factors, including Lrp6 polymorphisms (Chen et al., 2021). Addressing these issues will be critical in ensuring that flavonoid-probiotic combinations can be successfully used as personalized therapeutic strategies for MAFLD.

Despite the compelling preclinical evidence, the clinical translation of flavonoid-probiotic synbiotics encounters substantial hurdles. A particularly significant barrier is the regulatory gap whereby these combinations are typically classified as dietary supplements or probiotics rather than pharmaceuticals, subjecting them to considerably less stringent requirements for demonstrating efficacy, safety, and quality control compared to medicinal products—this permissive and inconsistent regulatory landscape contributes to variable product quality and weakened clinical evidence. A primary unmet need lies in establishing standardized formulations and dosages, given the immense diversity of flavonoid compounds and probiotic strains, which contributes to highly variable synergistic effects *in vivo* (Palencia-Argel et al., 2024). The intricate interplay among distinct flavonoid types, specific probiotic strains, individual host microbiomes, and dietary factors necessitates extensive and rigorous clinical trials to ascertain consistent efficacy and safety in MAFLD patients. Furthermore, the evolving regulatory requirement for synbiotic products presents additional challenges for their widespread clinical adoption and therapeutic standardization. Therefore, while these combinations hold considerable promise, achieving clinical readiness mandates overcoming these multifaceted translational gaps through meticulously designed, large-scale clinical studies and the establishment of clearer regulatory frameworks.

5.2 Nanotechnology for targeted delivery

Flavonoids exhibit broad-spectrum biological and pharmacological properties, but many of their key constituents is limited by physicochemical constraints such as poor dispersibility, and instability, as well as extensive gastrointestinal degradation, liver first-pass metabolism, and restricted membrane transport, all collectively contributing to reduced oral bioavailability (Bhia et al., 2021; Zhang et al., 2022). Nanotechnology, including flavonoid-loaded nanoparticles like chitosan, nanoliposomes, and solid lipid nanoparticles, has been developed to enhance the bioavailability, stability, solubility, and delivery of flavonoids such as EGCG (Hu L. et al., 2025; Prananda et al., 2025; Shi et al., 2018).

Chitosan nanoparticles, in particular, have been widely used to encapsulate flavonoids due to their biocompatibility, biodegradability, and mucoadhesive properties (Prananda et al., 2025; Seyam et al., 2020). These nanoparticles can be designed to provide controlled and sustained release of encapsulated flavonoids, ensuring a gradual and prolonged delivery to target cells or tissues (Stevens Barron et al., 2023). For example, one study focused on the effect of chitosan-modified, silymarin-loaded lipid-polymer hybrid nanoparticles (CS-LPNs) in enhancing the oral bioavailability of silymarin and improving its lipid-lowering efficacy for NAFLD treatment. The results showed that the relative bioavailability of CS-LPNs was 14.38 times higher than that of silymarin suspension, and it enhanced the uptake of the nanocarriers by fat-emulsion-treated HepG2 and Caco-2 cells. Meanwhile, the study confirmed that CS-LPNs inhibited lipid accumulation in the mouse liver and enhanced the therapeutic efficacy of silymarin in a transgenic mouse model of NAFLD. These clinical findings suggest that the improved uptake of CS-LPNs could be achieved *in vivo*, potentially increasing the oral bioavailability of silymarin (Talib et al., 2024; Liang et al., 2018). Also, in the field of lipid reduction, a recent study explored a novel strategy for obesity treatment by using hydroxy-α-sanshool-loaded adipose-targeted mesoporous silica nanoparticles (MSNs) to specifically induce the browning of white adipose tissue (WAT). This research demonstrated that the nanocarriers activate the transient receptor potential vanilloid 1 (TRPV1) channel, providing a potential therapeutic approach for MAFLD patients with hepatic lipid accumulation (Zhang Q. et al., 2025).

Nanoliposomes have also been used to encapsulate flavonoids, such as EGCG, to improve their bioavailability and stability (Talib et al., 2024; Hu L. et al., 2025). These nanoparticles can be designed

to provide targeted delivery of flavonoids to specific tissues, such as the myocardium or vascular endothelium, via endocytotic mechanisms (Prananda et al., 2025). Additionally, nanoliposomes have been shown to improve the therapeutic efficacy of flavonoids by enhancing their absorption and distribution in the body (Talib et al., 2024; Hu L. et al., 2025; Kasem et al., 2025).

While nanotechnology presents significant promise for overcoming the inherent limitations of flavonoid delivery, its clinical translation is fraught with several critical caveats and unmet needs. The vast majority of existing research remains confined to pre-clinical stages, with the long-term biosafety profile of these nanocarriers (including CS-LPNs and MSNs) representing a formidable "area of unknown" in clinical translation. Comprehensive assessments of their potential for long-term accumulation, immunogenicity, and unanticipated organ toxicity following chronic administration are notably lacking, necessitating extensive *in vivo* investigations to preclude unanticipated adverse effects, particularly with chronic administration (Bhia et al., 2021; Zhang et al., 2022; Hu L. et al., 2025). Furthermore, the challenges pertaining to reproducible synthesis, scalable manufacturing, and cost-effectiveness of nano formulations must be rigorously addressed for viable clinical applications (Shi et al., 2018). Most current protocols remain at the proof-of-concept stage, with little consideration given to Good Manufacturing Practice (GMP) compliance or industrial scalability, whilst stability issues during storage and transport present additional hurdles for real-world implementation. Meanwhile, the formidable biological barriers and complex *in vivo* interactions imply that targeted delivery, though conceptually appealing, is often less efficient than *in vitro* results. This disparity underscores the need for more sophisticated targeting strategies and meticulous preclinical validation.

6 Future perspective

The growing body of evidence highlights flavonoids as potential therapeutic agents for MAFLD, but significant challenges remain in translating these findings into effective clinical practice (Qian et al., 2024; Nie et al., 2025). A critical hurdle is addressing the significant inter-individual variability in response to flavonoid interventions. The complex interplay between flavonoids and the gut microbiota, shaped by host genetics, dictates their metabolism and bioavailability. Future research must move beyond a one-size-fits-all approach to investigate these genetic and microbial factors to develop more personalized strategies.

Another important caveat in developing flavonoid-based MAFLD drugs is that many natural flavonoids fall into the category of Pan-Assay Interference Compounds (PAINS) (Baell, 2016). These compounds often contain problematic motifs, such as redox-active catechols or aggregatory structures, which require cautious interpretation of data from high-throughput *in vitro* or cell-based assays. Therefore, structure-activity relationship (SAR) studies should aim to optimize flavonoids toward improved drug-like properties by reducing polyphenolic character while maintaining efficacy. Ultimately, demonstrating target engagement in physiologically relevant animal models is critical to distinguish true pharmacology from assay artifacts. Only rigorous, evidence-based approaches ensure that promising flavonoids advance as *bona fide* therapeutic candidates rather than as PAINS-misleading hits.

The translation of flavonoid-based therapies from bench to bedside for MAFLD management is primarily hampered by two interconnected barriers: the scarcity of robust long-term clinical evidence and the inherent challenges of bioavailability and consistent efficacy. The considerable inter-individual variation in flavonoid metabolism, heavily influenced by unique host genetics and gut microbial composition, necessitates a fundamental shift away from universal dosing regimens towards personalized, biomarker-guided approaches. Future clinical trials must therefore be larger, longer, and integratively designed to incorporate multi-omics analyses (metagenomics, metabolomics, genomics), enabling patient stratification and the identification of predictive biomarkers for treatment response.

To overcome the bioavailability barrier, innovative delivery systems such as nanotechnology and synbiotic formulations present promising avenues. However, their clinical translation mandates a critical focus on overcoming hurdles related to long-term safety, reproducible large-scale manufacturing, and the establishment of standardized regulatory frameworks. Ultimately, the future of flavonoid therapy lies in leveraging advanced delivery technologies and precision nutrition principles to transform the gut-liver axis paradigm into tangible, effective, and personalized clinical strategies for MAFLD.

To effectively translate these findings into clinical practice, a shift in perspective is required for both healthcare providers and researchers. Clinicians must move toward a personalized approach, using advanced diagnostic tools to screen for genetic predispositions and gut microbiome composition to identify which patients will benefit most. Researchers, in turn, should prioritize developing standardized, reproducible methods for assessing flavonoid bioavailability and metabolism in humans through well-powered, long-term randomized controlled trials. These efforts should focus on understanding dose-response relationships and developing safe, effective delivery systems. By overcoming these barriers, flavonoids could provide a novel, multi-targeted strategy to manage the complex pathophysiology of MAFLD/MASH, potentially revolutionizing treatment options.

Author contributions

MH: Investigation, Conceptualization, Funding acquisition, Methodology, Writing – original draft, Project administration, Visualization. ZW: Investigation, Writing – original draft. LW: Writing – original draft, Investigation. AY: Writing – review and editing. XS: Writing – review and editing. MZ: Writing – review and editing, Resources. XL: Writing – review and editing. QZ: Writing – review and editing. ZS: Funding acquisition, Writing – review and editing, Methodology, Supervision, Conceptualization.

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Conflict of interest

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

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References

Abdelnabi, M. N., Hassan, G. S., and Shoukry, N. H. (2024). Role of the type 3 cytokines IL-17 and IL-22 in modulating metabolic dysfunction-associated steatotic liver disease. *Front. Immunol.* 15, 1437046. doi:10.3389/fimmu.2024.1437046

Aneta, S., Sara, J.-C., and Rafał, F. (2024). Flavonoids and their role in preventing the development and progression of MAFLD by modifying the microbiota. *Int. J. Mol. Sci.* 25, 11187. doi:10.3390/ijms252011187

Axling, U., Olsson, C., Xu, J., Fernandez, C., Larsson, S., Ström, K., et al. (2012). Green tea powder and Lactobacillus plantarum affect gut microbiota, lipid metabolism and inflammation in high-fat fed C57BL/6J mice. *Nutr. Metab. (Lond).* 9, 105. doi:10.1186/1743-7075-9-105

Baell, J. B. (2016). Feeling nature's PAINS: natural products, natural product drugs, and pan assay interference compounds (PAINS). *J. Nat. Prod.* 79, 616–628. doi:10.1021/acs.jnatprod.5b00947

Baffy, G. (2009). Kupffer cells in non-alcoholic fatty liver disease: the emerging view. *J. Hepatol.* 51, 212–223. doi:10.1016/j.jhep.2009.03.008

Barber, T. M., Hanson, P., and Weickert, M. O. (2023). Metabolic-associated fatty liver disease and the gut microbiota. *Endocrinol. Metab. Clin. North Am.* 52, 485–496. doi:10.1016/j.ecl.2023.01.004

Barreca, D., Gattuso, G., Bellocco, E., Calderaro, A., Trombetta, D., Smeriglio, A., et al. (2017). Flavanones: citrus phytochemical with health-promoting properties. *BioFactors* 43, 495–506. doi:10.1002/biof.1363

Bhia, M., Motallebi, M., Abadi, B., Zarepour, A., Pereira-Silva, M., Saremnejad, F., et al. (2021). Naringenin nano-delivery systems and their therapeutic applications. *Pharmaceutics* 13, 291. doi:10.3390/pharmaceutics13020291

Braune, A., and Blaut, M. (2011). Deglycosylation of puerarin and other aromatic C-glucosides by a newly isolated human intestinal bacterium. *Environ. Microbiol.* 13, 482–494. doi:10.1111/j.1462-2920.2010.02352.x

Braune, A., and Blaut, M. (2016). Bacterial species involved in the conversion of dietary flavonoids in the human gut. *Gut Microbes* 7, 216–234. doi:10.1080/19490976.2016.1158395

Buisson, D., Quintin, J., and Lewin, G. (2007). Biotransformation of polymethoxylated flavonoids: access to their 4'-O-demethylated metabolites. *J. Nat. Prod.* 70, 1035–1038. doi:10.1021/np070084q

Cai, T., Song, X., Xu, X., Dong, L., Liang, S., Xin, M., et al. (2024). Effects of plant natural products on metabolic-associated fatty liver disease and the underlying mechanisms: a narrative review with a focus on the modulation of the gut microbiota. *Front. Cell Infect. Microbiol.* 14, 1323261. doi:10.3389/fcimb.2024.1323261

Cao, H., Chen, X., Jassbi, A. R., and Xiao, J. (2015). Microbial biotransformation of bioactive flavonoids. *Biotechnol. Adv.* 33, 214–223. doi:10.1016/j.biotechadv.2014.10.012

Carrasco-Pozo, C., Cires, M. J., and Gotteland, M. (2019). Quercetin and epigallocatechin gallate in the prevention and treatment of obesity: from molecular to clinical studies. *J. Med. Food* 22, 753–770. doi:10.1089/jmf.2018.0193

Chackelevicius, C. M., Gambaro, S. E., Tiribelli, C., and Rosso, N. (2016). Th17 involvement in nonalcoholic fatty liver disease progression to non-alcoholic steatohepatitis. *World J. Gastroenterol.* 22, 9096–9103. doi:10.3748/wjg.v22.i41.9096

Chen, Q. L., Zhu, L., Tang, Y. N., Kwan, H. Y., Zhao, Z. Z., Chen, H. B., et al. (2017). Comparative evaluation of chemical profiles of three representative 'snow lotus' herbs by UPLC-DAD-QTOF-MS combined with principal component and hierarchical cluster analyses. *Drug Test. Anal.* 9 (9), 1105–1115. doi:10.1002/dta.2123

Chen, L. J., Lin, X. X., Guo, J., Xu, Y., Zhang, S. X., Chen, D., et al. (2021). Lrp6 genotype affects individual susceptibility to nonalcoholic fatty liver disease and silibinin therapeutic response *via* Wnt/β-catenin-Cyp2e1 signaling. *Int. J. Biol. Sci.* 17, 3936–3953. doi:10.7150/ijbs.63732

Chen-Chen, J., Yue, R., Wenwen, X., Yiduo, H., Xin, L., and Feng-Jie, W. (2024). Effect of modified xiaoyao powder on intestinal barrier and intestinal flora in mice with metabolic associated fatty liver disease based on gut-liver axis. *Zhongguo Zhongyao Zazhi.* doi:10.19540/j.cnki.cjcmm.20240506.401

Cheng, Z., Chen, Y., Schnabl, B., Chu, H., and Yang, L. (2024). Bile acid and nonalcoholic steatohepatitis: molecular insights and therapeutic targets. *J. Adv. Res.* 59, 173–187. doi:10.1016/j.jare.2023.06.009

Cho, Y. J., Lee, H. G., Seo, K. H., Yokoyama, W., and Kim, H. (2018). Antiobesity effect of prebiotic polyphenol-rich grape seed flour supplemented with probiotic kefir-derived lactic acid bacteria. *J. Agric. Food Chem.* 66, 12498–12511. doi:10.1021/acs.jafc.8b03720

Chu, H., Khosravi, A., Kusumawardhani, I. P., Kwon, A. H., Vasconcelos, A. C., Cunha, L. D., et al. (2016). Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Science.* 352, 1116–1120. doi:10.1126/science.aad9948

Collins, S. L., Stine, J. G., Bisanz, J. E., Okafor, C. D., and Patterson, A. D. (2023). Bile acids and the gut microbiota: metabolic interactions and impacts on disease. *#N/A* 21, 236–247. doi:10.1038/s41579-022-00805-x

Constantinescu, T., and Lungu, C. N. (2021). Anticancer activity of natural and synthetic chalcones. *Int. J. Mol. Sci.* 22, 11306. doi:10.3390/ijms222111306

Dai, J., Liang, K., Zhao, S., Jia, W., Liu, Y., Wu, H., et al. (2018). Chemoproteomics reveals baicalin activates hepatic CPT1 to ameliorate diet-induced obesity and hepatic steatosis. *Proc. Natl. Acad. Sci. U. S. A.* 115, E5896–E905. doi:10.1073/pnas.1801745115

De Cól, J. P., de Lima, E. P., Pompeu, F. M., Cressoni Araújo, A., de Alvares Goulart, R., Bechara, M. D., et al. (2024). Underlying mechanisms behind the brain-gut-liver axis and metabolic-associated fatty liver disease (MAFLD): an update. *Int. J. Mol. Sci.* 25, 3694. doi:10.3390/ijms25073694

de Sousa Moraes, L. F., Sun, X., Peluzio, M., and Zhu, M. J. (2019). Anthocyanins/Anthocyanidins and colorectal cancer: what is behind the scenes? *Crit. Rev. Food Sci. Nutr.* 59, 59–71. doi:10.1080/10408398.2017.1357533

Déprez, S., Brezillon, C., Rabot, S., Philippe, C., Mila, I., Lapierre, C., et al. (2000). Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *J. Nutr.* 130, 2733–2738. doi:10.1093/jn/130.11.2733

Dey, P., Olmstead, B. D., Sasaki, G. Y., Vodovotz, Y., Yu, Z., and Bruno, R. S. (2020). Epigallocatechin gallate but not catechin prevents nonalcoholic steatohepatitis in mice similar to green tea extract while differentially affecting the gut microbiota. *#N/A* 84, 108455. doi:10.1016/j.jnutbio.2020.108455

Dias, M. C., Pinto, D., and Silva, A. M. S. (2021). Plant flavonoids: Chemical characteristics and biological activity. *Molecules* 26, 5377. doi:10.3390/molecules26175377

DiStefano, J. K. (2023). The role of choline, soy isoflavones, and probiotics as adjuvant treatments in the prevention and management of NAFLD in postmenopausal women. *Nutrients* 15 (12), 2670. doi:10.3390/nu15122670

Dong, L., Lou, W., Xu, C., and Wang, J. (2025). Naringenin cationic lipid-modified nanoparticles mitigate MASLD progression by modulating lipid homeostasis and gut microbiota. *J. Nanobiotechnology* 23, 168. doi:10.1186/s12951-025-03228-x

Fan, L., Zhao, X., Tong, Q., Zhou, X., Chen, J., Xiong, W., et al. (2018). Interactions of dihydromyricetin, a flavonoid from Vine tea (Ampelopsis grossedentata) with gut microbiota. *J. Food Sci.* 83, 1444–1453. doi:10.1111/1750-3841.14128

Fang, Y., Zhang, J., Zhu, S., He, M., Ma, S., Jia, Q., et al. (2021). Berberine ameliorates ovariectomy-induced anxiety-like behaviors by enrichment in equol generating gut microbiota. *Pharmacol. Res.* 165, 105439. doi:10.1016/j.phrs.2021.105439

Fang, Z., Shen, G., Wang, Y., Hong, F., Tang, X., Zeng, Y., et al. (2024). Elevated kallistatin promotes the occurrence and progression of non-alcoholic fatty liver disease. *Signal Transduct. Target Ther.* 9, 66. doi:10.1038/s41392-024-01781-9

Farhat, E. K., Sher, E. K., Džidić-Krivić, A., Banjari, I., and Sher, F. (2023). Functional biotransformation of phytoestrogens by gut microbiota with impact on cancer treatment. *#N/A* 118, 109368. doi:10.1016/j.jnutbio.2023.109368

Feng, W., Chengying, Z., Guifang, T., Xue, W., Zihan, M., Jiefen, C., et al. (2020). Naringin alleviates atherosclerosis in ApoE−/− mice by regulating cholesterol metabolism involved in gut microbiota remodeling. *J. Agric. Food Chem.* 68, 12651–12660. doi:10.1021/acs.jafc.0c05800

Feng, X. H., Xu, H. Y., Wang, J. Y., Duan, S., Wang, Y. C., and Ma, C. M. (2021). *In vivo* hepatoprotective activity and the underlying mechanism of chebulinic acid from Terminalia chebula fruit. *Phytomedicine* 83, 153479. doi:10.1016/j.phymed.2021.153479

Ferreira-Lazarte, A., Plaza-Vinuesa, L., de Las Rivas, B., Villamiel, M., Muñoz, R., and Moreno, F. J. (2021). Production of α-rhamnosidases from Lactobacillus plantarum WCFS1 and their role in deglycosylation of dietary flavonoids naringin and rutin. *Int. J. Biol. Macromol.* 193, 1093–1102. doi:10.1016/j.ijbiomac.2021.11.053

Fianchi, F., Liguori, A., Gasbarrini, A., Grieco, A., and Miele, L. (2021). Nonalcoholic fatty liver disease (NAFLD) as model of gut-liver axis interaction: from pathophysiology to potential target of treatment for personalized therapy. *Int. J. Mol. Sci.* 22, 6485. doi:10.3390/ijms22126485

Francesca, L., Stefano, G., Elisabetta, D. A., Alessandra, S., Josè Alberto, D. A., Pierluigi, M., et al. (2022). Metabolic-associated fatty liver disease from childhood to adulthood: state of art and future directions. *World J. Hepatology* 14, 1087–1098. doi:10.4254/wjh.v14.i6.1087

Fu, J., Zhao, Z., Zhou, D., and Liu, S. (2025). Therapeutic potential of polyphenols in targeting Th17/Treg balance for intestinal barrier maintenance. *Food Sci. Nutr.* 13, e70400. doi:10.1002/fsn3.70400

Gendrisch, F., Esser, P. R., Schempp, C. M., and Wölfle, U. (2021). Luteolin as a modulator of skin aging and inflammation. *BioFactors* 47, 170–180. doi:10.1002/biof.1699

Gnoni, A., Di Chiara Stanca, B., Giannotti, L., Gnoni, G. V., Siculella, L., and Damiano, F. (2022). Quercetin Reduces Lipid Accumulation in a Cell Model of NAFLD by Inhibiting *de novo* Fatty Acid Synthesis through the Acetyl-CoA Carboxylase 1/AMPK/PP2A Axis. *Int. J. Mol. Sci.* 23, 1044. doi:10.3390/ijms23031044

Gómez-Zorita, S., González-Arceo, M., Fernández-Quintela, A., Eseberri, I., Trepiana, J., and Portillo, M. P. (2020). Scientific evidence supporting the beneficial effects of isoflavones on human health. *#N/A*, 12. doi:10.3390/nu12123853

Guo, Q., Furuta, K., Islam, S., Caporarello, N., Kostallari, E., Dielis, K., et al. (2022). Liver sinusoidal endothelial cell expressed vascular cell adhesion molecule 1 promotes liver fibrosis. *Front. Immunol.* 13, 983255. doi:10.3389/fimmu.2022.983255

Hammerich, L., Heymann, F., and Tacke, F. (2011). Role of IL-17 and Th17 cells in liver diseases. *Clin. Dev. Immunol.* 2011, 345803. doi:10.1155/2011/345803

Hammoutene, A., and Rautou, P. E. (2019). Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. *J. Hepatol.* 70, 1278–1291. doi:10.1016/j.jhep.2019.02.012

Hammoutene, A., Biquard, L., Lasselin, J., Kheloufi, M., Tanguy, M., Vion, A. C., et al. (2020). A defect in endothelial autophagy occurs in patients with non-alcoholic steatohepatitis and promotes inflammation and fibrosis. *J. Hepatol.* 72, 528–538. doi:10.1016/j.jhep.2019.10.028

Hoek-van den Hil, E. F., Keijer, J., Bunschoten, A., Vervoort, J. J., Stankova, B., Bekkenkamp, M., et al. (2013). Quercetin induces hepatic lipid omega-oxidation and lowers serum lipid levels in mice. *PLoS One* 8, e51588. doi:10.1371/journal.pone.0051588

Hostetler, G. L., Ralston, R. A., and Schwartz, S. J. (2017). Flavones: food sources, bioavailability, metabolism, and bioactivity. *Adv. Nutr.* 8, 423–435. doi:10.3945/an.116.012948

Hu, Q., Zhang, W., Wu, Z., Tian, X., Xiang, J., Li, L., et al. (2021). Baicalin and the liver-gut system: pharmacological bases explaining its therapeutic effects. *Pharmacol. Res.* 165, 105444. doi:10.1016/j.phrs.2021.105444

Hu, Y., Hu, X., Jiang, L., Luo, J., Huang, J., Sun, Y., et al. (2025a). Microbiome and metabolomics reveal the effect of gut microbiota on liver regeneration of fatty liver disease. *EBioMedicine* 111, 105482. doi:10.1016/j.ebiom.2024.105482

Hu, L., Luo, Y., Yang, J., and Cheng, C. (2025b). Botanical flavonoids: efficacy, absorption, metabolism and advanced pharmaceutical technology for improving bioavailability. *Molecules* 30, 1184. doi:10.3390/molecules30051184

Huang, F., Zheng, X., Ma, X., Jiang, R., Zhou, W., Zhou, S., et al. (2019). Theabrownin from Pu-erh tea attenuates hypercholesterolemia *via* modulation of gut microbiota and bile acid metabolism. *Nat. Commun.* 10, 4971. doi:10.1038/s41467-019-12896-x

Huang, J., Li, W., Liao, W., Hao, Q., Tang, D., Wang, D., et al. (2020). Green tea polyphenol epigallocatechin-3-gallate alleviates nonalcoholic fatty liver disease and ameliorates intestinal immunity in mice fed a high-fat diet. *Food Funct.* 11, 9924–9935. doi:10.1039/d0fo02152k

Huang, D. Q., El-Serag, H. B., and Loomba, R. (2021). Global epidemiology of NAFLD-Related HCC: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* 18, 223–238. doi:10.1038/s41575-020-00381-6

Huiru, L., Wenwen, M., Dongsheng, Z., Zhen, M., Wenguang, Z., Zhi, C., et al. (2023). Study on mechanism of action of total flavonoids from cortex juglandis mandshuricae against alcoholic liver disease based on "gut-liver axis". *Front. Pharmacol.* 13, 1074286. doi:10.3389/fphar.2022.1074286

Iino, C., Endo, T., Iino, K., Tateda, T., Sato, S., Igarashi, G., et al. (2022). Reduced equol production and gut microbiota features in men with lean nonalcoholic fatty liver disease. *Am. J. Mens. Health* 16, 15579883221115598. doi:10.1177/15579883221115598

Ilaria, B., Flavia Agata, C., and Maria Gisella, C. (2020). Vitamin D and metabolic dysfunction-associated fatty liver disease (MAFLD): an update. *#N/A* 12, 3302. doi:10.3390/nu12113302

Ipsen, D. H., Lykkesfeldt, J., and Tveden-Nyborg, P. (2018). Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell Mol. Life Sci.* 75, 3313–3327. doi:10.1007/s00018-018-2860-6

Jazvinšćak, J. M., Oršolić, N., Karlović, D., and Peitl, V. (2023). Flavonols in action: targeting oxidative stress and neuroinflammation in major depressive disorder. *Int. J. Mol. Sci.*, 24. doi:10.3390/ijms24086888

Jeong, S. H., Kim, H. H., Ha, S. E., Park, M. Y., Bhosale, P. B., Abusaliya, A., et al. (2022). Flavones: six selected flavones and their related signaling pathways that induce apoptosis in cancer. *Int. J. Mol. Sci.* 23, 10965. doi:10.3390/ijms231810965

Jiang, L., Yi, R., Chen, H., and Wu, S. (2025a). Quercetin alleviates metabolic-associated fatty liver disease by tuning hepatic lipid metabolism, oxidative stress and inflammation. *Anim. Biotechnol.* 36, 2442351. doi:10.1080/10495398.2024.2442351

Jiang, Z., Lhamo, G., Ma, M., Ye, X., Chen, J., He, Y., et al. (2025b). Quercetin as a therapeutic agent for acute pancreatitis: a comprehensive review of antioxidant, anti-inflammatory, and immunomodulatory mechanisms. *Front. Pharmacol.* 16, 1587314–2025. doi:10.3389/fphar.2025.1587314

Jin, B., Sun, T., Yu, X. H., Yang, Y. X., and Yeo, A. E. (2012). The effects of TLR activation on T-cell development and differentiation. *Clin. Dev. Immunol.* 2012, 836485. doi:10.1155/2012/836485

Jin, Y., Wang, X., Chen, K., Chen, Y., Zhou, L., Zeng, Y., et al. (2024). Silymarin decreases liver stiffness associated with gut microbiota in patients with metabolic dysfunction-associated steatotic liver disease: a randomized, double-blind, placebo-controlled trial. *Lipids Health Dis.* 23, 239. doi:10.1186/s12944-024-02220-y

Juárez-Fernández, M., Porras, D., Petrov, P., Román-Sagüillo, S., García-Mediavilla, M. V., Soluyanova, P., et al. (2021). The synbiotic combination of Akkermansia muciniphila and quercetin ameliorates early obesity and NAFLD through gut microbiota reshaping and bile acid metabolism modulation. *Antioxidants (Basel)*, 10. doi:10.3390/antiox10122001

Kasem, E. A., Hamza, G., El-Shafai, N. M., Ghanem, N. F., Mahmoud, S., Sayed, S. M., et al. (2025). Thymoquinone-loaded chitosan nanoparticles combat testicular aging and oxidative stress through SIRT1/FOXO3a activation: an *in vivo* and *in vitro* study. *Pharmaceutics* 17, 210. doi:10.3390/pharmaceutics17020210

Katafuchi, T., and Makishima, M. (2022). Molecular basis of bile Acid-FXR-FGF15/19 signaling axis. *Int. J. Mol. Sci.* 23, 6046. doi:10.3390/ijms23116046

Kaushal, N., Singh, M., and Singh Sangwan, R. (2022). Flavonoids: food associations, therapeutic mechanisms, metabolism and nanoformulations. *Food Res. Int.* 157, 111442. doi:10.1016/j.foodres.2022.111442

Ke, X., Chen, Z., Wang, X., Kang, H., and Hong, S. (2023). Quercetin improves the imbalance of Th1/Th2 cells and Treg/Th17 cells to attenuate allergic rhinitis. *Autoimmunity* 56, 2189133. doi:10.1080/08916934.2023.2189133

Kelepouri, D., Mavropoulos, A., Bogdanos, D. P., and Sakkas, L. I. (2018). The role of flavonoids in inhibiting Th17 responses in inflammatory arthritis. *J. Immunol. Res.* 2018, 9324357. doi:10.1155/2018/9324357

Kim, N. Y., Kim, J. H., Lee, Y. H., Lee, E. J., Kim, J., Lim, Y., et al. (2007). O-methylation of flavonoids using DnrK based on molecular docking. *J. Microbiol. Biotechnol.* 17 (12), 1991–1995.

Kim, G., Jang, M. S., Son, Y. M., Seo, M. J., Ji, S. Y., Han, S. H., et al. (2013). Curcumin inhibits CD4+ T cell activation, but augments CD69 expression and TGF-β1-Mediated

generation of regulatory T cells at late phase. *PLoS One* 8, e62300. doi:10.1371/journal.pone.0062300

Kim, M., Kim, N., and Han, J. (2014). Metabolism of Kaempferia parviflora polymethoxyflavones by human intestinal bacterium bautia sp. MRG-PMF1. *J. Agric. Food Chem.* 62, 12377–12383. doi:10.1021/jf504074n

Kiriyama, Y., Tokumaru, H., Sadamoto, H., Kobayashi, S., and Nochi, H. (2024). Effects of phenolic acids produced from food-derived flavonoids and amino acids by the gut microbiota on health and disease. *Molecules* 29, 5102. doi:10.3390/molecules29215102

Kiyama, R. (2023). Estrogenic flavonoids and their molecular mechanisms of action. *#N/A* 114, 109250. doi:10.1016/j.jnutbio.2022.109250

Kliem, C., Merling, A., Giaisi, M., Köhler, R., Krammer, P. H., and Li-Weber, M. (2012). Curcumin suppresses T cell activation by blocking Ca2+ mobilization and nuclear factor of activated T cells (NFAT) activation. *J. Biol. Chem.* 287, 10200–10209. doi:10.1074/jbc.M111.318733

Křížová, L., Dadáková, K., Kašparovská, J., and Kašparovský, T. (2019). Isoflavones. *Molecules* 24, 1076. doi:10.3390/molecules24061076

Kumar, S., and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *ScientificWorldJournal* 2013, 162750. doi:10.1155/2013/162750

Kumari, N., Kumari, R., Dua, A., Singh, M., Kumar, R., Singh, P., et al. (2024). From gut to hormones: unraveling the role of gut microbiota in (phyto)Estrogen modulation in health and disease. *Mol. Nutr. Food Res.* 68, e2300688. doi:10.1002/mnfr.202300688

Kuziel, G. A., Lozano, G. L., Simian, C., Li, L., Manion, J., Stephen-Victor, E., et al. (2025). Functional diversification of dietary plant small molecules by the gut microbiome. *Cell* 188, 1967–83.e22. doi:10.1016/j.cell.2025.01.045

Kwon, J. H., Lee, H. G., Seo, K. H., and Kim, H. (2019). Combination of whole grapeseed flour and newly isolated kefir lactic acid bacteria reduces high-fat-induced hepatic steatosis. *Mol. Nutr. Food Res.* 63, e1801040. doi:10.1002/mnfr.201801040

Latos-Brozio, M., and Masek, A. (2019). Structure-activity relationships analysis of monomeric and polymeric polyphenols (quercetin, rutin and catechin) obtained by various polymerization methods. *Chem. Biodivers.* 16, e1900426. doi:10.1002/cbdv.201900426

Le Barz, M., Daniel, N., Varin, T. V., Naimi, S., Demers-Mathieu, V., Pilon, G., et al. (2019). *In vivo* screening of multiple bacterial strains identifies Lactobacillus rhamnosus Lb102 and Bifidobacterium animalis ssp. Lactis Bf141 as probiotics that improve metabolic disorders in a mouse model of obesity. *FASEB J.* 33, 4921–4935. doi:10.1096/fj.201801672R

Lee, Y. M., Yoon, Y., Yoon, H., Park, H. M., Song, S., and Yeum, K. J. (2017). Dietary anthocyanins against obesity and inflammation. *#N/A* 9, 1089. doi:10.3390/nu9101089

Li, L., and Somerset, S. (2018). Associations between flavonoid intakes and gut microbiota in a group of adults with cystic fibrosis. *#N/A* 10, 1264. doi:10.3390/nu10091264

Li, X., Jin, Q., Yao, Q., Xu, B., Li, L., Zhang, S., et al. (2018). The flavonoid quercetin ameliorates liver inflammation and fibrosis by regulating hepatic macrophages activation and polarization in mice. *Front. Pharmacol.* 9, 72. doi:10.3389/fphar.2018.00072

Li, B., Du, P., Du, Y., Zhao, D., Cai, Y., Yang, Q., et al. (2021a). Luteolin alleviates inflammation and modulates gut microbiota in ulcerative colitis rats. *Life Sci.* 269, 119008. doi:10.1016/j.lfs.2020.119008

Li, X., Yang, L., Li, J., Lin, L., and Zheng, G. (2021b). A flavonoid-rich smilax China L. extract prevents obesity by upregulating the adiponectin-receptor/AMPK signalling pathway and modulating the gut microbiota in mice. *Food Funct.* 12, 5862–5875. doi:10.1039/d1fo00282a

Li, P., Hu, J., Zhao, H., Feng, J., and Chai, B. (2022). Multi-omics reveals inhibitory effect of baicalein on non-alcoholic fatty liver disease in mice. *Front. Pharmacol.* 13, 925349. doi:10.3389/fphar.2022.925349

Li, C., Dai, T., Chen, J., Chen, M., Liang, R., Liu, C., et al. (2023a). Modification of flavonoids: methods and influences on biological activities. *Crit. Rev. Food Sci. Nutr.* 63, 10637–10658. doi:10.1080/10408398.2022.2083572

Li, L., Ji, K., Du, F., Jin, N., Boesch, C., Farag, M. A., et al. (2023b). Does flavonoid supplementation alleviate non-alcoholic fatty liver disease? A systematic review and meta-analysis of randomized controlled trials. *Mol. Nutr. Food Res.* 67, e2300480. doi:10.1002/mnfr.202300480

Li, N., Yin, L., Shang, J., Liang, M., Liu, Z., Yang, H., et al. (2023c). Kaempferol attenuates nonalcoholic fatty liver disease in type 2 diabetic mice *via* the Sirt1/AMPK signaling pathway. *Biomed. Pharmacother.* 165, 115113. doi:10.1016/j.biopha.2023.115113

Li, B., Yan, Y., Zhang, T., Xu, H., Wu, X., Yao, G., et al. (2024a). Quercetin reshapes gut microbiota homeostasis and modulates brain metabolic profile to regulate depression-like behaviors induced by CUMS in rats. *Front. Pharmacol.* 15, 1362464. doi:10.3389/fphar.2024.1362464

Li, B., Jiang, X. F., Dong, Y. J., Zhang, Y. P., He, X. L., Zhou, C. L., et al. (2024b). The effects of Atractylodes macrocephala extract BZEP self-microemulsion based on gut-liver axis HDL/LPS signaling pathway to ameliorate metabolic dysfunction-associated fatty liver disease in rats. *Biomed. Pharmacother.* 175, 116519. doi:10.1016/j.biopha.2024.116519

Li, N., Cui, C., Xu, J., Mi, M., Wang, J., and Qin, Y. (2024c). Quercetin intervention reduced hepatic fat deposition in patients with nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled crossover clinical trial. *Am. J. Clin. Nutr.* 120, 507–517. doi:10.1016/j.ajcnut.2024.07.013

Liang, J., Liu, Y., Liu, J., Li, Z., Fan, Q., Jiang, Z., et al. (2018). Chitosan-functionalized lipid-polymer hybrid nanoparticles for oral delivery of silymarin and enhanced lipid-lowering effect in NAFLD. *J. Nanobiotechnology* 16, 64. doi:10.1186/s12951-018-0391-9

Liang, A., Leonard, W., Beasley, J. T., Fang, Z., Zhang, P., and Ranadheera, C. S. (2024). Anthocyanins-gut microbiota-health axis: a review. *Crit. Rev. Food Sci. Nutr.* 64, 7563–7588. doi:10.1080/10408398.2023.2187212

Lin, Y., Wang, Z. Y., Wang, M. J., Jiang, Z. M., Qin, Y. Q., Huang, T. Q., et al. (2022). Baicalin attenuate diet-induced metabolic syndrome by improving abnormal metabolism and gut microbiota. *Eur. J. Pharmacol.* 925, 174996. doi:10.1016/j.ejphar.2022.174996

Liu, W., Zhou, Y., Qin, Y., Yu, L., Li, R., Chen, Y., et al. (2020). Quercetin intervention alleviates offspring's oxidative stress, inflammation, and tight junction damage in the colon induced by maternal fine particulate matter (PM2.5) exposure through the reduction of bacteroides. *Nutrients* 12 (10), 3095. doi:10.3390/nu12103095

Liu, Z., de Bruijn, W. J. C., Bruins, M. E., and Vincken, J. P. (2021a). Microbial metabolism of Theaflavin-3,3'-digallate and its gut microbiota composition modulatory effects. *J. Agric. Food Chem.* 69, 232–245. doi:10.1021/acs.jafc.0c06622

Liu, X., Sun, R., Li, Z., Xiao, R., Lv, P., Sun, X., et al. (2021b). Luteolin alleviates non-alcoholic fatty liver disease in rats *via* restoration of intestinal mucosal barrier damage and microbiota imbalance involving in gut-liver axis. *Arch. Biochem. Biophys.* 711, 109019. doi:10.1016/j.abb.2021.109019

Liu, C., Gan, R. Y., Chen, D., Zheng, L., Ng, S. B., and Rietjens, I. (2024a). Gut microbiota-mediated metabolism of green tea catechins and the biological consequences: an updated review. *Crit. Rev. Food Sci. Nutr.* 64, 7067–7084. doi:10.1080/10408398.2023.2180478

Liu, H., Li, Y., Jin, Y., Li, X., Wang, D., Yu, X., et al. (2024b). Effects of different natural products in patients with non-alcoholic fatty liver disease—A network meta-analysis of randomized controlled trials. *Phytother. Res.* 38, 3801–3824. doi:10.1002/ptr.8182

Liu, J., Liu, Y., Huang, C., He, C., Yang, T., Ren, R., et al. (2025). Quercetin-driven Akkermansia muciniphila alleviates obesity by modulating bile acid metabolism *via* an ILA/m(6)A/CYP8B1 signaling. *Adv. Sci. (Weinh)* 12, e2412865. doi:10.1002/advs.202412865

Long, J., Xu, Y., Zhang, X., Wu, B., and Wang, C. (2024). Role of FXR in the development of NAFLD and intervention strategies of small molecules. *Arch. Biochem. Biophys.* 757, 110024. doi:10.1016/j.abb.2024.110024

Loo, Y. T., Howell, K., Chan, M., Zhang, P., and Ng, K. (2020). Modulation of the human gut microbiota by phenolics and phenolic fiber-rich foods. *Compr. Rev. Food Sci. Food Saf.* 19 (4), 1268–1298. doi:10.1111/1541-4337.12563

Luo, Y., Jian, Y., Liu, Y., Jiang, S., Muhammad, D., and Wang, W. (2022). Flavanols from nature: a phytochemistry and biological activity review. *Molecules* 27, 719. doi:10.3390/molecules27030719

Luo, L., Chang, Y., and Sheng, L. (2023). Gut-liver axis in the progression of nonalcoholic fatty liver disease: from the microbial derivatives-centered perspective. *Life Sci.* 321, 121614. doi:10.1016/j.lfs.2023.121614

Ma, H. Y., Yamamoto, G., Xu, J., Liu, X., Karin, D., Kim, J. Y., et al. (2020). IL-17 signaling in steatotic hepatocytes and macrophages promotes hepatocellular carcinoma in alcohol-related liver disease. *J. Hepatol.* 72, 946–959. doi:10.1016/j.jhep.2019.12.016

Man, S., Deng, Y., Ma, Y., Fu, J., Bao, H., Yu, C., et al. (2023). Prevalence of liver steatosis and fibrosis in the general population and various high-risk populations: a nationwide study with 5.7 million adults in China. *Gastroenterology* 165, 1025–1040. doi:10.1053/j.gastro.2023.05.053

Martín, M., and Ramos, S. (2021). Impact of dietary flavanols on microbiota, immunity and inflammation in metabolic diseases. *N/A* 13, 850. doi:10.3390/nu13030850

Martín-Mateos, R., and Albillos, A. (2021). The role of the gut-liver axis in metabolic dysfunction-associated fatty liver disease. *Front. Immunol.* 12, 660179. doi:10.3389/fimmu.2021.660179

Martinez, G., Mijares Tussaint, M. R., and De Sanctis, J. (2019). Effects of flavonoids and its derivatives on immune cell responses. *Recent Pat. Inflamm. and Allergy Drug Discov.* 13, 84–104. doi:10.2174/1872213X13666190426164124

Meng, F., Wang, K., Aoyama, T., Grivennikov, S. I., Paik, Y., Scholten, D., et al. (2012). Interleukin-17 signaling in inflammatory, kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. *Gastroenterology* 143, 765–776. doi:10.1053/j.gastro.2012.05.049

Mi, W., Hu, Z., Xu, L., Bian, X., Lian, W., Yin, S., et al. (2022). Quercetin positively affects gene expression profiles and metabolic pathway of antibiotic-treated mouse gut microbiota. *Front. Microbiol.* 13, 983358. doi:10.3389/fmicb.2022.983358

Motallebi, M., Bhia, M., Rajani, H. F., Bhia, I., Tabarraei, H., Mohammadkhani, N., et al. (2022). Naringenin: a potential flavonoid phytochemical for cancer therapy. *Life Sci.* 305, 120752. doi:10.1016/j.lfs.2022.120752

Mu, H., Zhou, Q., Yang, R., Zeng, J., Li, X., Zhang, R., et al. (2020). Naringin attenuates high fat diet induced non-alcoholic fatty liver disease and gut bacterial dysbiosis in mice. *Front. Microbiol.* 11, 585066. doi:10.3389/fmicb.2020.585066

Murota, K., Nakamura, Y., and Uehara, M. (2018). Flavonoid metabolism: the interaction of metabolites and gut microbiota. *#N/A* 82, 600–610. doi:10.1080/09168451.2018.1444467

Namkhah, Z., Naeini, F., Mahdi Rezayat, S., Mehdi, Y., Mansouri, S., and Javad Hosseinzadeh-Attar, M. (2021). Does naringenin supplementation improve lipid profile, severity of hepatic steatosis and probability of liver fibrosis in overweight/obese patients with NAFLD? A randomised, double-blind, placebo-controlled, clinical trial. *Int. J. Clin. Pract.* 75, e14852. doi:10.1111/ijcp.14852

Naudhani, M., Thakur, K., Ni, Z. J., Zhang, J. G., and Wei, Z. J. (2021). Formononetin reshapes the gut microbiota, prevents progression of obesity and improves host metabolism. *Food Funct.* 12, 12303–12324. doi:10.1039/d1fo02942h

Nie, W. Y., Ye, Y., Tong, H. X., and Hu, J. Q. (2025). Herbal medicine as a potential treatment for non-alcoholic fatty liver disease. *World J. Gastroenterol.* 31, 100273. doi:10.3748/wjg.v31.i9.100273

Niwano, Y., Kohzaki, H., Shirato, M., Shishido, S., and Nakamura, K. (2022). Metabolic fate of orally ingested proanthocyanidins through the digestive tract. *Antioxidants (Basel)* 12, 17. doi:10.3390/antiox12010017

Niziński, P., Krajewska, A., Oniszczuk, T., Polak, B., and Oniszczuk, A. (2025). Hepatoprotective effect of Kaempferol-A review. *Molecules* 30, 1913. doi:10.3390/molecules30091913

Oh, K. K., Gupta, H., Ganesan, R., Sharma, S. P., Won, S. M., Jeong, J. J., et al. (2023). The seamless integration of dietary plant-derived natural flavonoids and gut microbiota May ameliorate non-alcoholic fatty liver disease: a network pharmacology analysis. *Artif. Cells Nanomed Biotechnol.* 51, 217–232. doi:10.1080/21691401.2023.2203734

Olveira, A., Augustin, S., Benlloch, S., Ampuero, J., Suárez-Pérez, J. A., Armesto, S., et al. (2023). The essential role of IL-17 as the pathogenetic link between psoriasis and metabolic-associated fatty liver disease. *Life (Basel)* 13, 419. doi:10.3390/life13020419

Ottaviani, J. I., Heiss, C., Spencer, J. P. E., Kelm, M., and Schroeter, H. (2018). Recommending flavanols and procyanidins for cardiovascular health: revisited. *Mol. Asp. Med.* 61, 63–75. doi:10.1016/j.mam.2018.02.001

Paik, J. M., Kabbara, K., Eberly, K. E., Younossi, Y., Henry, L., and Younossi, Z. M. (2022). Global burden of NAFLD and chronic liver disease among adolescents and young adults. *Hepatology* 75, 1204–1217. doi:10.1002/hep.32228

Palencia-Argel, M., Rodríguez-Villamil, H., Bernal-Castro, C., Díaz-Moreno, C., and Fuenmayor, C. A. (2024). Probiotics in anthocyanin-rich fruit beverages: research and development for novel synbiotic products. *Crit. Rev. Food Sci. Nutr.* 64, 110–126. doi:10.1080/10408398.2022.2104806

Pan, C. X., Tang, J., Wang, X. Y., Wu, F. R., Ge, J. F., and Chen, F. H. (2014). Role of interleukin-22 in liver diseases. *Inflamm. Res.* 63, 519–525. doi:10.1007/s00011-014-0727-3

Parafati, M., Lascala, A., La Russa, D., Mignogna, C., Trimboli, F., Morittu, V. M., et al. (2018). Bergamot polyphenols boost therapeutic effects of the diet on non-alcoholic steatohepatitis (NASH) induced by "Junk Food": evidence for anti-inflammatory activity. *Nutrients* 10 (11), 1604. doi:10.3390/nu10111604

Patanè, G. T., Putaggio, S., Tellone, E., Barreca, D., Ficarra, S., Maffei, C., et al. (2023). Catechins and proanthocyanidins involvement in metabolic syndrome. *Int. J. Mol. Sci.* 24, 9228. doi:10.3390/ijms24119228

Pei, R., Liu, X., and Bolling, B. (2020). Flavonoids and gut health. *Curr. Opin. Biotechnol.* 61, 153–159. doi:10.1016/j.copbio.2019.12.018

Peiseler, M., Schwabe, R., Hampe, J., Kubes, P., Heikenwälder, M., and Tacke, F. (2022). Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease - novel insights into cellular communication circuits. *J. Hepatol.* 77, 1136–1160. doi:10.1016/j.jhep.2022.06.012

Peng, M., Tabashsum, Z., Anderson, M., Truong, A., Houser, A. K., Padilla, J., et al. (2020). Effectiveness of probiotics, prebiotics, and prebiotic-like components in common functional foods. *#N/A* 19, 1908–1933. doi:10.1111/1541-4337.12565

Peng, L. Y., Shi, H. T., Tan, Y. R., Shen, S. Y., Yi, P. F., Shen, H. Q., et al. (2021). Baicalin inhibits APEC-Induced lung injury by regulating gut microbiota and SCFA production. *Food Funct.* 12, 12621–12633. doi:10.1039/d1fo02407h

Peng, W., He, C. X., Li, R. L., Qian, D., Wang, L. Y., Chen, W. W., et al. (2024). Zanthoxylum bungeanum amides ameliorates nonalcoholic fatty liver *via* regulating gut microbiota and activating AMPK/Nrf2 signaling. *J. Ethnopharmacol.* 318, 116848. doi:10.1016/j.jep.2023.116848

Piero, P., Mohamad, K., Laura, M., Valeria, P., Valeria, I., Annarita, G., et al. (2024). Metabolic dysfunction–associated steatotic liver disease: from pathogenesis to current therapeutic options. *Int. J. Mol. Sci.* 25, 5640. doi:10.3390/ijms25115640

Ponugoti, B., Kim, D. H., Xiao, Z., Smith, Z., Miao, J., Zang, M., et al. (2010). SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. *J. Biol. Chem.* 285, 33959–33970. doi:10.1074/jbc.M110.122978

Popiolek-Kalisz, J., and Fornal, E. (2022). The impact of flavonols on cardiovascular risk. *#N/A* 14, 1973. doi:10.3390/nu14091973

Porras, D., Nistal, E., Martínez-Flórez, S., Pisonero-Vaquero, S., Olcoz, J. L., Jover, R., et al. (2017). Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease in mice is mediated by modulating intestinal microbiota imbalance and related gut-liver axis activation. *Free Radic. Biol. Med.* 102, 188–202. doi:10.1016/j.freeradbiomed.2016.11.037

Prananda, A. T., Halim, P., and Syahputra, R. A. (2025). Targeting miRNA with flavonoids: unlocking novel pathways in cardiovascular disease management. *Front. Pharmacol.* 16, 1532986. doi:10.3389/fphar.2025.1532986

Pu, P., Wang, X. A., Salim, M., Zhu, L. H., Wang, L., Chen, K. J., et al. (2012). Baicalein, a natural product, selectively activating AMPKα(2) and ameliorates metabolic disorder in diet-induced mice. *Mol. Cell Endocrinol.* 362, 128–138. doi:10.1016/j.mce.2012.06.002

Qi, W., Cao, X., Chen, Y., Chen, H., Zhang, N., Liu, R., et al. (2025). JiGuCao capsule formula alleviates metabolic fatty liver disease by regulating the gut-liver axis and lipid metabolism. *Phytomedicine* 140, 156559. doi:10.1016/j.phymed.2025.156559

Qian, D., Zhang, Q., He, C. X., Guo, J., Huang, X. T., Zhao, J., et al. (2024). Haihonghua medicinal liquor is a reliable remedy for fracture by promotion of osteogenic differentiation *via* activation of PI3K/Akt pathway. *J. Ethnopharmacol.* 330, 118234. doi:10.1016/j.jep.2024.118234

Qu, Y., Li, X., Xu, F., Zhao, S., Wu, X., Wang, Y., et al. (2021). Kaempferol alleviates murine experimental colitis by restoring gut microbiota and inhibiting the LPS-TLR4-NF-κB axis. *Front. Immunol.* 12, 679897. doi:10.3389/fimmu.2021.679897

Rao, Y., Kuang, Z., Li, C., Guo, S., Xu, Y., Zhao, D., et al. (2021). Gut Akkermansia muciniphila ameliorates metabolic dysfunction-associated fatty liver disease by regulating the metabolism of L-aspartate *via* gut-liver axis. *Gut Microbes* 13, 1–19. doi:10.1080/19490976.2021.1927633

Riazi, K., Azhari, H., Charette, J. H., Underwood, F. E., King, J. A., Afshar, E. E., et al. (2022). The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol. Hepatol.* 7, 851–861. doi:10.1016/s2468-1253(22)00165-0

Ribeiro, A. E., Monteiro, N. E. S., Moraes, A. V. G., Costa-Paiva, L. H., and Pedro, A. O. (2018). Can the use of probiotics in association with isoflavone improve the symptoms of genitourinary syndrome of menopause? Results from a randomized controlled trial. *#N/A* 26, 643–652. doi:10.1097/gme.0000000000001279

Rong, L., Junyan, Z., Ran, W., Xinming, Q., Yaokai, C., Hongjuan, C., et al. (2023). Advancements in the treatment of non-alcoholic fatty liver disease (NAFLD). *Front. Endocrinol. (Lausanne)* 13, 1087260. doi:10.3389/fendo.2022.1087260

Round, J. L., Lee, S. M., Li, J., Tran, G., Jabri, B., Chatila, T. A., et al. (2011). The toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 332, 974–977. doi:10.1126/science.1206095

Sayin, S. I., Wahlström, A., Felin, J., Jäntti, S., Marschall, H. U., Bamberg, K., et al. (2013). Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab.* 17, 225–235. doi:10.1016/j.cmet.2013.01.003

Seo, K. H., Jeong, J., and Kim, H. (2020). Synergistic effects of heat-killed kefir paraprobiotics and flavonoid-rich prebiotics on Western diet-induced obesity. *#N/A* 12, 2465. doi:10.3390/nu12082465

Seyam, S., Nordin, N. A., and Alfatama, M. (2020). Recent progress of chitosan and chitosan derivatives-based nanoparticles: pharmaceutical perspectives of oral insulin delivery. *Pharm. (Basel)* 13, 307. doi:10.3390/ph13100307

Shabbir, U., Rubab, M., Daliri, E. B., Chelliah, R., Javed, A., and Oh, D. H. (2021). Curcumin, quercetin, catechins and metabolic diseases: the role of gut microbiota. *#N/A* 13, 206. doi:10.3390/nu13010206

Sharma, R., Kumari, M., Kumari, A., Sharma, A., Gulati, A., Gupta, M., et al. (2019). Diet supplemented with phytochemical epigallocatechin gallate and probiotic Lactobacillus fermentum confers second generation synbiotic effects by modulating cellular immune responses and antioxidant capacity in aging mice. *Eur. J. Nutr.* 58, 2943–2957. doi:10.1007/s00394-018-01890-6

Shen, N., Wang, T., Gan, Q., Liu, S., Wang, L., and Jin, B. (2022). Plant flavonoids: classification, distribution, biosynthesis, and antioxidant activity. *Food Chem.* 383, 132531. doi:10.1016/j.foodchem.2022.132531

Sherouk, F., and Joseph, M. P. (2023). Metabolic-associated fatty liver disease: a disastrous human health challenge. *Endocrinol. Metab. Clin. North Am.* 52. doi:10.1016/j.ecl.2023.03.001

Shi, M., Shi, Y. L., Li, X. M., Yang, R., Cai, Z. Y., Li, Q. S., et al. (2018). Food-grade encapsulation systems for (-)-Epigallocatechin gallate. *Molecules* 23, 445. doi:10.3390/molecules23020445

Shi, P. Z., Wang, J. W., Wang, P. C., Han, B., Lu, X. H., Ren, Y. X., et al. (2021). Urolithin a alleviates oxidative stress-induced senescence in nucleus pulposus-derived mesenchymal stem cells through SIRT1/PGC-1α pathway. *World J. Stem Cells* 13, 1928–1946. doi:10.4252/wjsc.v13.i12.1928

Shi, Z., Zhang, C., Lei, H., Chen, C., Cao, Z., Song, Y., et al. (2022). Structural insights into amelioration effects of quercetin and its glycoside derivatives on NAFLD in mice by modulating the gut microbiota and host metabolism. *J. Agric. Food Chem.* 70, 14732–14743. doi:10.1021/acs.jafc.2c06212

Shoji, T., Masumoto, S., and Miura, T. (2023). Mechanism of procyanidins for health functionality by improving the intestinal environment. *Biosci. Biotechnol. Biochem.* 88, 345–351. doi:10.1093/bbb/zbad174

Silva, A. K. S., and Peixoto, C. A. (2018). Role of peroxisome proliferator-activated receptors in non-alcoholic fatty liver disease inflammation. *Cell Mol. Life Sci.* 75, 2951–2961. doi:10.1007/s00018-018-2838-4

Song, H., Shen, X., Wang, F., Li, Y., and Zheng, X. (2021a). Black current anthocyanins improve lipid metabolism and modulate gut microbiota in high-fat diet-induced Obese mice. *Mol. Nutr. Food Res.* 65, e2001090. doi:10.1002/mnfr.202001090

Song, H., Shen, X., Zhou, Y., and Zheng, X. (2021b). Black rice anthocyanins alleviate hyperlipidemia, liver steatosis and insulin resistance by regulating lipid metabolism and gut microbiota in Obese mice. *Food Funct.* 12, 10160–10170. doi:10.1039/d1fo01394g

Stevens Barron, J. C., Chapa Gonzalez, C., Alvarez Parrilla, E., and De la Rosa, L. A. (2023). Nanoparticle-mediated delivery of flavonoids: impact on proinflammatory cytokine production: a systematic review. *Biomolecules* 13, 1158. doi:10.3390/biom13071158

Sun, W. L., Li, X. Y., Dou, H. Y., Wang, X. D., Li, J. D., Shen, L., et al. (2021). Myricetin supplementation decreases hepatic lipid synthesis and inflammation by modulating gut microbiota. *Cell Rep.* 36, 109641. doi:10.1016/j.celrep.2021.109641

Sun, X., Ge, N., Liang, Q., Wang, Q., Yu, H., and Jin, M. (2025). Effect of the total flavonoids of Dracocephalum moldavica L. on metabolic associated fatty liver disease in rats. *Front. Pharmacol.* 16, 1549515. doi:10.3389/fphar.2025.1549515

Talib, W. H., Awajan, D., Alqudah, A., Alsawwaf, R., Althunibat, R., Abu AlRoos, M., et al. (2024). Targeting cancer hallmarks with epigallocatechin gallate (EGCG): mechanistic basis and therapeutic targets. *Molecules* 29, 1373. doi:10.3390/molecules29061373

Tan, S., Caparros-Martin, J. A., Matthews, V. B., Koch, H., O'Gara, F., Croft, K. D., et al. (2018). Isoquercetin and inulin synergistically modulate the gut microbiome to prevent development of the metabolic syndrome in mice fed a high fat diet. *Sci. Rep.* 8, 10100. doi:10.1038/s41598-018-28521-8

Tan, P., Jin, L., Qin, X., and He, B. (2022). Natural flavonoids: potential therapeutic strategies for non-alcoholic fatty liver disease. *Front. Pharmacol.* 13, 1005312. doi:10.3389/fphar.2022.1005312

Tauil, R. B., Golono, P. T., de Lima, E. P., de Alvares Goulart, R., Guiguer, E. L., Bechara, M. D., et al. (2024). Metabolic-associated fatty liver disease: the influence of oxidative stress, inflammation, mitochondrial dysfunctions, and the role of polyphenols. *Pharm. (Basel).* 17, 1354. doi:10.3390/ph17101354

Thilakarathna, W., and Rupasinghe, H. P. V. (2024). Proanthocyanidins-based synbiotics as a novel strategy for nonalcoholic fatty liver disease (NAFLD) risk reduction. *Molecules* 29, 709. doi:10.3390/molecules29030709

Tian, L., Tan, Y., Chen, G., Wang, G., Sun, J., Ou, S., et al. (2019). Metabolism of anthocyanins and consequent effects on the gut microbiota. *Crit. Rev. Food Sci. Nutr.* 59, 982–991. doi:10.1080/10408398.2018.1533517

Ting, X., Liyong, L., Yan, L., Kang, S., Liyong, L., and Liang, Z. (2022). Hawk tea flavonoids as natural hepatoprotective agents alleviate acute liver damage by reshaping the intestinal microbiota and modulating the Nrf2 and NF-κB signaling pathways. *N/A* 14, 3662. doi:10.3390/nu14173662

Tong, J., Zeng, Y., Xie, J., Xiao, K., Li, M., and Cong, L. (2022). Association between flavonoid and subclasses intake and metabolic associated fatty liver disease in U.S. adults: results from national health and nutrition examination survey 2017-2018. *Front. Nutr.* 9, 1074494. doi:10.3389/fnut.2022.1074494

Vancamelbeke, M., and Vermeire, S. (2017). The intestinal barrier: a fundamental role in health and disease. *Expert Rev. Gastroenterol. Hepatol.* 11, 821–834. doi:10.1080/17474124.2017.1343143

Velliou, R. I., Legaki, A. I., Nikolakopoulou, P., Vlachogiannis, N. I., and Chatzigeorgiou, A. (2023). Liver endothelial cells in NAFLD and transition to NASH and HCC. *Cell Mol. Life Sci.* 80, 314. doi:10.1007/s00018-023-04966-7

Wallace, T. C., and Giusti, M. M. (2015). Anthocyanins. *Adv. Nutr.* 6, 620–622. doi:10.3945/an.115.009233

WalyEldeen, A. A., Sabet, S., El-Shorbagy, H. M., Abdelhamid, I. A., and Ibrahim, S. A. (2023). Chalcones: promising therapeutic agents targeting key players and signaling pathways regulating the hallmarks of cancer. *Chem-Biol. Interact.* 369, 110297. doi:10.1016/j.cbi.2022.110297

Wan, X., Ma, J., Bai, H., Hu, X., Ma, Y., Zhao, M., et al. (2025). Drug advances in NAFLD: individual and combination treatment strategies of natural products and small-synthetic-molecule drugs. *Biomolecules* 15, 140. doi:10.3390/biom15010140

Wang, H. K., Yeh, C. H., Iwamoto, T., Satsu, H., Shimizu, M., and Totsuka, M. (2012). Dietary flavonoid naringenin induces regulatory T cells *via* an Aryl hydrocarbon receptor mediated pathway. *J. Agric. Food Chem.* 60, 2171–2178. doi:10.1021/jf204625y

Wang, J., Niu, X., Wu, C., and Wu, D. (2018). Naringenin modifies the development of lineage-specific effector CD4(+) T cells. *Front. Immunol.* 9, 2267. doi:10.3389/fimmu.2018.02267

Wang, J. F., Liu, S. S., Song, Z. Q., Xu, T. C., Liu, C. S., Hou, Y. G., et al. (2020). Naturally occurring flavonoids and isoflavonoids and their microbial transformation: a review. *Molecules* 25, 5112. doi:10.3390/molecules25215112

Wang, S., Li, X. Y., Ji, H. F., and Shen, L. (2022a). Modulation of gut microbiota by glycyrrhizic acid May contribute to its anti-NAFLD effect in rats fed a high-fat diet. *Life Sci.* 310, 121110. doi:10.1016/j.lfs.2022.121110

Wang, M., Zhang, Z., Sun, H., He, S., Liu, S., Zhang, T., et al. (2022b). Research progress of anthocyanin prebiotic activity: a review. *Phytomedicine* 102, 154145. doi:10.1016/j.phymed.2022.154145

Wang, M., Lu, Y., Wu, Q., Chen, G., Zhao, H., Ho, C. T., et al. (2023). Biotransformation and gut microbiota-mediated bioactivity of flavonols. *J. Agric. Food Chem.* 71, 8317–8331. doi:10.1021/acs.jafc.3c01087

Wendlocha, D., Kubina, R., Krzykawski, K., and Mielczarek-Palacz, A. (2024). Selected flavonols targeting cell death pathways in cancer therapy: the latest achievements in research on apoptosis, autophagy, necroptosis, pyroptosis, ferroptosis, and. *Cuproptosis. #N/A*, 16. doi:10.3390/nu16081201

Wu, C., and Wang, G. (2025). Research progress on the mechanism of baicalein in Scutellaria baicalensis against liver fibrosis. *J. Contemp. Med. Pract.* 7 (7), 1–5. doi:10.53469/jcmp.2025.07(04).01

Wu, H. T., Lin, X. X., Yang, X. L., Ding, Y., Wang, J. L., Liu, C. L., et al. (2024). Kaempferol attenuates inflammation in lipopolysaccharide-induced gallbladder epithelial cells by inhibiting the MAPK/NF-κB signaling pathway. *Chem. Biol. Drug Des.* 103, e14519. doi:10.1111/cbdd.14519

Xiaopeng, Z., Mingfeng, X., and Xin, G. (2022). Update on genetics and epigenetics in metabolic associated fatty liver disease. *Ther. Adv. Endocrinol. Metabolism* 13, 20420188221132138. doi:10.1177/20420188221132138

Xiong, H.-H., Lin, S.-Y., Chen, L.-L., Ouyang, K.-H., and Wang, W.-J. (2023). The interaction between flavonoids and intestinal microbes: a review. *Foods* 12, 320. doi:10.3390/foods12020320

Xu, H., Yuan, M., Niu, K., Yang, W., Jiang, M., Zhang, L., et al. (2024). Involvement of bile acid metabolism and gut microbiota in the amelioration of experimental metabolism-associated fatty liver disease by nobiletin. *Molecules* 29, 976. doi:10.3390/molecules29050976

Xue, Y., Zhu, L., and Yi, T. (2017). Fingerprint analysis of resina draconis by ultra-performance liquid chromatography. *Chem. Cent. J.* 11, 67. doi:10.1186/s13065-017-0299-8

Yamagata, K., and Yamori, Y. (2021). Potential effects of soy isoflavones on the prevention of metabolic syndrome. *Molecules* 26, 5863. doi:10.3390/molecules26195863

Yang, Y., Zhang, X., Xu, M., Wu, X., Zhao, F., and Zhao, C. (2018). Quercetin attenuates collagen-induced arthritis by restoration of Th17/Treg balance and activation of heme oxygenase 1-mediated anti-inflammatory effect. *Int. Immunopharmacol.* 54, 153–162. doi:10.1016/j.intimp.2017.11.013

Yang, G., Hong, S., Yang, P., Sun, Y., Wang, Y., Zhang, P., et al. (2021). Discovery of an ene-reductase for initiating flavone and flavonol catabolism in gut bacteria. *Nat. Commun.* 12, 790. doi:10.1038/s41467-021-20974-2

Yang, S., Duan, H., Yan, Z., Xue, C., Niu, T., Cheng, W., et al. (2025). Luteolin alleviates ulcerative colitis in mice by modulating gut microbiota and plasma metabolism. *#N/A* 17, 203. doi:10.3390/nu17020203

Yi, T., Chen, H.-B., Zhao, Z.-Z., Jiang, Z.-H., Cai, S.-Q., and Wang, T.-M. (2009a). Comparative analysis of the major constituents in the traditional Tibetan medicinal plants Saussurea laniceps and S. medusa by LC–DAD–MS. *#N/A* 70, 957–962. doi:10.1365/s10337-009-1240-7

Yi, T., Chen, H.-B., Zhao, Z.-Z., Jiang, Z.-H., Cai, S.-Q., and Wang, T.-M. (2009b). Identification and determination of the major constituents in the traditional uighur medicinal plant Saussurea involucrata by LC-DAD-MS. *#N/A* 69, 537–542. doi:10.1365/s10337-008-0923-9

Yi, T., Tang, Y., Zhang, J., Zhao, Z., Yang, Z., and Chen, H. (2012). Characterization and determination of six flavonoids in the ethnomedicine "Dragon's Blood" by UPLC-PAD-MS. *Chem. Cent. J.* 6, 116. doi:10.1186/1752-153x-6-116

Yi, M., Manzoor, M., Yang, M., Zhang, H., Wang, L., Zhao, L., et al. (2024). Silymarin targets the FXR protein through microbial metabolite 7-keto-deoxycholic acid to treat MASLD in Obese mice. *Phytomedicine* 133, 155947. doi:10.1016/j.phymed.2024.155947

Yoon, Y. S., Seo, W. Y., Lee, M. W., Kim, S. T., and Koo, S. H. (2009). Salt-inducible kinase regulates hepatic lipogenesis by controlling SREBP-1c phosphorylation. *J. Biol. Chem.* 284, 10446–10452. doi:10.1074/jbc.M900096200

Younossi, Z. M., Golabi, P., Paik, J. M., Henry, A., Van Dongen, C., and Henry, L. (2023). The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology* 77, 1335–1347. doi:10.1097/hep.0000000000000004

Yu, Z., Xiaojia, L., Wei, Z., Jian, Z., Aiting, W., Jing, W., et al. (2024). Baicalin circumvents anti-PD-1 resistance by regulating the gut microbiota metabolite short-chain fatty acids. *Pharmacol. Res.* 199, 107033. doi:10.1016/j.phrs.2023.107033

Yuan, M., Sun, T., Zhang, Y., Guo, C., Wang, F., Yao, Z., et al. (2024). Quercetin alleviates insulin resistance and repairs intestinal barrier in Db/Db mice by modulating gut microbiota. *#N/A* 16, 1870. doi:10.3390/nu16121870

Zeng, S. L., Li, S. Z., Xiao, P. T., Cai, Y. Y., Chu, C., Chen, B. Z., et al. (2020). Citrus polymethoxyflavones attenuate metabolic syndrome by regulating gut microbiome and amino acid metabolism. *Sci. Adv.* 6, eaax6208. doi:10.1126/sciadv.aax6208

Zhang, Y., Shan, L., Hua, Y., Wang, D., Zeng, H., Liu, R., et al. (2013). Baicalein selectively induces apoptosis in activated lymphocytes and ameliorates concanavalin A-Induced hepatitis in mice. *PLoS One* 8, e69592. doi:10.1371/journal.pone.0069592

Zhang, Y., Bobe, G., Revel, J. S., Rodrigues, R. R., Sharpton, T. J., Fantacone, M. L., et al. (2020). Improvements in metabolic syndrome by xanthohumol derivatives are linked to altered gut microbiota and bile acid metabolism. *Mol. Nutr. Food Res.* 64, e1900789. doi:10.1002/mnfr.201900789

Zhang, Z., Li, X., Sang, S., McClements, D. J., Chen, L., Long, J., et al. (2022). A review of nanostructured delivery systems for the encapsulation, protection, and delivery of silymarin: an emerging nutraceutical. *Food Res. Int.* 156, 111314. doi:10.1016/j.foodres.2022.111314

Zhang, Y., Ren, L., Tian, Y., Guo, X., Wei, F., and Zhang, Y. (2024a). Signaling pathways that activate hepatic stellate cells during liver fibrosis. *Front. Med. (Lausanne)* 11, 1454980. doi:10.3389/fmed.2024.1454980

Zhang, H., Liang, S., Yin, K., Mo, Y., Li, Y., Lv, Y., et al. (2024b). Urinary equol and equol-predicting microbial genera are favorably associated with body fat measures among Chinese adults. *J. Nutr.* 154, 2843–2851. doi:10.1016/j.tjnut.2024.07.024

Zhang, S., Shen, C., Di, H., Wang, Y., and Guan, F. (2025a). Regulatory mechanisms of phenolic acids in metabolic dysfunction-associated steatotic liver disease: a review. *Antioxidants* 14, 760. doi:10.3390/antiox14070760

Zhang, Q., He, C., Guo, J., Tang, D., Qian, D., Zheng, C., et al. (2025b). Hydroxy-α-sanshool-loaded adipose-targeted mesoporous silica nanoparticles induce white adipose browning and reduce obesity by activating TRPV1. *Bio-Design Manuf.* 8, 288–309. doi:10.1631/bdm.2400248

Zhao, J., Sun, Y., Yuan, C., Li, T., Liang, Y., Zou, H., et al. (2023). Quercetin ameliorates hepatic fat accumulation in high-fat diet-induced Obese mice *via* PPARs. *Food Funct.* 14, 1674–1684. doi:10.1039/d2fo03013f

Zhou, M., Ma, J., Kang, M., Tang, W., Xia, S., Yin, J., et al. (2024). Flavonoids, gut microbiota, and host lipid metabolism. *Eng. Life Sci.* 24, 2300065. doi:10.1002/elsc.202300065

Zhu, H., Guo, L., Yu, D., and Du, X. (2022). New insights into immunomodulatory properties of lactic acid bacteria fermented herbal medicines. *Front. Microbiol.* 13, 1073922. doi:10.3389/fmicb.2022.1073922

Zhu, X., Dai, X., Zhao, L., Li, J., Zhu, Y., He, W., et al. (2024). Quercetin activates energy expenditure to combat metabolic syndrome through modulating gut microbiota-bile acids crosstalk in mice. *Gut Microbes* 16, 2390136. doi:10.1080/19490976.2024.2390136

Glossary

ACACA	Acetyl-CoA Carboxylase Alpha
ACC	Acetyl-CoA Carboxylase
ALK5	TGF-β type I receptor
AREs	antioxidant response elements
BA	bile acid
BMI	body mass index
BSH	bile salt hydrolases
ChREBP	carbohydrate-responsive element-binding protein
CPT1	Carnitine Palmitoyltransferase 1
CS-LPNs	chitosan-modified, silymarin-loaded lipid-polymer hybrid nanoparticles
CYP2E1	cytochrome P450 2E1
CYP7A1	cytochrome P450 7A1
DNL	*de novo* lipogenesis
ECM	extracellular matrix
EGCG	Epigallocatechin gallate
FA	fatty acid
FAO	fatty acid oxidation
FASN	Fatty Acid Synthase
FGF19	fibroblast growth factor 19
FGFR4	FGF19 receptor
FLR	flavone reductase
GCLC	glutamate-cysteine ligase catalytic subunit
GGT	γ-glutamyl transpeptidase
GPCRs	G-protein coupled receptors
GSH	glutathione
GSF	Grapeseed flour
HCC	hepatocellular carcinoma
HDACs	histone deacetylases
HDL	high-density lipoprotein
HFD	high-fat diet-fed
HO-1	Heme Oxygenase-1
HSCs	Hepatic Stellate Cells
IECs	Intestinal Epithelial Cells
IL-6	interleukin-6
JNK	c-Jun N-terminal kinase
KCs	Kupffer cells
LDL	low-density lipoprotein
LPS	lipopolysaccharides
LRP5/6	low-density lipoprotein receptor-related protein 5/6
MAFLD	Metabolic dysfunction-associated fatty liver disease
MAPK	mitogen-activated protein kinase
MASH	Metabolic dysfunction-associated steatohepatitis
MDA	malondialdehyde
MRI	magnetic resonance imaging
NFS	MAFLD fibrosis score
NHANES	National Health and Nutrition Examination Survey
NQO1	NAD(P)H quinone oxidoreductase 1
Nrf2	nuclear factor erythroid 2-related factor 2
PMFs	Polymethoxyflavones
PPARs	peroxisome proliferator-activated receptors
PPARα	Peroxisome Proliferator-Activated Receptor alpha
PSA	polysaccharide A
ROS	reactive oxygen species
SCFAs	Short-chain fatty acids
SFB	Segmentous filamentous bacteria
SREBP-1c	Sterol Regulatory Element-Binding Protein 1c
TC	total cholesterol
TG	triglyceride
TGF-β	Transforming growth factor-beta
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4
Treg	T regulatory

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