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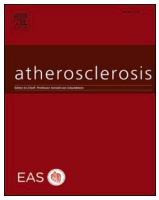

Lipotoxicity-driven metabolic dysfunction-associated steatotic liver disease (MASLD)

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ABSTRACT

Metabolic dysfunction-associated steatotic liver disease (MASLD) encompasses a spectrum of liver lesions, ranging from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH), that may further progress to cirrhosis. MASLD is estimated to affect more than one third of the general population and it represents a risk factor for end-stage liver failure and liver cancer, substantially contributing to liver-related morbidity and mortality. Although the pathogenesis of MASLD is incompletely understood, it is known to consist of a multifactorial process influenced by extrinsic and intrinsic factors such as metabolic, environmental and demographic features, gut microbiota and genetics. Dysregulation of both extracellular and intracellular lipid composition is known to promote the generation of toxic lipid species, thereby triggering lipotoxicity and cellular stress. These events ultimately lead to the activation of distinct cell death pathways, resulting in inflammation, fibrogenesis and, eventually, carcinogenesis. In this manuscript, we provide a comprehensive review of the role of lipotoxicity during MASLD pathogenesis, discussing the most relevant lipid species and related molecular mechanisms, summarizing the cell type-specific effects and highlighting the most promising putative therapeutic strategies for modulating lipotoxicity and lipid metabolism in MASLD.

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1. Introduction

Excessive high-fat and high-calorie food ingestion, combined with a sedentary lifestyle, has been contributing to the rising global prevalence of obesity and type 2 diabetes (T2DM), as well as metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD) [1]. MASLD prevalence is heterogeneously disseminated among different ethnic groups, although the estimated worldwide prevalence is ~38 %, making it the most prevalent chronic liver disease worldwide [2]. In fact, according to some studies, more than half of the general population will have MASLD by 2040 [3]. In addition, MASLD is emerging as the leading cause of cardiovascular-related deaths and liver transplantation among patients with chronic liver diseases [4,5].

MASLD encompasses a spectrum of liver lesions, ranging from simple steatosis (*i.e.*, accumulation of fat in the liver exceeding >5 % of hepatocytes), to metabolic dysfunction-associated steatohepatitis (MASH), which may be found in ~20 % of patients with MASLD [6]. Liver fibrosis may develop alongside MASLD progression, with ~15–20 % of patients with MASH progressing to cirrhosis, thus increasing the risk of end-stage liver failure and liver cancer (LC), and substantially contributing to liver-related morbidity and mortality [6–8]. Of note, up to one third of the tumors from patients with MASLD arise in the absence of cirrhosis, which could markedly increase the prevalence of primary liver tumors worldwide in the next decades [9,10]. Remarkably, lipid toxicity is particularly relevant in the development of MASLD and fibrogenesis. Consequently, understanding the key pathogenic pathways is essential for the development of novel treatment strategies as well as of new and more precise biomarkers that can help the implementation of proper risk stratification [11]. In this review, we focused on the role of lipid-induced toxicity, also known as lipotoxicity, in the development and progression of MASLD, providing the most recent findings in the field and highlighting novel potential therapeutic strategies.

2. MASLD pathogenesis

There are well-defined risk factors for developing MASLD, including Hispanic ethnicity, obesity, insulin resistance (IR), T2DM, dyslipidemia and hypertension, among others [6]. Development and progression of MASLD is further linked to multiple stimuli derived from the gut and adipose tissue acting simultaneously, which may promote chronic liver inflammation [12,13]. Overall, MASLD triggering and progression results from a multifactorial process influenced by extrinsic and intrinsic factors such as metabolic, environmental and demographic features, gut microbiota, and genetics [14,15].

Hepatic lipid accumulation is a hallmark of MASLD, which, in the presence of at least one cardiometabolic criteria (obesity, IR, T2DM, dyslipidemia or hypertension) and/or in the absence of other etiological causes for steatosis, confers the diagnosis of MASLD [1]. When free fatty acid (FFA) influx and synthesis in the liver exceeds the ability to metabolize or secret them back into circulation as triglycerides (TGs) in very-low-density lipoproteins (VLDL), they may accumulate, particularly inside the hepatocytes, which marks the onset of MASLD [16,17]. These FFAs are mainly obtained from fatty acids (FAs) derived from

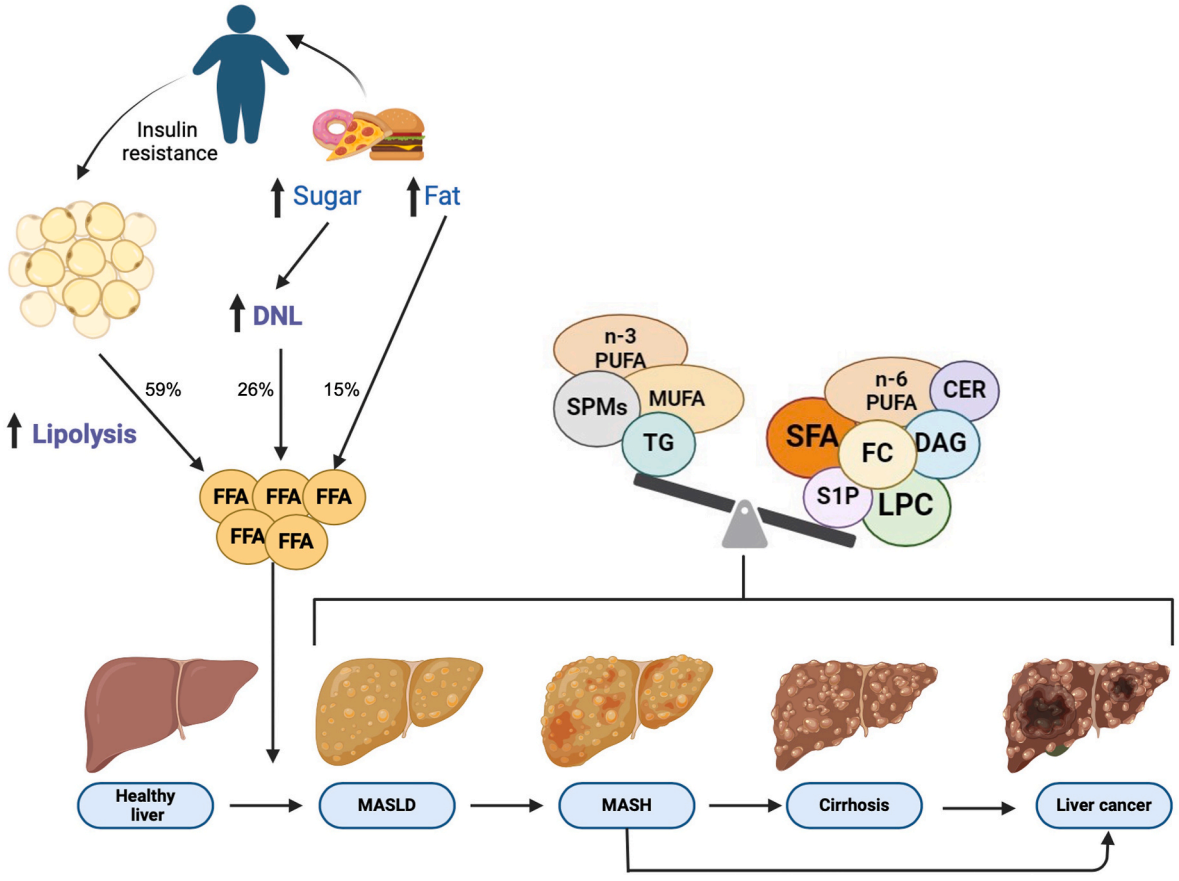

Fig. 1. Sources of fat in the liver and types of lipids changing during MASLD pathogenesis.
FFA can accumulate in the liver by three main mechanisms: derived from the diet, DNL and adipose tissue lipolysis. The transition from MASLD to MASH, cirrhosis and liver cancer is directly related to the dysfunction of lipid metabolism, which leads to accumulation of toxic lipid species in the liver and reduction of neutral or protective lipid species. Abbreviations: DNL, *de novo* lipogenesis; FFA, free fatty acid; LPC, lysophosphatidylcholine; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; MUFA, monounsaturated fatty acid; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid; S1P, sphingosine 1-phosphate; SFA, saturated fatty acid; SPM, specialized pro-resolving mediator.

adipose tissue lipolysis (59 %), but also from hepatic *de novo* lipogenesis (DNL; 26 %) and the diet (15 %; Fig. 1) [18]. Of note, hepatic DNL and related decrease in FA oxidation associate, among others, with consumption of sugar-rich diets and IR [19]. IR remodels whole body metabolic homeostasis and specifically in the adipose tissue, promotes the uninterrupted output of FAs by not adequately suppressing lipolysis, thus increasing the influx of FFAs into the liver [11,20].

In MASLD, lipid homeostasis and metabolism are highly perturbed. Lipid metabolism dysfunction may lead to deficiency on lipid species that are essential for cellular integrity and/or to formation of toxic lipid species, responsible for promoting injury (lipotoxicity). This damage triggers cellular stress, activation of distinct cell death pathways and inflammation, which in turn result in fibrogenesis and, eventually, carcinogenesis [14,20,21]. Lipotoxicity, among others, is considered one of the "multiple hits" responsible for disease progression and is commonly defined as the dysregulation of both extracellular and intracellular lipid composition, leading to an accumulation of toxic lipid species [20]. Nevertheless, not all lipid species are toxic and differential effects in distinct cell types might be observed depending on the lipid species that are accumulated in the liver [16].

3. Lipid species in MASLD

Lipids encompass a wide variety of molecules that play an important role in the structure and function of cells, participating in cellular homeostasis, cell-cell communication and regulation of inflammation and immunity [21]. The main lipid species that participate on lipid metabolism and lipotoxicity in the liver are TGs, free cholesterol (FC), saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), lysophosphatidylcholine (LPC) and sphingolipids, particularly ceramides [22]. The concentration of the different lipid species in the liver varies between healthy individuals and patients with MASLD. In particular, a higher concentration of SFAs, MUFAs and n-6 PUFA is observed in MASLD livers, in parallel with a lower concentration of n-3 PUFA, leading to a disproportion in the n-6/n-3 PUFAs ratio [23]. In patients with MASH, these differences seem to be accentuated, with greater increases of SFAs, MUFAs and n-6 PUFAs in MASH patients comparing to patients with simple steatosis (Fig. 1) [23].

3.1. Triglycerides

TGs are neutral lipids and represent the major form of lipid accumulation in the liver [24]. TGs are synthesized in the liver from the esterification of FFAs [25]. Despite accumulation of liver TGs being acknowledged as an initial step in MASLD, and several previous attempts tried to pharmacologically modulate TG accumulation as a way to improve disease progression, the increase in hepatic TG content is considered to be a protective factor against lipotoxicity [24]. In fact, it is now hypothesized that the liver increases TG accumulation, thus storing FAs in the liver in a harmless form, in an attempt to decrease the levels of more cytotoxic lipid species [16]. Therefore, elevated TG levels could be considered both a marker of aberrant hepatic lipid metabolism and a driving force of the disease progression. For instance, diacylglycerol (DAG) is a precursor of TG that can also accumulate in the liver, although, as opposed to TG, it associates with development of IR, as well as liver inflammation and an increased risk of MASLD progression [26]. In fact, TG synthesis dysfunction causes a shifting towards FFAs accumulation, which was shown to promote FFA-induced lipotoxicity [25]. In any case, as the origin of the disease lies on lipid accumulation, a potential therapeutic strategy would be the reduction of FFA synthesis and thereby the accumulation of TGs in the liver, through the inhibition of the fatty acid synthase (FAS) [27]. Of note, increased hepatic TG content is known to drive the production of large VLDL1 particles therefore increasing the susceptibility to develop atherosclerotic cardiovascular disease (ASCVD) [28,29]. In addition, despite the central role DNL plays in FA metabolism, inhibition of the rate-limiting enzyme acetyl-CoA carboxylase (ACC) may potentially be a viable therapeutic target. In this line, several clinical trials have tested the efficacy of the ACC inhibitor GS-0976 (Firsocostat) for the treatment of MASH, and although great debate has risen regarding its efficacy, positive results have been obtained, with a Phase II trial showing reduced steatosis and serum markers for fibrosis [30].

3.2. Free cholesterol

The liver has a key role in the production and processing of circulating lipoproteins. Severe dysregulation of hepatic cholesterol homeostasis has been reported in MASLD, resulting in increased FC levels within the liver [31,32]. A wide range of mechanisms associate with FC accumulation, namely increased levels and/or activity of 3-hydroxy-3--methyl-glutaryl-coenzyme A (HMG-CoA) reductase (HMGCR) and sterol regulatory element-binding protein 2 (SREBP-2) [33]. In agreement, HMGCR expression is increased in the liver of patients with simple steatosis and MASH, associating with disease severity [33]. Accumulation of FC in the liver of patients with MASLD may also result from increased absorption of cholesterol-rich lipoproteins and/or decreased cholesterol excretion into the bile [33]. At the cellular level, accumulation of FC in hepatocytes may induce endoplasmic reticulum (ER) stress and mitochondrial dysfunction, leading to accumulation of toxic oxysterols and crystallization of cholesterol into lipid droplets, promoting hepatocyte cell death through distinct mechanisms [34]. In parallel, FC may also activate non-parenchymal cells, namely Kupffer cells (KC) and hepatic stellate cells (HSC), contributing to inflammation and liver fibrosis [34]. Noteworthy, inhibition of intestinal cholesterol absorption by Ezetimibe was shown to improve hepatic steatosis in animal models of MASLD [35]. Similarly, a study of 45 patients with biopsy-proven MASLD showed that patients treated with Ezetimibe for 24 months presented with improved metabolic, biochemical and histopathological features, namely steatosis and inflammation [36]. This therapeutic approach holds potential, and more studies should be performed to test Ezetimibe's efficacy and safety on larger cohorts of patients. Of note, Ezetimibe targets NPC1L1, which is mainly expressed in human hepatocytes and therefore may reduce cholesterol reuptake from bile, avoiding the influx into hepatocytes and thus improving overall liver fitness [37].

3.3. Saturated fatty acids

SFAs represent the most common FFA accumulating in the liver of patients with MASLD, playing an important role in lipotoxicity [38]. In general, SFAs are more hepatotoxic than unsaturated lipid species, due to the ease of unsaturated FFAs to be esterified into neutral TGs [38]. The main SFAs found in the liver of individuals with MASLD are palmitate and stearate, with their levels positively correlating with the severity of MASLD [22]. Of note, the central role of DNL in MASLD pathogenesis may be mediated by the generation of the aforementioned SFAs. Still, it has been previously hypothesized that an increase in hepatic DNL could induce an atherogenic lipoprotein profile, thus promoting disease advancement [39]. SFA-mediated cytotoxicity encompasses activation of several pathways associated with inflammation, fibrogenesis and cell death [20]. In particular, SFA activate distinct cell death receptors, like tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptor 2 (TRAIL-R2) and damage-associated molecular pattern receptors like toll-like receptor 4 (TLR4), while also activating c-Jun N-terminal kinase (JNK)-associated cytotoxic pathways and ER stress [40]. Consequently, SFAs can induce cell damage in different liver cell types, including hepatocytes, cholangiocytes, KCs and HSCs [40–42].

3.4. Monounsaturated and polyunsaturated fatty acids

Some FFA species are less reactive and are thought to have a protective role against lipotoxicity. MUFAs, mostly comprised of palmitoleic acid and oleic acid, originate directly from SFAs, and despite also contributing to steatosis, they are much less harmful than SFAs due to their lower ability to trigger ER stress and apoptosis [43]. In turn, PUFAs are usually stored as part of the phospholipids that constitute the cell membrane, consequently impacting on membrane fluidity and permeability [44]. In the context of MASH, PUFAs can modulate the activity of genes involved in lipid metabolism, oxidative balance, inflammation and fibrogenesis through interaction with nuclear receptors, such as nucleotide-binding and oligomerization domain-like receptor pyrin domain-containing protein 3 (NLRP3) and nuclear factor kappa B (NF-κβ), and transcription factors, such as peroxisome proliferator activated receptors (PPARs) and SREBPs [22]. PUFAs are divided into 2 classes: n-6 PUFAs, including dihomo-Y-linolenic and arachidonic acid; and n-3 PUFAs, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [44]. n-3 PUFAs are protective against MASH by improving insulin sensitivity and being a substrate for PUFA-derived specialized pro-resolving mediators, namely protectins and resolvins, which have anti-inflammatory effects [45,46]. Additionally, the protective effect of n-3 PUFAs may also stem from their inhibitory effect on FA synthesis in the liver and by increasing β-oxidation [47,48]. In turn, n-6 PUFAs lead to the synthesis of eicosanoids, such as prostaglandins, thromboxanes and leukotrienes, that upregulate the production of both pro-inflammatory and anti-inflammatory cytokines, performing an important function in cellular signaling and inflammation, and being considered a key pathogenic factor in MASH [44]. Overall, since the metabolism of PUFAs is a very complex process that involves desaturation-, elongation- and ß-oxidation-enzymes, its dysregulation in MASLD leads to an increase in the ratio n-6/n-3 PUFAs, thus contributing to disease progression [45,49].

3.5. Lysophosphatidylcholine

Phospholipids represent an important class of lipids in the liver, especially due to its role in the formation and secretion of VLDL [22]. LPC is a plasma phospholipid that is found increased in patients with MASH comparing to simple steatosis [50]. LPC, generated from the catabolism of phosphatidylcholine (PC) and catalyzed intracellularly or extracellularly by phospholipase A2 or by lecithin-cholesterol acyltransferase respectively, represents an important mediator of SFA-induced lipotoxicity when transported into lipoproteins [51]. Most mechanisms of LPC-induced lipotoxicity overlap with the ones previously described for SFA, thus supporting the hypothesis that LPC mediates palmitate-induced lipotoxicity in hepatocytes [52]. At the molecular level, LPC-induced lipotoxicity encompasses activation of the JNK pathway and increased ER stress in hepatocytes [50]. Of note, LPC has also been found to induce cytotoxic effects in cholangiocytes [53]. In addition, when catalyzed by autotaxin, LPC is converted into lysophosphatidic acid, a potent pro-fibrogenic phospholipid that can activate HSCs and promote fibrogenesis [54].

3.6. Ceramides

Production of ceramides occurs through 3 distinct pathways: by *de novo* synthesis in the ER through a series of enzymatic steps; through the sphingomyelinase pathway; and by the recycling of complex sphingolipids in the plasma membrane or lysosomes [55]. Ceramides are sphingolipids with cell-signaling properties. They participate in the formation of glycosphingolipids and gangliosides, essential for cellular growth, differentiation, adhesion and cell signaling [56]. Nonetheless, they may accumulate in the liver during periods of increased hepatic influx of FFAs [52]. As such, hepatic ceramide levels are typically found increased in patients with MASLD and participate in the progression of simple steatosis to MASH [56]. Accumulation of ceramides within the liver impairs FA β-oxidation and promotes production of reactive oxygen species (ROS) in the mitochondria, while inducing ER stress, dysfunctional autophagy, and upregulation of hepatic hepcidin expression, leading to hepatic iron overload [56]. As a result, ceramide accumulation associates with inflammation and apoptosis [56,57]. Another important sphingolipid metabolite is sphingosine 1-phosphate (S1P), formed after ceramide deacylation by ceramidase and subsequent phosphorylation of sphingosine. Like ceramides, S1P associates with IR and MASLD development [57]. Noteworthy, ceramide toxicity depends greatly on the nature and chain length of the fatty acid conjugated to the sphingosine backbone. For instance, palmitate-conjugated ceramide has been described to induce apoptosis, heart failure, adipose tissue dysfunction, IR and fibrosis in the liver, whereas C24:0 conjugated ceramide is generally regarded as benign [58].

4. Molecular mechanisms of lipotoxicity

Toxic lipid species are known to cause damage by modifying the function of cellular organelles, such as the ER and mitochondria, and also by direct interaction with intracellular signaling pathways [11]. The most relevant mechanisms of lipotoxicity playing a role in MASLD development and progression (Fig. 2) are discussed below.

4.1. ER stress

The ER is a specialized organelle whose main function is the synthesis, folding and transport of most proteins in the cell. The correct function of this organelle is essential to ensure a proper lipid metabolism [59]. Nevertheless, different *stimuli* may negatively affect the homeostasis of this organelle, resulting in ER stress, with the subsequent activation of the unfolded protein response (UPR) [60,61]. Several signaling pathways are canonically associated with the UPR. Briefly, inositol-requiring enzyme type 1 (IRE1), protein kinase R-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6) are known to orchestrate the ER stress response [60,61]. Noteworthy, mice with genetic ablations of any of these three upstream regulatory factors showed a dysregulated ER stress response, leading to the development of microvesicular steatosis [62]. Of note, high-fat diet (HFD)-induced hepatic lipid deposition associates to activation of eukaryotic initiation factor-2α (eIF2α) during the UPR [63]. Indeed, the PERK/eIF2α axis was shown to play a key regulatory role in experimental steatosis and fibrosis in obese mice [64]. In particular, HFD-fed mice injected with salubrinal, an inhibitor of eIF2α dephosphorylation, were protected from ER stress-induced apoptosis due to its role in the regulation of lipogenic and hepatic steatosis [64].

4.2. Mitochondrial dysfunction and oxidative stress

Mitochondrial oxidative metabolism was found to be twice as high in MASLD patients compared to control individuals [65]. The impairment of physiological mitochondrial dynamics due to SFA accumulation triggers a variety of cellular responses that eventually lead to lipotoxic stress, thus activating a cascade of signaling pathways resulting on apoptosis [66]. Of note, mitochondrial β-oxidation of SFA in hepatocytes seems to be the main source of ROS within the liver, although peroxisomal β-oxidation, microsomal ω-oxidation, and increased generation of Krebs cycle byproducts are also a significant source of ROS [67–69].

Sirtuin 3 (SIRT3), a highly conserved histone deacetylase, has been identified as the main mitochondrial Sirtuin responsible for the protection from stress-induced mitochondrial activity and energy metabolism [70]. SIRT3 was observed to mitigate lipotoxicity in hepatocytes from mice with MASLD, by promoting lipid droplet macroautophagy, chaperon-mediated autophagy, and reducing expression of lipogenic stearoyl-CoA desaturase 1 [71]. Moreover, SIRT3 was found to be downregulated in mouse liver tissue in response to chronic HFD, and its

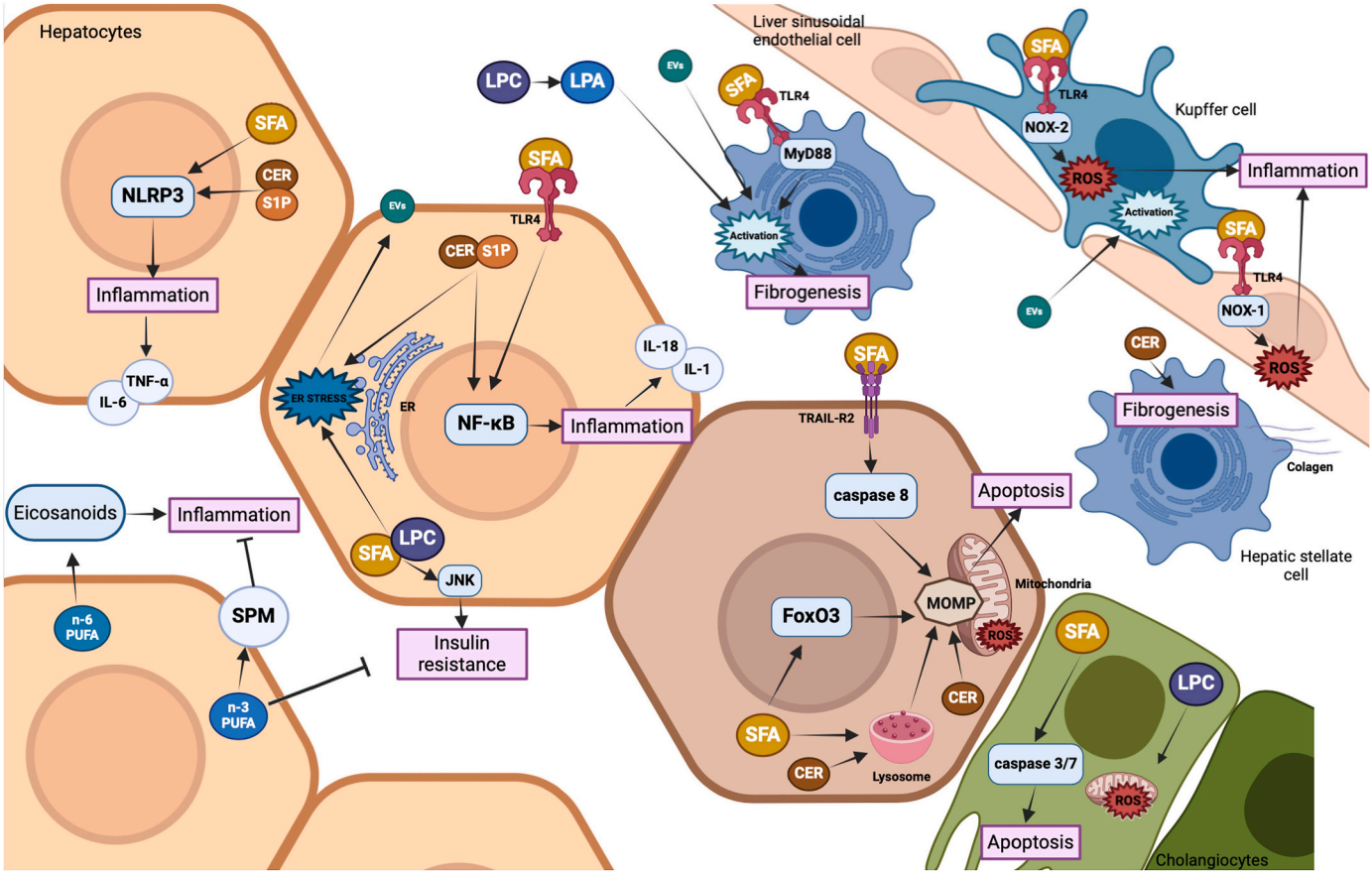

Fig. 2. Lipotoxicity mechanisms triggered by toxic lipid species in distinct liver cell types.
Lipid species can activate different pathways in different liver cells, promoting inflammation, insulin resistance, fibrogenesis and apoptosis. SFAs can induce inflammation through activation of the NLRP3 pathway in hepatocytes and through NF-κβ pathway activation by TLR4 receptor signaling in hepatocytes, LSECs and KCs. In hepatocytes, it can also lead to apoptosis *via* TRAIL-R2 receptor signaling pathway, activation of caspase 8, FoxO3 pathway and/or lysosome and ER stress, through the induction of mitochondrial outer membrane permeabilization (MOMP). In cholangiocytes, SFAs can activate caspase 3 and 7, leading to apoptosis. Overlapping with LPC, they can activate the JNK pathway, leading to apoptosis and insulin resistance in hepatocytes, and also induce inflammation, through ER stress with EV release in hepatocytes, and fibrogenesis by activation of HSCs. LPC can also induce inflammation in cholangiocytes through the generation of ROS. As important as LPC and SFAs, ceramides can induce inflammation, through activation of NLRP3 pathway and induction of ER stress with EV release, apoptosis, through ER stress and lysosomal pathway, and fibrogenesis, through HSC ECM deposition. PUFAs can either promote and protect against lipotoxicity. N-6 PUFAs can lead to inflammation by producing eicosanoids and n-3 PUFAs can protect against lipotoxicity by producing SPMs, which can attenuate the inflammatory response and improve insulin sensitivity. Abbreviations: CER, ceramide; ECM, extracellular matrix; ER, endoplasmic reticulum; EV, extracellular vesicle; FoxO3, Forkhead Box O3; HSC, hepatic stellate cell; IL, interleukin; KC, Kupffer cell; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LSEC, liver sinusoidal endothelial cell; MOMP, mitochondrial outer membrane permeabilization; NF-κβ, nuclear factor kappa B; NLRP3, nucleotide-binding and oligomerization domain-like receptor pyrin domain-containing protein 3; NOX, NADPH oxidase; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; S1P, sphingosine 1-phosphate; SFA, saturated fatty acid; SPM, specialized pro-resolving mediator; TLR4, toll-like receptor 4; TNF, tumor necrosis factor; TRAIL-R2, tumor necrosis factor-related apoptosis-inducing ligand receptor 2.

overexpression in isolated hepatocytes improved hepatic function, alleviating the inflammatory response, decreasing hepatocyte death and, therefore, attenuating liver fibrosis [72]. SIRT3 also appears to exert a protective effect against lipotoxicity. Nevertheless, its activity seems to be impaired in the presence of toxic lipid species. In the context of MASLD, a strong association between IR and SIRT3 has been found, with a marked downregulation of SIRT3 being detected in the skeletal muscle of streptozotocin-induced diabetic mice [73]. In addition, *Sirt3* KO mice display impaired glucose uptake in skeletal muscle upon insulin administration, confirming the relevance of this protein in regulating insulin homeostasis [74]. Therapies targeting SIRT3 for treating MASLD could potentially gain relevance in the following years, taking into consideration the implications of mitochondrial dynamics on disease pathogenesis. On this note, paricalcitol, a vitamin D receptor activator, was recently shown to attenuate oxidative stress and inflammatory response in the liver of MASLD, at least in part, by increasing SIRT3 expression [75]. In addition, strategies for the normalization of cellular NAD^+ levels, an essential component of the mitochondrial metabolism, have shown promising results in preclinical models, with patients showing improved mitochondrial function and decreased oxidative stress [76]. For instance, several studies have shown that repletion of NAD^+ precursors may potentially hinder MASLD development to MASH [76]. Modulators such as Urolithin A, spermidine and mitoquinone have also shown to ameliorate MASLD pathogenesis [77].

4.3. *JNK signaling pathway*

The JNK pathway is one of three MAPK pathways involved in cellular processes regulating inflammation and cancer progression [78]. The JNK pathway is a key regulator of hepatocyte lipoapoptosis, a crucial mechanism occurring during lipotoxicity. On this regard, JNK activation was found increased in the liver and skeletal muscle of patients with MASLD, associating with disease severity and paralleling engagement of apoptosis [79]. In fact, palmitate directly stimulates JNK activity,

resulting in activation of p53 upregulated modulator of apoptosis (PUMA), thus leading to BCL-2-associated X-protein (BAX) activation and apoptosis [80]. Additional studies including distinct murine models and MASLD patients have shown that the JNK pathway is overexpressed in the MASH liver, highlighting its relevance in disease pathogenesis [81,82]. Noteworthy, both pharmacological and genetic ablation of JNK reduced FFA-mediated hepatocyte lipoapoptosis by impacting on the mitochondrial apoptotic pathway [83].

JNK activity is also subjected to the TLR4/JNK axis. For instance, concomitant pharmacological inhibition of TLR4/JNK and activation of PI3K/Akt pathways ameliorated MASLD in mice models, with a significant reduction of body weight, liver weight, lipid accumulation and improved biochemical parameters [84]. Similarly, macrophage-specific epigenetic modulation of TLR4/JNK axis showed reduced inflammation and lipid accumulation in hepatocytes, and also reduced fibrogenic activation of HSCs [85].

4.4. Lipid-induced cell death

Lipotoxicity is an important driver of MASLD progression, typically resulting in selective activation of cell death pathways, depending on the cell nature, disease stage and cellular context [86]. Different cell death types have been extensively reviewed elsewhere [87–89], although an overview of their specific involvement in MASLD is provided below.

4.4.1. Apoptosis

The term "apoptosis" was first used in 1972 to describe a "controlled cell deletion" [90]. It is an exemplar type of cell death, extensively researched, and for a long time thought to be the only type of controlled cell death. Apoptosis can be activated through either the intrinsic pathway, also known as the mitochondrial pathway, or the extrinsic pathway (summarized elsewhere [87–89] and in Fig. 3). Noteworthy, apoptosis is considered a key event in MASLD development [91]. Caspase-2 and -3 activities, and consequently the number of apoptotic cells, are increased in the liver tissue from MASH patients, comparing to patients with simple steatosis [79]. SFAs such as stearate and palmitate

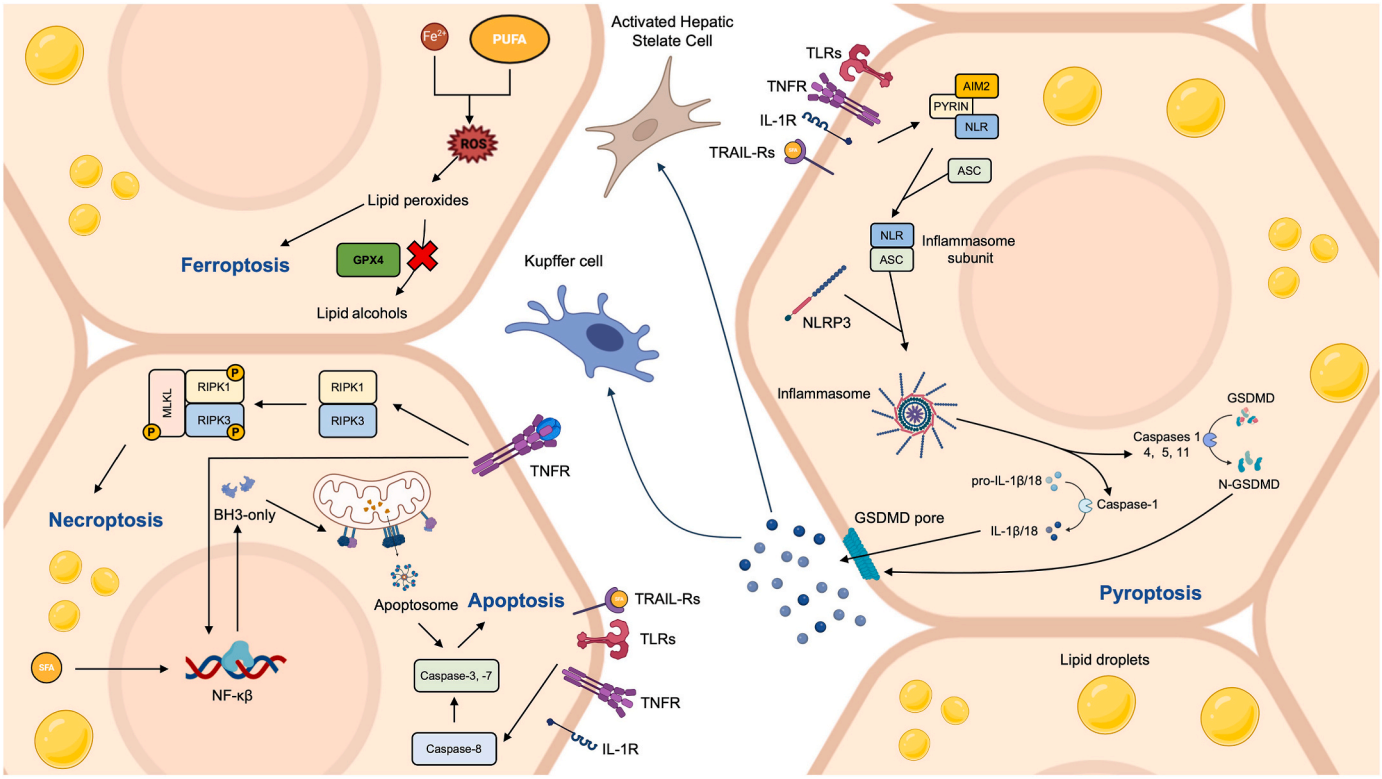

Fig. 3. SFA-induced lipoapoptosis, necroptosis, pyroptosis and ferroptosis.
SFAs can trigger apoptosis either by recognition by death receptors (TRAIL-R2, TNFR, TLRs) and subsequent activation of caspase cascade reactions or by directly influencing the expression of pro-apoptotic genes. In the first case, SFAs trigger the extrinsic apoptotic pathway, in which death receptors activate caspase-8 activity. Next, caspase-8 proteolytically activates caspases-3,7, thus initiating the activity of the apoptotic machinery. In the second case, cytoplasmatic SFAs promote the expression of BH3-only proteins. These subunits bind to BCL-2 mitochondrial proteins, thus releasing BAX/BAK proteins and allowing the formation of pores in the mitochondria, in a process known as mitochondrial outer membrane permeabilization (MOMP). This phenomenon allows for cytochrome *c* (Cyt *c*) release into the cytoplasm, leading to the formation of the apoptosome. This structure will activate caspases-3,7 and apoptosis will take place. Regarding necroptosis, binding of TNF and/or SFA to TNFR1 results on either the expression of pro-survival NF-κβ genes or formation of cytoplasmatic RIPK1/3 complex. Autophosphorylation of RIPK1/3 complex triggers bonding of MLKL to the RIPK1/3 complex, thus forming a subunit of the necrosome and promoting necroptosis. Ferroptosis results from dysfunctional metabolism of lipid peroxides into innocuous lipid alcohols catalyzed by GPX4. PUFAs from membrane phospholipids are particularly sensitive to oxidative stress, which results on a chain of autoperoxidation catalyzed by ferrous iron and ROS levels and consequent engagement of ferroptosis. Lastly, regarding pyroptosis, activation of death receptors results on oligomerization of AIM2, Pyrin receptor or NLR with ASC, thus forming the inflammasome subunit. Subsequent oligomerization with NLRP3 inflammasome will then initiate the pyroptosis reaction cascade. Proteolytical activation of caspase-1 allows for the release of active forms of IL-1β and IL-18, which will then be released to the outer medium through GSDMD pores formed by caspases-1, 4, 5, 11. Abbreviations: AIM2, DNA receptor absent in Melanoma 2; ASC, apoptosis-associated speck-like protein containing a C-terminal caspase recruitment; BAK, BCL-2-associated K protein; BAX, BCL-2-associated X protein; GSDMD, gasdermin D; MLKL, mixed lineage kinase domain like pseudokinase; NF-κβ, nuclear factor kappa β; NLR, Nod-like receptor; NLRP3, NLR family pyrin domain containing 3; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SFA, saturated fatty acid; TLR, toll-like receptor; TNF, tumor necrosis factor; TNFR1, TNF receptor 1; TRAIL-R, TNF-related apoptosis-inducing ligand receptor.

induce cytotoxicity, whereas MUFA, namely oleate and palmitoleate, have a protective role in FFA-induced apoptosis [43,92,93]. These FFAs can induce lysosomal membrane permeabilization in hepatocytes thus promoting apoptosis [86,94]. Regarding death receptors, palmitate was shown to induce expression of death receptor TRAIL-R2, subsequently promoting lipoapoptosis in a caspase-8-dependent manner. In the same study, TRAIL-R2-deficient primary hepatocytes and hepatocellular carcinoma (HCC) cells were observed to be protected against palmitate-induced cell death [95]. In the same line of evidence, TRAIL-R-deficient mice fed a western diet, rich in SFAs, are resistant to steatosis, inflammation, fibrosis and lipoapoptosis, as opposed to wild-type mice [96]. Palmitate has also been shown to activate intrinsic apoptosis: in primary mouse hepatocytes, PUMA was induced in a JNK-dependent manner upon palmitate exposure, and cells with genetic ablation of PUMA were protected against lipoapoptosis [80]. Caspase-2 has also been observed to play an important role in hepatocyte lipoapotosis. Using a metabolomics approach, caspase-2 was shown to be engaged in response to palmitate in hepatocytes, while downregulation of caspase-2 impaired cell death induced by FFAs, suggesting that it has an important role during lipotoxicity [97]. Additionally, mice genetically deficient for caspase-2 were shown to be protected from diet-induced obesity and MASLD [98].

4.4.2. Necroptosis

Necroptosis is a lytic form of cell death characterized by the release of pro-inflammatory cytokines [87]. It constitutes a type of regulated cell death morphologically identical to necrosis but occurring through well-established biochemical pathways, leading to plasma membrane integrity loss and cell shrinkage, and promoting the release of pro-inflammatory mediators [99]. The molecular machinery responsible for necroptosis does not differ much from that involved in the extrinsic apoptotic pathway. However, unlike the extrinsic apoptotic machinery, caspase-8 activity is inhibited during necroptosis, which hinders activation of caspases 3 and 7 [87]. In short, activation of either TNFR1, Fas/FasL, TLR3, TLR4, and/or cytosolic nucleic acid sensors such as retinoic acid-inducible gene I and stimulator of interferon genes, induce the production of type I interferon (IFN-1) and TNFα [88,100–106]. This will then result in the activation of pro-inflammatory signaling pathways such as NF-κβ and the formation of a downstream oligomeric complex composed of receptor-interacting protein kinase 1 (RIPK1), caspase-8 and caspase-10 [107,108]. Due to the lack of activity of caspase-8, RIPK3 is recruited and after phosphorylation of mixed lineage kinase domain like pseudokinase (MLKL), the necrosome forms, ensuing necroptosis (Fig. 3) [109].

Although the role of necroptosis in liver disease has been extensively studied, it remains incompletely understood [86]. Increased levels of RIPK3 and MLKL were observed in mice and human patients with MASLD, associating with activation of the JNK pathway and inflammation [110,111]. Additionally, *Rip3* deficient mice display reduced liver injury, inflammation and fibrosis compared to wild type mice [111]. In experimental MASH-HCC, *Ripk3*-deficient mice are also protected from progression of MASH towards LC [112]. Very long chain FAs, and potentially other FAs derived from these, were shown to promote necroptosis through membrane permeabilization [113]. Ceramides can also induce necroptosis through RIPK1 oligomerization and formation of large membrane pores [114]. Inhibition of necroptosis has been regarded as a potential novel therapeutic strategy against MASLD. Several small molecules have been identified as inhibitors of RIPK1, RIPK3, MLKL or a combination of these, being capable of reduing necroptosis [115,116]. Among these, necrostatin-1 (Nec-1) is a small molecule first identified in 2005 which inhibits RIPK1 activity and that has shown promising results for treating MASLD [117–119]. A more stable and specific form of Nec-1, Necrostatin-1 stable (Nec-1s), has recently been developed [117]. Both molecules have extensively been used to investigate the exact role that RIPK1 plays on necroptosis (Fig. 3) [120]. In the context of MASLD, mice deficient in the antioxidant enzyme Cu/Zn-superoxide dismutase were shown to present with increased liver necroptosis, which in turn induced MASH-like liver inflammation and fibrosis. Short term treatment Nec-1s reversed these detrimental effects [121].

4.4.3. Pyroptosis

Pyroptosis is an inflammatory type of cell death usually active against microbiological pathogens and characterized by the formation of gasdermin D (GSDMD) pores in cell membranes and the release of pro-inflammatory cytokines such as IL-1β and IL-18. Pyroptosis is triggered upon detection of specific damage-associated molecular pattern (DAMP) or pathogen associated molecular pattern (PAMP) signals [87–89]. This signals inflammasome sensors to oligomerize, namely Nod-like receptor proteins (NLR4), DNA receptor absent in Melanoma 2 (AIM2) and the Pyrin receptor; and recruit apoptosis-associated speck-like protein containing a C-terminal caspase recruitment (ASC) [122–124]. Upon inflammasome assembly, caspase-1 is activated and proteolytically cleaves interleukins pro-IL-1β and pro-IL-18. Additionally, caspase-1 activation allows for the oligomerization of GSDMD subunits, which will form transmembrane pores that induce membrane destabilization and cell lysis, as well as the release of pro-inflammatory mature cytokines IL-1β and IL-18 to the outer space [125].

Recent studies have shed light into the role of pyroptosis in MASLD [126]. For instance, NLRP3 overactive mutant mice display increased hepatocyte pyroptosis. In turn, NLRP3 activation contributes to MASLD progression by promoting severe liver inflammation [127]. Additionally, *Nlrp3* knockout mice were shown to be protected from diet-induced liver injury, inflammation, and fibrosis [128]. Remarkably, in mice models genetic and, interestingly, diet-induced PPARα loss induce upregulation of NLRP3, leading to increased pyroptosis [129].

4.4.4. Ferroptosis

Ferroptosis has extensively been reviewed elsewhere [130]. The role of this type of cell death in MASLD pathogenesis has only recently been brought under the spotlight. In short, PUFAs from membrane phospholipids are particularly sensitive to oxidative stress, which facilitates a chain of autoperoxidation catalyzed by ferrous iron, called lipid peroxidation, resulting in the generation of highly reactive hydroxiperoxides in phospholipids. Phenotypically, the effect of ferroptosis on the cells does not differ much from that of other type of cell deaths, as evidenced by the fact that for long it was thought that the death resulting from lipid peroxidation was either apoptosis or necrosis [130]. However, the discovery of ferroptosis as a novel and distinct type of cell death provides researchers with new therapeutic approaches for the treatment of MASLD. For instance, glutathione peroxidase 4 (GPX4) enhancers could potentially ameliorate MASLD pathogenesis, considering its key protective role against lipid peroxidation [130]. In addition, antioxidants that prevent lipid peroxidation and iron chelators to manage iron metabolism overload could also constitute a new therapeutic approach for MASLD treatment.

5. Differential effects of lipotoxicity in liver cells

5.1. Hepatocytes

The main toxic lipid species responsible for lipotoxicity in hepatocytes are FFAs, particularly SFAs [49,52]. The molecular mechanisms acting in hepatocytes have been highlighted above and are mainly related with the induction of stress and cell death. In short, SFAs trigger apoptosis *via*: 1) activation of TRAIL-R2 and caspase-8; 2) lysosome-mediated BAX activation with cathepsin B release; 3) Forkhead Box O3 (FoxO3) upregulation with Bim activation; and 4) induction of ER stress, promoting PUMA activation in a CHOP- and JNK-dependent manner [22]. SFAs can also induce inflammation and chemotaxis in hepatocytes through activation of the TLR4 receptor, thus engaging NF-κβ and NLRP3 signaling and leading to the production of

IL-6, TNF-α, IL-1 and IL-18 [16,127]. Last but not least, palmitate has been shown to induce both apoptosis and necroptosis in primary hepatocytes, highlighting the relevance of different types of cell death in MASLD-associated lipotoxicity [110].

5.2. Cholangiocytes

FFAs affect cholangiocytes in a similar way that they affect hepatocytes. Exposure of cholangiocytes to palmitate or stearate triggers the activation of caspase-3 and -7, likely as a result of PUMA upregulation, resulting in lipoapoptosis [42]. In parallel, palmitate also increases the expression of microRNA-34 (miR-34a), a key microRNA involved in MASLD pathogenesis, by activating FoxO3 [91,131,132]. Additionally, in human immortalized human cholangiocytes, LPC was shown to increase oxidative stress and global DNA hypomethylation, inducing the expression of components of the senescence-associated secretory phenotype (SASP) and establishing a potential link between LPC, lipotoxicity and the development of biliary cancer [53]. Still, the relevance of these processes in MASLD and progression towards liver cancer remains to be unveiled.

5.3. Non-parenchymal cells

Upon exposure to toxic lipid species, activated KCs increase the expression of inflammatory cytokines and alter the expression of genes associated with fibrosis and oxidative damage, ultimately leading to MASLD progression [40]. At the molecular level, TLR-mediated recognition of FFA by KCs activates the JNK and NF-κβ signaling pathways, with the subsequent upregulation of pro-inflammatory target genes [40,133,134]. In response to inflammation and parenchymal cell damage caused by lipotoxicity and KC activation, HSCs are also activated and converted into myofibroblast-like cells, secreting collagen and setting the ground for liver fibrosis [11,135,136]. Additionally, exposure of HSCs to FFA induces an inflammatory response mediated by the secretion of circulating cytokines, such as chemokine ligand 5 (CCL5) and chemokine ligand 20 (CCL20), which were shown to be increased in the serum of MASLD patients [137,138]. EV cargo released by hepatocytes undergoing lipoapoptosis may also have a role in the activation of non-parenchymal cells. For instance, EVs enriched with C-X-C motif chemokine ligand 10 (CXCL10) and ceramide induce macrophage and monocyte chemotaxis to the liver, TRAIL-enriched EVs induce macrophage activation; and miR-128-3p-enriched EVs activate HSCs [22].

Liver sinusoidal endothelial cells (LSEC) are a type of specialized liver cells characterized by the presence of fenestrae, forming a permeable barrier that facilitates the transport of oxygen and nutrients from the bloodstream [139]. During MASLD, LSEC cells lose their regulatory activity and become dysfunctional; toxic lipid species, specifically palmitate, were found to induce the expression of vascular cell adhesion molecule 1 (VCAM-1), in part, through MLK3 signaling, thus facilitating monocyte adhesion to LSEC cells and contributing to MASH [140]. Palmitate has also been shown to upregulate of NADPH oxidase 1 (NOX1) in LSECs through generation of ROS and hepatocellular injury [141].

6. Genetics

Several single nucleotide polymorphisms (SNPs), mainly related to alterations in specific amino acid residues of enzymes involved in lipid metabolism, have been linked to an increased susceptibility to develop MASLD [142]. The main genetic variants and their impact on lipid metabolism are summarized in Table 1.

The patatin-like phospholipase domain-containing 3 (PNPLA3) protein is an enzyme with lipase activity towards triglycerides and retinyl esters. The isoleucine to methionine substitution at position 148 is a genetic variant of the *PNPLA3* gene, leading to a morphological change in the catalytic domain of the enzyme that hinders substrate access, thus leading to macrovesicular steatosis [143,144]. Similarly, point mutations in the *MBOAT7, TM6SF2*, and *GCKR* loci also relate to abnormal lipid metabolism due to alterations in the encoded enzymes, thus increasing susceptibility to disease advancement [142]. In turn, the loss of function of some proteins can confer protective effects, as in the case of the *HSD17B13* gene, in which loss of function leads to inhibition of TG accumulation in lipid droplets [145]. Still, SNPs identified so far have been shown to aggravate MASLD pathogenesis, although the impact on lipid turnover and on activation of cell damage pathways is still largely unknown. Further research is needed to clarify the influence of SNPs in lipotoxicity and disease progression and to determine their potential therapeutic targeting [15,142]. Of note, a few trials exploring gene silencing strategies of *PNPLA3* and *HSD17B13* genes are underway [146]. Recently, administration of JNJ-75220795, a hepatocyte-targeted small interfering RNA against *PNPLA3*, showed a reduction in steatosis of up to 46 % in patients with MASLD patients harboring genetic alterations in this gene [147]. Overall, although data shows that MASLD represents a risk factor for the development of ASCVD, the development of MASLD associated with the presence of different mentioned polymorphisms may not be directly associated with ASCVD risk [148]. In fact, these patients less frequently present with IR and central obesity, which are currently the best predictors increased cardiovascular risk in steatotic liver disease, thus potentially explaining the decreased risk in genetic-driven MASLD [149].

7. Progression from MASH to LC

Irrespectively of disease stage, MASLD patients are at greater risk for developing both HCC and cholangiocarcinoma (CCA) [9,10]. The specific mechanisms responsible for the development of LC in a MASLD background are still unclear, although lipid metabolism rewiring has recently been found to play a central role in the pathogenesis of

Table 1
Main genetic variants and their impact on lipid metabolism.

Gene	Function	SNP	Protein function	Lipid metabolism	Impact
PNPLA3	Lipid droplets remodeling and VLDL production	rs738409 C > G	Loss	TG accumulation on hepatocytes and retinyl esters in HSCs	Risk of MASLD and MASH, fibrosis progression and HCC
MBOAT7	PI acyl-chain remodeling	rs641738 C > T	Loss	Decrease in PI containing arachidonic acid	Risk of MASLD, MASH and HCC
TM6SF2	VLDL secretion	rs58542926 C > T	Loss	TG accumulation and lower circulating lipoproteins	Risk of MASLD and MASH and fibrosis progression
GCKR	DNL regulation	rs1260326 C > T	Loss	TG accumulation and elevated plasma VLDL	Risk of MASLD and MASH and fibrosis progression
HSD17B13	Lipid droplet associated retinol dehydrogenase	rs72613567 T > TA	Loss	Inhibit TG accumulation in lipid droplets	Protection against MASH, liver fibrosis and HCC

GCKR, glucokinase regulator; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13; PNPLA3, patatin like phospholipase domain containing 3; MASH, metabolic-associated steatohepatitis; MASLD, metabolic-associated steatotic liver disease; MBOAT7, membrane-bound O-acyltransferase 7; PI, phosphatidylinositol; TG, triglyceride; TM6SF2, transmembrane 6 superfamily member 2; VLDL, very low-density lipoprotein.

Table 2
Therapeutic strategies for the treatment of MASLD aiming to modulate lipid metabolism and lipotoxicity.

Treatment	Mechanism	Trial phase
Resmetiron	β-selective agonist of the thyroid hormone receptor (THR-β)	Phase III [158]
Semaglutide	Glucagon-like peptide 1 receptor agonist	Phase II [159]
Survodutide	Glucagon receptor-GLP-1 receptor dual agonist	Phase II [160]
Tirzepatide	Glucagon receptor-GLP-1 receptor dual agonist	Phase III [161]
Pioglitazone	PPAR-γ agonist	Phase II [162]
n-3 PUFAs	Reduction of circulating triglyceride levels, thus hindering fatty acid influx to the liver	Phase II [163]
Obeticholic acid	Farnesoid X receptor agonist	Phase III [164]
Cilofexor	Farnesoid X receptor agonist	Phase II [165]
Tropifexor	Farnesoid X receptor agonist	Phase II [166]
Pegozafermin	FGF21 analogue	Phase II [167]
Denifanstat	Fatty acid synthase (FAS) inhibitor	Phase II [168]

MASLD-driven HCC. The transcription factors E2F, known regulators of cell-cycle, were found to also play a role in the regulation of lipid metabolism and to be key inductors of HCC development in MASLD [150]. Specifically, the expression of both *E2f1* and *E2f2* is increased in the liver of mice receiving a HFD and diethylnitrosamine (DEN). Furthermore, genetic ablation of either *E2f* was found to hinder HCC development in this model. In the same study, E2F2 was also found to be upregulated in human liver tumor tissue samples of MASLD-HCC patients, compared to the surrounding tissue, and its overexpression was also found to be positively correlated with disease severity [150]. Allegedly, E2Fs are thought to negatively impact on carnitine palmitoyltransferase 2 activity, thus decreasing fatty acid oxidation and facilitating hepatocarcinogenesis due to a lipid rich environment. Additionally, epigenetis, and specifically miRNAs, may also have an important role in the development and progression of MASLD towards HCC. In this sense, the levels of miR-21 were previously shown to be increased in patients with MASLD and MASLD-HCC, being directly associated with disease progression by targeting PPARα [151,152]. Noteworthy, genetic ablation of *Mir21* in mice not only ameliorated MASH pathogenesis but also prevented progression towards HCC [151,152].

MASLD-derived HCC development may also be a result of mutation buildup and is thought to be divided in three stages. The first stage corresponds to advanced MASH, characterized by exacerbated inflammation. During the second stage, hepatocytes accumulate genetic mutations as a result of the cellular stress experienced in the first stage. Eventually, accumulated mutations lead to development of a primary tumor [153]. The main stressor responsible for mutation buildup is lipotoxicity and the mechanisms involved in the tumorigenic process have been reviewed above. Among these, cell death, and specifically apoptosis, appear to significantly aid in tumor development. In a dietary mouse model of MASLD, hepatocyte-specific deletion of the anti-apoptotic regulator *Mcl1* resulted in increased hepatocarcinogenesis. Specifically, 78 % of mice with MASLD developed LC histologically classified as HCC, whereas only 38 % of the control mice grew HCC tumors. This study highlighted the tumor promoting ability of apoptosis in MASLD [154]. Necroptosis also appears to drive MASLD-LC progression, as reviewed [112,155]. Incidentally, lipotoxicity seems to play an important role not only in the development of HCC but also in that of CCA. On this regard, MASLD was found to exacerbate cholangitis in liver-specific *Cdh1* knockout mice, promoting CCA development [156]. Altogether, although it is clear that MASLD substantially increases the risk for LC development, the mechanisms are far from being entirely understood and deserve to be under the spotlight in the upcoming years.

8. Pharmacological strategies for modulating lipid metabolism and lipotoxicity

Several molecules have undergone clinical trials evaluating its therapeutic potential in various metabolic disorders, including obesity, T2DM, cardiovascular diseases (CVD), and MASLD [157]. The main therapeutic targets and drugs evaluated in clinical trials for MASLD treatment, including its impact on lipotoxicity, are described in Table 2 [158–168].

Recently, Resmetiron, a β-selective agonist of the thyroid hormone receptor (THR-β), has been approved by the FDA as the first medication for MASLD treatment [169]. A phase III trial found that treatment with Resmetiron was safe and well-tolerated, led to normalization of circulating levels of LDL-C, apoB, triglycerides, and reduced hepatic fat accumulation and liver stiffness [158]. Of note, studies in animal models have shown that THR-β, which is predominantly expressed in hepatocytes, plays a key role in regulating circulating triglycerides and cholesterol, thus improving insulin sensitivity and liver regeneration [170]. Glucagon like peptide-1 (GLP-1) receptor agonists are currently used as a treatment strategy for MASLD in patients with obesity and T2DM [157]. Although they can improve adipose tissue inflammation and steatosis, its direct impact on lipotoxicity needs to be clarified [16]. A possible mechanism would be the reduction of both macrophage infiltration and the expression of pro-inflammatory cytokines, such as IL-6 and TNF-α [171]. Pioglitazone, a PPAR-γ agonist, has been widely used in patients with MASLD without cirrhosis, although evidence to support its efficacy is not considered to be robust enough to support its recommendation [157]. Several randomized control trials have shown the efficacy of pioglitazone in improving histological features of steatohepatitis but not fibrosis [162]. Its incomplete efficacy, coupled with several side effects (weight gain, pedal oedema, hemodilution, bone loss in post-menopausal women and suspicion of increasing bladder cancer risk), has hindered the development of this treatment option [157]. Additionally, n-3 PUFAs were also shown to have a potential therapeutic effect in MASLD patients [22]. A randomized control trial showed decreased hepatic fat accumulation in patients treated with n-3 PUFAs, although no significant improvement in inflammation or fibrosis was found [163]. The therapeutic effect of n-3 PUFAs might be explained by the reduced circulating TG levels with a consequent liver FA influx reduction [163]. FXR agonists (obeticholic acid [164], Cilofexor [165] and Tropifexor [166]), FGF21 analogues (Pegozafermin [167]) and FAS inhibitors (Denifanstat [168]) also are promising modulators of lipid metabolism for MASLD treatment. In fact, in a recent multicentre, double-blind, randomized, placebo-controlled, phase 2b trial, Denifanstat treatment resulted in MASH resolution without a worsening of fibrosis in 26 % of the patients, thus being a promising drug for the treatment of MASH [168].

Additionally, statins have been regarded for a long time as a therapeutic option for patients with MASLD. Statins are widely used in patients with CVD due to their ability to lower plasma cholesterol by inhibiting the hydroxymethylglutaryl-CoA reductase enzyme, a key modulator of the sterol biosynthesis pathway [172]. Thus, many patients with concomitant MASLD and CVD may benefit from statin use. In a retrospective study, 7988 patients with confirmed MASLD by imaging techniques were analyzed to understand the effect of statin usage on long-term risk of all-cause mortality, liver-related clinical events and

liver stiffness progression [173]. Statin usage significantly improved the risk of all-cause mortality (adjusted HR = 0.233), liver-related clinical events (adjusted HR = 0.380) and liver stiffness progression rate (adjusted HR = 0.542) in these patients. Therefore, statins definitely represent a viable therapeutic option for MASLD patients by treating concomitant CVDs, although further research is needed including longer follow-up times [173].

9. Conclusions

MASLD is currently the most prevalent liver disease worldwide and represents one of the pandemics of this century. Affecting more than one third of the general population, MASLD represents a substantial risk factor for end-stage liver failure and cancer, both HCC and CCA, and its prevalence is expected to increase considerably in the upcoming years. Therefore, elucidating the molecular mechanisms responsible for MASLD development is key for bringing the best treatment and risk stratification strategies to affected individuals (Fig. 4). Noteworthy, lipotoxicity is regarded as a key process in MASLD triggering and driving disease progression. Further, unquestionable and increasing evidence suggests that the type of fat that accumulates within the liver is far more important than the quantity of fat. In this sense, different types of lipids may have different effects according to their structure and cell of origin/destiny. Several efforts are being conducted to develop effective drugs to tackle lipotoxicity and halt MASLD progression. Different pharmacological therapies are being assessed in clinical trials and results are warranted in the near future. Noteworthy, considering the relevance of lipotoxicity in promoting MASLD and knowing that MASLD patients have increased risk of developing LC, studying the lipotoxic mechanisms involved in the progression of MASLD to LC is of outmost importance and although the MASLD-HCC *continuum* remains poorly understood, different studies are now focused on identifying novel therapeutic targets and biomarkers for risk stratification. On the other hand, the study of MASLD-associated CCA is completely absent and considering the increased prevalence of MASLD worldwide, the number of CCA cases in a MASLD background is expected to rise significantly. Therefore, it is pivotal to deeply understand the effect of lipids and lipotoxicity not only in hepatocytes, but also in cholangiocytes, to clearly understand why some patients with MASLD develop HCC, while others progress to CCA. Overall, identifying novel lipotoxicity-related drugs may be a cornerstone in the treatment of MASLD.

Author contributions

All authors were involved in the discussion, writing and critical review of the manuscript.

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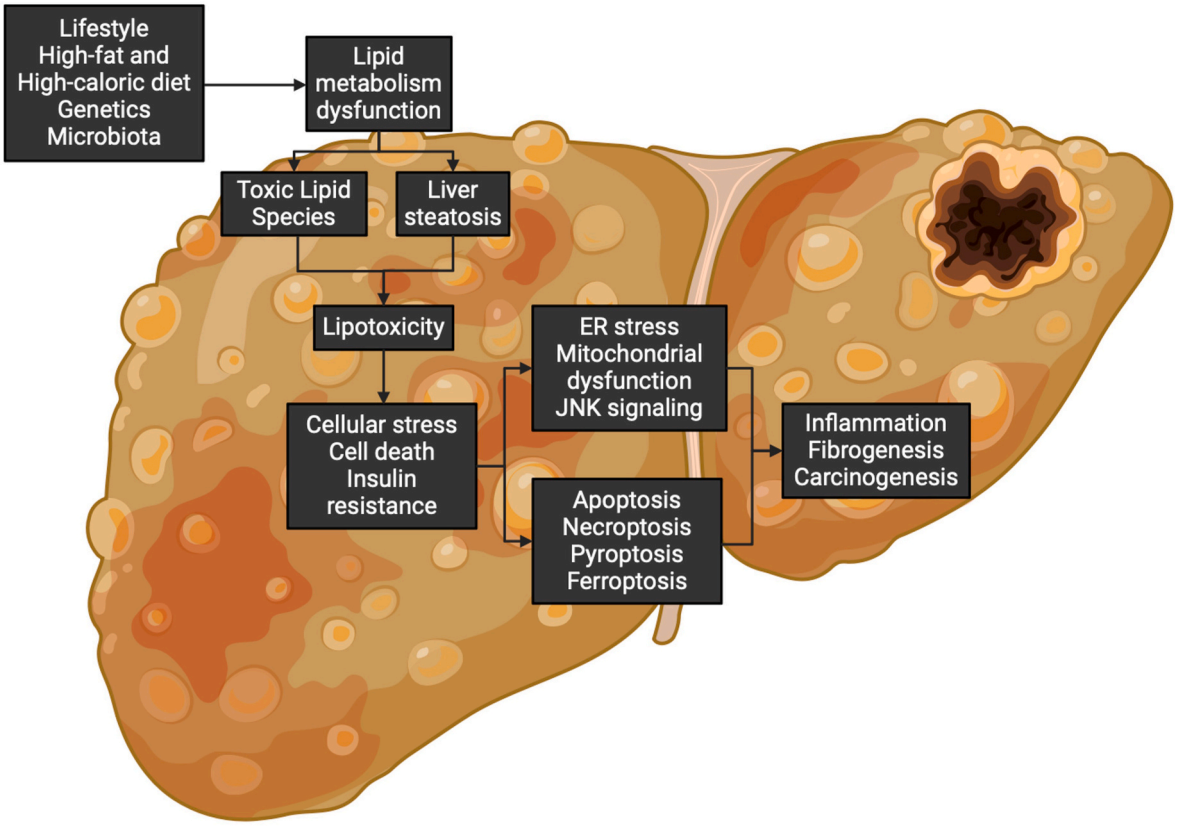

Fig. 4. Graphical abstract.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] M.E. Rinella, J.V. Lazarus, V. Ratziu, S.M. Francque, A.J. Sanyal, F. Kanwal, et al., A multi-society Delphi consensus statement on new fatty liver disease nomenclature, Hepatology (2023).
[2] Z.M. Younossi, P. Golabi, J.M. Paik, A. Henry, C. Van Dongen, L. Henry, The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review, Hepatology 77 (2023) 1335–1347.
[3] M.H. Le, Y.H. Yeo, B. Zou, S. Barnet, L. Henry, R. Cheung, et al., Forecasted 2040 global prevalence of nonalcoholic fatty liver disease using hierarchical bayesian approach, Clin. Mol. Hepatol. 28 (2022) 841–850.
[4] A.M. Allen, J.V. Lazarus, Z.M. Younossi, Healthcare and socioeconomic costs of NAFLD: a global framework to navigate the uncertainties, J. Hepatol. 79 (2023) 209–217.
[5] Z.M. Younossi, M. Stepanova, R. Al Shabeeb, K.E. Eberly, D. Shah, V. Nguyen, et al., The changing epidemiology of adult liver transplantation in the United States in 2013-2022: the dominance of metabolic dysfunction-associated steatotic liver disease and alcohol-associated liver disease, Hepatol Commun 8 (2024).
[6] R. Loomba, S.L. Friedman, G.I. Shulman, Mechanisms and disease consequences of nonalcoholic fatty liver disease, Cell 184 (2021) 2537–2564.
[7] D.Q. Huang, H.B. El-Serag, R. Loomba, Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention, Nat. Rev. Gastroenterol. Hepatol. 18 (2021) 223–238.
[8] M. Peiseler, R. Schwabe, J. Hampe, P. Kubes, M. Heikenwälder, F. Tacke, Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease - novel insights into cellular communication circuits, J. Hepatol. 77 (2022) 1136–1160.
[9] F. Kanwal, J.R. Kramer, S. Mapakshi, Y. Natarajan, M. Chayanupatkul, P. A. Richardson, et al., Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease, Gastroenterology 155 (2018) 1828–1837.e1822.
[10] S. Corrao, G. Natoli, C. Argano, Nonalcoholic fatty liver disease is associated with intrahepatic cholangiocarcinoma and not with extrahepatic form: definitive evidence from meta-analysis and trial sequential analysis, Eur. J. Gastroenterol. Hepatol. 33 (2021) 62–68.
[11] F. Nassir, NAFLD: mechanisms, treatments, and biomarkers, Biomolecules 12 (2022).
[12] H. Tilg, T.E. Adolph, A.R. Moschen, Multiple parallel hits hypothesis in nonalcoholic fatty liver disease: revisited after a decade, Hepatology 73 (2021) 833–842.
[13] J.P. Arab, M. Arrese, M. Trauner, Recent insights into the pathogenesis of nonalcoholic fatty liver disease, Annu. Rev. Pathol. 13 (2018) 321–350.
[14] E.E. Powell, V.W. Wong, M. Rinella, Non-alcoholic fatty liver disease, Lancet 397 (2021) 2212–2224.
[15] O. Juanola, S. Martínez-López, R. Francés, I. Gómez-Hurtado, Non-alcoholic fatty liver disease: metabolic, genetic, epigenetic and environmental risk factors, Int. J. Environ. Res. Publ. Health 18 (2021).
[16] E. Lee, H. Korf, A. Vidal-Puig, An adipocentric perspective on the development and progression of non-alcoholic fatty liver disease, J. Hepatol. 78 (2023) 1048–1062.
[17] S. Carotti, K. Aquilano, F. Valentini, S. Ruggiero, F. Alletto, S. Morini, et al., An overview of deregulated lipid metabolism in nonalcoholic fatty liver disease with special focus on lysosomal acid lipase, Am. J. Physiol. Gastrointest. Liver Physiol. 319 (2020) G469–G480.
[18] K.L. Donnelly, C.I. Smith, S.J. Schwarzenberg, J. Jessurun, M.D. Boldt, E.J. Parks, Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease, J. Clin. Invest. 115 (2005) 1343–1351.
[19] J.E. Lambert, M.A. Ramos-Roman, J.D. Browning, E.J. Parks, Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease, Gastroenterology 146 (2014) 726–735.
[20] F. Marra, G. Svegliati-Baroni, Lipotoxicity and the gut-liver axis in NASH pathogenesis, J. Hepatol. 68 (2018) 280–295.
[21] M.E. Ertunc, G.S. Hotamisligil, Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment, J. Lipid Res. 57 (2016) 2099–2114.
[22] G. Musso, M. Cassader, E. Paschetta, R. Gambino, Bioactive lipid species and metabolic pathways in progression and resolution of nonalcoholic steatohepatitis, Gastroenterology 155 (2018) 282–302.e288.
[23] S.A. Willis, S.J. Bawden, S. Malaikah, J.A. Sargeant, D.J. Stensel, G.P. Aithal, et al., The role of hepatic lipid composition in obesity-related metabolic disease, Liver Int. 41 (2021) 2819–2835.
[24] J. Jou, S.S. Choi, A.M. Diehl, Mechanisms of disease progression in nonalcoholic fatty liver disease, Semin. Liver Dis. 28 (2008) 370–379.
[25] S.S. Choi, A.M. Diehl, Hepatic triglyceride synthesis and nonalcoholic fatty liver disease, Curr. Opin. Lipidol. 19 (2008) 295–300.
[26] M.C. Petersen, G.I. Shulman, Roles of diacylglycerols and ceramides in hepatic insulin resistance, Trends Pharmacol. Sci. 38 (2017) 649–665.
[27] S. Xu, X. Wu, S. Wang, M. Xu, T. Fang, X. Ma, et al., TRIM56 protects against nonalcoholic fatty liver disease by promoting the degradation of fatty acid synthase, J. Clin. Invest. 134 (2024).
[28] M. Alves-Bezerra, D.E. Cohen, Triglyceride metabolism in the liver, Compr. Physiol. 8 (2017) 1–8.
[29] B.A. Ference, H.N. Ginsberg, I. Graham, K.K. Ray, C.J. Packard, E. Bruckert, et al., Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel, Eur. Heart J. 38 (2017) 2459–2472.
[30] N. Alkhouri, E. Lawitz, M. Noureddin, R. DeFronzo, G.I. Shulman, GS-0976 (Firsocostat): an investigational liver-directed acetyl-CoA carboxylase (ACC) inhibitor for the treatment of non-alcoholic steatohepatitis (NASH), Expet Opin. Invest. Drugs 29 (2020) 135–141.
[31] G.N. Ioannou, The role of cholesterol in the pathogenesis of NASH, Trends Endocrinol. Metabol. 27 (2016) 84–95.
[32] G. Arguello, E. Balboa, M. Arrese, S. Zanlungo, Recent insights on the role of cholesterol in non-alcoholic fatty liver disease, Biochim. Biophys. Acta 1852 (2015) 1765–1778.
[33] H.K. Min, A. Kapoor, M. Fuchs, F. Mirshahi, H. Zhou, J. Maher, et al., Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease, Cell Metabol. 15 (2012) 665–674.
[34] C.L. Horn, A.L. Morales, C. Savard, G.C. Farrell, G.N. Ioannou, Role of cholesterol-associated steatohepatitis in the development of NASH, Hepatol Commun 6 (2022) 12–35.
[35] M. Nguyen, A. Asgharpour, D.L. Dixon, A.J. Sanyal, A. Mehta, Emerging therapies for MASLD and their impact on plasma lipids, Am J Prev Cardiol 17 (2024) 100638.
[36] H. Park, T. Shima, K. Yamaguchi, H. Mitsuyoshi, M. Minami, K. Yasui, et al., Efficacy of long-term ezetimibe therapy in patients with nonalcoholic fatty liver disease, J. Gastroenterol. 46 (2011) 101–107.
[37] L. Ge, J. Wang, W. Qi, H.H. Miao, J. Cao, Y.X. Qu, et al., The cholesterol absorption inhibitor ezetimibe acts by blocking the sterol-induced internalization of NPC1L1, Cell Metabol. 7 (2008) 508–519.
[38] P. Rada, Á. González-Rodríguez, C. García-Monzón, Á. Valverde, Understanding lipotoxicity in NAFLD pathogenesis: is CD36 a key driver? Cell Death Dis. 11 (2020) 802.
[39] F. Westcott, D.J. Dearlove, L. Hodson, Hepatic fatty acid and glucose handling in metabolic disease: potential impact on cardiovascular disease risk, Atherosclerosis 394 (2024) 117237.
[40] S. Kumar, Q. Duan, R. Wu, E.N. Harris, Q. Su, Pathophysiological communication between hepatocytes and non-parenchymal cells in liver injury from NAFLD to liver fibrosis, Adv. Drug Deliv. Rev. 176 (2021) 113869.
[41] S. Win, T.A. Than, J. Zhang, C. Oo, R.W.M. Min, N. Kaplowitz, New insights into the role and mechanism of c-Jun-N-terminal kinase signaling in the pathobiology of liver diseases, Hepatology 67 (2018) 2013–2024.
[42] S.K. Natarajan, S.A. Ingham, A.M. Mohr, C.J. Wehrkamp, A. Ray, S. Roy, et al., Saturated free fatty acids induce cholangiocyte lipoapoptosis, Hepatology 60 (2014) 1942–1956.
[43] Y. Akazawa, S. Cazanave, J.L. Mott, N. Elmi, S.F. Bronk, S. Kohno, et al., Palmitoleate attenuates palmitate-induced Bim and PUMA up-regulation and hepatocyte lipoapoptosis, J. Hepatol. 52 (2010) 586–593.
[44] H.D. Le, J.A. Meisel, V.E. de Meijer, K.M. Gura, M. Puder, The essentiality of arachidonic acid and docosahexaenoic acid, Prostaglandins Leukot. Essent. Fatty Acids 81 (2009) 165–170.
[45] E. Scorletti, C.D. Byrne, Omega-3 fatty acids, hepatic lipid metabolism, and nonalcoholic fatty liver disease, Annu. Rev. Nutr. 33 (2013) 231–248.
[46] H.Q. Liu, Y. Qiu, Y. Mu, X.J. Zhang, L. Liu, X.H. Hou, et al., A high ratio of dietary n-3/n-6 polyunsaturated fatty acids improves obesity-linked inflammation and insulin resistance through suppressing activation of TLR4 in SD rats, Nutr. Res. 33 (2013) 849–858.
[47] J. Tian, J.L. Goldstein, S. Li, M.M. Schumacher, M.S. Brown, Phosphorylation of Insig-2 mediates inhibition of fatty acid synthesis by polyunsaturated fatty acids, Proc. Natl. Acad. Sci. U. S. A. 121 (2024) e2409262121.
[48] M. Wang, L.J. Ma, Y. Yang, Z. Xiao, J.B. Wan, n-3 Polyunsaturated fatty acids for the management of alcoholic liver disease: a critical review, Crit. Rev. Food Sci. Nutr. 59 (2019) S116–S129.
[49] F. Chiappini, C. Desterke, J. Bertrand-Michel, C. Guettier, F. Le Naour, Hepatic and serum lipid signatures specific to nonalcoholic steatohepatitis in murine models, Sci. Rep. 6 (2016) 31587.
[50] K. Kakisaka, S.C. Cazanave, C.D. Fingas, M.E. Guicciardi, S.F. Bronk, N. W. Werneburg, et al., Mechanisms of lysophosphatidylcholine-induced hepatocyte lipoapoptosis, Am. J. Physiol. Gastrointest. Liver Physiol. 302 (2012) G77–G84.

[51] G. Musso, R. Gambino, M. Cassader, Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD), Prog. Lipid Res. 48 (2009) 1–26.
[52] P. Hirsova, S.H. Ibrabim, G.J. Gores, H. Malhi, Lipotoxic lethal and sublethal stress signaling in hepatocytes: relevance to NASH pathogenesis, J. Lipid Res. 57 (2016) 1758–1770.
[53] R. Shimizu, K. Kanno, A. Sugiyama, H. Ohata, A. Araki, N. Kishikawa, et al., Cholangiocyte senescence caused by lysophosphatidylcholine as a potential implication in carcinogenesis, J Hepatobiliary Pancreat Sci 22 (2015) 675–682.
[54] E. Kaffe, A. Katsifa, N. Xylourgidis, I. Ninou, M. Zannikou, V. Harokopos, et al., Hepatocyte autotaxin expression promotes liver fibrosis and cancer, Hepatology 65 (2017) 1369–1383.
[55] C.R. Gault, L.M. Obeid, Y.A. Hannun, An overview of sphingolipid metabolism: from synthesis to breakdown, Adv. Exp. Med. Biol. 688 (2010) 1–23.
[56] M. Marí, J.C. Fernández-Checa, Sphingolipid signalling and liver diseases, Liver Int. 27 (2007) 440–450.
[57] X.D. Yu, J.W. Wang, Ceramide de novo synthesis in non-alcoholic fatty liver disease: Pathogenic mechanisms and therapeutic perspectives, Biochem. Pharmacol. 202 (2022) 115157.
[58] R.H. Choi, S.M. Tatum, J.D. Symons, S.A. Summers, W.L. Holland, Ceramides and other sphingolipids as drivers of cardiovascular disease, Nat. Rev. Cardiol. 18 (2021) 701–711.
[59] M. Wang, R.J. Kaufman, Protein misfolding in the endoplasmic reticulum as a conduit to human disease, Nature 529 (2016) 326–335.
[60] A. Ajoolabady, N. Kaplowitz, C. Lebeaupin, G. Kroemer, R.J. Kaufman, H. Malhi, et al., Endoplasmic reticulum stress in liver diseases, Hepatology 77 (2023) 619–639.
[61] H.J. Lu, N. Koju, R. Sheng, Mammalian integrated stress responses in stressed organelles and their functions, Acta Pharmacol. Sin. (2024).
[62] D.T. Rutkowski, J. Wu, S.H. Back, M.U. Callaghan, S.P. Ferris, J. Iqbal, et al., UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators, Dev. Cell 15 (2008) 829–840.
[63] S. Oyadomari, H.P. Harding, Y. Zhang, M. Oyadomari, D. Ron, Dephosphorylation of translation initiation factor 2alpha enhances glucose tolerance and attenuates hepatosteatosis in mice, Cell Metabol. 7 (2008) 520–532.
[64] J. Li, X. Li, D. Liu, S. Zhang, N. Tan, H. Yokota, et al., Phosphorylation of eIF2α signaling pathway attenuates obesity-induced non-alcoholic fatty liver disease in an ER stress and autophagy-dependent manner, Cell Death Dis. 11 (2020) 1069.
[65] N.E. Sunny, E.J. Parks, J.D. Browning, S.C. Burgess, Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease, Cell Metabol. 14 (2011) 804–810.
[66] M. Fuchs, A.J. Sanyal, Lipotoxicity in NASH, J. Hepatol. 56 (2012) 291–293.
[67] W. Liu, R.D. Baker, T. Bhatia, L. Zhu, S.S. Baker, Pathogenesis of nonalcoholic steatohepatitis, Cell. Mol. Life Sci. 73 (2016) 1969–1987.
[68] Y. Noguchi, J.D. Young, J.O. Aleman, M.E. Hansen, J.K. Kelleher, G. Stephanopoulos, Effect of anaplerotic fluxes and amino acid availability on hepatic lipoapoptosis, J. Biol. Chem. 284 (2009) 33425–33436.
[69] O. Tirosh, E. Ilan, S. Anavi, G. Ramadori, Z. Madar, Nutritional lipid-induced oxidative stress leads to mitochondrial dysfunction followed by necrotic death in FaO hepatocytes, Nutrition 25 (2009) 200–208.
[70] Khanna A. Anamika, P. Acharjee, A. Acharjee, S.K. Trigun, Mitochondrial SIRT3 and neurodegenerative brain disorders, J. Chem. Neuroanat. 95 (2019) 43–53.
[71] T. Zhang, J. Liu, S. Shen, Q. Tong, X. Ma, L. Lin, SIRT3 promotes lipophagy and chaperon-mediated autophagy to protect hepatocytes against lipotoxicity, Cell Death Differ. 27 (2020) 329–344.
[72] R. Li, T. Xin, D. Li, C. Wang, H. Zhu, H. Zhou, Therapeutic effect of Sirtuin 3 on ameliorating nonalcoholic fatty liver disease: the role of the ERK-CREB pathway and Bnip3-mediated mitophagy, Redox Biol. 18 (2018) 229–243.
[73] E. Jing, B. Emanuelli, M.D. Hirschey, J. Boucher, K.Y. Lee, D. Lombard, et al., Sirtuin-3 (Sirt3) regulates skeletal muscle metabolism and insulin signaling via altered mitochondrial oxidation and reactive oxygen species production, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 14608–14613.
[74] L. Lantier, A.S. Williams, I.M. Williams, K.K. Yang, D.P. Bracy, M. Goelzer, et al., SIRT3 is crucial for maintaining skeletal muscle insulin action and protects against severe insulin resistance in high-fat-fed mice, Diabetes 64 (2015) 3081–3092.
[75] N. Malladi, D. Lahamge, B.S. Somwanshi, V. Tiwari, K. Deshmukh, J.K. Balani, et al., Paricalcitol attenuates oxidative stress and inflammatory response in the liver of NAFLD rats by regulating FOXO3a and NFκB acetylation, Cell. Signal. 121 (2024) 111299.
[76] M. Dall, A.S. Hassing, J.T. Treebak, Nad, J. Physiol. 600 (2022) 1135–1154.
[77] S. Shin, J. Kim, J.Y. Lee, C.M. Oh, Mitochondrial quality control: its role in metabolic dysfunction-associated steatotic liver disease (MASLD), J Obes Metab Syndr 32 (2023) 289–302.
[78] Q. Wu, W. Wu, B. Fu, L. Shi, X. Wang, K. Kuca, JNK signaling in cancer cell survival, Med. Res. Rev. 39 (2019) 2082–2104.
[79] D.M. Ferreira, R.E. Castro, M.V. Machado, T. Evangelista, A. Silvestre, A. Costa, et al., Apoptosis and insulin resistance in liver and peripheral tissues of morbidly obese patients is associated with different stages of non-alcoholic fatty liver disease, Diabetologia 54 (2011) 1788–1798.
[80] S.C. Cazanave, J.L. Mott, N.A. Elmi, S.F. Bronk, N.W. Werneburg, Y. Akazawa, et al., JNK1-dependent PUMA expression contributes to hepatocyte lipoapoptosis, J. Biol. Chem. 284 (2009) 26591–26602.
[81] P. Puri, F. Mirshahi, O. Cheung, R. Natarajan, J.W. Maher, J.M. Kellum, et al., Activation and dysregulation of the unfolded protein response in nonalcoholic fatty liver disease, Gastroenterology 134 (2008) 568–576.
[82] R. Singh, Y. Wang, Y. Xiang, K.E. Tanaka, W.A. Gaarde, M.J. Czaja, Differential effects of JNK1 and JNK2 inhibition on murine steatohepatitis and insulin resistance, Hepatology 49 (2009) 87–96.
[83] L. Jin, M. Wang, B. Yang, L. Ye, W. Zhu, Q. Zhang, et al., A small-molecule JNK inhibitor JM-2 attenuates high-fat diet-induced non-alcoholic fatty liver disease in mice, Int. Immunopharm. 115 (2023) 109587.
[84] M. Pan, C. Cai, W. Li, T. Cao, Y. Liu, L. Yang, et al., Ebselen improves lipid metabolism by activating PI3K/Akt and inhibiting TLR4/JNK signaling pathway to alleviate nonalcoholic fatty liver, Cytokine 181 (2024) 156671.
[85] Z. Zou, X. Liu, J. Yu, T. Ban, Z. Zhang, P. Wang, et al., Nuclear miR-204-3p mitigates metabolic dysfunction-associated steatotic liver disease in mice, J. Hepatol. 80 (2024) 834–845.
[86] S. Schuster, D. Cabrera, M. Arrese, A.E. Feldstein, Triggering and resolution of inflammation in NASH, Nat. Rev. Gastroenterol. Hepatol. 15 (2018) 349–364.
[87] K. Newton, A. Strasser, N. Kayagaki, V.M. Dixit, Cell death, Cell 187 (2024) 235–256.
[88] D. Bertheloot, E. Latz, B.S. Franklin, Necroptosis, pyroptosis and apoptosis: an intricate game of cell death, Cell. Mol. Immunol. 18 (2021) 1106–1121.
[89] M.S. D'Arcy, Cell death: a review of the major forms of apoptosis, necrosis and autophagy, Cell Biol. Int. 43 (2019) 582–592.
[90] J.F. Kerr, A.H. Wyllie, A.R. Currie, Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics, Br. J. Cancer 26 (1972) 239–257.
[91] S.K. Natarajan, B.A. Stringham, A.M. Mohr, C.J. Wehrkamp, S. Lu, M.A. Phillippi, et al., FoxO3 increases miR-34a to cause palmitate-induced cholangiocyte lipoapoptosis, J. Lipid Res. 58 (2017) 866–875.
[92] H. Malhi, F.J. Barreyro, H. Isomoto, S.F. Bronk, G.J. Gores, Free fatty acids sensitise hepatocytes to TRAIL mediated cytotoxicity, Gut 56 (2007) 1124–1131.
[93] H. Malhi, S.F. Bronk, N.W. Werneburg, G.J. Gores, Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis, J. Biol. Chem. 281 (2006) 12093–12101.
[94] A.E. Feldstein, N.W. Werneburg, A. Canbay, M.E. Guicciardi, S.F. Bronk, R. Rydzewski, et al., Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway, Hepatology 40 (2004) 185–194.
[95] S.C. Cazanave, J.L. Mott, S.F. Bronk, N.W. Werneburg, C.D. Fingas, X.W. Meng, et al., Death receptor 5 signaling promotes hepatocyte lipoapoptosis, J. Biol. Chem. 286 (2011) 39336–39348.
[96] L. Idrissova, H. Malhi, N.W. Werneburg, N.K. LeBrasseur, S.F. Bronk, C. Fingas, et al., TRAIL receptor deletion in mice suppresses the inflammation of nutrient excess, J. Hepatol. 62 (2015) 1156–1163.
[97] E.S. Johnson, K.R. Lindblom, A. Robeson, R.D. Stevens, O.R. Ilkayeva, C. B. Newgard, et al., Metabolomic profiling reveals a role for caspase-2 in lipoapoptosis, J. Biol. Chem. 288 (2013) 14463–14475.
[98] M.V. Machado, G.A. Michelotti, M.L. Jewell, T.A. Pereira, G. Xie, R.T. Premont, et al., Caspase-2 promotes obesity, the metabolic syndrome and nonalcoholic fatty liver disease, Cell Death Dis. 7 (2016) e2096.
[99] M.B. Afonso, R.E. Castro, C.M.P. Rodrigues, Processes exacerbating apoptosis in non-alcoholic steatohepatitis, Clin. Sci. (Lond.) 133 (2019) 2245–2264.
[100] N. Holler, R. Zaru, O. Micheau, M. Thome, A. Attinger, S. Valitutti, et al., Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule, Nat. Immunol. 1 (2000) 489–495.
[101] S. He, Y. Liang, F. Shao, X. Wang, Toll-like receptors activate programmed necrosis in macrophages through a receptor-interacting kinase-3-mediated pathway, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 20054–20059.
[102] W.J. Kaiser, H. Sridharan, C. Huang, P. Mandal, J.W. Upton, P.J. Gough, et al., Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL, J. Biol. Chem. 288 (2013) 31268–31279.
[103] J. Lim, H. Park, J. Heisler, T. Maculins, M. Roose-Girma, M. Xu, et al., Autophagy regulates inflammatory programmed cell death via turnover of RHIM-domain proteins, Elife 8 (2019).
[104] S.N. Schock, N.V. Chandra, Y. Sun, T. Irie, Y. Kitagawa, B. Gotoh, et al., Induction of necroptotic cell death by viral activation of the RIG-I or STING pathway, Cell Death Differ. 24 (2017) 615–625.
[105] M. Brault, T.M. Olsen, J. Martinez, D.B. Stetson, A. Oberst, Intracellular nucleic acid sensing triggers necroptosis through synergistic type I IFN and TNF signaling, J. Immunol. 200 (2018) 2748–2756.
[106] D. Chen, J. Tong, L. Yang, L. Wei, D.B. Stolz, J. Yu, et al., PUMA amplifies necroptosis signaling by activating cytosolic DNA sensors, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 3930–3935.
[107] T. Tenev, K. Bianchi, M. Darding, M. Broemer, C. Langlais, F. Wallberg, et al., The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs, Mol. Cell. 43 (2011) 432–448.
[108] M. Feoktistova, P. Geserick, B. Kellert, D.P. Dimitrova, C. Langlais, M. Hupe, et al., cIAPs block Ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms, Mol. Cell. 43 (2011) 449–463.
[109] J.M. Murphy, P.E. Czabotar, J.M. Hildebrand, I.S. Lucet, J.G. Zhang, S. Alvarez-Diaz, et al., The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism, Immunity 39 (2013) 443–453.
[110] M.B. Afonso, P.M. Rodrigues, T. Carvalho, M. Caridade, P. Borralho, H. Cortez-Pinto, et al., Necroptosis is a key pathogenic event in human and experimental murine models of non-alcoholic steatohepatitis, Clin. Sci. (Lond.) 129 (2015) 721–739.
[111] J. Gautheron, M. Vucur, F. Reisinger, D.V. Cardenas, C. Roderburg, C. Koppe, et al., A positive feedback loop between RIP3 and JNK controls non-alcoholic steatohepatitis, EMBO Mol. Med. 6 (2014) 1062–1074.
[112] M.B. Afonso, P.M. Rodrigues, M. Mateus-Pinheiro, A.L. Simão, M.M. Gaspar, A. Majdi, et al., RIPK3 acts as a lipid metabolism regulator contributing to

inflammation and carcinogenesis in non-alcoholic fatty liver disease, Gut 70 (2021) 2359–2372.
[113] L.R. Parisi, N. Li, G.E. Atilla-Gokcumen, Very long chain fatty acids are functionally involved in necroptosis, Cell Chem. Biol. 24 (2017) 1445–1454. e1448.
[114] R. Nganga, N. Oleinik, J. Kim, S.P. Selvam, R. De Palma, K.A. Johnson, et al., Receptor-interacting Ser/Thr kinase 1 (RIPK1) and myosin IIA-dependent ceramidosomes form membrane pores that mediate blebbing and necroptosis, J. Biol. Chem. 294 (2019) 502–519.
[115] W.K. Saeed, D.W. Jun, K. Jang, D.H. Koh, Necroptosis signaling in liver diseases: an update, Pharmacol. Res. 148 (2019) 104439.
[116] H. Brito, V. Marques, M.B. Afonso, D.G. Brown, U. Börjesson, N. Selmi, et al., Phenotypic high-throughput screening platform identifies novel chemotypes for necroptosis inhibition, Cell Death Dis. 6 (2020) 6.
[117] A. Degterev, Z. Huang, M. Boyce, Y. Li, P. Jagtap, N. Mizushima, et al., Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury, Nat. Chem. Biol. 1 (2005) 112–119.
[118] A. Degterev, J. Hitomi, M. Germscheid, I.L. Ch'en, O. Korkina, X. Teng, et al., Identification of RIP1 kinase as a specific cellular target of necrostatins, Nat. Chem. Biol. 4 (2008) 313–321.
[119] H. Xu, X. Du, G. Liu, S. Huang, W. Du, S. Zou, et al., The pseudokinase MLKL regulates hepatic insulin sensitivity independently of inflammation, Mol. Metabol. 23 (2019) 14–23.
[120] Q. Wang, T. Zhou, Z. Liu, J. Ren, N. Phan, K. Gupta, et al., Inhibition of Receptor-Interacting Protein Kinase 1 with Necrostatin-1s ameliorates disease progression in elastase-induced mouse abdominal aortic aneurysm model, Sci. Rep. 7 (2017) 42159.
[121] S. Mohammed, E.H. Nicklas, N. Thadathil, R. Selvarani, G.H. Royce, M. Kinter, et al., Role of necroptosis in chronic hepatic inflammation and fibrosis in a mouse model of increased oxidative stress, Free Radic. Biol. Med. 164 (2021) 315–328.
[122] Y. Zhao, J. Yang, J. Shi, Y.N. Gong, Q. Lu, H. Xu, et al., The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus, Nature 477 (2011) 596–600.
[123] V.A. Rathinam, Z. Jiang, S.N. Waggoner, S. Sharma, L.E. Cole, L. Waggoner, et al., The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses, Nat. Immunol. 11 (2010) 395–402.
[124] H. Xu, J. Yang, W. Gao, L. Li, P. Li, L. Zhang, et al., Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome, Nature 513 (2014) 237–241.
[125] F. Alegre, P. Pelegrin, A.E. Feldstein, Inflammasomes in liver fibrosis, Semin. Liver Dis. 37 (2017) 119–127.
[126] S.J. Li, A.B. Liu, Y.Y. Yu, J.H. Ma, The role and mechanism of pyroptosis and potential therapeutic targets in non-alcoholic fatty liver disease (NAFLD), Front. Cell Dev. Biol. 12 (2024) 1407738.
[127] A. Wree, A. Eguchi, M.D. McGeough, C.A. Pena, C.D. Johnson, A. Canbay, et al., NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice, Hepatology 59 (2014) 898–910.
[128] A. Wree, M.D. McGeough, C.A. Peña, M. Schlattjan, H. Li, M.E. Inzaugarat, et al., NLRP3 inflammasome activation is required for fibrosis development in NAFLD, J. Mol. Med. (Berl.) 92 (2014) 1069–1082.
[129] C. Theys, T. Vanderhaeghen, E. Van Dijck, C. Peleman, A. Scheepers, J. Ibrahim, et al., Loss of PPARα function promotes epigenetic dysregulation of lipid homeostasis driving ferroptosis and pyroptosis lipotoxicity in metabolic dysfunction associated Steatotic liver disease (MASLD), Front Mol Med 3 (2023) 1283170.
[130] C. Peleman, S. Francque, T.V. Berghe, Emerging role of ferroptosis in metabolic dysfunction-associated steatotic liver disease: revisiting hepatic lipid peroxidation, EBioMedicine 102 (2024) 105088.
[131] A.L. Simão, M.B. Afonso, P.M. Rodrigues, M. Gama-Carvalho, M.V. Machado, H. Cortez-Pinto, et al., Skeletal muscle miR-34a/SIRT1:AMPK axis is activated in experimental and human non-alcoholic steatohepatitis, J. Mol. Med. (Berl.) 97 (2019) 1113–1126.
[132] R.E. Castro, D.M. Ferreira, M.B. Afonso, P.M. Borralho, M.V. Machado, H. Cortez-Pinto, et al., miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease, J. Hepatol. 58 (2013) 119–125.
[133] J.Y. Lee, D.H. Hwang, The modulation of inflammatory gene expression by lipids: mediation through Toll-like receptors, Mol. Cell. 21 (2006) 174–185.
[134] T. Suganami, J. Nishida, Y. Ogawa, A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 2062–2068.
[135] O. Khomich, A.V. Ivanov, B. Bartosch, Metabolic hallmarks of hepatic stellate cells in liver fibrosis, Cells 9 (2019).
[136] K.M. Peters, R.B. Wilson, N.M. Borradaile, Non-parenchymal hepatic cell lipotoxicity and the coordinated progression of non-alcoholic fatty liver disease and atherosclerosis, Curr. Opin. Lipidol. 29 (2018) 417–422.
[137] B.H. Li, F.P. He, X. Yang, Y.W. Chen, J.G. Fan, Steatosis induced CCL5 contributes to early-stage liver fibrosis in nonalcoholic fatty liver disease progress, Transl. Res. 180 (2017) 103–117.e104.
[138] X. Chu, Q. Jin, H. Chen, G.C. Wood, A. Petrick, W. Strodel, et al., CCL20 is up-regulated in non-alcoholic fatty liver disease fibrosis and is produced by hepatic stellate cells in response to fatty acid loading, J. Transl. Med. 16 (2018) 108.
[139] J. Gracia-Sancho, E. Caparrós, A. Fernández-Iglesias, R. Francés, Role of liver sinusoidal endothelial cells in liver diseases, Nat. Rev. Gastroenterol. Hepatol. 18 (2021) 411–431.
[140] K. Furuta, Q. Guo, K.D. Pavelko, J.H. Lee, K.D. Robertson, Y. Nakao, et al., Lipid-induced endothelial vascular cell adhesion molecule 1 promotes nonalcoholic steatohepatitis pathogenesis, J. Clin. Invest. 131 (2021).
[141] M. Matsumoto, J. Zhang, X. Zhang, J. Liu, J.X. Jiang, K. Yamaguchi, et al., The NOX1 isoform of NADPH oxidase is involved in dysfunction of liver sinusoids in nonalcoholic fatty liver disease, Free Radic. Biol. Med. 115 (2018) 412–420.
[142] M. Eslam, L. Valenti, S. Romeo, Genetics and epigenetics of NAFLD and NASH: clinical impact, J. Hepatol. 68 (2018) 268–279.
[143] P. Dongiovanni, B. Donati, R. Fares, R. Lombardi, R.M. Mancina, S. Romeo, et al., PNPLA3 I148M polymorphism and progressive liver disease, World J. Gastroenterol. 19 (2013) 6969–6978.
[144] S. Romeo, J. Kozlitina, C. Xing, A. Pertsemlidis, D. Cox, L.A. Pennacchio, et al., Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease, Nat. Genet. 40 (2008) 1461–1465.
[145] L. Alcober-Boquet, N. Kraus, L.S. Huber, R. Vutukuri, D.C. Fuhrmann, C. Stross, et al., BI-3231, an enzymatic inhibitor of HSD17B13, reduces lipotoxic effects induced by palmitic acid in murine and human hepatocytes, Am. J. Physiol.: Cell Physiol. 326 (2024) C880–C892.
[146] D. Lindén, S. Romeo, Therapeutic opportunities for the treatment of NASH with genetically validated targets, J. Hepatol. 79 (2023) 1056–1064.
[147] E. Fabbrini, B. Rady, A. Koshkina, J.Y. Jeon, V.S. Ayyar, C. Gargano, et al., Phase 1 trials of PNPLA3 siRNA in I148M homozygous patients with MAFLD, N. Engl. J. Med. 391 (2024) 475–476.
[148] H.H. Lee, H.A. Lee, E.J. Kim, H.Y. Kim, H.C. Kim, S.H. Ahn, et al., Metabolic dysfunction-associated steatotic liver disease and risk of cardiovascular disease, Gut 73 (2024) 533–540.
[149] G. Semmler, L. Balcar, S. Wernly, A. Völkerer, L. Semmler, L. Hauptmann, et al., Insulin resistance and central obesity determine hepatic steatosis and explain cardiovascular risk in steatotic liver disease, Front. Endocrinol. 14 (2023) 1244405.
[150] F. González-Romero, D. Mestre, I. Aurrekoetxea, C.J. O'Rourke, J.B. Andersen, A. Woodhoo, et al., E2F1 and E2F2-mediated repression of CPT2 establishes a lipid-rich tumor-promoting environment, Cancer Res. 81 (2021) 2874–2887.
[151] P.M. Rodrigues, M.B. Afonso, A.L. Simão, C.C. Carvalho, A. Trindade, A. Duarte, et al., miR-21 ablation and obeticholic acid ameliorate nonalcoholic steatohepatitis in mice, Cell Death Dis. 8 (2017) e2748.
[152] P.M. Rodrigues, M.B. Afonso, A.L. Simão, T. Islam, M.M. Gaspar, C.J. O'Rourke, et al., miR-21-5p promotes NASH-related hepatocarcinogenesis, Liver Int. 43 (2023) 2256–2274.
[153] X. Wang, L. Zhang, B. Dong, Molecular mechanisms in MASLD/MASH related HCC, Hepatology (2024).
[154] P. Hirsova, F. Bohm, E. Dohnalkova, B. Nozickova, M. Heikenwalder, G.J. Gores, et al., Hepatocyte apoptosis is tumor promoting in murine nonalcoholic steatohepatitis, Cell Death Dis. 11 (2020) 80.
[155] M.B. Afonso, T. Islam, J. Magusto, R. Amorim, V. Lenoir, R.F. Simões, et al., RIPK3 dampens mitochondrial bioenergetics and lipid droplet dynamics in metabolic liver disease, Hepatology 77 (2023) 1319–1334.
[156] S. Maeda, Y. Hikiba, H. Fujiwara, T. Ikenoue, S. Sue, M. Sugimori, et al., NAFLD exacerbates cholangitis and promotes cholangiocellular carcinoma in mice, Cancer Sci. 112 (2021) 1471–1480.
[157] European Association for the Study of the Liver (EASL) EAftSoDE, European Association for the Study of Obesity (EASO), et al., EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD), J. Hepatol. (2024).
[158] S.A. Harrison, P. Bedossa, C.D. Guy, J.M. Schattenberg, R. Loomba, R. Taub, et al., A phase 3, randomized, controlled trial of resmetirom in NASH with liver fibrosis, N. Engl. J. Med. 390 (2024) 497–509.
[159] P.N. Newsome, K. Buchholtz, K. Cusi, M. Linder, T. Okanoue, V. Ratziu, et al., A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis, N. Engl. J. Med. 384 (2021) 1113–1124.
[160] A.J. Sanyal, P. Bedossa, M. Fraessdorf, G.W. Neff, E. Lawitz, E. Bugianesi, et al., A phase 2 randomized trial of survodutide in MASH and fibrosis, N. Engl. J. Med. 391 (2024) 311–319.
[161] J. Rosenstock, C. Wysham, J.P. Frías, S. Kaneko, C.J. Lee, L. Fernández Landó, et al., Efficacy and safety of a novel dual GIP and GLP-1 receptor agonist tirzepatide in patients with type 2 diabetes (SURPASS-1): a double-blind, randomised, phase 3 trial, Lancet 398 (2021) 143–155.
[162] K. Cusi, B. Orsak, F. Bril, R. Lomonaco, J. Hecht, C. Ortiz-Lopez, et al., Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial, Ann. Intern. Med. 165 (2016) 305–315.
[163] C.K. Argo, J.T. Patrie, C. Lackner, T.D. Henry, E.E. de Lange, A.L. Weltman, et al., Effects of n-3 fish oil on metabolic and histological parameters in NASH: a double-blind, randomized, placebo-controlled trial, J. Hepatol. 62 (2015) 190–197.
[164] Z.M. Younossi, V. Ratziu, R. Loomba, M. Rinella, Q.M. Anstee, Z. Goodman, et al., Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial, Lancet 394 (2019) 2184–2196.
[165] K. Patel, S.A. Harrison, M. Elkhashab, J.F. Trotter, R. Herring, S.E. Rojter, et al., Cilofexor, a nonsteroidal FXR agonist, in patients with noncirrhotic NASH: a phase 2 randomized controlled trial, Hepatology 72 (2020) 58–71.
[166] A.J. Sanyal, P. Lopez, E.J. Lawitz, K.J. Lucas, J. Loeffler, W. Kim, et al., Tropifexor for nonalcoholic steatohepatitis: an adaptive, randomized, placebo-controlled phase 2a/b trial, Nat. Med. 29 (2023) 392–400.

[167] R. Loomba, A.J. Sanyal, K.V. Kowdley, D.L. Bhatt, N. Alkhouri, J.P. Frias, et al., Randomized, controlled trial of the FGF21 analogue pegozafermin in NASH, N. Engl. J. Med. 389 (2023) 998–1008.
[168] R. Loomba, P. Bedossa, K. Grimmer, G. Kemble, E. Bruno Martins, W. McCulloch, et al., Denifanstat for the treatment of metabolic dysfunction-associated steatohepatitis: a multicentre, double-blind, randomised, placebo-controlled, phase 2b trial, Lancet Gastroenterol Hepatol (2024).
[169] S. Petta, G. Targher, S. Romeo, U.B. Pajvani, M.H. Zheng, A. Aghemo, et al., The first MASH drug therapy on the horizon: current perspectives of resmetirom, Liver Int. 44 (2024) 1526–1536.
[170] O. Araki, H. Ying, X.G. Zhu, M.C. Willingham, S.Y. Cheng, Distinct dysregulation of lipid metabolism by unliganded thyroid hormone receptor isoforms, Mol. Endocrinol. 23 (2009) 308–315.
[171] Y.S. Lee, M.S. Park, J.S. Choung, S.S. Kim, H.H. Oh, C.S. Choi, et al., Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes, Diabetologia 55 (2012) 2456–2468.
[172] C.R. Sirtori, The pharmacology of statins, Pharmacol. Res. 88 (2014) 3–11.
[173] X.D. Zhou, S.U. Kim, T.C. Yip, S. Petta, A. Nakajima, E. Tsochatzis, et al., Long-term liver-related outcomes and liver stiffness progression of statin usage in steatotic liver disease, Gut 73 (2024) 1883–1892.