Metabolic dysfunction-associated steatotic liver disease: Pathogenesis, model and treatment (Review)

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Received May 19, 2025; Accepted September 22, 2025

DOI: 10.3892/ijmm.2025.5668

Abstract. Metabolic dysfunction-associated steatotic liver disease (MASLD) is caused by multiple factors that lead to the buildup of steatosis and fat deposition in hepatocytes. These changes are the primary hallmarks of the disease and result in significant impairment of liver function. Consequently, the quality of life of patients and their ability to work are adversely affected. The pathogenesis of MASLD involves both Western and Chinese medicines, with these mechanisms markedly influencing the onset and progression of MASLD; they are not independent but rather interrelated. Conducting histopathological diagnosis of MASLD in the liver is challenging in humans. Consequently, both *in vivo* and *in vitro* models are essential. Researchers must select appropriate methods and model types to establish MASLD models that most suitably mimic the human body. Currently, both pharmacological and non-pharmacological treatments have some efficacy in improving the condition of MASLD and the combination of the two is more helpful in providing more effective treatment for patients, but further research and clinical trials are needed to verify in the future. Therefore, the present review comprehensively summarized the pathogenesis, model and treatment of MASLD. It will provide an important basis for subsequent research on MASLD.

Contents

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) was proposed by Ludwig in 1986. It refers to a fatty liver disease characterized by excessive fat deposition in hepatocytes (with a fat content of ≥5%), excluding alcohol and other factors related to chronic liver diseases (1-4). In 2020, the Asian Pacific Association for the Study of the Liver proposed the term 'metabolic dysfunction-associated fatty liver disease (MAFLD)', emphasizing the role of metabolic disorders in the progression of fatty liver disease (5). In 2023, an international panel of experts reached a consensus and the European Association for the Study of the Liver, from an etiological perspective, renamed it as 'metabolic dysfunction-associated steatotic liver disease (MASLD)' (6,7). This change highlights the critical role of metabolic abnormalities in the occurrence and development of the disease (8). While avoiding 'stigmatization', this concept also provides explanations for the coexistence of MASLD and alcoholic-associated liver disease (9,10).

The etiology of MASLD is complex, with its core being the interplay between metabolic dysfunction (such

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDAA, choline deficient amino acid-defined; DXM, dexamethasone; ER, endoplasmic reticulum; FFA, free fatty acid; IR, insulin resistance; MCD, methionine choline deficiency; MSG, monosodium glutamate; mTOR, mammalian target of rapamycin; NO, nitric oxide; OA, oleic acid; PHH, primary human hepatocytes; ROS, reactive oxygen species; SOD, superoxide dismutase; TG, triglyceride; THR-α, thyroid hormone receptor-α; TNF-α, tumor necrosis factor-α

Key words: metabolic dysfunction-associated steatotic liver disease, pathogenesis, model, treatment

as insulin resistance and dyslipidemia) and multifactorial elements (including genetics, environment and lifestyle), rather than being attributed to a single factor. Researchers have established various animal models of MASLD using diverse methodologies to investigate its pathophysiological characteristics. Currently, common interventional drugs mainly include: Farnesoid X receptor agonists, peroxisome proliferator-activated receptor (PPAR)α/γ/δ agonists, GLP-1 agonists and fibroblast growth factor 19/21 analogs (11). For individuals with a body mass index >35, bariatric surgery is primarily recommended. In the treatment of MASLD, although chemical drugs have advanced rapidly, their efficacy remains limited with notable adverse effects. By contrast, traditional Chinese medicine (TCM) not only enables comprehensive regulation targeting the multifactorial pathogenesis of MASLD, but also strives to improve the quality of life of patients, thereby opening up new possibilities for the management of MASLD (12).

In recent years, influenced by unhealthy lifestyles and dietary habits, the prevalence of MASLD has been increasing annually. The global incidence rate has reached 25-35%, with a prevalence of 29.2% in China (13). It is projected that the number of MASLD patients in China will reach 315 million by 2030, imposing a heavy economic burden on society (14). Even mild fatty liver disease increases the risk of death by 71% and this risk is positively associated with the severity of the disease (14). Currently, while the incidence of viral liver diseases is on the decline, the incidence of MASLD is rising, making early intervention for MASLD an urgent priority. The present study analyzed the pathogenesis, experimental models and therapeutic approaches of MASLD, aiming to provide novel scientific insights and strategies for future research, clinical treatment and drug development.

2. Methods

To compile the research progress on the pathogenesis, model and treatment of MASLD as comprehensively as possible, the present study searched for 'pathogenesis', 'model', 'treatment' and 'traditional Chinese medicine' in existing scientific databases. The references related to MASLD were obtained from both online and offline databases, spanning the period from 1980-2025 with a total of 385 references. Online databases included PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (https://www.webofscience.com/), Elsevier (https://www.elsever.com/), Sci-Hub (https://sci-hub.st/), Wiley (https://www.wiley.com/), SpringerLink (https://link.springer.com/), Google Scholar (https://scholar.google.com/), EMBASE (https://www.embase.com/), Cochrane Library and China National Knowledge Infrastructure (CNKI) (https://www.cnki.net/). Other references were obtained from ancient Chinese books, pharmacopoeias and other articles. The present authors used BioGDP (https://biogdp.com/) as well as Adobe Illustrator (Adobe Systems, Inc.) for graphing.

3. Pathogenesis of MASLD

Research on the pathogenesis of MASLD in chemical pharmaceuticals. The pathogenesis of MASLD remains unclear due to its complexity and diversity. As the main metabolic organ, the liver's metabolism is closely connected with multiple organs and systems in the human body. There are intricate links between these organs and systems. Some consider that the hypothesis of 'multiple parallel strikes' could be an improved explanation of the causes of MASLD development and these strikes included oxidative stress, endoplasmic reticulum (ER) stress, lipid metabolism disorders, abnormal adipokines and cytokines production and mitochondrial dysfunction (15). One study found that mitochondrial dysfunction might be a central factor in the development of MASLD and other factors that cause disease progression also involve mitochondrial dysfunction (16). The pathogenesis of MASLD is now being investigated by combining different pathogenic pathways for an improved understanding of metabolic diseases.

The 'multiple strikes' hypothesis. The 'first strike' mainly refers to insulin resistance (IR) caused by obesity and type 2 diabetes mellitus (T2DM) and hepatic steatosis caused by excessive accumulation of triglyceride (TG) and cholesterol in the liver parenchymal cells in the form of lipid droplets. The 'Two-hit' hypothesis includes inflammatory cytokines, lipid peroxidation, mitochondrial dysfunction and oxidative stress (17), of which oxidative stress is the primary driver (18). This hypothesis posits that with steatosis, the liver undergoes ER stress, oxidative stress and mitochondrial dysfunction, leading to an increase in oxidative metabolites, hepatocellular damage and secretion of a large number of inflammatory factors, which ultimately leads to further deterioration into liver disease, liver fibrosis, cirrhosis, inflammatory necrosis and other reactions (19,20). In the early stages of MASLD, classical activation of macrophage M1 in the liver can promote further inflammation and IR, as well as steatosis (21). Among them, inflammatory cytokines such as interleukin (IL)-6, IL-8, tumor necrosis factor-α (TNF-α), IL-1β and cyclooxygenase-2 contribute to the development of chronic inflammatory diseases (22). IL-6 has been shown to have both anti-inflammatory and pro-inflammatory effects, but its role in MASLD is controversial. IL-10, produced primarily by monocytes and B cells, is an anti-inflammatory factor with immunomodulatory properties (22). Other inflammatory cells of the innate immune system, such as natural killer T cell and natural killer cells, also play the important roles in the pathogenesis of MASLD and MASH (21). However, the factors that induce MASLD in clinical practice are diverse and as research has deepened, the two-hit hypothesis has already shown certain limitations and cannot fully explain the pathogenesis of MASLD. Recently, the 'three-strike' and 'multiple-strike' hypotheses have been proposed, which include gut microbiota toxins, IR, cytokines and inflammation, oxidative stress due to mitochondrial dysfunction, lipid peroxidation, ER stress, intrinsic immune regulation, circadian rhythm disruption and immune system development. Imbalances, circadian patterns and genetics are all parallel and mutually reinforcing factors that contribute to the onset and development of MASLD (23,24) (Fig. 1).

LSEC with abnormal window aperture. The role of liver sinusoidal endothelial cell (LSEC) in the development and progression of MASLD is being gradually recognized. LSEC is the most predominant liver nonparenchymal cell (NPC), accounting for 15-20% of the total number of hepatocytes

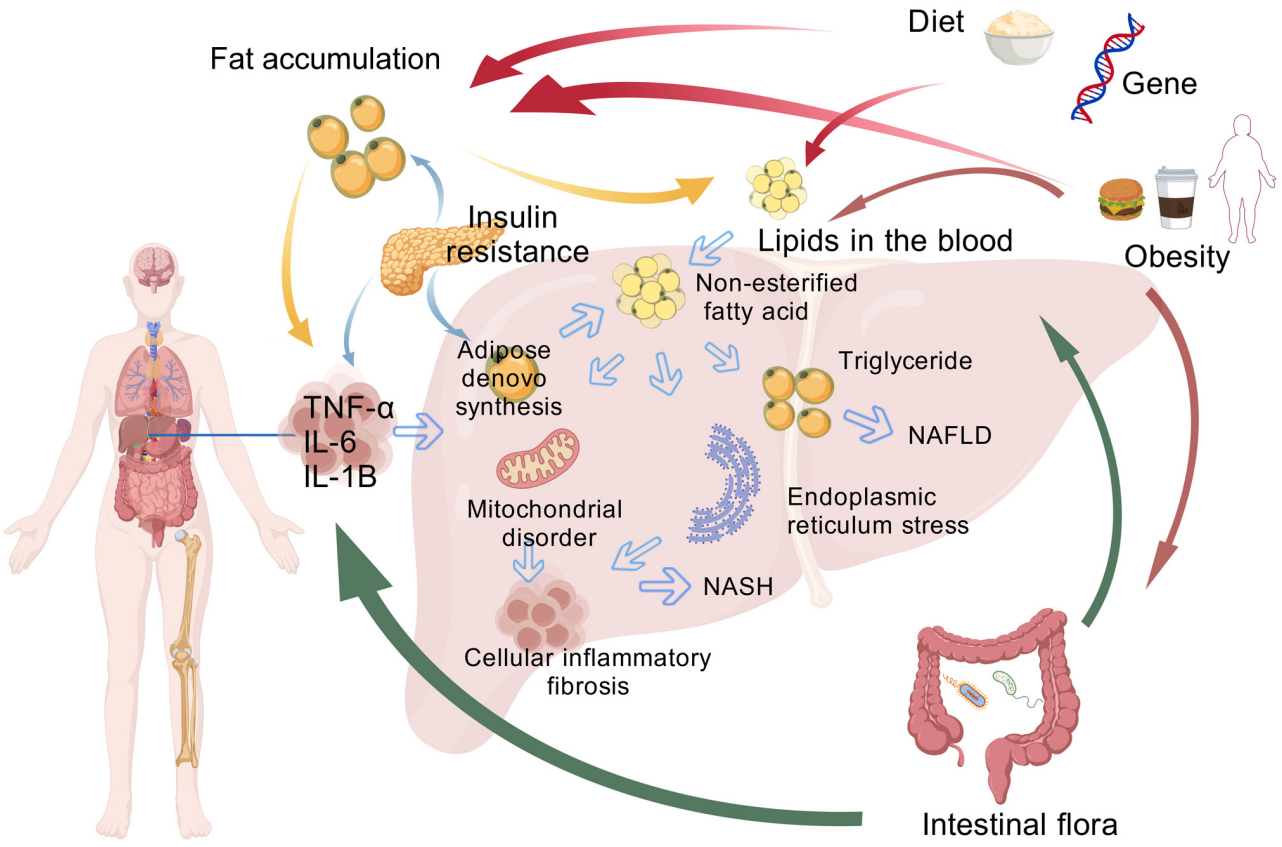

Figure 1. The mechanism 'Multiple Strike' hypothesis of MASLD. MASLD, metabolic dysfunction-associated steatotic liver disease; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

but only 3% of the total volume of the liver (25). The biggest difference between LSECs and other vascular endothelial cells is the presence of window pores and the lack of a basement membrane. LSECs are considered the most permeable endothelial cells in mammals (25). Window pores are small pores with diameters ranging from 50-200 nm, accounting for 2-20% of the endothelial surface area (26). MASLD manifests as NAFL in the early stage, which is not accompanied, or only accompanied by mild, inflammation. LSEC acts as the first line of defense in the initiation of MASLD (27), exerting anti-inflammatory effects, preventing the over-activation of the hepatic immune system and maintaining the microenvironmental homeostasis of hepatic sinusoids. It has been shown that the unique window pore structure of LSECs in a physiological state serves an important role in maintaining the stability of the liver, which manifests pro-vasodilatory, strong endocytosis, anti-inflammatory and anti-fibrotic effects (28). This window pore regulates the act in the endothelial cell skeleton of LSECs through the form of calcium-calmodulin-actin complex to adjust and control the size of the window pore on the endothelial cells of LSEC; if the size, number, and permeability of window pores in LSECs could not be effectively improved, they would disappear in the course of time. From the initial manifestation of fat accumulation to inflammatory symptoms, as well as hepatic fibrosis and in severe cases, cirrhosis, LSEC regulates blood flow through the secretion of two substances, nitric oxide (NO) and endothelin-1 (ET-1) (27). NO promotes vasodilatation and ET-1 induces vasoconstriction. Reduced NO bioavailability induces LSEC capillarization characterized by loss of the LSEC window pore and basement membrane formation and leads to an imbalance in its secretion of vasoactive substances, which contributes to MASLD progression. With the progression of MASLD, LSEC undergoes capillarization and hepatic sinusoidal endothelial dysfunction, which gradually shifts from an anti-inflammatory to a pro-inflammatory phenotype and promotes the progression of inflammation, fibrosis and angiogenesis. Hepatic sinusoidal endothelial dysfunction precedes the development of hepatic inflammation or fibrosis. It is a major feature and early event in MASLD pathogenesis (29).

Insulin resistance. IR refers to the phenomenon of reduced sensitivity of tissues and organs to insulin, which is the initiation and central link in the progression of MASLD (30). It has been found that the degree of IR in MASLD patients is positively associated with the severity of the disease (31). IR acts on target organs mainly the liver, skeletal muscle and adipose tissue; the body's sensitivity to insulin is reduced, so that the biological effect of insulin on target organs is reduced and glucose uptake and utilization are reduced, thus failing to maintain blood glucose stability. Compensatory overproduction of pancreatic islets and eventual development of hyperinsulinemia, result in elevated hepatic synthesis and accumulation of total cholesterol (TC) and elevated low-density lipoprotein (LDL) levels, leading to inactivation of the enzyme LDL lipase, which is critical for cholesterol clearance. Large amounts of TG cannot be cleared and IR-induced hyperinsulinemia inhibits apolipoprotein B100 synthesis

and reduces very low-density lipoprotein (VLDL) related lipid output from hepatocytes (32). In addition, IR leads to decreasing lipid metabolism, increasing lipolysis and hepatic uptake of large amounts of free fatty acid (FFA). By contrast, fatty acid β-oxidation is inhibited by hyperinsulinemia and large amounts of FFA are deposited in the liver, exacerbating hepatocellular steatosis (33). Excessive deposition of lipids further exacerbates IR and they are mutually influencing and reinforcing processes (34). MASLD also leads to lower levels of adiponectin (ADPN), a protein released from adipocytes. ADPN aggravates steatosis in hepatocytes (35) and plays a role in regulating glucose and lipid metabolism, as well as exhibiting anti-inflammatory and anti-insulin resistance effects (36). In addition, Shah and Fonseca (37) found that iron overload could reduce insulin sensitivity and IR, which are closely related to the occurrence of MASLD. The main reason is that iron can activate the nuclear factor-kappa B (NF-κB) signaling pathway. Activation of this pathway causes hepatocytes to produce an inflammatory response, which accelerates the formation of MASLD. Although iron is an essential trace element in the human body, a study found that iron overload is closely related to the occurrence of MASLD (37). Iron overload can generate reactive oxygen species (ROS) and cause oxidative stress. Iron overload can also affect lipid metabolism and insulin signaling, which can accelerate the progression of MASLD (38). Using hepatic puncture biopsy, Nelson *et al* (39) discovered that 34.5% of 849 patients with MASLD had intrahepatic iron deposition, which suggested that iron deposition was a risk factor for the progression of MASLD patients to advanced liver diseases.

Adipokines. Adipokines are small-molecule proteins secreted by tissues to regulate adipocytes, mainly including leptin (LP) and ADPN. A study (40) found that IR is closely related to the secretion of LP, resistin and ADPN. ADPN is an adipokine that affects hepatic fat and glucose metabolism, which interacts with adiponectin receptor1 and adiponectin receptor 2. Adiponectin receptor 2 is mainly expressed in liver tissues. Clinical studies have shown that the serum level of ADPN in patients with MASLD is low and negatively is associated with the severity of fatty liver (40). In addition, serum lipocalin levels are reduced in MASLD patients and are associated with the degree of hepatic necroinflammation, leading to the suggestion that hypo-lipocalinemia will lead to the progression of MASLD, which may be related to the fact that ADPN acts on the sterol regulatory element-binding protein-1C to inhibit lipolysis of fats (41). Studies have shown a close relationship between LP and the development of MASLD and that LP can lead to the activation of hepatic LP and can lead to the activation of hepatic stellate cells in MASLD and promote the development of MASLD to liver fibrosis (41). Moreover, LP is also associated with IR; when the feedback regulation mechanism between pancreatic islets-adipocytes is damaged, the sensitivity of insulin to LP decreases. It makes the fatty acid content of hepatocytes rise, which promotes the increase in the synthesis of TGs in the hepatocytes, leading to the formation of MASLD (42). Wang *et al* (43) found that the amount of ADPN expression can be used to predict the occurrence of MASLD to a certain extent. Resistin is a prepeptide adipocytokine containing a 108-amino-acid segment, named after its anti-insulin effect. In another study, RES was found to be highly expressed in adipose tissue and serum of both high-fat diet-induced obese rats and transgenic obese rats (44). Activation of resistin can induce the release of inflammatory factors and promote the development of liver fibrosis (45).

Lipotoxicity. Lipotoxicity refers to lipid metabolism disorders leading to an increase in FFA, excessive FFA makes pancreatic islet β-cells dysfunctional, inducing them to produce a large amount of NO, causing a series of cytotoxic injuries. Markedly increased plasma FFA level in MASLD patients is the main reason for increased IR in the body. In addition, increasing plasma FFA content leads to fatty acid oxidation overload in hepatocytes, induces mitochondrial damage and generates large amounts of ROS, causing oxidative stress, ER stress and inflammatory response, which advances the process of disease progression. The mechanisms of hepatic fatty acid metabolism on MASLD are shown in Fig. 2.

Lipotoxicity promotes hepatocyte apoptosis. The normal number of hepatocytes is controlled by hepatocyte apoptosis, which maintains the liver at a normal size and plays an important role in the development of the liver and the maintenance of internal homeostasis, it is the defender of hepatocytes against infections, tumors and autoimmune reactions; under pathological conditions apoptosis of hepatocytes is the central link in the basis of injury and other liver diseases. Lipotoxicity induces apoptosis in hepatocytes, called lipoapoptosis. It has been demonstrated that FFA-treated hepatocytes show an increase in the expression of pro-apoptotic proteins and apoptosis regulators upregulated by tumor suppressor genes, a process that is accompanied by a decrease in the expression of the anti-apoptotic protein B-cell lymphoma-2. The aforementioned process initiates the mitochondria-induced apoptosis pathway, which activates caspase-3,6,7 leading to apoptosis (46). Hepatocyte apoptosis is closely related to the pathogenesis of MASLD (47).

Lipotoxicity-induced liver mitochondrial damage. Mitochondria are an important site for hepatic FFA oxidation. When FFA and related metabolites in the liver increase in excess, it causes mitochondrial microstructure swelling and mitochondrial dysfunction, resulting in impaired β-oxidation, altering the permeability of the ER membrane, inducing the production of a large number of ROS. ATP generation is reduced, causing oxidative stress and mitochondrial damage (48). In the meantime, ROS generated by oxidative stress in the mitochondrial membrane react with unsaturated fatty acids of mitochondrial membrane phospholipids, nucleic acids and other macromolecules to cause lipid peroxidation and the products of lipid peroxidation produce endogenous ROS and O^{2-}, which can damage mitochondrial DNA structure and further diminish the anti-oxidant effect of mitochondria (49). In addition, ROS generated by oxidative stress in hepatocytes can trigger an inflammatory response. This response induces neutrophil infiltration and activates Kupffer cells (KCs) to secrete ROS and TNF-α. These events create a vicious cycle of oxidative stress, leading to VLDL deposition in the liver and the development of MASLD. Further hepatocyte necrosis may occur

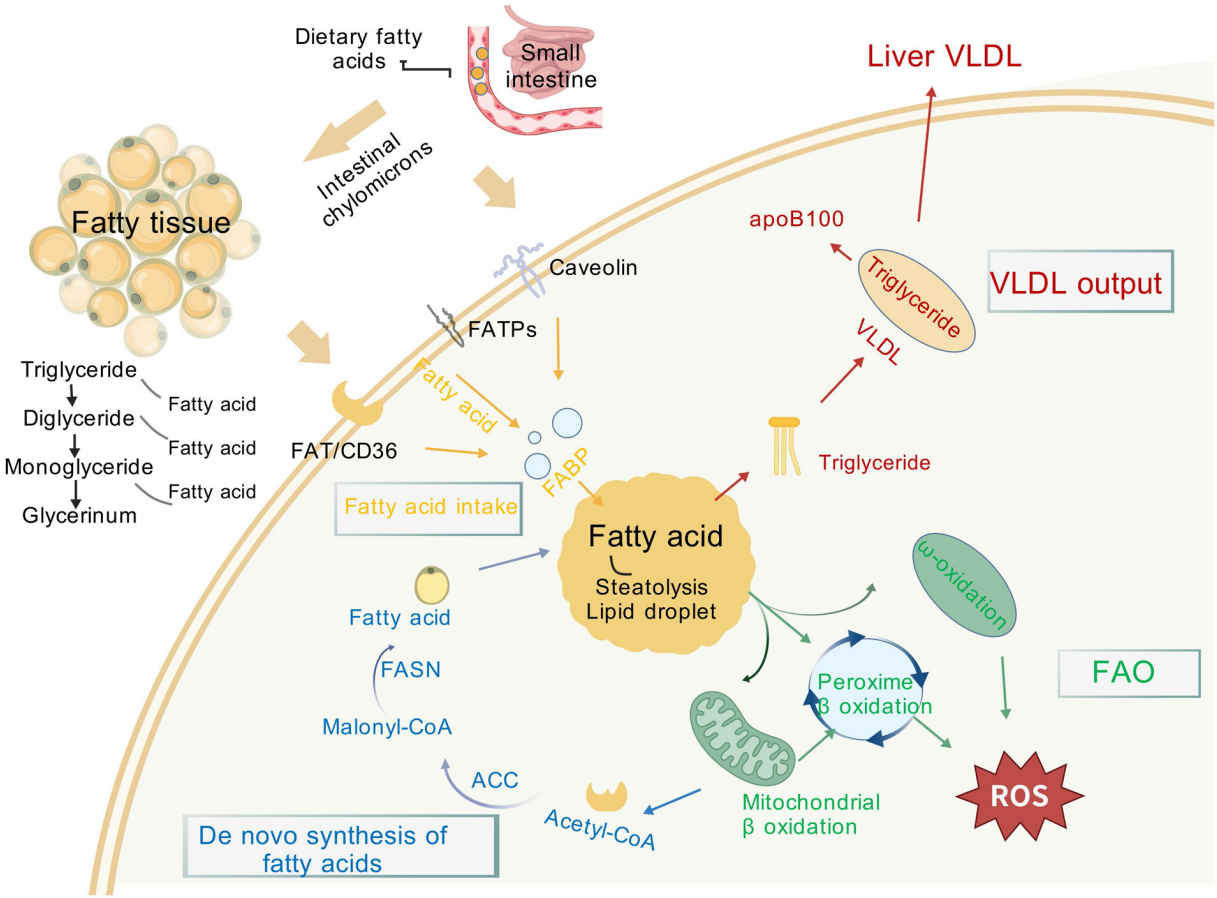

Figure 2. The mechanism of hepatic fatty acid metabolism on MASLD. FAO, fatty acid beta-oxidation; FATP, fatty acid transport protein; MASLD, metabolic dysfunction-associated steatotic liver disease; VLDL, very low-density lipoprotein.

due to other cytokines, such as transforming growth factor-β (TGF-β), IL-6 and C-reactive protein (49,50).

Lipotoxicity-induced inflammation promotes MASLD. Persistent inflammation is the main driver of MASLD progression to MASH and fibrosis and activation of toll-like receptors (TLRs) is thought to be a key factor in triggering hepatic steatotic inflammation. The liver, as a central immune organ, possesses the largest number of resident macrophages, known as KCs. KCs account for 35% of the liver's NPC and 80-90% of all tissue macrophages in the body. KCs are considered to be the first immune cells to come into contact with intestinal or hepatic autoimmune reactive substances and are rich in the expression of TLRs (51). Accumulation of lipids in hepatocytes will result in lipotoxicity, which will lead to hepatocyte damage or death. Damaged or dead hepatocytes release damage-associated pattern molecules, including mitochondrial DNA (mtDNA) and high mobility group protein-1 (HMGB1) (52). Among them, mtDNA can directly activate recombinant TLR9 in KCs, triggering an inflammatory cascade (53). Recombinant toll-like receptor 4 (TLR4) (Fig. 3) is another TLR family member upregulated in MASLD and lipopolysaccharide (LPS), ROS, HMGB1 and various damage-associated molecular patterns can bind to TLR4 in KCs to promote TNF-α, IL-1β, IL-6 and interferon-γ (IFN-γ) production of various pro-inflammatory cytokines, thereby contributing to hepatic inflammation and the progression of MASLD (54). In addition to triggering the inflammatory response through lipotoxicity that causes hepatocyte injury or death, excess FFA in the liver can also exacerbate the inflammatory response through direct or indirect activation of TLR4. Circulating FFA, especially saturated fatty acid (SFA), acts as non-microbial TLR4 agonists and triggers inflammatory responses. SFA is thought to have a similar recognition pathway to LPS, a natural ligand of TLR4, in order to activate the TLR4 signaling pathway, thereby triggering downstream inflammatory pathways (55). Furthermore, to activate TLR4, SFA is also thought to activate recombinant TLR2 to promote the development of inflammatory responses (56). It has been shown that SFA can activate TLR by interacting with TLR co-receptors, such as cluster of differentiation36 (CD36) or LDL receptors, to promote inflammation (57). In addition, FFA can interact with various other cytokines, such as hepatocyte nuclear factor 4-α (HNF-4α), leading to overall changes in signaling pathways that regulate metabolism and stress (58). Lipotoxicity can also further induce ER stress, impair autophagy and promote aseptic inflammatory responses, thereby exacerbating hepatocyte injury and death (59,60). Cholesterol synthesis is markedly increased in patients with MASLD, suggesting that cholesterol may also be one of the important driving forces in its development (61). Cholesterol not only promotes *de novo* lipid synthesis but also induces lipid peroxidation (62). Moreover, it has been found that increased dietary cholesterol intake leads to hepatic inflammation and oxidative stress in

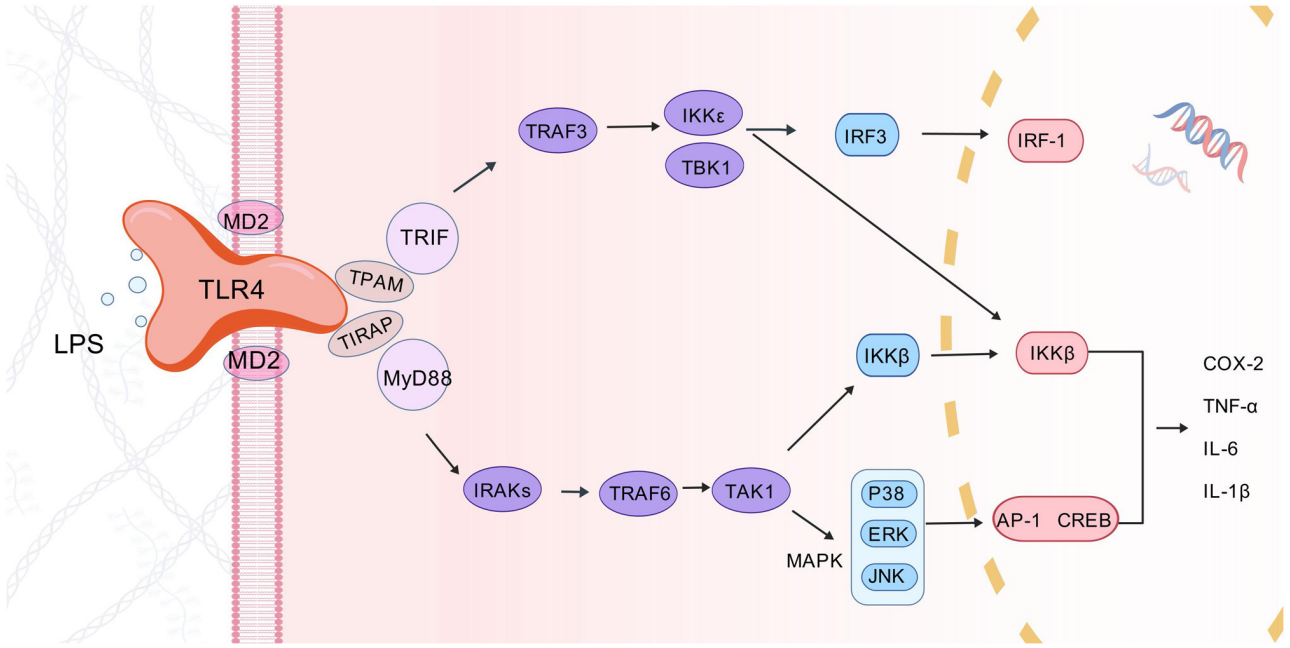

Figure 3. The mechanism of lipotoxicity-induced inflammation promotes MASLD. LPS, lipopolysaccharide; MASLD, metabolic dysfunction-associated steatotic liver disease; MD2, myeloid differentiation factor 2; TLR4, Toll-like receptor 4.

mice, suggesting that cholesterol probably plays a contributory role in the disease progression of MASLD (63).

ER stress contributes to the development of MASLD. Lipid accumulation in hepatocytes can affect mitochondrial function, ER membrane fluidity and calcium ion homeostasis. It has been found that thioesterase superfamily member 2 (THEM2) promotes the uptake of saturated fatty acids by ER phospholipid membranes, reduces ER membrane fluidity and affects the activity of ER Ca^{2+}associated ATPase. The dysregulation of Ca^{2+} signaling can cause protein misfolding and secretion (64). Non-esterified fatty acids can expand the membrane structure of the endoplasmic reticulum-mitochondria association. Changes in the membrane structure of the ER allow Ca^{2+} to flow into the cytoplasm or mitochondria, which in turn alters the open channels of the mitochondria and cytochrome *c* is released into the cytoplasm. Ca^{2+} entering the cytoplasm can activate the Ca^{2+} dependent protein kinase, which together with cytochrome *c* induces the onset of apoptosis (65). ROS generated by mitochondria can enter the ER and trigger the unfolded protein response (UPR), which can activate three types of ER transmembrane protein receptors: Protein kinase RNA-like endoplasmic reticulum kinase (PERK), activating transcription factor-6α (ATF-6α) and inositol-requiring enzyme-1α (IRE1α), which mediate three signaling pathways. The three transmembrane proteins mediate three signaling pathways, which can maintain the stability of ER protein synthesis to a certain extent in the early stage of steatosis, but under the continuous stimulation of lipids and ROS, the three transmembrane proteins mediate the signaling pathways that can activate cellular inflammation and apoptosis, accelerating the progression of hepatic steatosis (66).

In the process of hepatocellular steatosis, one of the hallmarks of MASLD is the appearance of the UPR in hepatocytes, which can inhibit mitochondrial lipid oxidation through the UPR-induced ATF-6α pathway and promote fatty acid synthesis via the PERK, ATF-6α and IRE1α signaling pathways, which together promote intracellular lipid accumulation (67,68). Hepatocyte lipid accumulation and ER stress form a positive feedback loop that continuously exacerbates hepatocyte steatosis. The occurrence of chronic inflammation in hepatocytes is also one of the main features of metabolic disorders. The inflammatory response can activate the UPR through three major signaling pathways (PERK, ATF-6α and IRE1α). The UPR can also interact with NF-κB and N-terminal kinase/activator protein 1-regulated pro-inflammatory pathways to accelerate disease progression (66,69).

Imbalance of the intestinal flora

Effects of intestinal permeability. The intestinal flora is a symbiotic group of microorganisms that act synergistically with the host. Under normal circumstances, the intestinal flora of the human body is in a state of equilibrium. Imbalances in the intestinal flora can be caused by environmental factors, immune levels, bile secretion, changes in gastric acidity and alkalinity and impaired intestinal peristalsis. In recent years, it has been found that dysbiosis is one of the causative factors of MASLD (70). The intestinal flora mainly influences the liver through the 'gut-liver axis'. Normally, there is a balance between the barrier function of the intestine and the detoxification capacity of the liver and trace amounts of intestinal bacteria cross the mucosa and enter the venous bloodstream and are cleared by the liver (71). When intestinal flora dysbiosis occurs, metabolic disorders will occur, the permeability of the intestinal tract will increase and a large amount of intestinal bacterial metabolites, bacterial components and other harmful substances will enter the liver through the portal vein via the intestinal-hepatic axis and through the enterohepatic circulation reach the liver and bind to the

TLR4 of hepatocytes and exacerbate the hepatic inflammatory response, oxidative stress and lipid accumulation, which further stimulate the inflammatory response. Intestinal microorganisms change their phenotype and virulence by sensing the various adverse signals generated during intestinal stress, shifting from a commensal to a pathogenic mode and causing damage to the organism (72). Ley *et al* (73) found that changes in the composition of the gut microbial community associated with obesity as well as IR were observed after the adoption of two different dietary patterns. IR, an important feature of MASLD, can be improved after antibiotic treatment (74). A study conducted by Le *et al* (75) demonstrated that when gut microbes from successfully modeled MASLD mice were transplanted into normal control mice, the normal control mice developed MASLD and this study strongly confirmed that the development of MASLD is associated with the development of MASLD and its effects on the gut microbial community. This study also confirms the correlation between the development of MASLD and gut microbes. A Chinese cohort study reported that high-alcohol-producing Klebsiella Pneumoniae (HiAlc-Kpn), which produces large amounts of alcohol, was detected in 61% of MASLD patients. In order to investigate the relationship between HiAlc bacteria and fatty liver disease, they fed specific pathogen free (SPF) mice with HiAlc-Kpn. It is noteworthy that HiAlc-Kpn feeding induced chronic hepatic steatosis. They investigated the relationship between intestinal flora imbalance and severe MASLD lesions (MASH and fibrosis) and showed that an increase in the number of *Ruminococcus* was positively associated with an aggravation of the degree of fibrosis (76). In addition, LPS is an important component of the cell wall of Gram-negative bacilli. It is recognized by pattern receptors and mediates the LPS-CD14-TLR4 signaling pathway, which activates intrahepatic KCs. These KCs release large amounts of cytokines, such as IL-6, CD68 and TNF-α. LPS also causes inflammation and metabolic disorders *in vivo*, including increased fat burning, elevated circulating free FFA and TG and deposition of FFA in the liver. This process may contribute to inflammation and trigger IR, which can ultimately lead to MASLD. The deposition of FFA in the liver may also promote inflammation, leading to IR, which in turn triggers the development of MASLD (77). Dysbiosis may also contribute to MASH through other mechanisms, such as ethanol production and interference with choline metabolism (22). Overall, intestinal flora and its harmful metabolites (including EtOH, SFA, polyamines and H_2S) may be drivers of liver injury (76).

Effects of Bile acid (BA) metabolism. BA is synthesized in the liver and the main raw material for synthesis is cholesterol. BA regulates glucose and lipid metabolism. BA, along with its downstream receptors farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5), plays an important role in lipid metabolism in the liver (78). BA also regulates the cholesterol metabolic pathway, allowing cholesterol to be eliminated as a water-soluble product and impaired regulation can lead to an inflammatory response that can exacerbate the development of MASLD (79). In a MASLD mouse model, Jiang *et al* (80) found that the administration of antibiotics to mice on a high-fat diet reduced triacylglycerol accumulation in the liver. Activation of TGR5 by BA in brown adipose tissue and muscle increases energy expenditure and reduces diet-induced obesity (81). Activated TGR5 can downregulate the expression level of inflammatory factors, promote energy expenditure in adipose and muscle tissues and regulate the body's immunity, which has a positive effect on improving MASLD (82). Watanabe *et al* (83) found in an animal experiment that cholesterol and TG levels were markedly elevated in mice lacking FXR *in vivo*. FXR has a positive effect on lipid regulation and also reduces the generation of inflammatory responses; FXR plays a key role in the regulation of BA and regulates lipid metabolism. In addition, BA can affect the growth of bacteria and is a bacteriostatic substance. Conversely, intestinal flora can also regulate BA, intestinal flora and BA metabolism affect each other. However, the effects of different flora are inconsistent. For example, *Aspergillus* and *Anaplastic bacilli* in the intestine will reduce the synthesis of BA and aggravate inflammatory reactions, while *Actinobacteria* will increase the synthesis of BA, reduce the inflammatory response and reduce the damage of hepatocytes (84). The normal metabolism of BA plays a crucial role in maintaining the balance of intestinal flora. Gut microorganisms can also accelerate the metabolism of primary BA and produce secondary BA, increasing BA types (85). LPS produced by intestinal flora can stimulate NF-κB to recruit inflammatory factors and increase the level of inflammation in the body. These studies suggest that influencing the gut flora by modulating the signaling pathways between BA and their controlled receptors is a promising new therapeutic approach for the treatment of MASLD (86).

Cellular autophagy. Phenotypic changes in LSEC are one of the key events in the progression of MASLD in humans, suggesting that the progression of MASLD inflammation, hepatic fibrosis and impaired hepatic lipid metabolism may affect the expression of Fc γ receptor IIb (FcγR IIb) and macrophage function in LSEC (87). Autophagy is an intracellular process that maintains homeostasis *in vivo* by forming double-membrane autophagosomes that encapsulate to-be-degraded material and bind to lysosomes to self-digest excessive or defective organelles. Autophagy can be categorized into three types: Macroautophagy, microautophagy and molecular chaperone-mediated autophagy, of which the most common is macroautophagy. Autophagy is related to MASLD and macroautophagy is the main type of autophagy that regulates MASLD. Liver autophagy is defective and autophagy level is reduced in MASLD patients and autophagy shows different functions at different stages of MASLD (87). In the early stage of MASLD, autophagy inhibits apoptosis (87), while in the late stage, autophagy is pro-apoptotic, which also indicates that autophagy is involved in the whole stage of MASLD development (87). Recombinant autophagy-related protein 7 (Atg7), a core member of the anti-thymocyte globulin (ATG) gene family, is responsible for driving the classical degradation and the major stages of autophagy, which is a key component of the autophagy-associated gene family. Abnormal expression of Atg7, a core member of the ATG gene family, can drive the main stage of classical degradation of autophagy, resulting in defective autophagy in cells. In Atg7 knockout mice, hepatic autophagy activity was markedly reduced and intracellular lipid accumulation was increased, leading to the formation of MASLD (88). Phosphatidylinositol-3 kinase

(PI3K) is a key regulator of the activation of the mammalian target of rapamycin (mTOR), which is considered an important regulator upstream of the ATG gene. The PI3K inhibitor 3-methyladenine blocked the formation of autophagosomes, increased the lipid content of hepatocytes and promoted the formation of MASLD (89).

Inflammatory response induced by immune system dysfunction. Inflammatory response induced by immune system dysfunction has been found to be one of the hallmarks of MASH and a progressive form of MASLD (90). Under normal conditions, the liver has immune defense and immunoregulatory functions and when the intensity of the immune system's response to autoantigens exceeds the limits of immune regulation and affects its physiological functions, it will cause inflammatory damage to its tissues or organs, leading to the occurrence of autoimmune diseases. The liver has a large number of immune cells involved in the immune response and it has been found that macrophages are the largest proportion of immune cells (80-90%) in the human liver and they are closely related to the development and severity of MASLD (91). In patients with MASLD, macrophage infiltration occurs around the portal vein and is observed at an early stage before evidence of inflammation appears. In addition, macrophages are capable of producing a variety of inflammatory factors, such as NF-κB, TNF-α, IL-2, IL-6 and IL-8 and activating nuclear transcription factors like NF-κB, which also play an important role in the progression of MASLD (92). NF-κB, as a nuclear transcriptional regulator of inflammatory genes, which is activated, enters the nucleus and promotes the transcription and expression of inflammatory factors, releasing a large number of inflammatory factors. After entering the liver, NF-κB leads to oxidative stress in hepatocytes, activates the expression of pro-apoptotic proteins in mitochondria, promotes hepatocyte apoptosis and exacerbates the progression of MASLD (93). TNF-α is one of the important regulators mediating hepatocyte injury and is also a bridge between inflammation and metabolism that plays a key role in the development of MASLD. TNF-α regulates the inflammatory signaling pathway and exacerbates the inflammatory response in MASLD through pathways such as NF-κB and p38 mitogen-activated kinase (94). IL-2 can promote the proliferation of activated B cells and is also a growth factor for all T cell subpopulations and can positively feedback regulate the production of cytokines such as TNF-α, leading to a further increase in IL-2 in MASLD livers (95). IL-6 can inhibit the activity of lipoprotein esterase, which can reduce the ability to catabolize lipids and promote the formation of hepatic lipids. IL-8 can cause inflammatory cells to accumulate in the liver and cause inflammation and lipid accumulation in hepatocytes can also stimulate the inflammation of MASLD hepatocytes. The accumulation of lipids in hepatocytes can also stimulate KCs to release TNF-α, which further promotes the production of IL-8 and participates in the inflammatory response of hepatocytes, leading to the aggravation of hepatocyte injury (96).

Law of genetics. Genetic biomarkers for MASLD include DNA sequence variants, such as single nucleotide polymorphism (SNP) and the most studied SNPs in MASLD are rs738409 and rs58542926, which are located in patatin-like phospholipase domain containing protein 3 (PNPLA3) and transmembrane 6 superfamily member 2 (TM6SF2), respectively. The risk of developing MASLD has been found to be influenced by single nucleotide gene polymorphisms. For example, SNPs in the PNPLA3 gene and mutations in the PNPLA3 gene are associated with MASLD. PAPLA3 is one of the members of the patatin-like phospholipase family that have been identified as being closely associated with the development of MASLD (97,98), PNPLA3 was found to be expressed mainly in the liver and adipocytes, with non-specific TG lipase and acylglycerol transacylase activities involved in TG hydrolysis in hepatocytes. Mutations in PNPLA3 antagonize normal proteasomal degradation and increase the risk of MASLD and its severity. TM6SF2 is a multiple transmembrane protein that is involved in lipid transfer, VLDL secretion and TC synthesis. For the rs58542926 variant of the TM6SF2 gene (E167K), a mutation in the TM6SF2 allele results in decreased VLDL secretion and fatty acid retention in the liver, leading to hepatic steatosis and progression of MASLD (99). Hydroxysteroid (17-β) dehydrogenase 13 (HSD17B13) is a gene encoding the hepatic lipid droplet protein 17β-hydroxysteroid dehydrogenase type 13 and it has been found that HSD17B13 expression is markedly upregulated in patients and mice with MASLD, suggesting that HSD17B13 usually produces a product that promotes hepatocyte injury (100). Other SNP genes associated with MASLD include neuromucin and glucokinase regulatory protein. In addition, the level of immunity and metabolic rate of an individual are genetically related.

MicroRNA factors. MicroRNAs are a class of RNA regulators, ~22 nucleotides in length and a growing number of studies on diet-induced and genetic (ob/ob) models of obese rodents and patients with severe MASLD suggest a potential role for miRNAs in MASLD pathogenesis and IR (101). A number of miRNAs have been identified, including miR-122 and miR-21 and their expression levels in both peripheral blood and liver have been associated with the development of MASLD (102). One study found that serum miR-122 was upregulated 7.2-fold in patients with MASH compared with healthy subjects and 3.1-fold compared with patients with simple steatosis. This suggests that serum miR-122 is an extra-hepatic feature of MASH. Moreover, in a mouse model without elevated alanine aminotransferase (ALT), the elevated serum miR-122 levels were positively associated with the severity of MASLD (103). It has been observed that the expression level of miR-21 in the liver of diet-induced MASH mice progressively decreased with disease progression, compared with the control group (103). It has been found that miR-27a attenuates neoplastic liver lipogenesis and obesity-induced MASLD by inhibiting the fatty acid synthase (FAS) gene and stearoyl coenzyme A desaturase-1 in the liver. Histological analyses also showed reduced lipid accumulation in the livers of mice with hepatic miR-27a overexpression, suggesting reduced hepatic steatosis; furthermore, trichrome staining of miR-27a overexpressed livers showed markedly reduced fibrosis and lower MASLD activity scores, reflecting improved development of MASLD (104). miR-155 is one of the miRNAs that can regulate KCs, which are involved in inflammatory processes that control innate and adaptive immunity in alcoholic and MASLD diseases (103). Szabo and Csak (105) proposed that miR-155 was a major

regulator of inflammation and mice lacking miR-155 on methionine choline deficiency (MCD) with attenuated steatosis but no change in serum ALT or inflammation indicative of liver damage after diet-induced steatohepatitis. In another MASH model, however, miR-155 deficiency resulted in enhanced hepatic steatosis (105). A study has found miR-16 to be elevated in MASLD patients with simple steatosis and others have found that serum miR-16 is elevated in MASH patients and is associated with the stage of fibrosis (106). It has been observed that the expression of miR-197, miR-146b, miR-181d and miR-99a is markedly decreased in MASLD patients. Additionally, the hepatic levels of miR-301a-3p and miR-34a-5p increase monotonically from simple steatosis to MASH to cirrhosis. Conversely, miR-375 levels decrease monotonically during this progression (107). Some MASLD-related miRNAs, such as miR-149, had elevated expression levels in both FA-treated human hepatocellular carcinomas cells and MASLD animal models (102).

H_2S factors. H_2S is a mammalian endogenous signaling molecule that plays an important role in the pathophysiology of the liver. H_2S has been reported to prevent the elevation of lipid peroxides malondialdehyde induced by MCD feeding (108). Wu *et al* (109) found that a high-fat diet reduced hepatic anti-oxidant defenses by downregulating glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities. H_2S effectively restored the activities of these enzymes and these results suggested that H_2S could provide anti-oxidant effects against high-fat diet-induced hepatotoxicity. It was likewise demonstrated that H_2S could inhibit hepatic fat accumulation through downregulation of FAS and upregulation of CPT-1, thus allowing the liver to recover from steatosis. In addition, H_2S attenuated MASLD by upregulating the activities of anti-oxidant enzymes (GPx and SOD) and concluded that H_2S could alleviate MASLD. It was concluded that H_2S administration could alleviate the inhibition of MASLD accumulation by downregulating the expression of FAS and upregulating CPT-1 in obese mice fed a high-fat diet, thus ameliorating MASLD. In addition, H_2S inhibited oxidative stress by increasing the activity of anti-oxidant enzymes such as GPx and SOD. These results suggested that H_2S played an important role in regulating lipid and anti-oxidant metabolism (109).

Circadian pattern. Some studies have found a large number of circadian rhythmically expressed genes in the liver, which are involved in maintaining the metabolic balance of the body. MASLD is closely related to daily lifestyle. Irregular lifestyle may cause liver overload, including lack of sleep and little exercise. In addition, long-term intake of high-fat and high-protein foods, omitting breakfast, adding meals before bedtime and other bad dietary habits are also risk factors for MASLD (110). Sleep deprivation leads to an increased risk of morbidity and mortality (111). Epidemiologic studies have shown that sleep deprivation leads to altered glucose homeostasis, IR, weight gain, obesity, metabolic syndrome and DM, all of which are associated with MASLD. In experimental studies, it has been found that sleep disorders may induce MASLD through pro-inflammatory markers such as TNF-α, IL-1β and IL-6. In addition, sleep deprivation increases growth hormone-releasing peptide levels and decreases LEP levels, which increases appetite and further contributes to obesity and chronic insomnia activates the hypothalamo-pituitary-adrenal axis, which increases stress hormones, exacerbates IR and promotes the development of MASLD (112,113). Another study found that increased mRNA expression in hepatic biological clock genes, a decrease in levels of key metabolism-regulating enzymes, hepatic inflammation and steatosis can occur, which is associated with glucose and fatty acid metabolism (111). In the case of liver-specific knockout of BMAL1, which results in the loss of key metabolic gene oscillations in the liver and subsequently exacerbates oxidative damage to hepatocytes and induces IR. The glucocorticoid rhythm plays a key role in coordinating glucose, lipid and protein metabolism and it is an entrainment signal for the systemic circadian rhythm through the hypothalamic suprachiasmatic nucleus, the peripheral clock in the liver and adipose tissue. The kidney is regulated by the autonomic nervous system and rhythmic entrainment signals. It has been noted that the oscillation of the cellular redox state, independently of the biological clock transcriptional feedback loop and in the metabolic process, may control the circadian rhythm (114). Circadian rhythm disruption will lead to cellular dysfunction, which in turn affects the metabolic function of the liver (115). It induces metabolic dysfunction and the occurrence of obesity, fatty liver, metabolic syndrome and other conditions.

Psychosocial factors. Social stress is closely related to emotional and physical health and one of the factors that can trigger the development of MASLD is excessive stress (116). Some studies suggest that emotional problems such as anxiety and depression may have an effect on the progression of chronic liver diseases, including MASLD (117). A study by Youssef *et al* (118) confirmed the association of depression and anxiety with the severity of histologic features of MASLD by examining 567 patients, demonstrating that depression was markedly dose-dependent with more severe hepatocellular ballooning and that patients with subclinical depression were 2.1 times more likely to develop more severe hepatocellular ballooning than patients without depressive symptoms.

Diet and lifestyle-related factors. Studies have shown that hyperinsulinemia, lipid and lipoprotein metabolism changes caused by high-fat diet (HFD), high-carbohydrate diet, or high-fat and high-carbohydrate diets may promote the occurrence and development of MASLD (119). Yu *et al* (120) explored the relationship between dietary choline and MASLD, choline deficiency stimulated hepatic fat accumulation and increased dietary choline intake in normal-weight Chinese women associated with a reduced risk of MASLD. Soft drinks and meat intake were markedly associated with the increased risks of MASLD (121,122). High salt intake may affect MASLD by increasing plasma levels of triglyceride (TG) and it intake also stimulates endogenous fructose production. Lanaspa *et al* (123) found that a high salt diet activated the aldose reductase pathway in the liver, leading to endogenous fructose production, which induced LEP resistance with the development of the metabolic syndrome and MASLD. In a study based on a northern Chinese population, the correlation between salt intake and MASLD in DM patients was analyzed by Spearman analysis, which showed a positive correlation

between salt intake and the incidence of MASLD, suggesting that the likelihood of MASLD in DM patients increases with increasing salt intake (124). A variety of mechanisms, including the anti-oxidant, anti-inflammatory and anti-fibrotic effects of coffee, may be related to the protective effects of coffee against MASLD (125). Studies have shown that caffeine may stimulate the hepatic autophagy-lysosomal pathway and induce fatty acid oxidation (126). However, how caffeine activates autophagic flux is still unclear (125). Active smoking has also been associated with a high risk of MASLD and a study has demonstrated the association of smoking history with advanced liver fibrosis in patients with MASLD (127). A cross-sectional study of 2,691 Chinese men found that ex-smokers and heavy smokers (≥40 cigarettes/day) had a higher prevalence of MASLD than never-smokers and some studies have reported that smoking is an independent risk factor for MASLD, which may exacerbate MASLD (128,129).

4. Experimental models of MASLD

Animal models. Commonly used laboratory animals include rats, mice and rabbits. Among them, rats and mice are the most widely used. Rats are represented by Wistar and Sprague Dawley (SD). There are more diverse mouse strains, with C57BL/6J being the most common. The present study summarized rat, mouse, rabbit, monkey, chicken, hamster and guinea pig models. The models summarized include diet-induced models, drug-induced models, special models and spontaneous models. Diet-induced models include HFD, MCD, choline-deficient amino acid-defined (CDAA) and atherogenic diet. It has the advantages of simple operation, low cost, repeatability and low mortality, but the disadvantage is that it is time-consuming. Drug-induced models usually use drugs such as streptozotocin (STZ), carbon tetrachloride (CCl_4), LPS and tetracycline. Their advantages are short modeling time and economy, while its disadvantages are that the drugs have high toxicity. Special models mainly use gene knockout methods to make model animals prone to fatty liver. Compared with other models, the modeling time of spontaneous formation of fatty liver in special models is short, but it has requirements for the variety of animals and high cost. Spontaneous models mainly include monkeys. Monkeys are similar to humans in genes and can develop MASLD in old age without the need for a high-fat diet or drug induction. The modeling method of the nutritional model is to feed laboratory animals with high-sugar, high-fat and high-calorie diets or to create an MCD. The drug-induced model aims to build the model by administering drugs such as CCl_4, tetracycline, polychlorinated biphenyl 118, so that the drugs exert their effects or even cause poisoning. The special strain model is to select certain diseases that can spontaneously form fatty liver, hyperlipidemia and other diseases closely related to MASLD or can spontaneously present symptoms relates to MASLD. The aforementioned modeling methods can be used independently and the pathological characteristics or disease phenotypes of the model can be reasonably selected according to different experimental requirements, they can also be used in combination to reduce the modeling time and improve the success rate of the model. The pathogenesis of MASLD is complex and a single animal model cannot fully mimic human MASLD. The ideal animal experimental model is a composite model formed by combining gene mutation or specific target gene modification with diet and drug/toxin induction. The phenotype of such a MASLD animal model is closer to that of human MASLD and the experimental results are more applicable to humans.

MASLD model in rats. Rats are of moderate size and have strong fertility and blood collection abilities. Currently, commonly used rat models for constructing MASLD diseases in laboratories include Wistar rats, SD rats and Zucker rats (Table I).

MASLD model in mice. It is found that 93% of genomic regions of mice are arranged in an order similar to that of human beings and they are characterized by inexpensive feeding, rapid reproduction, easy modeling and small inter-individual differences that facilitate the observation of parallel experiments. MASLD mouse models can simulate different pathogenic factors and the development of MASLD at each stage of the disease, guide the search for the pathogenesis of MASLD and its potential therapeutic targets and also be used for the screening and evaluation of MASLD drugs, which are closely related to the research of MASLD. Mouse models have been induced by high-fat and high-sugar diets, subcutaneous injections of CCl_4 and gastrointestinal nutritional solutions, with high-fat diets being the main modality. Mice commonly used in current experimental studies include C57BL/6J mice, Kunming mice and gene-deficient mice (Table II).

MASLD model in other animals. In addition to rodents, other animals are commonly used to establish MASLD models, including rabbits, monkeys and chickens (Table III). These models are helpful for studying the disease progression and providing crucial evidence for exploring the pathogenesis of MASLD and evaluating the efficacy of drugs (Table IV).

Cell models. At present, animal models are the most commonly used in the research of MASLD. However, animal models have some unfavorable factors, such as large individual differences, long model-building cycles, difficult control of experimental conditions and differences between animals and humans. By contrast, cell models are superior in overcoming the aforementioned factors. According to experimental requirements, cell viability can be maintained all the time, which is close to the process of human diseases and the cellular mechanisms of diseases can be studied in a targeted manner. Therefore, establishing a cell model of MASLD *in vitro* has important theoretical significance and broad application value for studying its pathogenesis and disease development and further preventing and treating MASLD.

Existing model cells can be classified into human primary hepatocytes, animal primary hepatocytes, human hepatocytes, hepatocyte-like cells (HLCs), induced pluripotent stem cells (iPSCs) and liver slices. The culture forms can be divided into monoculture, co-culture and three-dimensional culture. Advantages and disadvantages of different cell lines and different culture methods were shown in Fig. 4.

Single cell culture. Monoculture refers to the adherent culture of one type of cell, which is the most basic culture method.

Table I. Metabolic dysfunction-associated steatotic liver disease models in different strains of rats.

Authors, year	Species	Time	Inducer	Sample	Testing	Mechanism	(Refs.)
Salman *et al*, 2022	Wistar rats	14 weeks	HFD	Blood from heart, liver tissue	Serum biochemical indicators, serum ELISA, real-time RNA	Hepatic steatosis with abnormal liver function and lipid profile	(130)
Zhou *et al*, 2019	Wistar rats	13 weeks	PCB118	Liver tissue	Histopathology, serum biochemical indicators	ALT↑, TG↑, TC↑, GLU↑, LDL-C↑, HDL-C↑, IL-1β↑, TNF-α↑, TGF-β1↑, MMP2↑, α-SMA mRNA↑, hepatic steatosis, inflammatory infiltration, structural disorders of liver lobules and fibrosis	(131)
Stephen Robert *et al*, 2021	Wistar rats	8 weeks	Vegetable oil-CCl_4	Liver tissue	Histopathology, serum biochemical indicators, oxidative stress indicators, gene expression analysis	Body weight↑, ALT↑, ALP↑, AST↑, GGT-sensitive enzymes↑, liver injury, pathology showing hepatotoxicity of hepatocytes	(132)
Zhang *et al*, 2018	SD rats	12 weeks	HFD	Blood from vena cava, liver tissue	Serum biochemistry indicators, histopathology, ultrastructural observation	Lipids↑, blood glucose↑, high-density lipoprotein cholesterol↑, LDL-C↑, ALT↑, glutamine aminotransferase↑, insulin↑	(133)
Lin *et al*, 2019	SD rats	6 weeks	HFD	Blood from heart	Serum biochemical indicators, liver histopathology	Body weight↑, liver weight↑, liver index↑, GLU↑, HOMA-IR↑	(134)
Jin *et al*, 2018	SD rats	9 weeks	CCl_4	Blood from abdominal aorta	Serum biochemical indicators, histopathology	Yellowish liver surface, size↑, weight↑	(135)
Chen *et al*, 2017	SD rats	10 weeks	CDAA	Liver tissue	Serum biochemical indicators, serum fibrosis indicators; histopathology	Degeneration, necrosis, regeneration, fatty liver changes, hepatic fibrosis	(136)
Yan *et al*, 2015	SD rats	8 weeks	HFD+STZ	Blood from abdominal aorta, liver tissue	Histopathology, serum biochemical indicators	Blood glucose↑, blood lipids↑, liver function↓, lipidosis	(137)

Table I. Continued.

Authors, year	Species	Time	Inducer	Sample	Testing	Mechanism	(Refs.)
Li and Li 2017	SD rats	12 weeks	High fat solution	Liver tissue	Serum biochemical indicators, histopathology	AST↑, ALT↑, AKP↑, TC↑, TG↑, LDL↑, fat infiltration, inflammatory response, liver weight↑	(138)
Yong *et al*, 2020	SD rats	2 days	0.9% tetracycline	Blood from abdominal aorta, liver tissue	Histopathology	Steatosis, hepatocellular damage	(139)
Che and Han 2018	SD rats	32 days	MSG	Blood from heart, liver tissue	Histopathology	Liver weight↑, ALT↑, AST↑, ALP↑, hepatic steatosis	(140)
Hakkak *et al*, 2022	Zucker rats	9 weeks	AIN-93G diet	Liver tissue	Serum biochemical indicators, histopathology, liver weight	ALT↑, AST↑, body weight↑, fatty degeneration	(141)
Matsumoto *et al*, 2021	Zucker rats	10 weeks	53% corn amylum	Blood from heart	Serum biochemical indicators, histopathology	Lipid droplets↑, ALP↑, ALT↑, AST↑	(142)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GLU, glucose; HFD, high-fat diet; IL-1β, HOMA-IR, homeostatic model assessment for iInsulin resistance; interleukin-1β; LDL, low-density lipoprotein; STZ, streptozotocin; TC, total cholesterol; TG, triglyceride; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

Compared with co-culture, monoculture has the advantages of simple operation, unlimited subculture and short model-building time, which is conducive to high-throughput drug screening experiments. Therefore, it is widely used in the research of fatty liver. Human hepatocyte cell lines, primary human hepatocytes (PHH), animal primary hepatocytes and HLC can be used for induction culture. The excessive deposition of extracellular matrix (ECM) leads to the occurrence of fibrosis (194), which is a major cause of liver function impairment. The limitation of monoculture is that it cannot explore the cell-ECM interaction and cannot well mimic the phenotype of liver fibrosis. The three-dimensional liver fibrosis cell model mentioned below can solve this problem and can also well simulate the human ECM structure *in vitro* (195).

PHH. PHH is generally extracted from surgically resected tissues and separated from the tissues by two-step collagenase perfusion or magnetic cell separation techniques (196), which can well mimic the physiological situation of the human liver. However, due to its rapid dedifferentiation *in vitro* and the need to obtain ethical permission for specimen acquisition, these reasons limit the use of primary hepatocytes. The improved medium supplemented with various chemicals solves the problem of limited traditional primary hepatocyte culture time. The PHH cultured with this medium can be comparable to PHH spheroids for at least 4 weeks and has the potential to simulate steatosis disease models (197).

Due to individual differences among different donors and the influence of various factors in the cell separation process, the experimental results may be unstable and have poor reproducibility. In addition, the culture conditions of human primary hepatocytes are relatively harsh, they can only be cultured in the short term and cannot be subcultured indefinitely, which is not conducive to the progress of the experiment. The scarcity of human liver samples and ethical issues also hinder the wide application of human primary hepatocytes.

Animal primary hepatocytes. Animal primary hepatocytes are often derived from the livers of rodents and the hepatocytes are isolated and extracted by perfusion or non-perfusion methods. The non-perfusion method mechanically separates and digests with collagenase or trypsin on small pieces of liver tissue. The non-perfusion method is simple to operate but often has the problem of incomplete digestion, while the perfusion method can greatly improve the viability of the isolated hepatocytes, with a survival rate as high as 80% (198). The two-step collagenase perfusion method is the standard procedure for isolating primary hepatocytes. The improved standard procedure can show higher cell viability (85-95%) through multi-parameter perfusion control (199). Animal primary cells solve the ethical and quantitative restriction

Table II. Metabolic dysfunction-associated steatotic liver disease models in different strains of mice.

Authors, year	Species	Time	Inducer	Sample	Testing	Mechanism	(Refs.)
Xia *et al*, 2022	C57BL/6N mice	12 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology	Body weight↑, TG↑, TC↑, FFA↑, hepatic steatosis	(143)
Shi *et al*, 2022	C57BL/6 mice	24 weeks	HFD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Lipid droplet deposits↑, vacuolate lesions	(144)
Yu *et al*, 2022	C57BL/6 mice	8 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology	Liver steatosis, ALT↑, AST↑, TC↑, TG↑	(145)
De Minicis *et al*, 2017	C57BL/6 mice	4 weeks	CDAA+CCl_4	Liver tissue	Serum biochemical indicators, histopathology	Liver steatosis, liver fibrosis, tumours, IGF-2↑, SPP-1↑	(146)
Li *et al*, 2013	C57BL/6 mice	8 weeks	HFD+CCl_4	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Hepatic steatosis, fibrosis, abundant vacuolar hepatocytes	(147)
Feng *et al*, 2021	C57BL/6 mice	23 days	DXM + HFD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	ALT↑, AST↑, TG↑, TC↑, LDL↑, HDL↓	(148)
Asgharpour *et al*, 2016	C57BL/6J mice	8 weeks	HFD	Liver tissue	Histopathology	Body weight↑, IR↑, TG↑, LDL-C↑	(149)
Ma *et al*, 2021	C57BL/6J mice	16 weeks	Nutrition Solution	Liver tissue, spleen, abdominal fat	Serum biochemical indicators, histopathology, immune factor	TG↑, TC↑, liver deposits↑, lipid vacuoles↑	(150)
Jahn *et al*, 2019	KM mice	16 weeks	HFD/HFSD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Liver steatosis, liver fibrosis	(151)
Wang *et al*, 2011	KM mice	8 weeks	HFD+5% CCl_4	Blood from heart, liver tissue	Liver morphology, histopathology	Fatty liver disease, liver fibrosis	(152)
Liu *et al*, 2020	KM mice	8 weeks	CCl_4	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	TG↑, TC↑; inflammatory cell infiltration	(153)
Malloy *et al*, 2013	ob/ob mice	14 weeks	MR	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Body weight↑, TG↑, IR/HOMA ratio↑	(154)
Son *et al*, 2021	db/db mice	3 weeks	MCD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Liver steatosis, inflammatory cell infiltration and fibrosis, TG↑, TNF-α↑, IL-6↑	(155)
Yu *et al*, 2022	$ApoE^{-/-}$ mice	7 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology	Liver steatosis, liver weight↑, liver index↑, TG↑, TC↑	(156)
Song *et al*, 2020	$ApoE^{-/-}$ mice	4 weeks	HFD+LPS	Blood from abdominal aorta, liver tissue	Serum biochemical indicators, histopathology	Hepatic tissue inflammatory cell infiltration, hepatic tissue steatosis, inflammatory factors↑	(157)
Yang *et al*, 2020	LivKO mice	14 weeks	Western diet feeding	Liver tissue	Serum biochemical indicators, histopathology	Hepatomegaly, liver steatosis, fat droplets↑, inflammatory cell infiltration, TG↑, TC↑, body	(158)

Table II. Continued.

Authors, year	Species	Time	Inducer	Sample	Testing	Mechanism	(Refs.)
Piguet *et al*, 2015	$PTEN^{L-/-}$ mice	32 weeks	SD	Liver tissue	Histopathology	weight↑ BS↑, insulin↑, TG↑, TC↑	(159)
Semba *et al*, 2013	FLS mice	12 weeks	SD	Liver tissue	Serum biochemical indicators, histopathology, western blotting	Lipocalin-2, CXCL1 and CXCL9 expression, hepatic inflammatory cell infiltration, hepatocyte injury, liver steatosis	(160)
Li *et al*, 2012	PNPLA3 transgenic mice	12 weeks	Regular feed	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	TC↑, TG↑	(161)
Yin *et al*, 2006	ICR mice	72 h	SD+Tetracycline	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Liver steatosis, TC↑, TG↑, β-oxidation of fatty acids↓	(162)
Li *et al*, 2016.	ICR mice	8 weeks	HFD diet	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	TG↑, TC↑, HDL↑	(163)
Yu *et al*, 2016	FVB/N mice	3 weeks	AKT/SB plasmid+ saline	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Fat vacuoles, cytoplasmic fat deposition, hepatocyte swelling	(164)
López-Lemus *et al*, 2018	BALB/c mice	6 months	HFD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Hepatic steatosis, degree of germinal epithelial loss changes	(165)
Musolino *et al*, 2020	DIAMOND mice	27 weeks	WD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology, western blotting	ALT↑, TG↑, LDL-C↑, dyslipidaemia	(166)
Chow *et al*, 2011	ArKO mice	6 weeks	Soy-free diet	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Hepatic steatosis, TG↑	(167)
Komatsu *et al*, 2019	$AIM^{-/-}$ mice	12 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology	Fatty degeneration, inflammation, hepatic fibrosis	(168)
Nguyen *et al*, 2021	SREBP-1c KO mouse	12 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology, GTT, western blotting, RNA, H_2S	Liver steatosis	(169)
Deguise *et al*, 2021	$Smn^{2b/-}$ mice	7 days	SD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Protein output, complement, coagulation, iron homeostasis, IGF-1 metabolism disorders,	(170)
Cano *et al*, 2011	MAT1A-KO mice	7 days	Protein rodent diet	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	VLDL↑, TG↑, TC↑	(171)
Heintz *et al*, 2019	Cyp2b-null mice	10 weeks	HFD	Liver tissue, lipid	Lipidomics, lipid quantification, lipid annotation	Ketosis↑, LP↑, TC↑, TG↑	(172)
Edmunds *et al*, 2020	LKO mice	12 weeks	HFD	Liver tissue, lipid	Serum biochemical indicators, histopathology; western blotting	Hepatic fat deposits↑, hepatic steatosis, TG↑, TC↑, blood glucose↑, decreased insulin sensitivity↑	(173)

Table II. Continued.

Authors, year	Species	Time	Inducer	Sample	Testing	Mechanism	(Refs.)
Yang *et al*, 2025	$Alb^{Smad4-/-}$ mice	16 weeks	HFD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	ALT↓, TG↓, NEFA↓, ASK1-P38-JNK↓, CXCL1↓	(174)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDAA, choline deficent aminoacid-defined; DXM, dexamethasone; FFA, free fatty acid; HFD, high-fat diet; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; LPS, lipopolysaccharide; MCD, methionine choline deficiency; MR, methionine restriction; LP, leptin; SD, Sprague Dawley; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α; VLDL, very low-density lipoprotein; WD, western diet.

problems of human primary hepatocytes. However, they are affected by different animal sources and batches, have poor reproducibility, are relatively sensitive and have harsh cultural conditions.

HLC. HLC can be derived from embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells, endodermal cells and hepatic progenitor cells. Hepatocyte nuclear and epidermal growth factors are often used to promote cell-directed differentiation. Oncostatin M (OSM) helps to promote the maturation of fetal hepatocytes and OSM is often added during the maturation stage to promote complete cell differentiation. Although the subsequent culture time of HLC is longer than that of traditional PHH, its induction time is long (2-4 weeks) and the cost is high (200). There is also no unified standard for the types and ratios of cytokines and nutrients during the differentiation process and these factors limit the application prospects of HLC.

Human hepatocyte cell lines. Hepatocyte cell lines have become high-quality *in vitro* models due to their advantages, such as strong repeatability, stable subculture and controllable conditions. Commonly used human hepatocyte cell lines include hepatocellular carcinoma cell lines such as HepG2, human hepatoma cells and protein phospholipase C, which are taken from human liver cancer tissues and normal hepatocyte cell lines such as LO2. Compared with primary hepatocytes, immortal cell lines have the following advantages: Stable growth, unlimited lifespan, stable phenotype and simpler culture conditions than primary hepatocytes and they are easy to standardize among different laboratories. FFA are lipotoxic substances and the most commonly used stimulants for inducing MASLD *in vitro* models (201). In addition, oleic acid (OA) and palmitic acid (PA) are also commonly used in the experiments. When studying MASLD, two hepatocyte cell lines can be selected for mutual verification to increase the credibility of the experimental results. Zhao *et al* (202) induced LO2 cells with 3% ethanol for 48 h and the levels of aspartate aminotransferase (AST), ALT, TG content and ROS markedly increased, proving that the cells acquired the characteristics of fatty liver and oxidative stress. Yu *et al* (203) induced HepG2 cells with 0.5 mmol/l OA for 24 h and TG and ROS markedly increased and the ability of cells to take up glucose markedly decreased, indicating that the cells showed symptoms of steatosis, oxidative stress and IR.

Induced pluripotent stem cell-derived hepatocyte-like cells. Induced pluripotent stem cells can differentiate into various somatic cells without causing immune rejection and ethical issues. The HLCs differentiated from human iPSCs can be comparable to primary hepatocytes in terms of morphological and functional characteristics and have the advantages of wide sources, large cultivation quantities and stable phenotypes, making up for the deficiencies of primary hepatocytes (204). However, the induced pluripotent hepatocyte technology still has problems, such as low efficiency of inducing cell transformation, possible gene mutations during the induction process and tumorigenic risks, which limit its wide application.

Liver slices. Precision liver slices are an *in vitro* culture technology between the organ and cell levels. Compared with cell models, liver slices are closer to the complex structure and composition of the human liver. Liver slices contain hepatocytes, KCs and hepatic stellate cells, creating a multi-cellular environment. The interaction among various hepatocytes can offer an improved simulation of the liver tissue environment. However, its disadvantage is that the survival time is short and it cannot be cultured for a long time.

Co-culture. The co-culture model can make up for the defects of the monoculture model. Introducing a second type of cell into the model increases the interaction between cells and can offer an improved simulation of the physiological functions of the liver. The advantages of the co-culture model are that it compensates for the shortcomings of the monoculture model, has simple culture conditions and enables high-throughput experiments. However, there are not a great number of existing co-culture model types and there is great potential for future development. Chen and Ma (205) established a co-culture model of HepG2 cells and THP-1 macrophages. Firstly, the macrophages adhered to a sterile glass slide and then the slide was placed into a 6-well plate inoculated with HepG2 cells. A mixed fatty acid with a concentration of 1 mmol/l (the ratio of PA:OA was 1:2) was added and an MASLD model was established after 24-h induction. Giraudi *et al* (206) co-cultured HuH7 and human hepatic stellate cells. After 24-h induction with fatty acids, the intracellular lipid accumulation increased and the expression of α-smooth muscle actin increased, indicating that the hepatic stellate cells were activated and the cells showed the characteristics of steatosis and fibrosis. In

Table III. Metabolic dysfunction-associated steatotic liver disease models models in other rodents.

Authors, year	Species	Time	Inducer	Sample	Testing	Mechanism	(Refs.)
Yang *et al*, 2013	*Microtus fortis*	12 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology	Hepatocellular steatosis, diffuse steatosis	(175)
Yu *et al*, 2021	*Meriones unguieulataus*	4 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology	Punctate and focal necrosis of hepatocytes	(176)
Bhathena *et al*, 2011	Bio F1B hamster	5 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology	Fat deposition↑, fatty liver, mild diabetes	(177)
Cui *et al*, 2017	Hamsters	2 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology	TG↑, hepatic adipogenesis from head↑, FFA↑, FFA oxidation↓, p-AMPKα↓, Sirt1↓	(178)
Zhao *et al*, 2022	Golden hamster	2 weeks	HFFCD	Liver tissue	Serum biochemical indicators, histopathology, western blotting	Liver steatosis, lipid accumulation, abnormal serum, liver lipid markers	(179)
Skat-Rørdam *et al*, 2021	Guinea pig	16 weeks	HFD	Liver tissue	Serum biochemical indicators, histopathology	Liver steatosis, inflammation, significant abnormalities in serum, liver biochemical indicators	(180)
Jin *et al*, 2022	Guinea pig	6 weeks	HCD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology, western blotting	Pathological changes in the liver, lipid accumulation and pathological changes in the aortic wall	(181)
Ipsen *et al*, 2019	Guinea pig	25 weeks	HFHSD	Blood from heart, liver tissue	RT-qPCR	Liver fibrosis and cirrhosis	(182)
Pedersen *et al*, 2023	Guinea pig	32 weeks	HFD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	AUC↑, body weight↑, AST↑, TC↑, TG↑, fatty degeneration↑, inflammation↑	(183)

AST, aspartate aminotransferase; AUC, area under curve; FFA, free fatty acid; HCD, high-carbohydrate diet; HFD, high-fat diet; RT-qPCR, reverse transcription-polymerase chain reaction; SD, Sprague Dawley; TC, total cholesterol; TG, triglyceride.

another study, human primary hepatocytes, hepatic stellate cells and KCs were co-cultured. The cells were stimulated with fatty acids, glucose, insulin and inflammatory cytokines to simulate MASH. In this model, the *de novo* lipogenesis in the cells was enhanced and the cells showed symptoms of oxidative stress, inflammation, fibrosis and activation of hepatic stellate cells (207). The co-culture of human primary hepatocytes and endothelial cells can support hepatocytes to maintain their phenotypic morphology, improve their specific functions and form capillary-like structures, which is more conducive to simulate the *in vivo* environment and studying the pathological mechanisms of fatty liver disease (208).

Three-dimensional culture. In the living liver tissue, there is substance transport and signal transduction between hepatocytes and the ECM and the ECM also plays a role in supporting the three-dimensional structure (209). When human primary hepatocytes are cultured in the traditional two-dimensional system for a long time, morphological changes occur due to epithelial-mesenchymal transition, resulting in the loss of hepatocyte polarity and related liver functions (210). The construction of a three-dimensional model is more complicated. For the three-dimensional model of PHH, KCs and hepatic stellate cells co-cultured with the help of a three-dimensional microphysiological system, at least two weeks of induction with FFA is required (211). The co-culture mode with multiple hepatocytes can more accurately simulate the liver microenvironment, but it requires more stringent culture conditions. In the case of few or no precedents, researchers are required to preliminarily explore

Table IV. Metabolic dysfunction-associated steatotic liver disease models in other different animals.

Authors, year	Species	Time	Inducer	Sample	Testing	Mechanism	(Refs.)
Wang *et al*, 2018	Japanese white rabbit	12 weeks	HFCD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	TC↑, TG↑, FFA↑	(184)
Sturzeneker *et al*, 2019	New Zealand rabbit	8 weeks	Specific diet	Blood from heart, liver tissue	Serum biochemical indicators, histopathology, ICH	Inflammation, lipid deposition↑, hepatocyte damage, hepatic fibrosis↑	(185)
Nguyen *et al*, 2019	New Zealand rabbit	11 weeks	HFD	Liver tissue	Liver fat	Liver steatosis	(186)
Zheng *et al*, 2018	*Macaca mulatta*	8 weeks	Specific diet	Liver tissue	Ultrasound imaging of liver	Fat droplets↑, inflammatory cell infiltration, hepatocyte ballooning, hepatic fibrosis	(187)
Cydylo *et al*, 2017	Macaque	7 years	HFD	Liver tissue	H&E staining, MTC staining	Disorders of glucose-lipid metabolism, inflammatory response, hepatic fat deposition, hepatic fibrosis	(188)
Kramer *et al*, 2015	Marmoset	6 years	HFD	Blood from heart, liver tissue	Serum biochemical indicators, histopathology, ICH, liver lipid	Hepatomegaly with cytoplasmic vacuolation, IR, multifocal inflammation with ballooning hepatocyte degeneration	(189)
Hamid *et al*, 2019	Hy-Line Brown chicken	32 weeks	Specific diet	Blood from heart, liver tissue	Cecal microbiota	Dysbiosis of cecal microbiota, AST↑, ALP↑, UA↑	(190)
Zhu *et al*, 2022	Hy-Line Brown chicken	52 weeks	Corn-soybean diets	Liver tissue	Histopathology; western blotting	Changes in the expression levels of miR-216, miR-217, TG, TC	(191)
Tian *et al*, 2019	Lu's blue-shelled egg-laying hens	30 weeks	Specific diet	Liver tissue	Serum biochemical indicators, histopathology, western blotting	TG↑, TC↑, miR-34a-5p positively associated with ACSL1 protein expression	(192)
Song *et al*, 2022	Rhode island white hen	80 weeks	Specific diet	Blood from heart, liver tissue	Serum biochemical indicators, histopathology	Dyslipidaemia, severe degree of hepatic steatosis, significant atherosclerotic plaque formation	(193)

AST, aspartate aminotransferase; HFD, high-fat diet; FFA, free fatty acid; IR, insulin resistance; TC, total cholesterol; TG, triglyceride; UA, uric acid.

the culture medium suitable for the survival and proliferation of multiple cells, at least considering the effects of pH value, osmotic pressure and gas environment. Three-dimensional culture is divided into three-dimensional monoculture and three-dimensional co-culture.

Three-dimensional monoculture. Kostrzewski *et al* (212) established a three-dimensional human primary hepatocyte model. The model group contained 0.6 mmol/l of fatty acids and both the control group and the model group were supplemented with appropriate concentrations of insulin and

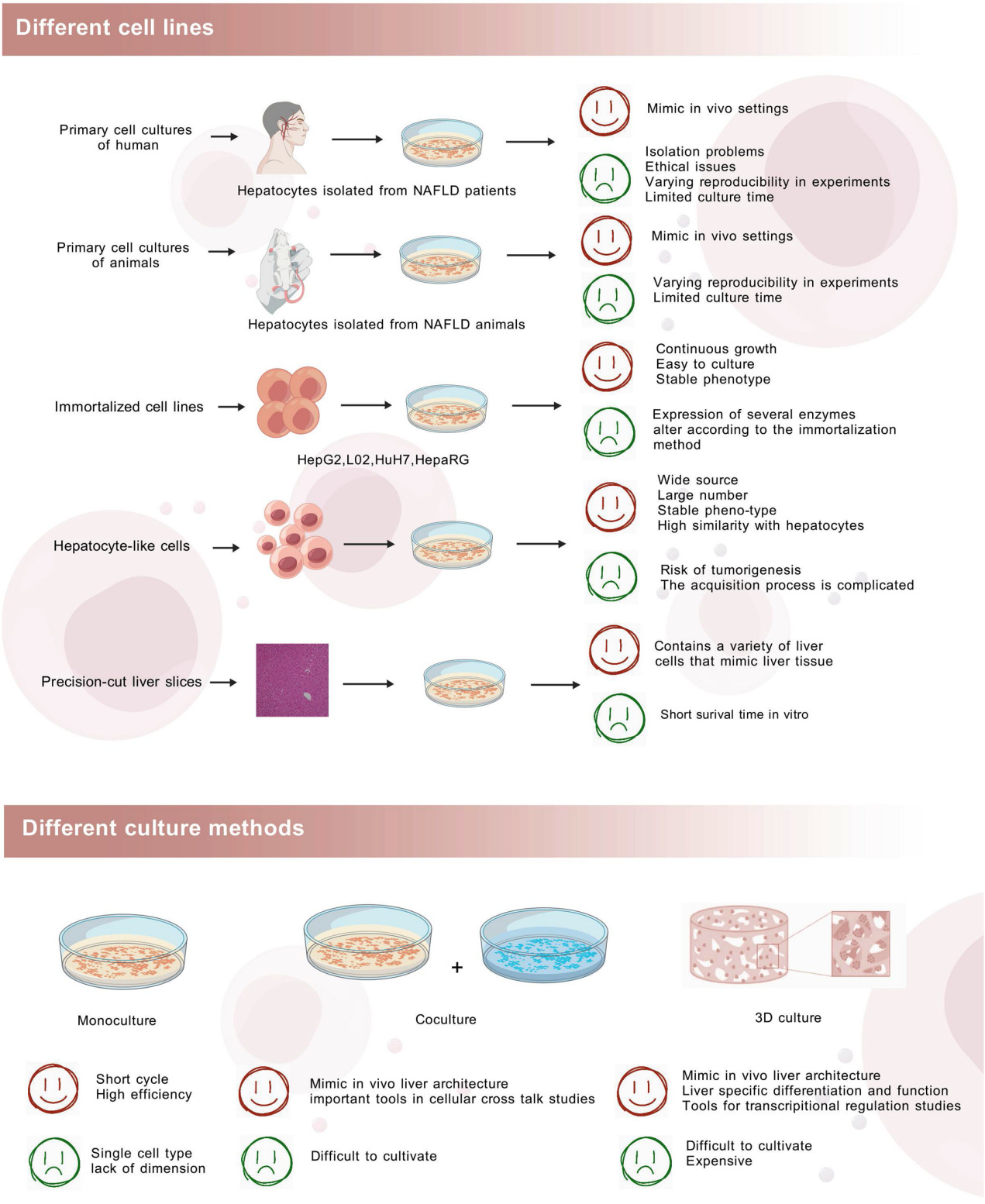

Figure 4. The advantages and disadvantages of different cell lines and different culture methods.

glucose. After 7 days of culture, the lipid accumulation in the model group increased markedly and further increased after 14 days. The fatty acids consumed by the model group were more than four times that of the control group. After model establishment, there was no significant change in the leakage of AST and ALT in the cells, indicating that the cell activity was normal and not affected by the modeling solution. The intracellular glutathione level, lactate dehydrogenase release, urea production and mitochondrial activity were also not affected by the modeling solution, proving that the cells in this three-dimensional model had complete functions. The expressions of genes CYP2E1, IGFBI, PDK4 and cholesterol 7 alpha-hydroxylase were all markedly increased, indicating that the model had the characteristics of steatosis. This model

had a significant effect in detecting anti-fatty liver drugs such as metformin. The three-dimensional model can offer an improved simulation of the *in vivo* environment, has a longer culture time and is conducive to repeated induction experiments. The spherical three-dimensional model can be used for high-throughput screening experiments with high efficiency. The disadvantage is that it is very time-consuming and expensive to establish a three-dimensional model. Since it is still in the early stage of use, some functions remain to be verified.

Three-dimensional co-culture model. The three-dimensional co-culture model can be used to study more complex fatty liver phenotypes, such as inflammation and liver fibrosis. In the three-dimensional co-culture model, human hepatocytes are cultured together with non-parenchymal cells, which can simulate the interactions between various types of cells in the liver in three-dimensional space. The advantage of the three-dimensional co-culture model is that it can maintain important metabolic functions for a long time and induce fatty liver pathological phenotypes under relevant conditions.

Hepatic spheroids. Hepatic spheroids are spheres formed by the self-aggregation of hepatocytes cultured in suspension without a substrate-promoting cell attachment. The size of the spheroids can be controlled by changing the number of starting cells and this controllability provides convenience for standardized measurement. The cell sources for hepatic spheroid culture can be primary hepatocytes, hepatocyte cell lines and induced pluripotent stem cells. Single-cell and multi-cell cultures can both form hepatic spheroids. Compared with the planar culture of cells, the three-dimensional structure presents superior characteristics of the liver. HepG2 cell spheroids are more sensitive to hepatotoxic substances. Compared with HepG2 cells cultured in a monolayer, the half-maximal effective concentration values of a number of drugs are markedly reduced (213). Hepatic spheroids cultured from PHH can survive up to 7 weeks (214) and can achieve 100% specificity and 69% sensitivity in distinguishing hepatotoxic substances (215). Compared with hepatic spheroids derived from single-cell culture, multi-cell co-culture spheroids may have more development potential. Hepatic spheroids constructed by co-culturing PHH and NPC perform outstandingly in glycogen storage (216) and albumin synthesis, indicating that co-culture hepatic spheroids can offer an improved imitation of the structure and function of the human liver (217).

Organoids. Organoids have a self-renewing stem cell population and can exhibit some organ characteristics, which contain single or multiple cell types. It has been reported that organoids from adult hepatocytes can be cultured for up to 2.5 months (218). PHH and pluripotent stem cells can both simulate the adult liver. For the induction of pluripotent stem cells, a three-dimensional model can be constructed after monolayer culture induction to maturity or directly cultured in three dimensions at the undifferentiated stage (219). The induction of MASLD requires the addition of FFA to the culture medium. Liver organoids in this hepatotoxic environment for a long time show lipid droplet formation and TG accumulation and the upregulation of the expressions of genes related to lipid metabolism, inflammatory cytokines and fibrosis markers, showing the typical biochemical characteristics of MASLD progression (220). In addition, liver organoids can also be derived from liver cancer cells to simulate the cancer microenvironment, playing an important role in the field of anti-cancer research.

Liver-on-a-chip. Liver-on-a-chip technology is used to simulate the minimum functional unit of liver tissue, mainly constructed with the assistance of computer aided design software. When designing a microfluidic chip, factors such as flow rate, design size and aspect ratio need to be considered. In a three-dimensional culture system, microfluidic devices can reproduce the characteristics of the multi-cell microenvironment by controlling various parameters (221) and conducting cell culture and sample secretions. Although liver-on-a-chip technology is costly and the chip manufacturing process is complex, it can mimic the complex *in vivo* liver microenvironment, precisely control chemical gradients and manipulate parameters such as time, so it has broad development prospects in the research field of cell-matrix and cell-cell interactions.

3D-bioprinting. 3D-bioprinting technology polymerizes various biological materials through inkjet, extrusion, laser polymerization, or digital light processing. In the field of liver model construction, a vascularized hepatic lobule model containing hepatocytes and endothelial cells has been developed through extrusion technology. Compared with a simple mixture of hepatocytes and endothelial cells, this model has increased albumin and urea secretion and a superior imitation of liver functions (222). With the development of 3D-bioprinting technology, the optimization of 'ink' has also been put on the agenda. Type I collagen is the main component of the ECM. When collagen I is mixed with thiolated hyaluronic acid in different ratios, it was found that the ratio of 3:1 can offer an improved maintenance of biological activity (223). The bioprinting method for MASLD needs to be further explored. One possible idea is to culture monolayers of steatotic hepatocytes as 'ink' to print three-dimensional structures or to induce lipotoxic substances after printing a liver model (224,225).

Chronic stress method. The chronic stress method uses one or several stress factors to change the emotional state of experimental animals (226). Although this method has a good effect, it takes a long time and only one emotional stimulus is applied to the rats for a long time, which is likely to cause the rats to adapt to the stimulus, resulting in insignificant emotional changes. To improve the errors caused by this method, unpredictable stress is now mostly used in the experiment, which can minimize errors and prevent rats from adapting to the stimulus. Sun *et al* (227) used fixed-time foot electroshock combined with noise stimulation to increase the diversity of chronic stress methods.

Chronic restraint method. The chronic restraint method is a way to restrict the activities of experimental animals by placing them in restraint devices, thus triggering emotional changes in the experimental animals. Some scholars have established the MASLD model by using long-term chronic restraint stress.

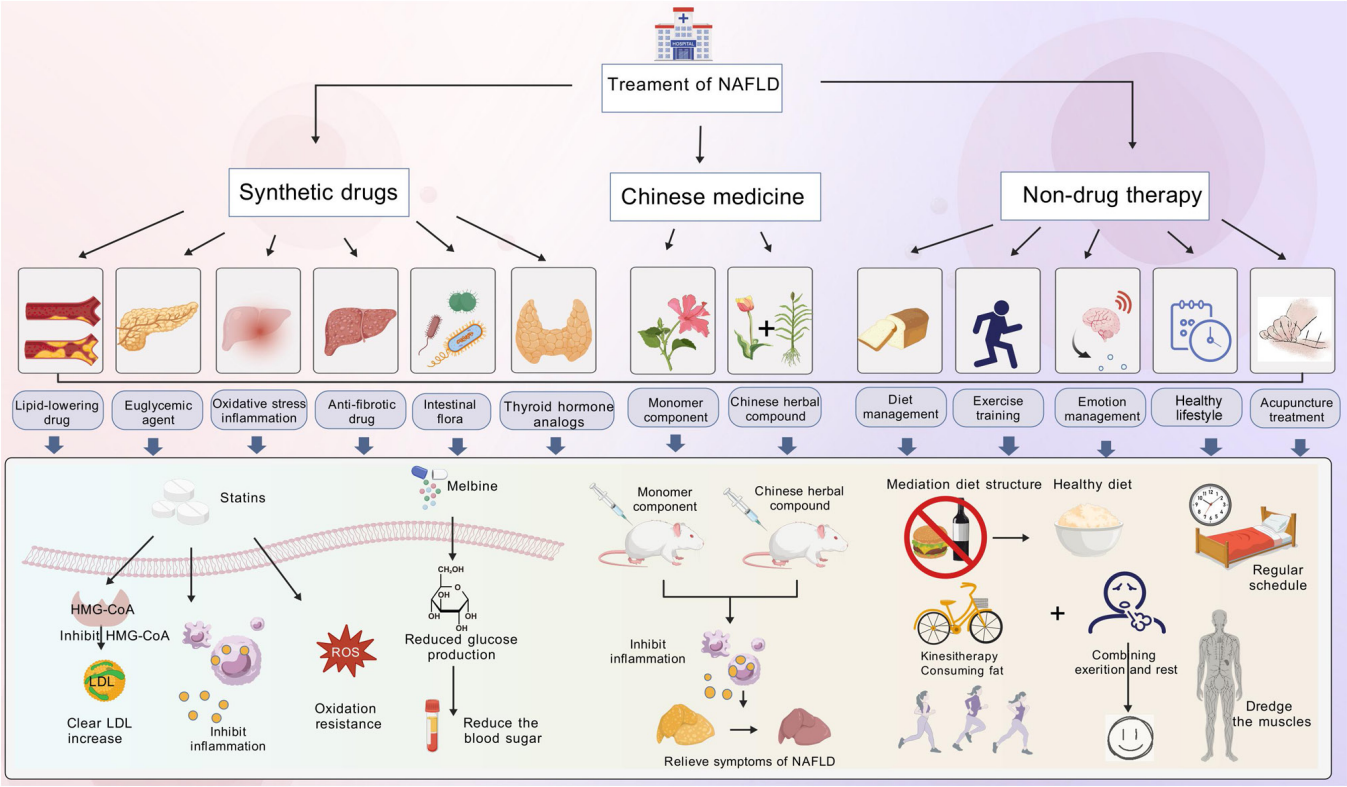

Figure 5. The means of drug therapy and non-drug therapy of MASLD. HMG-CoA, hydroxy methylglutaryl coenzyme A; LDL, low-density lipoprotein; MASLD, metabolic dysfunction-associated steatotic liver disease; NAFLD, non-alcoholic fatty liver disease; ROS, reactive oxygen species.

They mainly applied restraint stress to rats by using plastic restrainers. To prevent the rats from breaking free, they also carried out punctures multiple times to make the restrainers fit closely with the rats. The rats were restrained for 6 h every day and this lasted for 9 weeks (228-231).

Model of blood stasis syndrome. Since MASLD is a chronic disease, according to the theory that chronic diseases often lead to deficiency and blood stasis, patients with this disease usually present with various syndromes of deficiency and blood stasis, among which the syndrome of blood stasis is the most common. Patients with this syndrome show symptoms such as masses in the right hypochondrium, loss of appetite, abdominal distension, weakness, loose stools, dull complexion and a pale and dark tongue. Liu *et al* (232) prepared the MASLD model of blood stasis syndrome by feeding rats with a high-fat and HFFD and intraperitoneally injecting 30 mg/kg STZ. The results showed that the rats' body weight firstly increased then decreased. Their fur was yellowish, dull, wilting and lacking luster. Their mental state was poor, they were inactive and easily startled and their claws were dull and had a low temperature. Moreover, the levels of ALT, AST, TC, TG and LDL-C in rats were increased and there was inflammatory infiltration in the cytoplasm of hepatocytes. Researchers have prepared the MASLD model of blood stasis type by using high-fat feed combined with leg-binding stimulation and reagent intervention methods. After 8 weeks, the rats showed manifestations such as dull hair and irritability, an increased liver index, diffuse fatty hepatocytes, varying degrees of macrovesicular and microvesicular steatosis and occasionally inflammatory cell infiltration. Meanwhile, they also had pathological manifestations consistent with MASLD (233).

5. Treatment for MASLD

Treatments for MASLD of synthetic drugs. In the process of treating MASLD, since the changes in liver tissue of most patients are at the stage of simple steatosis, the priority of treatment should be to solve the problem of overweight and improve the patient's IR. The secondary aim is to avoid 'additional blows' leading to MASH and acute liver failure and to reduce hepatic fat deposition in patients (234). Currently, the main clinical drugs for MASLD are lipid-lowering drugs, insulin sensitizers, anti-fibrotic and thyroid hormone drugs. Moreover, inhibition or reduction of microRNA activity by targeted drugs can alleviate the condition, which provides a new idea for the proposal of MASH typing and the development of individualized treatment modalities (235,236). The precise treatment of different subtypes of MASH patients with targeted drugs is also the development trend of future drug research (Fig. 5). The drugs for the targets in the treatment of MASLD disease are shown in Table V.

Lipid-lowering drugs. Lipid metabolism disorder is the main clinical characteristic of MASLD patients and it is an important factor in the development of MASLD to hepatitis and cirrhosis, so lipid-lowering has become an important method of treatment for MASLD patients. At present, the best lipid-lowering effect of drugs is from statins, their mechanisms are mainly through the inhibition of hydroxy methylglutaryl

Table V. Treatments for metabolic dysfunction-associated steatotic liver disease of synthetic drugs.

Authors, year	Name	Target	Clinical Progress	Classification	(Refs.)
Abenavoli *et al*, 2018	OCA	FXR	Phase III	Insulin sensitizer	(249)
Mudaliar *et al*, 2013	GS-9674	FXR	Phase I	Insulin sensitizer	(244)
Cui *et al*, 2020	LJN452 FXR	FXR	Phase II	Insulin sensitizer	(242)
Abenavoli *et al*, 2018	LMB763	FXR	Phase II	Insulin sensitizer	(249)
Lewis *et al*, 2015	Pioglitazone	PPARγ	Phase III	Insulin sensitizer	(253)
Pawlak *et al*, 2015	Elafibranor	PPARα/δ	Phase III	Insulin sensitizer	(270)
Chen *et al*, 2015	Saroglitazar	PPARα/γ	Phase III	Insulin sensitizer	(252)
Harrison *et al*, 2020	IVA337	PPARα/δ	Phase II	Insulin sensitizer	(254)
Knudsen and Lau, 2019	Liraglutide	GLP-1	Phase II	Insulin sensitizer	(246)
Lewis *et al*, 2015	Somatostatin	GLP-1	Phase II	Insulin sensitizer	(253)
Sumida *et al*, 2018	INT-767	FXR/TGR5	Preclinical study	Insulin sensitizer	(251)
Sanyal *et al*, 2010	Vitamin E	FXR	Phase III	Oxidative stress and inflammation	(257)
Sanyal *et al*, 2010	Pentoxifylline	TNF-α	Phase II	Oxidative stress and inflammation	(257)
Kruger *et al*, 2018	Cenicriviroc	CCR2/CCR5	Phase II	Oxidative stress and inflammation	(267)
Zhu *et al*, 2021	Amlexanox	IKKε/TBK1	Phase II	Oxidative stress and inflammation	(261)
Hu *et al*, 2023	PXS-4728A	VAP-1	Phase I	Oxidative stress and inflammation	(262)
Xiang *et al*, 2016	Nalmefene	TLR4	Phase II	Oxidative stress and inflammation	(263)
Fernandes *et al*, 2014	Solithromycin	Intestinal lipase	Phase II	Intestinal targeted preparation	(279)
Chan *et al*, 2017	Orlistat	Intestinal lipase	Phase II	Intestinal targeted preparation	(260)
Chalasani *et al*, 2020	GR-MD-02	Galectin-3	Phase II	Anti-fibrosis drug	(273)
Bi *et al*, 2022	Simtuzumab	LOXL2	Phase II	Anti-fibrosis drug	(274)
Chalasani *et al*, 2020	Pirfenidone	TGF-β	Preclinical study	Anti-fibrosis drug	(273)
Dai *et al*, 2021	Oltipraz	LXRα	Phase II	Anti-fibrosis drug	(288)

CCR2/5, C-C motif chemokine receptor 2/5; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; OCA, obeticholic acid; PPAR, peroxisome proliferator-activated receptor; TGF-β, transforming growth factor-β; TGR 5, takeda G protein-coupled receptor 5; TLR 4, Toll like receptor 4; TNF-α, tumor necrosis factor-α.

coenzyme A (HMG-CoA), thereby blocking the synthesis of hepatic cholesterol and causing hepatic compensatory LDL receptor synthesis, so that the plasma LDL and LDL clearance increases. At the same time, statins can also exert anti-oxidant and anti-inflammatory effects (237). However, there are more problems in the use of statins, such as producing hepatotoxicity and muscle toxicity (238). In recent years, it has been found that inhibition of proprotein convertase-subtilisin-kexin type 9 (PCSK9) can markedly reduce LDL levels. Another experimental study found that the combination of statins with PCSK9 inhibitors was more effective than statins alone (239). In addition, ezetimibe and PCSK9 inhibitors (IIb, C) can be used in combination in statin-intolerant patients (240).

Insulin sensitizers. IR is a key link in the pathogenesis of MASLD, insulin sensitizers can effectively target the 'one strike' and increase insulin sensitivity to improve IR. Thus, enhancing the sensitivity of effector organs to insulin has become an important way to treat MASLD. Metformin, glucagon-like peptide-1 (GLP-1), thiazolidinediones and other drugs are currently more popular.

Metformin. Metformin contains two guanidinium groups in its structure and is a common oral metformin hypoglycemic drug in clinical practice. It lowers blood glucose by decreasing glucose production in the liver, increasing glucose metabolism and thus improving IR and has a certain effect on weight loss and regulation of lipid metabolism (241). However, metformin is generally not used alone in the treatment of MASLD (242). Some studies have shown that metformin can markedly reduce the levels of ghrelin, insulin and C-peptide in MASLD patients, but the improvement of liver fibrosis and liver inflammation is not significant (243,244). Although metformin is not the first choice for the treatment of MASLD, it is effective in improving body weight, lipids and glucose metabolism in patients with MASLD or MASLD combined with T2DM (245).

Agonists. GLP-1 is a well-established target in the field of diabetes. Liraglutide is a GLP-1 agonist used for the treatment of type 2 diabetes (1.2 to 1.8 mg/day) and obesity (3 mg/day). In addition to acting on the pancreas, GLP-1 can improve peripheral insulin sensitivity, participate in the physiological regulation of blood glucose, increase hepatic glucose uptake and glycogen synthesis, delay gastric emptying and reduce appetite and reduce the occurrence of atherosclerosis (246). Several GLP-1 drugs have been studied in clinical trials for their ability to promote insulin secretion, induce β-cell proliferation, inhibit postprandial glycogen release and delay gastric

emptying. It has been shown that GLP-1 receptor agonists can enhance insulin sensitivity in effector organs, promote fatty acid oxidation, reduce lipogenesis and improve glucose metabolism after binding to the receptor (247).

Polyene phosphatidylcholine has anti-oxidant, anti-inflammatory properties, reduces hepatocyte damage and apoptosis and can effectively target the pathological symptoms caused by MASLD and its combination with metformin drugs for the treatment of patients with MASLD as well as MASH is markedly more effective than monotherapy (248). Obeticholic acid (OCA), as a nuclear transcription factor FXR agonist, has good application in the treatment of MASLD by affecting the gene expression of various metabolic and cellular damage pathways, such as lipid metabolism, IR and oxidative stress (249).

Thiazolidinediones. Pioglitazones are first-generation insulin sensitizers, which can specifically bind and activate PPAR-γ, improve IR, participate in glucose and lipid metabolism and inhibit the expression of LP and the expression of TNF-α, so as to reduce the hepatic lipid deposition and inhibit liver inflammation and fibrosis formation (250). Sumida and Yoneda (251) found that pioglitazone not only improved IR but also improved hepatic glucose-lipid metabolism, steatosis and inflammatory necrosis in patients with MASLD by activating PPAR-γ. The American Academy of Liver Diseases recommended pioglitazone in 2017 guidelines for the diagnosis and treatment of MASLD (252). Pioglitazone is not widely available because of safety concerns, including congestive heart failure, bone fractures in women and the possibility that pioglitazone may lead to an increased risk of bladder cancer (253). The second-generation insulin sensitizer MSDC-0602K did not show the side effects associated with the first-generation insulin sensitizers. According to the report of the latest phase IIb 52-week double-blind study (254), MSDC-0602K markedly reduced fasting glucose, insulin, glycosylated hemoglobin and markers of liver injury without dose-limiting side effects. However, it did not show a significant effect on the liver histology of the biopsy technique used. The information gained from this trial may provide a pre-basis for future study.

Drugs that regulate oxidative stress and inflammation

Anti-oxidants. In the disease process of MASLD, increased oxidative stress and defective anti-oxidant defense mechanisms promote liver injury as well as disease progression to MASH (255). Therefore, drugs with anti-oxidant activity can be used in the treatment of MASLD. Vitamin E is present in the phospholipid bilayer of cell membranes and helps to prevent oxidative damage caused by free radicals (256). The randomized controlled trial of PIVENS selected non-diabetic and non-cirrhotic MASH patients, conducted a 2-year trial of vitamin E (800 IU/d), pioglitazone and placebo and showed that vitamin E had significant histological improvements and that 36% of patients treated with vitamin E had improved steatosis, inflammation and remission of MASH (257). This suggested that vitamin E is favorable for the treatment of MASLD and MASH (258). However, long-term use of vitamin E may increase the risk of prostate cancer and hemorrhagic shock, so vitamin E should not be used for long-term treatment of MASH and MASLD (259). Another study found that the active ingredient in silymarin could improve liver fibrosis and reverse MASLD by reducing oxidative stress and inflammation (260). Although silymarin did not meet the primary endpoint in MASH patients, it markedly improved liver fibrosis compared with placebo (260).

ZSP1601 is a pan-phosphodiesterase inhibitor, which is the first small molecule innovative drug to obtain clinical trial approval for the treatment of MASH in China. Phase Ia clinical trials were conducted in healthy populations and ZSP1601 had a peak time of 1.5-2.5 h and a half-life of 6.34-8.64 h. The drug was well tolerated when compared with the placebo group (261). Its safety and pharmacokinetic profile supports further efficacy assessment in MASH patients. In the Ib/IIa clinical trial, ZSP601 was shown to have a favorable safety and tolerability profile in MASH patients, with a pharmacokinetic profile consistent with that of healthy subjects. After receiving 28 days of treatment, different dose groups of ZSP601 showed significant improvements in metabolism, hepatic fat content, inflammation and fibrosis biomarker indices (262), supporting the evaluation of its long-term safety and efficacy in a larger sample size of MASH patients.

Apoptosis inhibitors. Apoptosis is closely related to the formation of hepatic fibrosis, hepatocyte apoptosis releases excessive DNA fragments, which stimulates hepatic stellate cell activation and promotes the formation of fibrosis. In the liver, TNF mediates a number of biological reactions, reduces oxidative stress and fights against fibrosis and it is a potential therapeutic drug for MASLD. Pentoxifylline has been suggested as a potential therapeutic agent for MASH because of its ability to reduce oxidative stress production and its anti-fibrotic effects. Researchers treated patients with biopsy-proven MASH with either placebo or pentoxifylline 400 mg/day for 1 year in a small RDBPCT clinical trial (263). The results showed an improvement in histologic features in 38.5% of the subjects in the pentoxifylline treatment group (13.8% in the control group, P=0.036).

Apoptosis signal-regulating kinase 1 (ASK1) is a kinase in the MAP3 kinase family that activates the p38/JNK pathway downstream of TNF-α, leading to apoptosis and fibrosis. Studies in mice have shown that ASK-1 promotes TNF-α-mediated IR and steatosis (263), whereas inhibition of ASK1 ameliorates diet-induced steatosis and fibrosis (264). GS-4997 is an oral ASK1 inhibitor and a randomized, double-blind, open phase II clinical trial in patients with MASH and fibrosis is currently underway to evaluate the efficacy of 24 weeks of treatment with GS-4997 or GS-4997 and simtuzumab. ASK-1 inhibitors have been shown to improve inflammation and fibrosis in animal models of MASH, but Selonsertib's phase III trial does not meet its primary endpoint (265).

Immunomodulators. C-C motif chemokine receptor 2/5 (CCR2/5) are inflammatory chemokine receptors that are highly expressed in MASH. Chemokines induce cell migration in the direction of increased chemokine concentration and exert their biological effects by interacting with G-protein-linked transmembrane receptors. CCR2 on the surface of macrophages promotes inflammation, neovascularization and activation of hepatic stellate cells, exacerbating hepatic fibrosis, whereas CCR5 is mainly expressed by hepatic stellate cells, causing hepatic fibrosis (266). Inhibition of the activity

of such receptors can reduce the inflammatory response in the liver and prevent or slow down liver fibrosis. Cenicriviroc (CVC) is an orally potent dual inhibitor of CCR2/5, which has been shown to ameliorate the progression of hepatic fibrosis in MASH mice (267). A phase IIb clinical trial with a sample of MASH patients showed that the anti-fibrotic results of CVC in the second year were consistent with those of the first year and remained significant in the two-year treatment group, demonstrating good tolerability and efficacy (268). CVC was in a phase III clinical study (AURORA, NCT03028740) aimed at evaluating its efficacy and safety in adult MASH patients with hepatic fibrosis (269). However, the AURORA phase III trial was forced to be terminated prematurely due to the failure to meet the objectives of its first phase results.

A total of two kinases, ικb kinase ε (IKKε) and recombinant tank binding kinase 1 (TBK1), are closely associated with obesity and inflammation and results from animal experiments demonstrated that the expression of IKKε and TBK1 was upregulated in adipose tissue of diet-induced obese animals, but IR, obesity and adiposity were not observed in IKKε and TBK1-deficient animals. During drug screening experiments for amlexanox, a drug used in the treatment of asthma and aphthous ulcers, it was discovered that amlexanox inhibits both IKKε and TBK12 kinases and reduces body weight in diet-induced or genetically obese animals by increasing energy expenditure. Amlexanox improves insulin sensitivity and reduces hepatic steatosis and inflammatory gene expression. A Phase II RDBPCT clinical trial is underway to evaluate the effect of amlexanox on liver steatosis in patients with diabetes, obesity and fatty liver disease (270).

Anti-inflammatory agents. Elafibranor is an agonist of PPAR-α and PPAR-δ. Studies have shown that elafibranor has the effect of improving insulin sensitivity, balancing blood glucose, regulating lipid metabolism and reducing inflammatory response (270). Ratziu *et al* (271) conducted an international, multicenter, randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of elafibranor in patients with MASH. MASH patients without cirrhosis were randomized in a 1:1:1 ratio to receive elafibranor 80, 120 mg, or placebo for 52 weeks. Liver biopsies, as well as clinical and laboratory evaluations, were performed on patients every 2 months. The results of the available studies suggest that elafibranor 120 mg/day for 1 year in moderate-to-severe patients may alleviate MASH fibrosis.

Anti-fibrotic agents. The degree of hepatic fibrosis is used to determine the progression of MASLD and most targeted clinical trials end in the improvement of fibrosis. In addition to acting on the pathogenesis of MASLD, some anti-fibrotic drugs have great potential. It has been shown that activation of HSCs can exacerbate the degree of hepatic fibrosis (272). Galactose lectin-3 (Gal-3) protein is closely associated with inflammation and liver fibrosis. Inhibiting Gal-3 can markedly reduce the symptoms of liver fibrosis in animals and clinical trials have also shown that it has an improved therapeutic effect on subjects with MASH and bridging fibrosis (273). Pirfenidone is a TGF-β inhibitor, clinically used in the treatment of idiopathic pulmonary fibrosis and another study found that it markedly improved the pathological features of liver fibrosis in animals (273). Belapectin (GR-MD-02) is a Gal-3 inhibitor developed by Galectin Therapeutics. Gal-3 protein is involved in a variety of inflammatory and fibrotic processes. Animal studies have demonstrated that Belapectin reduces fibrosis. Clinical trials have also demonstrated that it is well tolerated in subjects with MASH with bridging fibrosis and has beneficial prevention and treatment of esophageal varices in patients with MASH liver cirrhosis (273).

Regulation of intestinal flora. Dysbiosis of intestinal flora is closely associated with liver disease and cancer (274). Thus, manipulation of the microbiome through diet, probiotics, prebiotics and other medications is a viable line of research for the treatment of liver diseases, including MASLD. It has been found that prebiotics can reduce the accumulation of TG in the liver by decreasing the production of fat and decreasing the expression of genes such as FAS (275). In addition, probiotics can repair leaky gut and delay the progression of NAFL to MASH by modulating nuclear receptor expression and improving IR in liver and adipose tissue (276). In a clinical study by Malaguarnera *et al* (277), *Bifidobacterium bifidum* was found to markedly improve lipids, IR index, liver tissue inflammation and liver fibrosis in patients with MASH. Wang *et al* (278) found that *Bifidobacterium trifidum* tablets markedly reduced the levels of lipids, inflammatory factors, LPS and lipocalin in a rat model of MASH. Triclosan is a widely used antibiotic that ameliorates the ecological dysbiosis of the gut microbiome associated with MASLD by inhibiting pathogenic gram-negative bacteria. Solithromycin is a new-generation macrolide antibiotic clinically used to treat bacterial infections. In a mouse model of MASH induced by both diet and STZ, solithromycin was shown to ameliorate hepatocellular ballooning and inflammation, reduce blood glucose levels and downregulate hepatic gluconeogenic enzyme expression (279). Based on preclinical results, a Phase II open clinical trial study is currently underway to evaluate whether 13 weeks of solithromycin treatment in non-cirrhotic MASH patients can improve liver histologic characteristics.

Thyroid hormone analogs. Thyroid hormone receptor (THR-β) is highly expressed in hepatocytes and plays a key role in regulating the impaired metabolic pathway of MASH (280). The degree of hepatic hypothyroidism is higher in patients with MASH (281). This suggests that hepatic hypothyroidism may contribute to the development of MASH to some extent. Moreover, drugs targeting this condition are currently approved for MASH treatment.

Resmetirom (MGL-3196). Resmetirom is an oral, liver-targeted THR agonist, which is 28-fold more selective for THR-β than triiodothyronine for THR-α (282). In MASH, Resmetirom's selectivity for THR-β contributes to hepatic-mediated thyroid hormone metabolism while avoiding the adverse effects of excess thyroid hormones in the heart and bone mediated through THR-α. Resmetirom and other thyroid hormone analogs reduce hepatic TG, inflammatory factors, fibrosis markers and ALT levels and reduce hepatic steatosis lipid peroxidation (283). In a multicenter, randomized, double-blind, placebo-controlled Phase III clinical trial of MAESTRO-MASH, the proportions of patients who achieved

the primary endpoint of MASH symptom resolution without worsening of hepatic fibrosis at 52 weeks were 25.9 and 29.9% in the Resmetirom 80 and 100 mg dosage groups, respectively, compared with 9.7% in the placebo group (P<0.001). In addition, there were significant reductions in several lipid and lipoprotein metrics, including the key secondary endpoint of LDL-C (284). This trial was the first phase III clinical trial to achieve the two primary endpoints proposed by the FDA in patients with MASH. Accordingly, based on these key clinical data, the FDA approved the drug on March 15, 2024, for the treatment of patients with non-cirrhotic MASH with moderate to advanced liver fibrosis. This is the first MASH treatment approved by the FDA and represents a landmark breakthrough in the field.

VK2809. VK2809 is another THR-β selective agonist with specificity for liver tissue and good therapeutic potential. It belongs to a family of prodrugs that cleave and release potent thyroxine analogs *in vivo*. A randomized, double-blind, multicenter and placebo-controlled phase II study was conducted to evaluate the efficacy, safety and tolerability of VK2809 in reducing LDL-C and liver fat content in patients with primary hypercholesterolemia and MASLD (285). Patients were dosed for 12 weeks and subsequently discontinued for 4 weeks. Results showed a statistically significant 53.8% median reduction in liver fat in patients using 5 mg of VK2809 daily, 88% of cases in patients using VK2809 showed ≥30% reduction in liver fat at 12 weeks. In all dose groups, VK2809 had a favorable safety and tolerability profile, with no adverse reactions reported.

Treatment of MASLD with medicinal plants. Medicinal plants have important advantages in preventing and improving MASLD, so treatment of MASLD by medicinal plants is a potential therapeutic means (286). Medicinal plants have a history of several hundred years in the prevention and treatment of liver diseases. Compared with chemical pharmaceuticals, medicinal plants have a holistic concept and the idea of diagnosis and treatment, which is the most significant and basic feature of medicinal plants and shows its advantages in the treatment of MASLD (287). The treatment of MASLD by medicinal plants focuses on the holistic theory of liver preservation, which manifests itself in a variety of mechanistic forms, including anti-oxidative stress, lipid metabolism regulation, anti-inflammation, anti-fibrosis and intestinal microbiota regulation (288). It can be seen that the treatment of MASLD by medicinal plants is more individualized and comprehensive, which is in line with the characteristics of MASLD (Fig. 5).

Mechanisms of components of unitary medicines in treating MASLD. Single medicinal plants have multi-component, multi-target and multi-dimensional effects in anti-MASLD. Exploring the monomers of medicinal plants for the treatment of MASLD studies have found that natural compounds such as polysaccharides, terpenoids, glycosides, alkaloids, flavonoids, phenols and other natural compounds have a wide range of biological activities. By regulating various signaling pathways, they can exert comprehensive benefits such as inhibiting inflammatory response, improving lipid metabolism, reducing IR, then effectively alleviating the symptoms of MASLD. A summary of some studies on the prevention and treatment of MASLD by medicinal plants is shown in Table VI.

Mechanism of Chinese traditional patent medicine in treating MASLD. Based on evidence-based treatment, medicinal plants combine different chemical components for specific etiology and pathogenesis and exert the advantages of multi-targeting and synergistic effects in the prevention and treatment of MASLD. The mechanisms are summarized in the following Table VII.

Non-pharmacological treatments. Non-pharmacological treatments for MASLD are mainly life-related initiatives. In general, weight control, improved dietary patterns and lifestyle modifications can prevent the development of metabolic syndrome and MASLD (374). Proper aerobic exercise with intermittent high-intensity exercise can effectively reduce excess body fat. Hannah and Harrison (375) found that MASLD patients' weight loss of 3-5% slowed down the process of fatty liver lesions, weight loss of 5-7% may result in reversal of fibrosis. Obese and diabetic patients are more likely to plan their diets reasonably, adjust their daily routines and reduce their calorie intake to prevent MASLD at source (Fig. 5).

Dietary management. Dietary management should follow the principles of nutritional balance, limiting energy intake and adjusting dietary structure. Firstly, to supplement dietary fiber and minerals: Eat more food rich in low calorie, high protein and high fiber, mix coarse and fine grains and choose more vegetables, fruits, fungi and seaweed to ensure sufficient fiber intake and maintain intestinal microbial homeostasis. Secondly, to supplement vitamins and appropriate amounts of micronutrients: eat more food rich in vitamins and micronutrients selenium (purple cabbage, beans, seafood and seaweed) can accelerate the decomposition of lipid peroxidation and prevent liver fibrosis (376). Reduce the intake of carbohydrates: Eat less high sugar, high fat, high cholesterol, spicy, fried and salty food. In order to reduce the degree of obesity and promote the oxidation and decomposition of fatty acids, reduce the content of FFA and the burden of fat accumulation on the liver (377). In addition, fasting therapy: Patients only drink a moderate amount of water and eat low-calorie food for a certain period of time, plus a moderate amount of exercise to prevent and control MASLD, usually not more than 10 days. After the fasting period, the use of food from soft and easy to digest food gradually over to ordinary meals. For patients with fatty liver caused by excessive weight loss, food containing unsaturated fatty acids can be given to increase satiety and nutrition according to the patient's own situation and their fat intake should not exceed 30% (378). In other aspects: Develop good dietary habits, eat three meals a day regularly and quantitatively, avoid overeating diet, regular breakfast, reduce the number of fast food and dinner, drink more tea and coffee.

Exercise training. Exercise training therapy is an effective means to promote the recovery of MASLD patients, which has advantages of being green and inexpensive. Aerobic exercise can improve the rate of fat oxidation, increase fat

Table VI. Treatments for metabolic dysfunction-associated steatotic liver disease from natural products.

Authors, year	Name	Mechanism	Classification	(Refs.)
Deng *et al*, 2021	Polygonin	Hepatic steatosis↓, IR↓, glycolipid metabolism↓, oxidative stress↓, maintain the stability of liver lysosomes; fatty acids↓	Glycoside	(289)
Pan *et al*, 2016	Ginsenoside Re	IR↓, regulate lipid metabolism, mitochondrial dysfunction↓, oxidative stress↓, NF-κB ↓	Glycoside	(290)
Li *et al*, 2021	*Panax notoginseng* saponin	AST↓, GPT↓, LDL↓, TC↓, TG↓, intestinal permeability↓, LPS↓, TLR4↓	Glycoside	(291)
Liu *et al*, 2020	Astragaloside IV	TLR4↓, MyD88↓, NF-κB↓, AMPK↑, SREBP-1c↓, IR↓, fat deposition↓	Glycoside	(292)
Zhao *et al*, 2019	*Cassia obtusifolia* anthraquinone glycoside	TLR4↓, NF-κB↓, skin inflammation↓, blood lipids↓, lipid peroxidation reaction↓	Glycoside	(293)
Paudel *et al*, 2018	Cassiaside	Affects Nrf2, HepG2 cells death↓, regulating NF-κB, liver inflammation response↑	Glycoside	(294)
Zhang *et al*, 2015	Geniposide	AST↓, GPT↓, LDL↓, TC↓, TG↓, HDL↑, TNF-α↓, IL-6↓, liver damage↓, clear oxygen free radicals↑	Glycoside	(295)
Lee *et al*, 2014	Salidroside	ACCase↓, Malonyl CoA↓, CPT-1↑, oxidation of fatty acids↑, hepatic steatosis↓	Glycoside	(296)
Xu *et al*, 2020	Gentiopicroside	pAMPKα↑, oxidative stress↓, regulate Nrf2, TLR4↓, NF-κB↓, regulate inflammatory response and lipid metabolism	Glycoside	(297)
Molteni *et al*, 2016	Didymin	TLR/NF-κB↓, release of cytokines↓	Glycoside	(298)
Wang *et al*, 2016	Baicalin	Inflammatory cytokines↓, FFA↓	Glycoside	(299)
Zhen *et al*, 2018	Total glucosides of paeony	TLR4↓, C-Jun N-terminal kinase↓, TG↓, TC↓	Glycoside	(300)
Shen *et al*, 2020	Gypenoside	IL-17↓, TNF-α↓, IL-10↑, regulate Treg/Th17 immune dysfunction	Glycoside	(301)
Li *et al*, 2020	Hesperidin	SREBP-1c↓, PPARα↑, TLR4↓, IL-1↓, TNF-α↓	Glycoside	(302)
Park *et al*, 2010	Oleuropein	Hepatic steatosis↓, PPARγ↓	Glycoside	(303)
Chen *et al*, 2025	Buddleoside	Regulates AMPK-TFEB, lipid accumulation↓, inflammatory response↓, improve liver function	Glycoside	(304)
Chitturi *et al*, 2018	Quercetin	Regulates TC metabolism, CYP7A1 activity↑, cytochrome overexpression↓	Flavonoid	(234)
Yin *et al*, 2019	Genistein	TLR4/NF-κB↓, IR↓, TNF-α↓	Flavonoid	(305)
Liu *et al*, 2021	Luteolin	LPS↓, TLR4↓, inflammatory cytokines↓	Flavonoid	(306)
Kou *et al*, 2024	Chuanpianin Nobiletin	Lipid metabolism, lipid accumulation↓	Flavonoid	(307)
He *et al*, 2018	Naringenin	Blood lipids↓, obesity↓, IL-1β↓, TNF-α↓, IL-6↓, lipid toxicity↓	Flavonoid	(308)
Pan *et al*, 2017	Puerarin	AMPK↑, PPARα↑, FFA↓, IR↓	Flavonoid	(309)
Liang *et al*, 2016	Ginkgo flavone	Reduce levels of NF-κB and TNF-α	Flavonoid	(310)
Huang *et al*, 2019	Tanshinone	TLR4/NF-κB↓, liver fat accumulation↓, regulate oxidative stress	Terpenoid	(311)
Zheng *et al*, 2020	Curcumol	TLR4/NF-κB↓, liver function↑, lipid levels↑	Terpenoid	(312)
Han *et al*, 2018	Celastrol	TLR4↑, MyD88↑, NF-κB↑, steatosis↓, inflammatory response↓	Terpenoid	(313)
Ye *et al*, 2024	Asiatic acid	TLR4/NF-κB/NLRP3↓, liver inflammation↓	Terpenoid	(314)
Malekinejad *et al*, 2023	Lupeol	TLR4↓, IR↓, oxidative stress↓	Terpenoid	(315)
Brusotti *et al*, 2017	Betulinic acid	Mediates synthesis of phospholipids and TG, hepatic steatosis↓, LPL↓, cellular lipid accumulation↓	Terpenoid	(316)
Hou *et al*, 2015	Ursolic acid	Lipolysis, TG↓, TC↓	Terpenoid	(317)

Table VI. Continued.

Authors, year	Name	Mechanism	Classification	(Refs.)
Zhang *et al*, 2012	Andrographolide	Expression of adipogenic genes↓, cellular lipid accumulation↓, lipid metabolism↑	Terpenoid	(318)
Zhang *et al*, 2019	Diosgenin	AMPK↑, PPARα↑, FFA↓, IR↓	Terpenoid	(319)
Zhang *et al*, 2019	Hawthorn acid	Stress response↓, NF-κB↓	Terpenoid	(319)
Zhou *et al*, 2019	Alisol A	ABCA1/ABCG1↑, SERBP-1c↓ ACC↓, Fas↓, CPT1↑, ACOX1↑, AMPKα↑, TG↓, blood lipid↓, IR↓, liver steatosis↓	Terpenoid	(320)
Ho *et al*, 2019	Alisol B	Cell lipid toxicity↓ FXR↑, lipid accumulation↓, hepatic lobular inflammation↓, peri cellular fibrosis↓	Terpenoid	(321)
Li *et al*, 2022	Resveratrol	TLR4/NF-κB↓, lipid deposition↓, oxidative stress↓, mitochondrial activity↑, TG↓, regulate intestinal microbiota composition	Phenol	(322)
Feng *et al*, 2019	Curcumin	LPS↓, TLR4/NF-κB↓, TNF-α↓, IL-1β↓, improve intestinal oxidative stress, intestinal inflammatory response↓	Phenol	(323)
Zhang *et al*, 2024	Gastrodin	SREBP1c↓, TLR4↓, lipid synthesis↓	Phenol	(324)
Xiao *et al*, 2017	Litchi pulp phenolics	Clear free radicals, regulate mitochondrial dysfunction	Phenol	(325)
Aithal *et al*, 2008	Proanthocyanidins	Lipid accumulation↓, hepatic steatosis↓, oxidative stress↓	Phenol	(326)
Castellino *et al*, 2019	Chlorogenic acid	JNK↓, IR↓, relieve liver metabolic index	Phenol	(327)
Tan *et al*, 2017	Green tea polyphenols	AMPK↑, ACC↑, SREBP-1c↑, BG↓, lipid↓	Phenol	(328)
Chen *et al*, 2018	Salvianolic acid B	Regulates SIRT3 and SOD2 pathways, improve liver lipid deposition, oxidative stress response↓	Phenol	(329)
Zhang *et al*, 2021	Berberine hydrochloride	TLR4↓, IκBα↓, liver inflammation↓	Alkaloid	(330)
Kim *et al*, 2015	Total alkaloids of corydalis yanhusuo	TLR4/NF-κB↓, inflammation↓, hepatic lipid↓	Alkaloid	(331)
Sun *et al*, 2021	Matrine	Lipogenesis↓, mitochondrial dysfunction↓, ER stress↓	Alkaloid	(332)
Cani and Delzenne 2009	Berberine	Improves mitochondrial swelling, promote mitochondrial fusion↑, liver lipid content↓, liver inflammation↓	Alkaloid	(333)
Zhang *et al*, 2015	Nuciferine	PASK↓, improve abnormal accumulation of glycerophospholipid and linoleic acid, regulate FFA permeation, oxidative stress	Alkaloid	(334)
Rahban *et al*, 2022	*Atractylodes macrocephala* polysaccharide	TLR4↓, IL-1β↓, IL-6↓, TNF-α↓	Polysaccharide	(335)
Zhong *et al*, 2022	Astragalus polysaccharide	TLR4↓, NF-κB↓, NLRP3↓, liver inflammation↓, lipid accumulation↓	Polysaccharide	(336)
Wang *et al*, 2024	*Pericarpium citri* reticulatae polysaccharide	Interferes with tlr4/md2 signaling pathway, macrophage infiltration↓, LPS↓, inflammation↓	Polysaccharide	(337)
Yu *et al*, 2021	*Dendrobium nobile* polysaccharide	TLR4↓, HO-1↓, inflammatory↓	Polysaccharide	(338)
Liu *et al*, 2024	*Polygala fallax* Hemsl Polysaccharide	TLR-4↓, NF-κB↓, peroxidation↓, inflammatory↓	Polysaccharide	(339)

Table VI. Continued.

Authors, year	Name	Mechanism	Classification	(Refs.)
Zhang and Jin, 2019	*Dicliptera chinensis* polysaccharide	PPAR-γ↑, TLR-4↓, NF-κB↓, TNF-α↓, IL-6↓, IL-1β↓	Polysaccharide	(340)
Zhao *et al*, 2022	Sodium alginate	LPS↓, TLR4↓, inflammatory↓	Polysaccharide	(341)
Liu *et al*, 2017	*Bupleurum chinense*	Activation of hepatic stellate cells↓, injury of hepatocytes↓	Polysaccharide	(342)
Xie *et al*, 2022	Zingerone	Regulates TLR4, Nrf2, mRNA, intervene lipid metabolism, oxidative stress	Other chemicals	(343)
Lv *et al*, 2018; Wei *et al*, 2016	Rhein	MDA↓, SOD↑, GSH-Px↑, oxidative stress↓, fat metabolism↓, fat infiltration of hepatocytes↓, inflammatory factors↓	Anthraquinone	(344,345)
Dong *et al*, 2016	Emodin	IRE1α↓, TNF-α↓, IL-6↓, IL-1β↓, regulate PPAR-γ, IR↓, FXR↑, blood lipid content↓, obesity↓, fat deposition↓	Anthraquinone	(346)
Ye *et al*, 2022	Fucoxanthin	Regulates AMPK/NRF2/TLR4, lipid metabolism, oxidative stress, inflammation	Alkene	(347)
Borstlap *et al*, 2018	Sulforaphane	LPS↓, LPS/TLR4↓, fat metabolism↓, inflammatory↓	Isothiocyanate	(348)
Yu *et al*, 2022	Schisandrin A	LPS↓, TLR4↓, fat metabolism↓, inflammatory↓	Lignan	(349)
Gu *et al*, 2021	Coix seed oil	p-AMPK↓, sepp1↓, apoer2↓, fat accumulation↓	Volatile oil	(350)

AMP, adenosine 5'-monophosphate; AMPK, AMP-activated protein kinase; AST, aspartate aminotransferase; FFA, free fatty acid; FXR, farnesoid X receptor; GSH, glutathione; HFD, high-fat diet; IL-6, interleukin-6; IR, insulin resistance; IRE1α, inositol-requiring enzyme-1α; JNK, Jun n-terminal kinase; LDL, low-density lipoprotein; LPS, lipopolysaccharide; NF-κB, nuclear factor-kappa B; PPAR-α, peroxisome proliferator-activated receptor-α; SOD, superoxide dismutase; SREBP-1c, sterol regulatory element binding protein-1c; TC, total cholesterol; TG, triglyceride; TLR 4, Toll like receptor 4; TNF-α, tumor necrosis factor-α.

consumption and reduce the accumulation of liver fat (379). At present, with the social and economic development and the increase of pressure at work, people are physically and mentally exhausted. It is easy to occur a variety of chronic diseases due to less exercise training or insufficient continuity, so it is more important to guide the MASLD patients to engage actively in exercise training. Li *et al* (380) used exercise training to intervene in MASLD, 3 times a week, each time lasting 3-4 times, lasting 40-60 min and the continuous intervention for 24 weeks, which could effectively improve the lipid metabolism level of patients with mild, moderate and severe MASLD, reduce blood glucose and improve the function of the liver.

Emotion management. Anxiety, depression and other negative emotions will increase the risk of MASLD (381). Therefore, patients should always maintain a stable state of mind, guard against arrogance and impatience, combine work and rest and learn to apply exercise, music, muscle relaxation in daily life and other methods to alleviate their own negative emotions, relax the body and mind and ultimately to achieve the effect of liver detoxification and restoration of liver function.

Maintain healthy lifestyle. Circadian rhythm disorders can cause MASLD, so patients should develop good living habits, including go to bed early, get up early, not stay up late, regular personal work and rest. At the same time, regular checks of blood glucose, blood lipids, blood pressure and abdominal ultrasound, in order to timely detection of the occurrence of MASLD and its risk factors (381).

6. Conclusion and future perspectives

As a complex metabolic disease involving multiple liver injuries, the incidence of MASLD is not only increasing year by year, but also tends affect younger and younger individuals, so its prevention and treatment should not be postponed. The pathogenesis of MASLD is complex and progressive, the liver histopathological diagnosis of MASLD is difficult to achieve in humans and suitable experimental models must be established to study MASLD, thus posing great challenges to its prevention and drug development. The present study reviewed the complex pathogenesis of MASLD, including IR, lipotoxicity, immune system disorders inducing inflammatory responses, intestinal flora disorders and genetic factors, as well as the study of Chinese medicine on the pathogenesis of MASLD (Fig. 6). MASLD arises from the interconnection and influence of multiple organs and systems, which can not only act individually, but also interact and synergize with each other to promote the development of MASLD and the specific triggering mechanisms still need to be explored at a deeper level.

In recent years, the study of the molecular mechanism of MASLD has attracted much attention. The notch

Table VII. Treatments for metabolic dysfunction-associated steatotic liver disease of Chinese traditional patent medicines.

Authors, year	Name	Source	Mechanism	Classification	(Refs.)
Pan *et al*, 2021	Shen-Ling-Bai-Zhu-San	Prescriptions People's Welfare Pharmacy	LPS↓, TLR4/NLRP3↓, inflammatory factors↓	Classic prescription	(351)
Du *et al*, 2024	Fu-Zi-Li-Zhong-Wan	Sanyin Ji Yi Bingzheng Fang Lun	TLR4↓, TRAM/TRAF3↑, LDL-C↓, FFA↓, HDL-C↑	Classic prescription	(352)
Liu *et al*, 2021	Er-Chen-Tang	Prescriptions People's Welfare Pharmacy	LPS↓, TLR4↓, inflammatory factor↓	Classic prescription	(353)
Zhang *et al*, 2020	Si-Wu-Tang	Secrets of treating wounds and bone-setting	TLR4/JNK↓, inflammatory↓, hepatic steatosis↓	Classic prescription	(354)
Su *et al*, 2024	Xiao-Yao-Wan	Prescriptions People's Welfare Pharmacy	Regulates TLR4-NF-κB, IL-6↓, TNF- α↓	Classic prescription	(355)
Feng *et al*, 2023	Astragalus powder	Sheng Ji Zong Lu	AMPK/TLR4↑, hepatic steatosis↓	Classic prescription	(356)
Xia *et al*, 2016	Zhi-Gan-Tang	Hospital preparation	Improves ALT, AST, LDL-C, TG	Self-formulated prescription	(357)
Lu *et al*, 2019	Qing-Hua-Tang	Hospital preparation	Hepatic steatosis↓	Self-formulated prescription	(358)
Xie *et al*, 2021	Chai-Hu-Bao-Gan-San	Hospital preparation	ALT↓, AST↓, TC↓, IL-1↓, IL-6↓, TNF-α↓	Self-formulated prescription	(359)
Xu *et al*, 2019	Yin-Chen-Hao-Tang	Hospital preparation	Regulates lipid metabolism and intestinal microecological balance	Self-formulated prescription	(360)
Zhan *et al*, 2021	Ban-Xia-Yang-Xin-Tang	Hospital preparation	TC↓, TG↓, LDL-C↓, FGP↓, FINS↓, HOMA-IR↓, ISI↑, HOMA-β↑, fat accumulation↓, improve IR	Self-formulated prescription	(361)
Wan *et al*, 2020	Bu-Shen-Tiao-Gan-Tang	Hospital preparation	Blood lipid↓, improves IR	Self-formulated prescription	(362)
Chen *et al*, 2020	Wu-Cao-Fen	Hospital preparation	Improves liver function and blood lipid indicators	Self-formulated prescription	(363)
Mian and Yang, 2018	Huo-Xue-Jiang-Zhi-Tang	Hospital preparation	Improve TNF-α, FFA	Self-formulated prescription	(364)
Zhang *et al*, 2024	Jian-Pi-Jiang-Zhi-Tang	Hospital preparation	HMGB1/TLR4/NF-κB↓, improve hepatic inflammatory response	Empirical effective prescription	(365)
Wang *et al*, 2023	Zhi-Mu-Shan-Zha-Yin	Hospital preparation	TLR4/MyD88/NF-κB↓, hepatocyte apoptosis↓, lipid metabolism disorder↓, oxidative stress↓	Empirical effective prescription	(366)
Wang *et al*, 2024	Tong-Ping-Zhi-Fang-Tang	Hospital preparation	TLR4↓, MyD88↓, NF-κB↓, liver injury↓	Empirical effective prescription	(367)
Ding *et al*, 2021	Qing-Gan-Jiang-Zhuo-Tang	Hospital preparation	TLR4↓, NF-κB↓, MyD88↓, liver fat accumulation↓	Empirical effective prescription	(368)
Chen *et al*, 2020	Hu-Gan-Qing-Zhi-Pian	Hospital preparation	TLR4/MyD88↓, inflammatory factor↓	Empirical effective prescription	(369)
Ye *et al*, 2021	Hua-Zhi-Fu-Gan granule	Hospital preparation	Regulate the expression of TLR4 and NF-κB	Chinese patent drug	(370)
Guo *et al*, 2022	Deng-Zhan-Sheng-Mai capsule	Hospital preparation	LPS↓, TLR4/NF-κB↓, inflammatory response↓	Chinese patent drug	(371)
Liao *et al*, 2019	Zhi-Bi-Tai capsule	Hospital preparation	MAPK-ERK-TLR4↓, inflammatory response↓	Chinese patent drug	(372)

Table VII. Continued.

Authors, year	Name	Source	Mechanism	Classification	(Refs.)
Yang *et al*, 2022	Ku-Huang granule	Hospital preparation	TLR4↓, MyD88↓, regulate IR	Chinese patent drug	(373)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FFA, free fatty acid; IL-6, interleukin-6; IR, insulin resistance; LDL, low-density lipoprotein; LPS, lipopolysaccharide; NF-κB, nuclear factor-kappa B; TC, total cholesterol; TG, triglyceride; TLR 4, Toll like receptor 4; TNF-α, tumor necrosis factor-α.

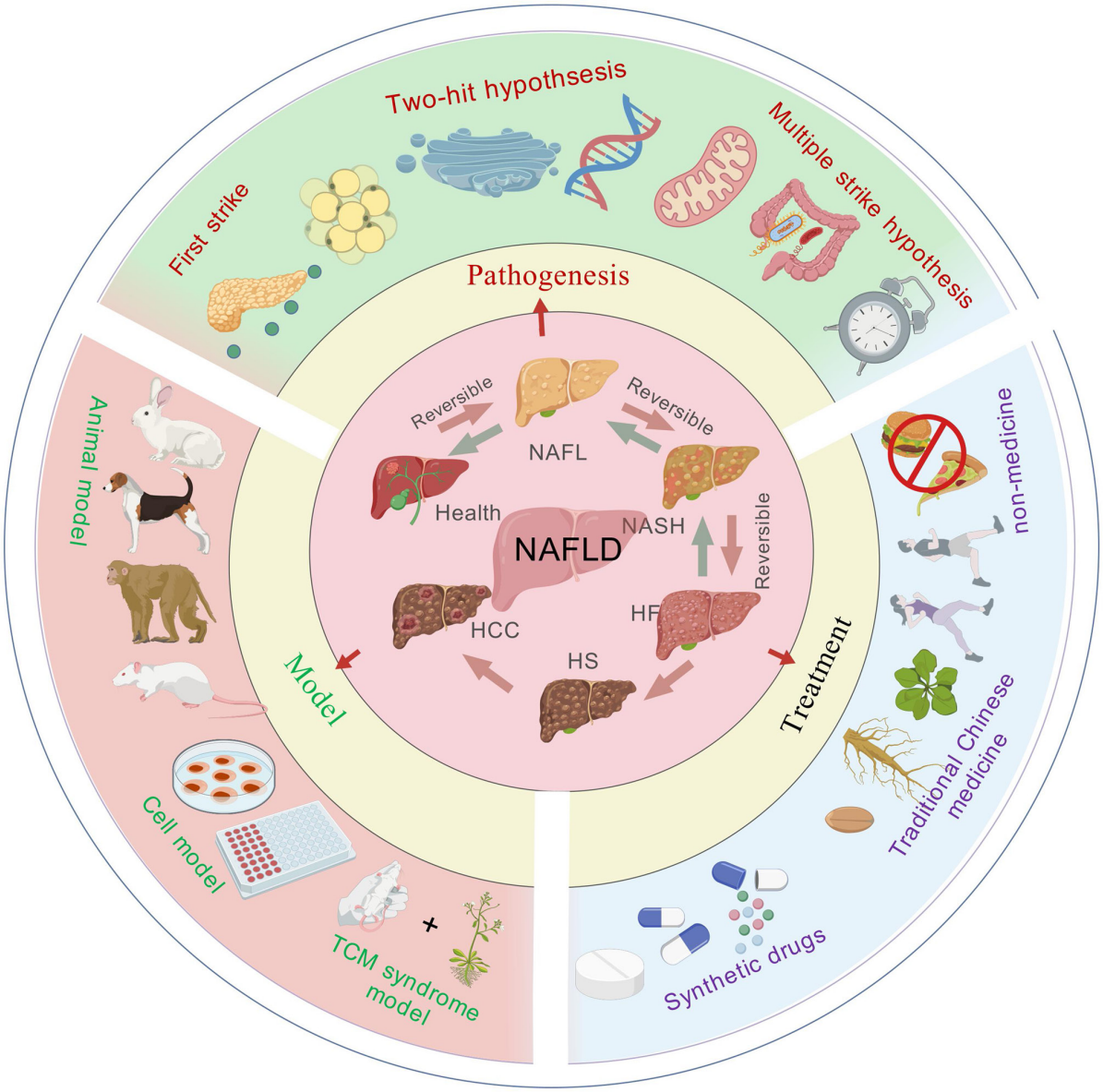

Figure 6. The pathogenesis, model and treatments of MASLD. HCC, hepatocellular carcinoma; HF, hepatic fibrosis; HS, hepatic steatosis; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease.

signaling pathway is a highly conserved transmembrane signaling pathway. It activates signal transduction through receptor-ligand interactions, which affected gene expression. The Notch signaling pathway plays a key role in hepatic lipid metabolism disorders, inflammatory responses and liver fibrosis (382). adenosine 5'-monophosphate-activated protein kinase (AMPK), as a core hub of energy metabolism, plays a crucial role in MASLD by regulating fatty acid synthesis, oxidation, autophagy and inflammation. Researches showed that various monomers of medicinal plants by inhibiting

key factors of lipid synthesis (SREBP-1c, FAS and ACC), promoting fatty acid oxidation (PPARα and CPT-1), improving insulin resistance, enhancing autophagy (ULK1 and LC3) and inhibiting inflammation (NF-κB) and oxidative stress (Nrf2) (383). A large amount of basic researches shows that the NF-κB pathway is a crucial pathway for mediating inflammatory and oxidative stress responses and also plays an important role in the occurrence and development of MASLD. When the internal environment of the body changed, the levels of inflammatory factors in the body increases markedly. The NF-κB pathway is activated by activating the expression of inflammation and immune related receptors on the surface of liver cell membranes. The NF-κB pathway regulates various factors upstream and downstream, further mediating the inflammatory response, oxidative stress response, liver fibrosis, cell apoptosis, autophagy and pyroptosis of liver tissue to promote the process of MASLD (384). Chitosan markedly improves symptoms of MASLD by inhibiting lipid production, regulating inflammatory responses, alleviating oxidative stress, improving insulin resistance and regulating gut microbiota through multiple mechanisms. The specific mechanisms included that it inhibits the expression of transcription factors, activates the AMPK signaling pathway to promote fatty acid oxidation and inhibits the activity of signaling pathways such as PI3K/AKT/mTOR (385).

Through the continuous efforts of researchers at home and abroad, the study of experimental models of MASLD disease (including animal models and cellular models) has made great progress. Ideal disease models should be similar to human pathogenesis, simple, inexpensive, fast modelling, low animal mortality, high replication rate, good reproducibility and easy to use. In the process of practical application, researchers should understand the species differences between experimental animals and human beings, the formation mechanism of MASLD model and pathological changes are very different from those of human beings when they suffer from the disease and they need to select appropriate models according to specific research purposes and needs and consider the complications of the MASLD model. The TCM syndrome models, guided by TCM theory and based on the basic principle of evidence-based treatment, has obvious advantages in the treatment of fatty liver, but the formation principle, method and judgement index of the model are difficult to be standardized and need to be further verified (Fig. 6). Future research should establish a unified standard for the models and on the basis of improving the existing models, more efforts should be devoted to exploring new modelling methods, so that the experimental models can be more closely related to the characteristics of human MASLD, with a view to carrying out more in-depth research on the disease, elucidating its pathogenesis and an improved chance of preventing and treating MASLD.

Due to the complex pathogenesis of MASLD, there is no specific clinical drug and the use of a single treatment is limited. In the light of the current research results, individualized dietary control and lifestyle changes are the basic treatment for MASLD. A healthy lifestyle with good dietary habits, active treatment of the primary disease and selection of appropriate medication as an adjuvant therapy are conducive to intervening and controlling the development of MASLD and preventing further deterioration of the disease. Currently, effective therapeutic agents for MASLD are focused on multiple potential targets, with multi-target, multi-pathway therapeutic measures and targeted agents for key aspects of the target genes or metabolic pathways will also be applied in the clinic. However, various drugs are still in the research stage and in the future, clinical attention should not only be paid to the efficacy and safety of such drugs, but also need to further evaluate the effects of combining drugs with different mechanisms, so as to ultimately provide patients with a reasonable individualized medication plan (Fig. 6). In addition to synthetic drugs, natural medicines (TCM, Chinese medicine compounds), are characterized by multiple components and multiple targets of action, so the development of new natural medicines with multiple targets of action, maintaining a healthy composition of intestinal flora and promoting energy metabolism of the body, play a therapeutic role in MASLD from another angle.

Acknowledgements

Not applicable.

Funding

The present study was supported by National Natural Science Foundation of China (grant nos 82360682 and 82360825), Major Science and Technology Research Projects of Nanchang City [grant no. Hongkezi(2023)137-2], Training Project of Ganpo Juncai Support Plan for High level and High skilled Leading Talent in 2024 (grant no. 20240003188), 'Chunhui Plan' Collaborative Research Project of Ministry of Education of the People's Republic of China (grant nos. H ZKY20220385 and 202200200), Jiangxi Provincial Natural Science Foundation (grant nos. 20242BAB26172, 20224BA B206104 and 20224BAB206112) and Jiangxi University of Chinese Medicine Science and Technology Innovation Team Development Program (grant no. CXTD-22002).

Availability of data and materials

Not applicable.

Authors' contributions

KL was responsible for data curation and writing the original draft. CC, LW and ZS were responsible for formal analysis and methodology. JL, MA and QL were responsible for conceptualization and investigation. HH, QM, XW and RW were responsible for supervision, funding acquisition, writing, reviewing and editing. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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