



The hepatic lipidome: From basic science to clinical translation

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ABSTRACT

The liver is the key organ involved in lipid metabolism and transport. Excessive lipid accumulation due to dysregulated lipid metabolism predisposes the liver to steatosis, cirrhosis, and hepatocellular carcinoma. Lipids are generally compartmentalized in specialized organelles called lipid droplets that enable cells to store and release lipids in a regulated manner. However, during flux-in and flux-out of droplets, lipids are converted into toxic species leading to lipid-mediated liver damage. Lipids are categorized into 'toxic' or 'healthy' lipids that are involved in liver disease pathogenesis or resolution, respectively. Lipidomic analysis have revealed unique lipid signature that correlates with the disease progression therefore being used for disease diagnosis. In this comprehensive review, we provide an overview on hepatic lipid homeostasis, lipid compartmentalization mechanisms and lipidomic profiles in different liver diseases. We further discuss promising therapeutics targeting the hepatic lipidome including pro-resolving lipids, liposomes, and small-molecule inhibitors for the treatment of liver diseases.

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Abbreviations: LDs, lipid droplets; TG, triglycerides; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; AFLD, alcoholic fatty liver disease; ASH, alcoholic steatohepatitis; HCV/HBV, hepatitis B virus/hepatitis C virus; DIS, drug-induced steatosis; DISH, drug-induced steatohepatitis; HCC, hepatocellular carcinoma.

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1. Introduction

The liver plays a major role in metabolic homeostasis as it is responsible for the synthesis, storage, and redistribution of different biomolecules like lipids, proteins, and carbohydrates. In addition, the liver is responsible for the detoxification of blood [1]. Multiple etiologies are responsible for a dysfunctional liver, which can develop into different kinds of liver diseases. The most common etiologies of liver diseases are fatty liver disease, alcoholic liver disease, viral hepatitis, drug-induced liver injury, cirrhosis, and liver cancer [2,3].

Among others, fatty liver (steatosis) is a growing health problem worldwide in both adults and children, in which lipids play a substantial role. Fatty liver diseases are clinically divided into two subtypes: alcoholic fatty liver diseases (AFLD) and non-alcoholic fatty liver diseases (NAFLD). In contrast to AFLD, the prevalence of NAFLD has been increasing exponentially and has become the leading cause of liver transplantation worldwide [4]. Globally, the prevalence of NAFLD is estimated at 25% with the highest rates reported from South America and the Middle East, followed by Asia, the US, and Europe. 75–92% of morbidly obese patients and 76% of patients with type 2 diabetes mellitus (T2DM) develop NAFLD [5,6]. Compared to other liver diseases, a larger percentage (35–50%) of hepatocellular carcinomas (HCCs) arise from non-alcoholic steatohepatitis (NASH). These NASH-associated HCCs are larger and less amenable to curative therapies [7]. Notably, NASH also doubles the risk of non-liver related outcomes, such as cardiovascular diseases and malignancy [8].

Steatosis, including alcoholic and non-alcoholic steatosis, is mainly caused by the accumulation of lipids in hepatic cells due to increased lipogenesis, dysregulated lipid metabolism and/or reduced lipolysis. Lipid accumulation can lead to toxic effects, also known as lipotoxicity. A dysregulated lipid metabolism/lipotoxicity can lead to liver diseases with different phenotypes and severities varying from mild steatosis, steatohepatitis, cirrhosis, and HCC. So far, there are no FDA-approved therapies available for the management of liver diseases except for the removal of the underlying cause of the disease [9]. Besides

liver-related pathologies, impaired lipid metabolism can affect normal bodily functions and result in other clinical manifestations including cardiomyopathy, acute renal failure, insulin resistance and pancreatic β-cells dysfunction [10].

Hepatic cells possess mechanism(s) to protect the cells from lipotoxicity by compartmentalizing lipids, in so-called lipid droplets (LDs), to prevent cellular toxicity. However, during steatosis, the LD-associated proteins are altered, leading to altered in- and out-flux of lipids from the LDs, which results in the conversion of lipids to so-called ‘toxic’ lipids. These toxic lipids are unique molecular signatures that can be identified using lipidomic analysis and therefore can be used as biomarkers for non-invasive diagnosis of a specific liver disease. While excessive accumulation of lipids can result in liver damage, lipids can also be used as potential therapeutics such as pro-resolving lipids and as nanocarriers e.g. drug-encapsulated liposomes/lipid nanoparticles, for the treatment of chronic liver diseases. In this review, we summarize the recent insights about the role of lipids in the development and progression of liver diseases of different etiologies and provide an overview of the lipids that can be used as biomarkers as well as therapeutics. Furthermore, we provide an outlook into the therapies that have been developed to remodel abnormal hepatic lipidome and to maintain lipid homeostasis.

2. Role of the liver in lipid metabolism

Lipids are biomolecules that are insoluble in water or soluble in organic solvents. In the body, they function as energy reservoir, structural component of cell membranes, chemical messenger mediating cell-to-cell communication, as a part of the immune system regulating inflammatory processes, and regulate protein function via active interaction [11]. According to the comprehensive LIPID MAPS classification system (<http://www.lipidmaps.org>), lipids are classified into eight categories: (a) fatty acyls including fatty acids and fatty acyl coenzymes As (CoAs); (b) glycerolipids; (c) glycerophospholipids; (d) sphingolipids;

(e) sterol lipids; (f) prenol lipids; (g) saccharolipids; and (h) polyketides. These categories can be further subdivided into different classes and subclasses [12,13]. The liver is a major metabolic organ and plays a crucial role in lipid metabolism. Based on lipidomic analysis, the liver contains the following lipid categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, and prenol lipids [12,13].

Fatty acyls are one of the most diversified lipid categories in the mammalian lipidome, and fatty acids and fatty acyl CoAs are the most fundamental and essential building blocks incorporated into more complex lipids. Fatty acids in the liver can originate from diverse routes such as the direct uptake from circulation (dietary intake), *de novo* lipogenesis, peripheral lipolysis (from adipose tissue), or hydrolysis from triglycerides (TGs) from LDs [1]. 15–25% of the fatty acids are derived from the circulation. They are absorbed from the diet in the bloodstream followed by liver uptake. Moreover, these molecules can emerge from *de novo* lipogenesis that accounts for 5–30% in the liver [14]. During lipogenesis, glucose is metabolized into pyruvic acid and then reduced to acetyl-CoA via glycolysis, which is needed for the synthesis of TGs used as substrates [15]. This process is regulated by endoplasmic reticulum (ER) bound transcription factors [16]. Finally, fatty acids can be hydrolyzed from TGs of LDs which are localized in adipose tissue or liver cells. If originated from adipose tissue, these compounds are transported into the plasma for the uptake by the liver (Fig. 1) [17].

The oxidation of fatty acids is regulated by blood glucose levels [5]. When glucose levels are low, fatty acids are converted into acetyl-CoA that serves as a substrate for the tricarboxylic acid (TCA) cycle. During the TCA cycle, acetyl-CoA is oxidized into water and carbon dioxide (CO_2), and results in the production of adenosine triphosphate (ATP). Alternatively, acetyl-CoA can be converted into ketones via ketogenesis by oxidation to acetone and CO_2 as a substitutable energy source for

glucose in other tissues [4,18]. Furthermore, transport of acetyl-CoA from mitochondria to the cytosol can initiate cholesterol biosynthesis regulated by 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (Fig. 1).

In hepatic cells, there are two types of oxidation: β -oxidation which takes place in the mitochondria and peroxisomes, and ω -oxidation that occurs in the ER. β -oxidation results in the shortening of fatty acids to acetyl-CoA. In the case of ω -oxidation, fatty acids are hydroxylated into dicarboxylic acids that are converted to dicarboxyl-CoA (DCA), a substrate for the β -oxidation pathway. In contrast to the β -oxidation, ω -oxidation is only a minor pathway [4,18]. Moreover, chronic ER stress during liver damage, via β - and ω -oxidation, results in the production of reactive oxygen species (ROS) that promote deoxyribonucleic acid (DNA) damage in mitochondria (and nuclei) leading to cell apoptosis (Fig. 1). An equally important factor contributing to liver metabolism is represented by a nuclear receptor, peroxisome proliferator-activated receptor alpha (PPAR- α). PPAR- α is activated by fatty acids, which in turn leads to the transcription of PPAR- α regulated genes that further induce enzymes that are involved in both oxidation pathways [4,19,20].

In addition, the liver exports fatty acids in order to prevent intra-hepatic accumulation. In general, the liver plays an important role in the distribution of lipids throughout the body. In hepatic cells, fatty acids are converted into TGs. Eventually, these TGs fuse with apolipoprotein B-100 resulting in the formation of very low-density lipoprotein (VLDL) particles, which are secreted by the hepatic cells into the bloodstream [16,21] (Fig. 1). Ideally, the liver in-flux of fatty acids should be in balance with the out-flux. Therefore, dysregulation or disruption of the hepatic lipid metabolism can lead to the accumulation of fatty acids in hepatic cells resulting in multiple hepatic pathologies [19].

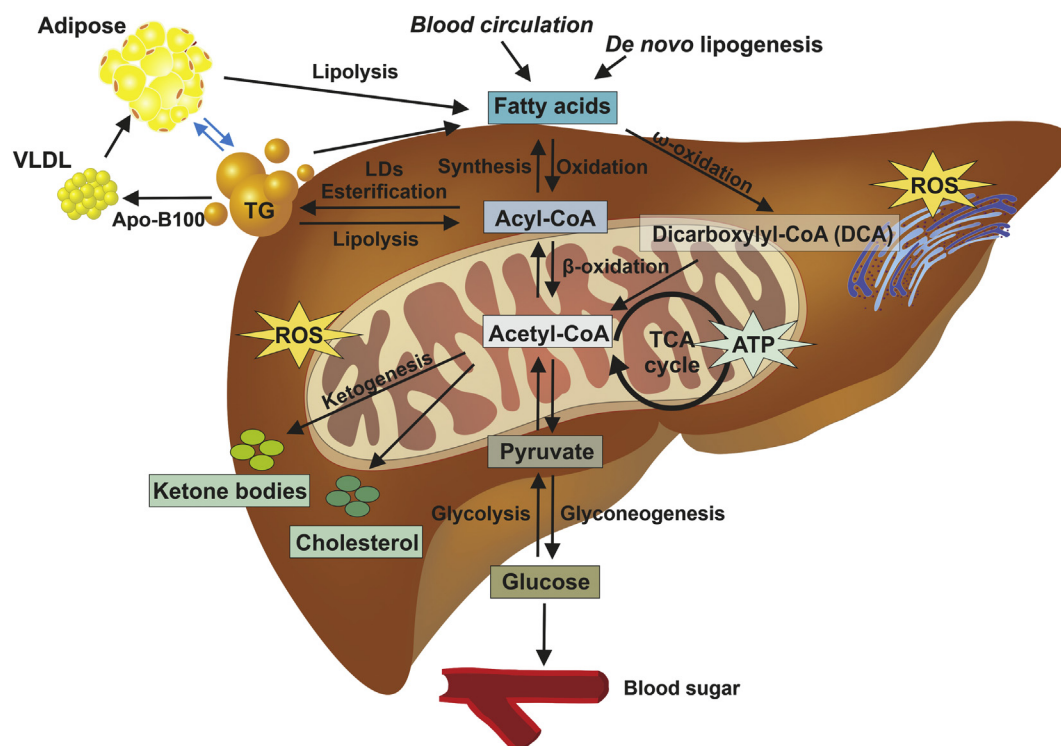


Fig. 1. Hepatic lipid metabolism. Schematic showing the hepatic lipid metabolism. Fatty acids derived from adipose tissue due to lipolysis, triglycerides, blood circulation, or *de novo* lipogenesis, can be oxidized via β -oxidation or ω -oxidation into acetyl-CoA. Acetyl-CoA can be processed in the mitochondria by the tricarboxylic acid (TCA) cycle resulting in the production of adenosine triphosphate (ATP). Acetyl-CoA serves also as a substrate for the biogenesis of ketone bodies, cholesterol, or glucose. Glucose can be transformed into fatty acids via glycolysis. Fatty acids in the cells can be esterified into triglycerides (TGs), which, together with apolipoprotein (Apo)B-100, form very low-density lipoproteins (VLDLs), which can be excreted from the liver. Chronic endoplasmic reticulum (ER) stress, due to the liver damage, results in the production of reactive oxygen species (ROS) via β - and ω -oxidation and promotes DNA damage in nuclei and mitochondria activating the cell apoptosis pathways.

3. Lipids involved in the liver disease pathogenesis

3.1. Lipid droplets

3.1.1. LDs structure and formation

LDs are specialized and dynamic cytosolic organelles found in nearly all the cells. LDs mainly consist of a phospholipid monolayer with a core of neutral hydrophobic lipids, mostly TGs and cholesterol esters (CEs), but less abundant species such as ether lipids and waxes can be also integrated [21,22]. Especially phosphatidylcholines (PCs) as part of the surrounding monolayer serve as surfactants that sustain the enclosed droplets by reduction of the surface tension between (non)-organic phases [21]. Almost all cell types can form small LDs about 300–800 nm in size, albeit enlarged droplets with a diameter bigger than 1 μ m can evolve even towards tremendous dimensions in hepatocytes and adipocytes [21]. Intriguingly, so-called giant or supersized LDs form either by the coalescence of two LDs into one or by the diffusion of lipids from one LD to the other by the process called ripening.

Fusion or formation of the large LDs that occurs in hepatocytes or adipocytes is most likely mediated by the members of the cell death-inducing DNA-fragmentation-factor 45 (DFF45)-like effector (CIDE) protein family [21]. When there is an excess of lipids in a cell, the lipids accumulate in the bilayer of the ER. Once a critical concentration is reached, the phase separation of the bilayer ensues until the LDs are budded and excreted into the cytoplasm where they can continue to grow in size. During the formation of the LDs, proteins like perilipins (PLINs) are involved, and enzymes such as diacylglycerol O-acyltransferase 1,2 (DGAT1,2) and glycerol-3-phosphate acyltransferase 4 (GPAT4) localize around the droplet surface and synthesize TGs that are stored in the LDs. Besides triglycerides, hepatocytes express acyl CoA:cholesterol acyltransferase 1 and 2 (ACAT1 and ACAT2) enzymes that are involved in CEs synthesis and therefore possess an ability to synthesize and store CEs. However, their contributions to CEs synthesis in the human liver has been debated [21]. A recent study has revealed that LDs evolve in tandem with peroxisomes at conserved ER subdomains, reticulon-like Pex30/multiple C2 domain containing transmembrane protein 2 (MCTP2), suggesting a link between LDs and peroxisomal biogenesis, and mediating intracellular signaling between the ER, peroxisomes and LDs [23].

3.1.2. LDs proteomics

The most frequent proteins found in LDs are PLINs, in particular PLIN2 and PLIN3 [21]. Protein targeting is a crucial process that ensures LD expansion and transport, for instance to other organelles in the cytoplasm and is mainly directed by PLINs and GPAT4. LDs are connected to the ER via membrane bridges involving the ADP ribosylation factor 1 (ARF1)-coat protein complex I (COPI) vesicles, so-called ARF1-COPI machinery, which is involved in vesicle trafficking, and required for targeted TG-synthesis and catabolism via specific enzymes localized at the LD surfaces. Although targeting mechanisms are still poorly understood, recent studies indicate remodeling of the LD surface as potential mechanism that increases the surface tension by phospholipid removal [24].

Proteomic analyses have revealed that diverse proteins at the LD surfaces are cross-species and cross-tissues/cells dependent. Fujimoto et al. compared LD proteins from yeast *Saccharomyces cerevisiae* with human samples. Several proteins were found to be conserved in yeast and human. Conversely, some differences e.g. a homolog of PAT family proteins [PLIN/ADRP (adipose differentiation-related protein)/TIP47 (tail-interacting protein of 47 kDa)] was not found in yeast. Furthermore, ADRP was the most abundant LD protein present in humans, while sterol 24-C-methyltransferase was found in yeast [25,26]. Fujimoto et al. further demonstrated that ADRP was the most abundant protein in Huh7 and J774 cells, but rather a minor component of lipid droplets in adipocytes. In addition, the expression of PAT family proteins is tissue-specific. While ADRP and TIP47 are expressed ubiquitously, the

expression of PLIN is restricted to adipocytes and steroidogenic cells [25]. Moreover, Wu et al. compared LD proteins of mammary epithelial cells with hepatocytes in mice and found different LD proteome among both cell types. In particular, LD proteins of mammary epithelial cells possess a unique function and are involved in the secretion of lipids from the mammary gland [27]. Bersuker et al. investigated the LD proteome of other two human cell lines: U2OS, an osteosarcoma cell line, and Huh7 cells. Authors found 26 Rab guanosine triphosphate proteins (GTPases) in both cell types, however, 10 Rab-GTPases were exclusively found, either in U2OS or in Huh7 cells [28,29]. LD proteomes are currently been examined from different species, tissues, and cells subjected to different nutritional states [29]. Other proteins implicated in LDs are CIDE proteins and adipose triglyceride lipases (ATGLs) [21]. With respect to LD biogenesis, four protein families seem to be involved, namely SEIPINs, PLINs, fat storage-inducing transmembrane (FIT) proteins, and LDs shaping ER proteins [30]. These studies suggested that the LD proteins can vary among different species, tissues and cell types, and, most likely, possess diverse functions based on the respective expression.

3.1.3. LDs as functional units

LDs function as storage reservoirs that regulate the in- and out-flux of lipids in a controlled manner to prevent their conversion to a toxic species [22]. PPAR- α is responsible for the induction of enzymes that are required for remodeling and detoxification of toxic lipids during oxidation processes [31]. Additionally, the lipids in LDs can be used as an energy source. Cellular lipases are transported to the LDs to hydrolyze lipids or the LDs interact with other organelles like peroxisomes, endosomes, ER, plasma membranes, and mitochondria to catalyze lipids into free fatty acids (FFAs) via β -oxidation and release ATP [32]. The purpose of lipolysis is to mobilize fatty acids as well as to provide substrates for the generation of VLDLs [21]. The basic understanding of lipases correlates with the lipolysis in adipocytes. Enzymes that are mainly expressed in the liver or involved in hepatic lipolysis include patatin-like phospholipase domain containing 2 (PNPLA2) or ATGLs, hormone-sensitive lipase (HSL), monoglyceride lipase (MGL) as well as PNPLA3 that affect TGs metabolism, and ER luminal triacylglycerol lipase as a crucial factor for VLDL secretion. Lipolysis also affects recruited LD-associated surface proteins that could either be removed due to macromolecular protein crowding or via chaperone-mediated autophagy [21]. Besides lipolysis, lipids can also be generated via lipophagy (autophagic degradation of LDs) in which LDs are engulfed by autophagosomes that subsequently fuse with lysosomes and form autolysosomes. This lipophagic process is essential for mitochondrial respiration during starvation [21]. Although underlying mechanisms involved in lipophagy are still unknown, recent research reported the interaction between two GTPases - dynamin 2 (Dyn 2) and small regulatory Rab GTPase (Rab10) - that seem to induce synergistic effects important for accumulating and catabolizing LDs potentially linked to hepatocellular metabolism [33].

3.2. Oxidized lipids

Lipids or fatty acids can also be oxidized into different metabolites with diverse molecular configurations such as double bonds, the chain length as well as the degree and location of desaturation. During cellular stress responses triggered by different factors, enzymes are upregulated that alter the oxidation of FFAs leading to the accumulation of saturated fatty acids (SFAs) and a decreased excretion of these products. SFAs such as ceramides (Cers), palmitate, and lysophosphatidyl choline (LPC) trigger ER stress by upregulation of the c-Jun N-terminal kinase (JNK) pathway that has been associated with pathological processes and shown to play a crucial role in apoptosis in cell death paradigms. SFAs can also activate hepatic stellate cells (HSCs) and macrophages, thereby promoting steatohepatitis and fibrosis. Cers belong to cell membranes as part of the lipid bilayer and presumed to play a critical

role in the regulation of hepatic steatosis via an unknown mechanism. Palmitate or palmitic acids are converted into palmitoyl CoA that are integrated in other lipids and stimulate *de novo* synthesis of saturated phospholipids including Cers, resulting in their cellular accumulation. Finally, LPCs incorporated in lipid bilayers, cell membranes, LD membranes, and VLDLs, critical for maintaining the cell membrane integrity, can also impair the mitochondrial oxidative phosphorylation leading to ER stress [16,34,35].

4. Hepatic lipid metabolism imbalance during different liver pathologies

A dysregulated hepatic lipid metabolism mostly results in the intracellular and extracellular accumulation of lipids. Such imbalances in lipid homeostasis can be caused by increased *de novo* lipid biogenesis, decreased oxidation of lipids, or impaired secretion of lipids [36]. Imbalances in hepatic lipid metabolism may result from other pathological conditions (e.g. hyperinsulinemia, insulin resistance), and can result in life-threatening diseases including liver diseases as described here.

Excessive accumulation of lipids and their toxic metabolites leading to cellular responses including mitochondrial dysfunction, ER stress, oxidative stress, inflammation, and cell death is referred to as lipotoxicity [34]. Although underlying mechanisms are still under investigation, several theories about toxic lipids have emerged over the last decade. Potential toxic lipids can be characterized, for instance, by the degree of saturation or number of double bonds in fatty acids or their relative positions or configuration (*cis* or *trans*) [37]. Understanding the effects and mechanisms of specific lipids is essential for gaining insights into cellular lipotoxicity and liver diseases.

Excess of lipids has been shown to increase cell-cell adhesion that promotes hepatic infiltration and the proximity of such species to hepatic macrophages and Kupffer cells influences their plasticity [38]. For example, SFAs like palmitate tend to polarize Kupffer cells towards the pro-inflammatory M1 phenotype leading to the activation of several inflammatory processes. Furthermore, lipid accumulation in hepatocytes acts as mechanical initiator resulting in hepatocyte ballooning, dysfunction, and capillarization of other cell types due to compression, increased intrahepatic vascular resistance, and shear stress [38]. Cholesterol crystals have been identified on hepatocytic LD surfaces that facilitated Kupffer cell activation [39]. In addition to the impact on inflammatory mechanisms, palmitate has been suggested to impair structure and function of the ER causing compromised organelle morphology and integrity including membrane dilation [40,41]. Both cholesterol and palmitate are examples of toxic lipids that also affect

mitochondrial permeabilization, contributing to oxidative stress and apoptosis [42]. Toxic lipids also trigger the unfolded protein response (UPR), an acute cellular stress response that regulates ER homeostasis [16]. Chronic ER stress due to intrahepatic accumulation of fatty acids results in the production of ROS via β - and ω -oxidation pathways. Besides triggering the release of pro-inflammatory cytokines and hepatic inflammation, ROS promotes DNA damage in nuclei and mitochondria activating the apoptosis pathway resulting in cell death [19,43].

Fatty liver (steatosis) arises due to the accumulation of the lipids, primarily TGs in form of small or large LDs (technically 5–10% of the liver weight) [44]. Large LDs or macrovascular steatosis are mostly associated with obesity, T2DM, and alcoholism in which hepatic cells contain one large vacuole of lipids, whereas small LDs or microvascular steatosis are caused by genetic disorders or toxic damage [4]. Hereunder, we describe the impaired lipid metabolism in different hepatic pathologies: i) NAFLD/NASH, ii) AFLD/alcoholic steatohepatitis (ASH), iii) hepatitis C virus (HCV) infection, iv) drug-induced steatosis/drug-induced steatohepatitis (DIS/DISH), v) cirrhosis and vi) HCC (Fig. 2).

4.1. NAFLD/NASH

Currently, NAFLD is a global health problem affecting millions of people worldwide [5]. NAFLD is closely associated with morbid obesity and/or T2DM, and display spectrum of pathologies ranging from steatosis to severe NASH, cirrhosis and HCC. Morbidly obese people have lipid overload due to increased unhealthy diet intake, whereas patients with T2DM suffer from insulin resistance resulting in an increase in fatty acid release from adipocytes into the bloodstream. In general, accumulation of fatty acids is caused by the high availability of lipids or other energy sources in the bloodstream. Eventually, these lipids are taken up by the liver, in which they assemble in the form of LDs [4,5]. During lipid peroxidation, ROS are generated that trigger the production of inflammatory cytokines, driving innate immune system and local inflammation [4]. During fasting, mitochondria interact with LDs to initiate β -oxidation. PLIN1, an LD-associated protein, promotes the LD-mitochondrial interactions responsible for the mitochondrial transfer of fatty acids. It has been reported that in NAFLD, the expression of PLIN1 is altered leading to a decreased LD-mitochondrial interaction and corresponding reduction in β -oxidation (Fig. 2). Consequently, fatty acids are transported to adjacent cellular organelles instead of the mitochondria, and contribute to steatosis [21]. Furthermore, mutations in the LD lipase PNPLA3 and VLDL-mediator transmembrane 6 superfamily member 2 (TM6SF2) protein have been correlated with NAFLD progression [45,46]. According to the lipid signature

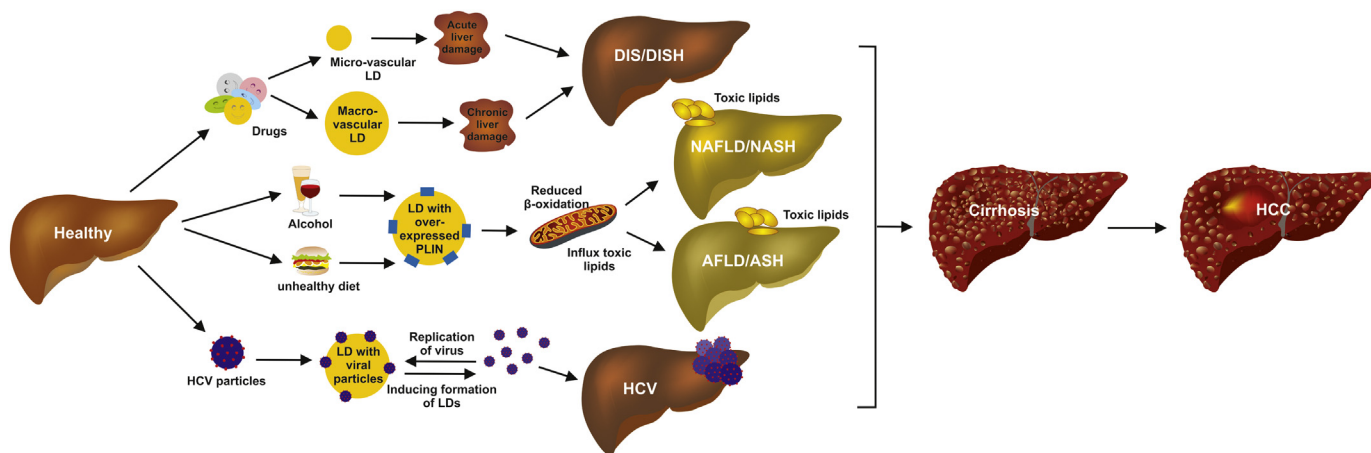


Fig. 2. Lipid droplets (LDs) and liver disease pathogenesis. Different etiologies exhibit varying effects on LDs in the liver, which leads to liver disease pathogenesis including (non)-alcoholic fatty liver diseases (NAFLD/AFLD), (non)-alcoholic steatohepatitis (NASH/ASH), hepatitis C-virus (HCV) infection and drug-induced steatosis or steatohepatitis (DIS/DISH) via diverse mechanisms. During chronic injury, these liver diseases can develop into cirrhosis and/or hepatocellular cancer (HCC).

Table 1

Overview of lipidomic profiles in different hepatic pathologies. Highlighted disease-related lipid biomarkers including respective models/samples, disease/conditions, analytical methods as well as the main findings. The following lipids and lipid clusters were investigated with respect to non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH), alcoholic fatty liver disease/alcoholic steatohepatitis (AFLD/ASH), hepatitis B/C virus (HBV/HCV), hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC): lysophospholipids/phospholipids (LPLs, PLs) including phosphatidylethanolamines (PEs), phosphatidylcholines (PCs), phosphoinositols (PIs), phosphatidylserines (PSs), cholesterol species and ceramides (C, CE, Cer), triglycerides (TGs), bis-(monoacylglycerol)-phosphate (BMP), prostaglandins (PGs), 5-, 8-, 11-hydroxyeicosatetraenoic acids (5-, 8-, 11-HETEs), 9- and 13-hydroxyoctadecadienoic acids (9-, 13-HODEs), 11,12-dihydroxyeicosatrienoic acids (11,12-DHETs), 13,14-dihydro-15-keto prostaglandin D2 (dhk PGD2), 20-carboxy arachidonic acid (20-COOH AA), sphingomyelins (SMs), phosphatidylglycerol, N-lauroglycine (NLG), decatrienoic acid (DTEA), glycerol backbones, fatty acyl chains, fatty acid methyl esters (FAMES), phosphatidic acids, cardiolipins, sterols, glycerophosphocholines (GPCs), glycerophosphates (GPs), fatty acids and conjugates, glycerophosphoserines (GPSs) and glycerophosphoinositols (GPIs), vaccenic acid, oleic acid, palmitoleic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acids (LAs), free fatty acids (FFAs), diacylglycerols (DGs), triacylglycerols (TAGs), galactosylceramides (GalCers) and lactosylceramides (LacCers). The techniques involve accurate mass time of flight mass spectrometry (TOF/MS), gas chromatography (GC), gas chromatography and liquid chromatography – mass spectrometry (GC/LC-MS), high performance liquid chromatography (HPLC), ion-mobility spectrometry (IMS), liquid chromatography – mass spectrometry (LC-MS), liquid chromatography with tandem mass spectrometry (LC/MS/MS), matrix-assisted laser desorption/ionization – mass spectrometry (MALDI-MS), matrix-assisted laser desorption/ionization – mass spectrometric imaging (MALDI-MSI), mass spectrometry (MS), normal phase high performance liquid chromatography mass spectrometry (NP HPLC/MS), normal phase/reversed phase two dimensional liquid chromatography-quadrupole time-of-flight mass spectrometry (NP/RP, 2D LC-QTOF/MS), Proton (¹H)/ phosphorus (³¹P) nuclear magnetic resonance (NMR), quadrupole time-of-flight (QTOF), tandem mass spectrometry (MS/MS), thin-layer chromatography (TLC), tip-contact sampling/ionization – mass spectrometry (TCSI-MS), ultra-fast liquid chromatography/ion trap-time of flight mass spectrometry (UFLC/IT-TOF MS), ultra-performance liquid chromatography – high resolution mass spectrometry/mass spectrometry (UHPLC-HRMS/MS), ultra-performance liquid chromatography – mass spectrometry (UPLC-MS).

	Biomarker(s)	Model/Sample	Disease/Condition	Analytical method(s)	Main findings	Ref.
NAFLD/NASH	LPLs	French-Tsukamoto mouse model	Obesity	MS	Increased levels	[47]
	PGE2; 5-, 8-, 11-HETEs; 9- and 13-HODEs	French-Tsukamoto mouse model	Obesity and alcohol	MS	Increased levels	[47]
	Short- and medium-chain TAGs and Cers	ob/ob mouse	Obesity	UPLC-MS	Correlation with elevated TAGs and Cers	[48]
	BMP	C57BL/6J mice, hepatic lipid extract	Long-term high-fat diet	High-resolution MS	Enormous accumulation of hepatic BMP	[49]
	AA-containing intracellular phospholipids: PI and PE species	Fresh frozen human liver biopsies	Obese patients	MALDI-MSI, MS/MS	Loss of AA-containing phospholipids, predominantly PI and PE species	[50]
	PE (16:0/22:6), PE (8:0/22:6), TG (58:5), TG (58:6), PE (16:0/20:4), PC (16:0/20:4), PC (40:6), TG (56:5), TG (54:2), TG (56:4), TG (56:5)	C57BL/6 mice	NAFLD	UHPLC-HRMS/MS	Decreased PEs/PCs and increased TG species - association with disease progression	[51]
	11,12-DHETs, dhk PGD2, 20-COOH AAs	Human plasma samples	Patients with NAFLD (n=10), NASH (n=9), and non-NAFLD (n=10)	LC-MS/MS	Differentiating NAFLD from NASH	[52]
	C14:0, C16:0, C16:1n-7, C18:1n-7, C18:1n-9, C18:2n-6, CE (C16), CE (C18), DG (16/16), TG (14/16/16), TG (16/16/16), TG (16/16/18), TG (16/18/18), TG (18/18/18), Cer (d18:1/16:0), Cer (d18:1/24:0), PC (16:0/18:0), PC (18:0/18:1), PC (18:0/20:3), PC (16:0/18:1), PC (18:0/20:4), PC (18:2/18:2), PE (16:1/16:1), PI (18:0/18:0), PI (18:0/22:3), PI (18:1/20:4), PS (16:0/16:0), PS (16:0/18:1), PS (18:0/18:1), SM (18:1/14:0), SM (18:1/16:0)	Human liver biopsies	Healthy control (n = 7), NAFLD patients (n = 39) and NASH patients (n = 15)	GC/LC-MS	9 lipids decreased in NASH, mainly Cers and PLs, 23 lipids showed a significant increase in NAFLD/NASH including 6 fatty acids, 2 CEs, 1 DG, 5 TGs and 9 PLs; 100% sensitivity and specificity as NASH lipid signature.	[53]
AFLD/ASH	PLs, phosphatidylglycerol, LPLs	French-Tsukamoto mouse model	Alcohol	MS	High levels	[47]
	PGE2; 5-, 8-, 11-HETEs; 9-,13-HODEs	French-Tsukamoto mouse model	Obesity and alcohol	MS	High levels	[47]
	PCs, glycerol backbone, fatty acyl chains, cholesterol	Male Fischer 344 rats, liver, and plasma samples	Fed 5% alcohol in a Lieber-DeCarli diet	¹ H/ ³¹ P NMR	Decrease in PC production and fatty acyl chains; increase in glycerol backbone and cholesterol	[61]
	NLG, DTEA	Human serum	30 patients of different alcoholic liver disease stages, ongoing alcohol consumption	LC-MS	NLG determines cirrhosis with 100% sensitivity and 90% negative predictive value, and DTEA determines disease severity with 100% sensitivity and 100% negative predictive value	[56]
	PCs, TGs, CEs, fatty acyl chains, FAMES	ADH-deer mice, liver samples	1, 2 or 3.5% ethanol in the liquid diet for 2 months	¹ H/ ³¹ P NMR	Decreased PCs, increased total cholesterol, CEs, FAMES, TGs, and decreased free cholesterol and PLs	[62]
Cirrhosis	SMs, PCs, LPCs, TGs, CEs	Human serum	Patients with HBV-induced cirrhosis	UFLC/IT-TOF MS	Cirrhosis and HCC patients had similar serum lipid profile while different as compared to hepatitis patients; downregulated levels of SMs, PCs, LPCs, TGs and CEs	[99]
	Total plasma lipid content, CEs, PCs, PEs	Human plasma	40 de-compensated cirrhotic patients with different etiologies	HPLC	Decreased total plasma lipid content of cirrhotic patients vs control subjects with diminished CEs and PCs; lipid profile of cirrhosis showed alterations in the PC/PE plasma ratio	[100]
	Sterols, GPCs, GPs, fatty acids and conjugates, GPSs, GPIs	Human plasma samples	25 patients with HCV-induced	MALDI-MS	HCC vs. liver cirrhosis: 76–80% accuracy, 88–100% sensitivity and	[101]

(continued on next page)

Table 1 (continued)

	Biomarker(s)	Model/Sample	Disease/Condition	Analytical method(s)	Main findings	Ref.
HCC	GPCs, GPSs, GPLs	Human serum	cirrhosis Patients with HBV induced cirrhosis and/or HCC	UPLC-MS	53–60% specificity Increased accuracy with 78% sensitivity and 64% specificity; HCC differentiated from HBV-induced cirrhosis with 100% sensitivity and specificity	[82]
	Vaccenic, oleic acids, LA, EPA, DHA, n6-C22 over n3-C22 ratio	Mice liver and tumors, human plasma samples	Mice with hepatocytic deletion of <i>Pten</i> with developed NASH and HCC patients with NASH-induced HCC	TLC and GC	Increased hepatic and circulating vaccenic and oleic acids in HCC; reduced hepatic and circulating LA in HCC; decreased EPA and DHA in NASH with negative correlation of DHA with tumor burden; n6-C22/n3-C22 ratio positively correlated with tumor burden	[103]
	PC species with palmitoleic acid or oleic acid at the sn-2-position, LPC with palmitic acid at the sn-1-position	Human HCC tissues	HCC specimens	IMS	Increased PC with palmitoleic or oleic acid at the sn-2-position and reduced LPC with palmitic acid at the sn-1-position	[104]
	Palmitic acyl (C16:0)-containing GPLs	Hep3B, SW480, SW620, AGS, BGC-823, HGC-27, L02, MHCC97H (97H), HCCLM3 (LM3) cells and HCC specimens	HCC cells and patients with primary HCC	NP HPLC/MS	Decreased palmitic acyl (C16:0)-containing GPLs are positively associated with metastatic abilities of HCC cells	[105]
	GalCer (36:5), FFA (20:4), PE (40:6), PE (38:6), DG (40:5), DG (44:2), LacCer (40:3), PC (40:6), FFA (22:5) PE (19:0/0:0), PE (18:2(9Z,12Z)/0:0), PC (14:0/0:0), PC (18:0/0:0)	Human plasma	Patients with HCC	NP/RP 2D, LC-ToF/MS	Excellent diagnostic ability to compare HCC patients with healthy controls	[106]
	C18:1/C18:2, C20:3/C20:4, C22:4/C22:5, PE36:3/PE36:4, PE38:5/PE38:6, PI34:1/PI34:2, PI36:3/PI36:4, PI36:1/PI36:2, PI38:3/PI38:4, SM16:1/SM16:2, PC36:4/PC36:5, TG50:1/TG50:2, TG52:2/TG52:3, TG54:3/TG54:4, TG54:2/TG54:3, C16:0/C16:1, PI36:2/PI36:3, PI38:4/PI38:5, PI38:1/PI38:2, TG52:4/TG52:5, TG54:5/TG54:6, TG54:4/TG54:5	Blood samples	Patients with ICC or HCC	Mass TOF/MS	Metabolites used to distinguish HCC and ICC as potential early-warning biomarker	[107]
		Liver tissue	Patients with HCC	TCSI-MS	Primary HCC tissues can be discriminated from the non-tumor parts with a high accuracy (91.7–98.3%) for tumor identification	[108]

characteristic for NAFLD/NASH, accumulation or alteration in lipid concentrations, for instance, different lysophospholipids/phospholipids (LPLs/PLs) including phosphatidylethanolamines (PEs), PCs, phosphoinositols (PIs), phosphatidylserines (PSs), cholesterol, cholesterol ester species and ceramides (C, CE, Cers), TGs, bis-(monoacylglycerol)-phosphate (BMP), prostaglandins (PGs), 5-, 8-, 11-hydroxyeicosatetraenoic acids (5-, 8-, 11-HETEs), 9-, 13-hydroxyoctadecadienoic acids (9-, 13-HODEs), 11,12-dihydroxyeicosatrienoic acids (11,12-DHETs), 13,14-dihydro-15-keto prostaglandins D_2 (13,14-dhk PGD₂), 20-carboxy arachidonic acid (20-COOH AA) and sphingomyelins (SMs) have been observed [47–53] (Table 1).

4.2. AFLD/ASH

Excessive alcohol consumption, characterized by individual intake of 60 g or more alcohol per day, is one of the main reasons for chronic liver diseases and the second leading cause of liver-related mortality [54,55]. Similar to NAFLD, AFLD also display disease spectrum ranging from hepatic steatosis, alcoholic steatohepatitis (ASH) to cirrhosis and HCC. 60–90% of the AFLD patients, most likely, develop hepatic steatosis. About 20% of these cases progress to steatohepatitis whereas 20–40% fibrosis, 8–20% develop cirrhosis and 1.5–2% develop HCC [56,57]. PPAR- α and sterol regulatory element-binding protein (SREBP) regulate fatty acid and cholesterol homeostasis via fatty acid oxidation and lipogenesis, respectively. During AFLD, ethanol exhibits inhibitory effects on PPAR- α while activating SREBP1c, thereby inhibits fatty acid oxidation and increases lipogenesis. Besides, alcohol mediates ER stress and

mitochondrial damage further inhibiting fatty acid oxidation. These processes lead to the accumulation of lipids in hepatocytes [4,20,58]. Ethanol also hinders the activity of fatty acid desaturases (FADS) that are involved in the formation of arachidonic acids (AAs), eicosapentaenoic acids (EPAs) and docosahexaenoic acids (DHAs). This suppression impacts the balance between inflammatory and pro-resolving mediators wherein AA, EPA, and DHA are the key determinants of disease progression to liver cirrhosis [59]. Ethanol also increases expression of LD-associated proteins such as PLIN2 that regulate the influx of toxic FFAs (Fig. 2). Increased intracellular FFAs lead to ER stress and ROS generation resulting in lipotoxicity [54,55,60]. Characteristic lipotoxic species include LPLs/PLs, phosphatidylglycerol, N-lauroglycine (NLG), decatrienoic acid (DTEA), PGs, 5-, 8-, 11-HETEs, 9-, 13-HODEs, glycerol backbones, fatty acyl chains, fatty acid methyl esters (FAMES), Cs/CEs, PCs and TGs (Table 1) [47,56,61,62].

4.3. HCV

HCV causes liver inflammation due to hepatitis C viral infections. 50% of HCV patients develop macrovascular hepatic steatosis [63], around 20% of all patients develop acute hepatitis that can lead to chronic hepatitis. 25% of the patients with chronic hepatitis develop cirrhosis and 50% of these patients suffer from HCC [64]. HCV patients in combination with obesity or T2DM can also develop steatosis due to comorbidities [65,66]. In HCV-infected hepatocytes, the HCV proteins localize around the ER membrane. The core proteins of HCV variants interfere with the intracellular lipid metabolism resulting in lipid

accumulation. Core proteins interact with mitochondria resulting in decreased fatty acid oxidation and increased fatty acid uptake generating ROS and leading to oxidative stress, hence lipid-mediated toxicity. Studies have demonstrated that HCV-related steatosis is regulated by a direct interaction between HCV and microsomal triglyceride transfer protein (MTP). It has been suggested that MTP is critical for the secretion of HCV particles and MTP inhibition reduces HCV production. During the acute phase, HCV upregulates the transcription and activity of MTP thereby facilitating HCV propagation, whereas in the chronic phase MTP protein is downregulated. This results in the disability of TG secretion and accumulation of TGs in the LDs thereby favoring the “storage” of virus particles for persistent infection. HCV also influences PPAR- α and upregulates SREBP-1c, leading to enhanced lipid synthesis and formation of LDs [64,66,67]. For replication, the virus requires nuclear pore complex (NPC)- and replication complex (RC)-rich structures that are located in the ER membrane and around the LDs. The core proteins lure the other viral components to the membrane of the LDs, where they can generate new viral particles. HCV interferes with the host lipid metabolism to initiate the generation of LDs which are used for viral propagation (Fig. 2) [64].

4.4. DIS/DISH

DIS/DISH belong to a class of drug-induced liver injuries (DILIs) with comparatively low prevalence. Only about 2% of NASH is considered to be drug-induced [68,69]. Both evolve due to long-term drug-mediated toxicities in the liver [68]. Harmful effects are based on dose-dependencies or adverse effects of the drug when used at or above the therapeutic level. Furthermore, DIS can be categorized into acute microvascular and chronic macrovascular subtypes [70]. Microvascular DIS leads to acute liver damage, resulting in inhibition of β -oxidation with subsequent fatty acid aggregation (Fig. 2). The oxidation inhibition further impairs glucogenesis leading to hypoglycemia. Besides, the generation of reactive lipids and ROS contributes to DNA damage. Taken together, microvascular DIS can lead to liver injury with life-threatening consequences [71]. In particular, common drugs such as acetylsalicylic acid, tetracycline, valproic acid, amiodarone, dexamethasone, 5-fluorouracil-based adjuvant therapy, or margosa oil induce microvascular DIS [72,73]. Equally important, macrovascular DIS is related to a slowly developing chronic liver damage which can potentially lead to fibrosis, cirrhosis, and liver failure (Fig. 2). For instance, drugs like 5-fluorouracil, methotrexate, irinotecan, cisplatin, and tamoxifen are known to promote macrovascular DIS [72,73]. However, there is a thin line between these two subtypes, so several drugs can induce both types of steatosis [72,73].

4.5. Cirrhosis

The liver is characterized by its extraordinary ability to regenerate aided by hepatocytes. Injured hepatocytes mediate an important function by triggering an inflammatory response that leads to the activation, proliferation, and trans-differentiation of HSCs into activated HSCs or myofibroblasts [74,75]. As a response, HSCs secrete extracellular matrix (ECM) to initiate a wound healing process that is also influenced by lipid metabolism imbalances [21]. However, during chronic liver injury, HSCs are persistently activated leading to fibrosis that causes alterations of the liver structure, loss of functioning, and cirrhosis and/or end-stage liver failure [74,75]. The prevalence of liver cirrhosis is estimated to be 1% of the global population [76]. Liver cirrhosis is often associated with the distortion of liver vasculature, which leads to shunting of the blood supply directly from the inflow to the outflow resulting in a blocked exchange of molecules. This leads to impaired function of the liver, portal hypertension, and may progress to liver decompensation as well as HCC development [76–78]. It is important to note that lipid imbalances are a cause rather than the consequence in liver cirrhosis [21,79]. Aforementioned driving forces can include, for instance, ROS

and inflammation, which are often associated with hepatic damage or toxic lipids. As a result, pro-fibrotic processes are activated, for instance, by overproduction of transforming growth factor beta (TGF- β) due to lipid peroxidation products [21]. Regardless of the etiology, cirrhosis can develop due to various factors and pre-existing conditions like fatty liver disorders. Therefore, underlying causes of predispositions involving lipotoxicity play a role in the path towards cirrhosis [21,79]. Although some *in vitro* data regarding lipotoxicity does not coincide with cirrhosis, correlations were found regarding altered plasma levels of lipids like DHA, AA, or linoleic acid (LA) [80]. Potential cirrhosis-related lipid signatures in plasma are discussed in Section 5.1.5 and presented in Table 1.

4.6. HCC

HCC is the third most common cause of cancer-related mortality with a 5-year survival rate of less than 7% [81,82]. Patients with advanced cirrhosis are likely to develop HCC [83]. Apoptotic cells and released DNA fragments activate the wound healing responses resulting in excessive accumulations of ECM proteins by HSCs supporting fibrosis, which is also affected by lipid dysregulations [21,44]. Since cirrhosis (pre-cancerous stage) usually precedes HCC, correlated pathological processes are therefore responsible for tumor development. However, it has been reported that cancer cells, including HCC, make use of mechanisms that avoid accumulation of lipids with tumor-suppressive character like Cers or polyunsaturated PLs [84]. Altered lipid contents have been detected in HCC patient samples that showed mainly reduced levels, which is further elaborated in Section 5.1.6. Recently, Hall et al. investigated the lipidome of proliferating hepatocytes, and suggested that the specific metabolic pathways i.e. lipogenesis, FA desaturation and de novo synthesis of PC, are involved in the transition of hepatocytes to proliferation during hepatocarcinogenesis [85]. Hall and co-workers used multiple models to study the reprogramming of hepatic lipid metabolism involving different datasets, species and biological processes, which implies a broader association of those lipid profiles not necessarily limited to HCC [85].

5. Lipidome for diagnosis of liver diseases

Liver biopsy is yet the traditional way to diagnose patients with liver disease. Using liver biopsy, factors like the disease grade, stage, and inflammation can be determined with high accuracy. However, this procedure is invasive, expensive, time-consuming and the biopsy only represents a small part of the liver including the risk of sampling error [43,86,87].

Besides biopsies, there are several advanced non-invasive diagnostic tests. The first category of non-invasive techniques is based on imaging: magnetic resonance imaging (MRI), computerized tomography (CT), ultrasonography, and elastography [88]. Although MRI is associated with high costs, it is highly sensitive and offers the possibility to quantitate fat tissue. On the other hand, fat tissue cannot be clearly observed by CT and radiation is required for the imaging procedure. By use of ultrasonography, accumulated lipids can be detected due to an increased echogenicity, however, the thickness of peripheral tissue contributes as limiting factor. Furthermore, elastography determines the elastic properties of the liver but is also associated with high costs [43].

Additionally, there are other diagnostic markers detectable in the blood. One of the most common biomarkers is alanine aminotransferase (ALT) representing the functioning of the liver, however, it is a ubiquitous factor that reveals limited information due to its low specificity. In case of steatosis, ALT is insufficiently predictive as a diagnostic tool [43]. Although several biomarkers can be detected in the blood for diagnosis of steatosis or fibrosis, the accuracy is relatively low compared to the liver biopsy [43]. Over the last decades, non-invasive diagnostic tests have become available, but until now they cannot completely replace biopsies as the gold standard [89].

5.1. Lipidomic profiling as a diagnostic tool

Technically, the lipidome covers the entirety of lipids present in a defined system like cells, tissues, or organisms [90]. Accordingly, lipidomics represents a promising tool for the identification and quantification of lipids in such sample types (Fig. 3). However, lipid complexity and variety due to their physico-chemical properties and dynamic nature concerning metabolic events as well as physiological and pathological conditions, remain major issues in the field of lipidomics [90]. Despite recent technological advancements in the field of lipidomics/lipid analysis, particularly in mass spectrometry-related methods, full lipid mapping approaches are still lacking or are severely limited [90–93]. Potential lipid characterization methods include liquid chromatography-mass spectrometry (LC-MS), shotgun, and nuclear magnetic resonance (NMR) spectroscopy [11,105].

The initial step of a lipidomic procedure is the extraction of the lipids from the sample. Subsequently, the lipid sample is analyzed with one of the three lipidomic techniques. To begin with, LC-MS is a two-step procedure. First, the LC separates the lipids, which are then analyzed via MS. The use of LC makes it possible to study and quantify complex samples with high sensitivity. However, potential drawbacks include requirement of bigger sample size and time ineffectiveness. On the contrary, shotgun lipidomics analyze the lipid samples directly with the MS, therefore this approach is useful for smaller sample volumes and is time-effective. However, MS cannot distinguish isobaric lipids within the same class of lipids. Although NMR accurately analyzes lipids in human plasma, it possesses only a moderate sensitivity with limited resolving power. The obtained data, which is based on peak intensity or area, is used for lipid identification or quantification. Eventually, using these methods, the obtained outcomes can be assigned to biological context based on lipid pathways related to hepatic pathologies [11, 105]. Quehenberger and co-workers presented in-depth characterization of lipids in human plasma by the use of various mass spectrometric

methods for specific lipid classes [94]. In order to identify toxic lipids in a reproducible manner resembling the total lipidome, several factors including accuracy, precision, linear dynamic range, sensitivity, selectivity, and throughput play an important role and hence should be considered during analysis [90–93].

The application of lipidomics in hepatic diseases is investigated in a few studies, mostly by use of *in vivo* models. Moreover, most research has been conducted using non-ideal samples such as blood or whole liver extract. It is questionable, whether liver tissues in combination with blood are qualified as adequate samples as lipids retrieved from the blood do not totally reflect the hepatic lipidome, however, the majority of toxic lipids have been assessed and correlated in this manner [80]. In addition, liver tissue is composed of a heterogeneous cell population, so that liver extract can lack cell-specific information. Consequently, the isolation of specific cell types, respectively, involved in certain hepatic disorders could improve reproducibility [50,79]. It should be, however, taken into consideration that most of the mentioned lipid signatures are based on the studies with different experimental settings i.e. different lipidomic identification techniques (mainly based on mass spectrometry), sample type and preparation etc. In addition to analytical variation, lipidome profiles can be affected by several biological factors including age, ethnicity, gender, diet, gut microbiome, lifestyle, circadian rhythm etc. Besides biological factors that have been commonly considered while interpreting lipidomic profile, other factors such as environmental exposure to chemicals, pollutants, drugs etc. can also have a marked impact on the lipidome. To completely understand the impact of lipids in health and disease, and to make data more comparable across different studies and populations, it is essential to systematically assess the influence of different internal and external factors. Although deciphering the entire hepatic lipidome remains a challenge, surrogate indicators have been found to predict hepatology-related lipid fingerprints [80]. In the following sections, hepatic disease-related diagnosis is reviewed in more detail, especially in context to lipidomics.

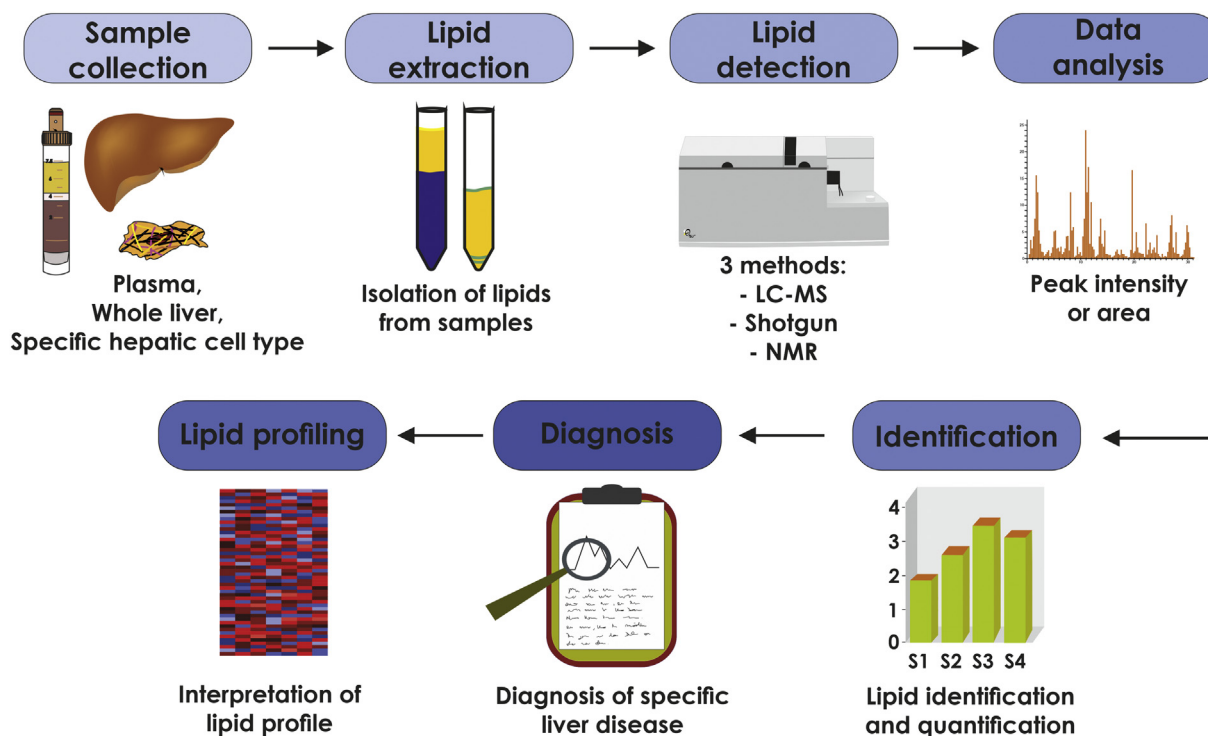


Fig. 3. Hepatic lipidomic analysis workflow. The general workflow of lipidomic analysis illustrated as a methodical sequence in which lipid profiles are identified and quantified for the diagnosis of liver diseases.

5.1.1. NAFLD/NASH

For the diagnosis of NAFLD, it is important to exclude other causes e.g. excessive alcohol consumption or medication. ALT is the most common biomarker to diagnose NAFLD, although it shows a low specificity, whereas cytokeratin-18 (CK18) is a biomarker used to diagnose NASH. Besides laboratory testing, different imaging modalities are used for NAFLD diagnosis including MRI, elastography, ultrasonography, and CT. However, liver biopsy remains the gold standard for a reliable diagnosis and classification of NAFLD and NASH [6,43]. Consequently, there is an urgent need for a non-invasive method to diagnose and distinguish NAFLD from other liver diseases. As a novel approach, lipidomics would contribute towards that goal by the identification of a specific signature based on elevated or declined hepatic concentrations of various lipids that are characteristic for steatotic versus non-steatotic livers (Table 1) [56]. Both, obese animal models and clinical trials with NAFLD and NASH patients revealed alterations in lipid-based biomarkers as detected by different mass spectrometric methods. While increased levels of several lipids i.e. LPLs, TGs, hepatic BMP, 5-,8-,11-hydroxy-eicosatetraenoic acids, 9-,13-hydroxyoctadecadienoic acids, short- and medium-chain triacylglycerols (TAGs), and Cers were evidenced, reduced levels of AA-containing intracellular PLs like phosphoinositols (PIs), phosphatidylethanolamines (PE) and PCs were found [47–51]. Loomba et al. examined lipid profiles to distinguish between NAFLD and NASH, and revealed NASH-related decrease of Cers and PLs, whereas significant elevations were observed in several fatty acids, CEs, diacylglycerols (DGs), TGs and PLs, as further confirmed by Suci and co-workers [52,56].

5.1.2. AFLD/ASH

The diagnosis of AFLD/ASH is based on the patient's history with excessive alcohol consumption combined with clinical, laboratory, and histological findings. A liver biopsy can diagnose ASH, however, there are no reliable, non-invasive biomarkers to diagnose and stage patients with AFLD/ASH [95]. Lipidomics is a relatively recent approach that identifies non-invasive biomarkers in which the increase or decrease of specific lipid molecules can be used as profile to distinguish subjects with AFLD from healthy subjects or subjects with other liver diseases. For instance, Carr et al. revealed the substantial elevation of several ceramide species in an ethanol-induced mouse model. Using a cohort of 20 human patients, it has been observed that alcoholic liver diseases are associated with significantly higher levels of specific Cers [55]. An isoform of LPC was identified as a predictor of liver-related decompensation and death in patients with severe alcoholic hepatitis [56]. Furthermore, different *in vivo* studies showed increase, for example, in PLs, LPLs, phosphatidylglycerol, cholesterol, 5-,8-,11-HETEs, 9-,13-HODEs, CEs and TGs, while levels of PCs, fatty acyl chains, free cholesterol, and PLs were reduced in some studies [47,56,61,62]. Besides, lipid profiles with specific concentration alterations are recognized as markers for AFLD/ASH progression e.g. NLG and DTEA revealed high sensitivities for disease identification or for the assessment of disease severity (Table 1) [47,56,61,62].

5.1.3. HCV

HCV can be diagnosed using serological assays detecting either components of the HCV virus such as HCV RNA or core antigen (direct) or antibodies against HCV components (indirect). Quantitative nucleic acid amplification tests (NAT), which detects the amount of HCV RNA in the serum is considered to be the golden standard, due to its high sensitivity (99%) and specificity (98–99%) [96]. The amount of HCV RNA in the serum has been also correlated with the degree of liver steatosis [56]. Steatotic lipid signatures therefore could support the disease pathology based on RNA levels in the serum. In HCV, current diagnosis techniques already exhibit high standard, therefore novel biomarkers are not necessarily needed. However, lipid profiles associated with more severe forms of hepatic disorders like cirrhosis could be helpful for predicting liver disease staging and progression.

5.1.4. DIS/DISH

The diagnosis of DIS and DISH is based on the history of the patient, especially the medication list and the information obtained from clinical, histological, and laboratory data [68]. The process is based on the following characteristics: (a) the biochemical and histologic data obtained from the liver; (b), the time between the intake of the suspected drug and the appearance of steatosis; and (c) the indication of improvement of the liver is taken into account after stopping the drug administration. However, the diagnosis remains difficult as in most cases due to the lack of specific biomarkers. Besides, macrovascular DIS is often mistaken for NAFLD which is more common [97]. In order to obtain an accurate diagnosis, NAFLD and/or AFLD have to be excluded [68,97]. Accordingly, lipidomic profiling could be useful by refining the exclusion criteria.

5.1.5. Cirrhosis

Liver fibrosis/cirrhosis can be diagnosed using FibroScan, a commonly used elastography-related technique that possesses some limitations. Blood or plasma-derived biomarkers are also used but are difficult to interpret, especially when patients have other clinically-related manifestations. Unfortunately, the only reliable way to diagnose cirrhosis relies on histological analyses of an invasive liver biopsy [98]. The diagnosis and risk of disease progression are based on the grade of inflammation and the stage of liver disease [76]. In addition to the identification of abnormal lipid content diagnosing cirrhosis, lipidomic analysis could be of great help to distinguish cirrhosis from HCC [99–101]. Accordingly, several clinical studies showed altered levels of the total lipid content in plasma such as downregulated SMs, PCs, LPCs, TGs and CEs as well as concentration variations in sterols, glycerophosphocholines (GPCs), glycerophosphates (GPs), fatty acids and conjugates, glycerophosphoserines (GPSs) and glycerophosphoinositols (GPIs), which could be considered as cirrhosis-related lipid signatures (Table 1) [99–101].

5.1.6. HCC

HCC is usually diagnosed using imaging techniques. However, imaging is not sensitive enough to distinguish between small cancerous lesions and cirrhotic tissue. Furthermore, alpha-fetoprotein (AFP) is used as a biomarker for HCC, however, the sensitivity is only 60%, serum AFP levels are not reliable when the tumor is smaller than 3 cm and elevated AFP levels can also be detected in patients with cirrhosis [82,102]. It is important to diagnose HCC at an early stage since there are treatment options available for early HCC such as surgery or ablative therapy. Unfortunately, in most cases, HCC is detected in an advanced stage that makes surgery impossible. So far, there is no effective and accurate biomarker for the detection of HCC in an early stage [82]. The field of lipidomics can help with the standardization of a lipid profile that could be characteristic for HCC, even in an initial state. Several toxic lipids, both circulating and intrahepatic, have been observed as potential biomarkers, positively or negatively correlated with HCC diagnosis with increased specificities and sensitivities, metastatic potential, and tumor burden (Table 1) [82,103–108]. Potential lipid signatures characteristic for HCC include, for instance, GPCs, GPSs, GPIs, fatty acids including vaccenic, oleic or palmitoleic acids, EPAs, DHAs, LAs, FFAs, PEs, PIs, SMs, TGs, Cs, PCs, DGs, galactosylceramides (GalCers) and lactosylceramides (LacCers), that allow identification of cancerous tissues compared to healthy tissues [82,103–108]. Recently, using an integrated multiple omics approach, Hall et al. detected upregulated levels of monounsaturated-PCs that correlated with hepatocyte proliferation and other HCC markers [85].

Taken together, lipidomic profiling represents a promising approach to identify lipids associated with certain disease profiles that enable us to distinguish closely related hepatic disorders with accurate detection in early or curable stages that paves the way for novel treatment strategies.

6. Therapeutics targeting the hepatic lipidome

Currently, there are no FDA-approved therapies available that target disrupted hepatic lipidome. Non-clinical treatment strategies for hepatic diseases include lifestyle modifications that seem to induce some beneficial effects at the early stage of the disease [43]. Patients suffering from NAFLD and NASH have shown beneficial effects with the change of diet and exercising, resulting in weight loss accompanied by improvement in liver histology and inflammation as well as the reduction of steatosis and fibrosis [43]. Treatments that are available mainly focus on the comorbidities that are often combined with steatosis, such as T2DM, cardiovascular diseases, or obesity [89,109]. Pioglitazone (PIO), belonging to the thiazolidinedione class, is usually used for the treatment of T2DM lowering blood glucose levels. Although this drug ameliorates steatosis and inflammation, it showed no impact on fibrosis and induces common adverse effects such as heart failure, bone fracture, edema, and weight gain. Alternatively, vitamin E, a fat-soluble antioxidant, can be administered to steatosis patients. This vitamin is associated with improvements in steatosis and inflammation, however, individual treatment outcomes are highly variable. Until now, the therapeutic performance of vitamin E is not completely verified inclusive potentially severe side effects owing to high doses [89,110]. Accordingly, target-oriented treatments against steatosis are desperately needed.

The most effective treatment against AFLD/ASH is to stop the intake of alcohol [54]. However, patients with poor liver prognosis due to excessive alcohol consumption should be treated with other therapies. For these patients there are two first-line therapeutics available: prednisolone and pentoxifylline. Unfortunately, many patients do not respond to these therapies implicating the need for alternative treatment options [95]. Until now, liver transplantation is the only option for severe AFLD/ASH [111].

As discussed before, HCV infections can be a reason for the development of steatosis. HCV is treated with interferon, peginterferon, ribavirin, and/or HCV direct-acting antiviral (DAA) therapy. Furthermore, HCV treatment guidelines are now available for the effective management of patients with acute and chronic HCV infections. Once the virus is eliminated, the hepatic steatosis seems to regress [64]. For the treatment of DIS and DISH, there exist no specific guidelines. However, it is crucial to identify and eliminate the drug that is responsible for the initiation of steatosis. Alternative therapy could be considered to replace harmful drugs. Principally, both DIS and DISH should improve after discontinued drug administration [68,97].

Liver fibrosis is a dynamic condition balanced by fibrogenic and fibrolytic processes. It is generally reversible with the treatment or removal of the underlying cause, or by reducing hepatocyte apoptosis, inflammation or proliferation and activation of HSCs or by increasing ECM-degrading enzymes for ECM breakdown or by promoting liver regeneration [74,75]. In contrast, cirrhosis inevitably implies irreversibility. However, the fate of this disease depends on the underlying cause and treatment options. If the cause of cirrhosis is established, further disease progression can be impaired. Nevertheless, the 5-year mortality of cirrhosis is 85% and the ultimate therapy, especially for advanced cirrhosis, is liver transplantation. Therefore, drug development is urgently needed in order to prevent disease progression [76].

When HCC is diagnosed at an early stage, surgical excision, ablative therapies, or liver transplantation are the suitable options [82]. However, there is a high recurrence of HCC after such treatments. The 5-year survival rate accounts for only 47–53% of the patient after surgery. Along with other hepatic disorders, the investigation of novel therapeutics is necessary in order to treat HCC [108]. In case of end-stage liver disease, patients suffer from severe, irreversible damage that cannot be regressed by lifestyle adjustments making them suitable for a liver transplant. Liver transplantations, however, limited due to lack of donor livers, are invasive, generally associated with high costs,

life-long immunosuppression, and might cause a graft-loss or posttransplant mortality [112].

6.1. Pro-resolving lipids

Pro-resolving lipids are considered as highly promising therapeutic candidates (Table 2). Lipids play a bilateral role in hepatic disorders with 'toxic' lipids resulting in liver damage, while 'healthy' lipids possess pro-resolving properties therefore involved in disease resolution. The most important class of pro-resolving lipids consists of the omega-3 polyunsaturated fatty acids (ω -3 PUFAs). Particularly EPA and DHA are important precursors of the specialized pro-resolving mediators (SPMs): resolvins, protectins, and maresins.

ω -3 PUFAs possess multiple properties such as PPAR- α upregulation that results in the activation of β -oxidation pathways and suppress SREBP-1c thereby inhibiting *de novo* lipogenesis. As a source of ω -3 PUFAs, fish oil treatment was given in ethanol-induced steatosis models that resulted in reductions in SREBP-1c activity and stearoyl-CoA desaturase 1 (SCD-1) expression while significant increase in fatty acid oxidation was observed [113,114]. Parenteral fish oil administration for the management of patients with parenteral nutrition-associated liver disease (PNALD) reversed preexisting PNALD, stabilized cirrhosis, and attenuated cholestasis with no need for liver transplantation [115]. In addition, ω -3 PUFAs reduced the secretion of VLDLs and TGs from hepatic cells and inactivated nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway, reducing intrahepatic inflammation and fibrosis in a carbon tetrachloride-induced cirrhosis model with ω -3 PUFAs daily treatment [116]. Moreover, ω -3 PUFAs induce macrophage phenotype polarization to a pro-resolution state resulting in phagocytosis of dead cells. Supplementation of pure EPA and DHA for a prolonged period has been demonstrated as an attractive therapy to prevent further hepatic complications. However, a clinical study with Opti-EPA (concentrated marine fish oil containing EPA and DHA) showed no beneficial effects in NASH patients with diabetes [117], probably due to the advanced stage of the disease. Clinical studies using FDA-approved Epanova (omega-3 carboxylic acids) showed liver fat reductions up to 15% as well as a decrease in TGs up to 26% in NAFLD and NASH patients. TG concentrations were also lowered by treatment of EPA-ethyl ester (EPA-E) [119].

Several pro-resolving lipids such as AA, EPA, DHA, alpha-linolenic acid (ALA), gamma-linolenic acid (GLA), phosphoglyceric acid (PGA), prostaglandin E1 (PGE1), prostaglandin J2 (PGJ2), and LA promote therapeutic effects in HCV-infected patients. ω -3 PUFA supplementation reduced insulin resistance in HCV patients. EPA treatment also prevented depression as a serious side effect of the concurrent IFN- α therapy in HCV-infected patients [118]. In summary, the intake of ω -3 PUFAs (and pro-resolving lipids) are effective therapeutics, safe and well-tolerated with very few or no side effects [120–123].

6.2. Small-molecule inhibitors

Fatty liver diseases, especially in advanced stages, are marked by various pathological phenomenon such as steatosis, inflammation, and fibrosis involving multiple cell types and mechanisms. It is therefore imperative that a drug has an ability to act on multi-faceted pathologies [80]. Toxic lipids, in particular excessive lipid accumulation, in hepatic tissue instigate the consequent processes like inflammation and fibrosis suggesting that the abnormal hepatic lipidome is a promising therapeutic target. However, other factors such as disease stage, hepatic inflammation, fibrosis and drug-induced hepatotoxicity, should be considered during drug designing [8,68,125].

Intriguingly, a study by Papazyan et al., demonstrated that lipogenesis can have opposing functions depending on the severity of hepatic steatosis. Authors observed an acute onset of death in knockout mouse model with impaired lipogenesis and fatty acid oxidation (FAO) suggesting the cytoprotective role of lipids. The results

Table 2

Overview of recent treatment strategies targeting hepatic diseases using pro-resolving lipids. Pro-resolving lipids listed include Epanova, Opti-EPA (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), EPA-ethyl ester (EPA-E), lipo-eicosapentaenoic acid (lipo-EPA) and lipo-docosahexaenoic acid (lipo-DHA), fish oil and omega-3 polyunsaturated fatty acids (ω -3 PUFA/fatty acids) in context to non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH), hepatitis C virus (HCV) and parental nutrition associated liver disease (PNALD). Dose and administration, subject and duration as well as main findings are represented, respectively.

	Drug/Formulation	Dose & administration	Subjects & duration of treatment in brackets	Main findings	Ref.
NAFLD/NASH	Epanova	4 g/day, oral administration	15 individuals (drugs), 20 individuals (placebo), patients with type 2 diabetes and NAFLD, (12 weeks)	Liver fat reduction: -15%	[139]
	Epanova	4 g/day, oral administration	23 individuals (drugs), 23 individuals (placebo), obese patients with NAFLD and hypertriglyceridemia, (15 weeks)	Liver fat reduction (-2%) and decrease serum triglycerides (-26%)	[156]
	Opti-EPA	360 mg EPA & 240 mg DHA per capsule, 3 capsules 2x per day, oral administration	18 individuals (drugs), 18 individuals (placebo), patients with NASH and T2DM (48 weeks)	Opti-EPA provided no benefit compared to placebo in NASH patients with diabetes	[117]
	EPA-E	1.8 g/day or 2.7 g/day	75 individuals (placebo), 82 individuals (low dose), 86 individuals (high dose), Adult patients with NASH, (365 days)	EPA-E showed no significant effect on the histological features of NASH, but reduced triglyceride levels compared to placebo without adverse effects.	[119]
	Liposomal EPA (Lipo-EPA)	10 g/kg, oral administration	Orotic acid-induced NAFLD rats, n=7, (10 days)	Improvement due to protection of hepatic function and the inhibition of lipid/ cholesterol synthesis	[124]
	Liposomal DHA (Lipo-DHA)	10 g/kg, oral administration	Orotic acid-induced NAFLD rats, n=7, (10 days)	Somewhat amelioration of NAFLD by enhancing lipolysis and cholesterol efflux	[124]
AFLD/ASH	Fish oil	25 % or 57 % substitution via diet	38-week-old male Wistar rats, (8 weeks)	Improvement in fatty acid oxidation by increased mRNA levels of downstream transcription factors that inhibited ethanol-induced hepatic steatosis in rats	[113]
	Fish oil	30 % of total energy	8-week-old male C57BL/6 mice fed with ethanol (3 g/kg body weight), (1h)	Prior ingestion prevented ethanol-induced fatty liver, reduced sterol regulatory element-binding protein-1c activity and stearyl-CoA desaturase-1 levels	[114]
HCV	EPA	3.5 g/day, 5x capsules per day	162 patients with HCV treated with interferon-alpha, (2 weeks)	EPA prevented depression in HCV patients who received interferon-alpha therapy	[118]
	ω -3 PUFA	6000 mg/day of fish oil, dietary supplementation	Patients with HCV, (12 weeks)	ω -3 PUFA supplementation reduced insulin resistance in genotype 1 HCV infected patients	[157]
Cirrhosis	Fish oil	1 g/kg, 1x per day	Children with cirrhosis due to PNALD, (8 years)	Cirrhosis from PNALD might be stable rather than progressive (once cholestasis resolves), no clinical evidence of liver disease progression	[115]
	ω -3 fatty acids	0.4 g/kg, sc. Injection 1x per day	Adult male Balb/c mice with carbon tetrachloride-induced cirrhosis, (2 weeks)	ω -3 fatty acids show an anti-inflammatory and anti-fibrotic effect	[116]

conclusively suggested that during an early course of hepatic steatosis, lipogenesis and FAO prevent lipotoxicity by sequestering toxic lipids, however at the late disease stages, storage of lipids become saturated leading to the accumulation of toxic lipids resulting in lipotoxicity [127]. Furthermore, during advanced stages of liver diseases, activated HSCs secrete excessive amount of ECM which leads to scar tissue formation, end-stage liver cirrhosis and cancer development [80]. This implies the limitation of treatment approaches that specifically (and only) focus on the hepatic lipidome at the advanced disease stages. Contrastingly, since hepatic steatosis, inflammation and fibrosis are interconnected pathways, inhibiting progressive steatosis (abnormal hepatic lipidome) may result in attenuation of disease progression.

Compared to monotherapy, combination therapy combining drugs with different modes-of-action i.e. a drug with metabolic/steatotic mechanism of action (targeting disrupted lipidome) with an anti-inflammatory or anti-fibrosis drug represents an attractive approach for the treatment of fatty liver diseases. Different strategies/drugs have been explored to reprogram disrupted lipid homeostasis through inhibition of *de novo* lipogenesis, increased β -oxidation, metabolic enzyme inhibition etc. Besides, several potential drugs targeting abnormal hepatic lipidome that are currently being examined in *in vitro* and preclinical studies, significant number of promising therapeutic candidates have been already evaluated in the clinical trials as summarized in Table 3 and schematically presented in Fig. 4 and reviewed here.

6.2.1. Farnesoid X receptor (FXR) agonists

FXR is a transcription factor regulated by bile acids, mediates bile acid signaling and regulates hepatic lipid metabolism. *In vitro* and *in vivo* studies using FXR agonists showed promising findings resulted in clinical trials evaluating FXR agonists alone or in combination. FXR agonists have been shown to antagonize steatosis via decreased hepatic

lipogenesis and increased fatty acid β -oxidation, NF- κ B-related inflammation, and fibrosis with the risk of adverse effects related to abnormal serum lipid profiles [128–131].

6.2.2. Glucagon-like peptide-1 (GLP-1) agonists

GLP-1 regulates glucose levels by stimulating insulin secretion and inhibiting glucagon release, and promotes weight loss and satiation in patients with T2DM. GLP-1 analogs exert multi-target effects during NAFLD and NASH. GLP-1 agonists stimulate anti-inflammatory macrophage polarization, suppress HSCs activation and hepatic fibrogenesis, and increase FAO as well as decrease hepatic *de novo* lipogenesis that further reduces lipid content, potentially leading to attenuations in ER and oxidative stress [132,133].

6.2.3. Mitogen-activated protein kinase kinase 4 (MKK4) inhibitors

MKK4 has shown to regulate hepatocyte regeneration in damaged liver [134]. MKK4 is also integrated in the apoptosis signal-regulating kinase 1 (ASK1) signaling cascade triggered by hepatic lipotoxicity, and therefore regulates inflammation, fibrosis, and cell death. However, further studies are warranted to understand the underlying molecular mechanism and its impact on the hepatic lipidome [135]. MKK4 inhibitors are currently in early clinical development and therefore limited published information is available for these inhibitors.

6.2.4. PPAR agonists

As described previously, PPARs (PPAR- α , PPAR- γ and PPAR- δ) are involved in fatty acid metabolism. PIO is a first-generation PPAR- γ agonist with promising therapeutic effects and recommended as a therapy for NASH patients. However, PIO possesses several deleterious adverse effects thereby restricting its therapeutic use. Elafibranor is a PPAR- α and PPAR- δ agonist and enhances fatty acid transport and oxidation,

Table 3

Overview of therapeutics targeting the hepatic lipidome. In this table, class of therapeutics, related drug names, clinical trials, and their effect on the hepatic lipidome are presented. Class of therapeutics comprises of farnesoid X receptor (FXR) agonists, glucagon-like peptide-1 (GLP-1) agonists, peroxisome proliferator-activated receptor (PPAR) agonists, ketohexokinase (KHK) inhibitors, sodium/glucose transport protein 2 (SGLT2) inhibitors, thyroid hormone receptor beta (THR β) agonists, sterol-CoA desaturase-1 (SCD-1) inhibitors, fibroblast growth factor 21 (FGF21) agonists, and acetyl-coenzyme A carboxylase (ACC) inhibitors.

Therapeutics	Drugs	Effect on hepatic lipidome	Phase	Clinical trials	Ref.
FXR agonists	Obeticholic acid	Decreased hepatic <i>de novo</i> lipogenesis and increased fatty acid β -oxidation	Phase 3	NCT02548351	[128–131,150,151]
	Tropifexor		Phase 2	NCT02855164	
	Cilofexor (mono/combined)		Phase 2	NCT03449446	
	EDP-305		Phase 2	NCT03421431	
	EYP001a		Phase 2	NCT03812029	
	Nidufexor (LMB763)		Phase 2	NCT02913105	
GLP-1 agonists	Liraglutide	Increased fatty acid β -oxidation and decreased hepatic <i>de novo</i> lipogenesis	Phase 4	NCT01237119	[152–154,128,132,133]
	Dulaglutide		Phase 4	NCT03648554	
	Semaglutide		Phase 2	NCT02970942	
	Tirzepatide		Phase 2	NCT04166773	
	Cotadutide		Phase 2	NCT04019561	
PPAR agonists	Pioglitazone (PPAR- γ)	Enhanced fatty acid transport and oxidation	Phase 3	NCT00063622	[128,136,155]
	Elafibraror (PPAR- δ)		Phase 2	NCT01694849	
	Saroglitazar (dual PPAR- α/γ)		Phase 2	NCT03061721	
	Lanifibranor (Pan-PPAR)		Phase 2	NCT03008070	
	Seladelpar (PPAR- δ)		Phase 2	NCT03551522	
KHK inhibitors	PF-06835919	Inhibition of fructose phosphorylation and <i>de novo</i> lipogenesis	Phase 2	NCT03969719	[128,137]
SGLT2 inhibitors	Dapagliflozin	Improved hepatic insulin sensitivity and increased uptake of non-esterified fatty acids (NEFAs)	Phase 2	NCT02279407	[128,138–140]
			Phase 3	NCT03723252	
THR β agonists	VK2809	Reduction of triglycerides and cholesterol levels	Phase 2	NCT02927184	[128,141]
	Resmetirom		Phase 2	NCT04173065	
			Phase 3	NCT03900429	
			Phase 3	NCT04197479	
SCD-1 inhibitors	Aramchol	Inhibited conversion of saturated fatty acids into mono-unsaturated fatty acids, reduced hepatic lipogenesis and enhanced lipid oxidation	Phase 3	NCT04104321	[128,142]
			Phase 2/3	NCT02279524	
			Phase 2	NCT01094158	
FGF21 agonists	Pegbelfermin (BMS-986036)	Stimulated mitochondrial β -oxidation	Phase 2	NCT02413372	[128,143]
ACC inhibitors	Firsocostat (mono/combined)	Inhibited conversion of acetyl-CoA into malonyl-CoA, <i>de novo</i> lipogenesis and increased transfer of fatty acids for mitochondrial β -oxidation	Phase 2	NCT03486912	[128,144]
	Firsocostat (mono)		Phase 2	NCT02781584	
	PF-05221304		Phase 2	NCT02856555	
			Phase 2	NCT03248882	

reducing steatosis. Ratziu et al. tested the safety and efficacy of elafibraror in a clinical trial with NASH patients (NCT01694849) and evidenced NASH resolution without fibrosis worsening [136].

6.2.5. Ketohexokinase (KHK) inhibitors

KHK or hepatic fructokinase inhibitors are another group of promising therapeutics targeting hepatic metabolism. Excessive intake of fructose leads to the activation of Carbohydrate Response Element Binding Protein (ChREBP), which is a lipogenic transcription factor, promoting steatosis. KHK is involved in the phosphorylation of fructose, and contributes to *de novo* lipogenesis, dyslipidemia, hepatic LD formation and steatosis. Inhibiting KHK leads to a decrease in *de novo* lipogenesis and steatosis. Calle et al. evaluated a KHK inhibitor, PF-06835919, in a clinical trial (NCT03256526) in NAFLD patients and showed reduction in steatosis [137].

6.2.6. Sodium/glucose transport protein 2 (SGLT2) inhibitors

SGLT2 inhibitors are glucose-lowering agents that are used to treat T2DM by inhibiting the reabsorption of glucose in the kidneys. SGLT2 inhibitors have the potential to reduce hepatic fat accumulation, by improving hepatic insulin sensitivity and increasing uptake of non-esterified fatty acids (NEFAs) [138]. Dapagliflozin and luseogliflozin are both SGLT2 inhibitors that are also used to treat

the hepatic lipidome. Eriksson et al. investigated the effects of dapagliflozin and omega-3 carboxylic acids in a clinical trial (NCT02279407) on patients with NAFLD and T2DM. The combination treatment led to significantly reduced liver fat content [139]. Sumida et al. evaluated the effects of luseogliflozin in T2DM patients with NAFLD in the LEAD trial, which also resulted in a significant decrease in hepatic fat content [140].

6.2.7. Thyroid hormone receptor beta (THR β) agonists

THR β agonists regulate hepatic lipid metabolism by reducing triglycerides and cholesterol levels. THR β pathway is frequently impaired in NAFLD and NASH due to diminished liver thyroid hormone levels or hepatic hypothyroidism. Harrison et al. assessed the effects of Resmetirom, a THR β agonist, in a clinical trial (NCT02912260) in patients with NASH and showed significant reduction in hepatic fat after treatment [141].

6.2.8. Steroyl-CoA desaturase-1 (SCD-1) inhibitors

SCD-1 converts saturated fatty acids into mono-unsaturated fatty acids. SCD-1 inhibition results in reduced lipogenesis and enhanced lipid oxidation. Ratziu et al. investigated the effects of Aramchol, a SCD-1 inhibitor, in a phase 2b clinical trial (NCT02279524) in patients

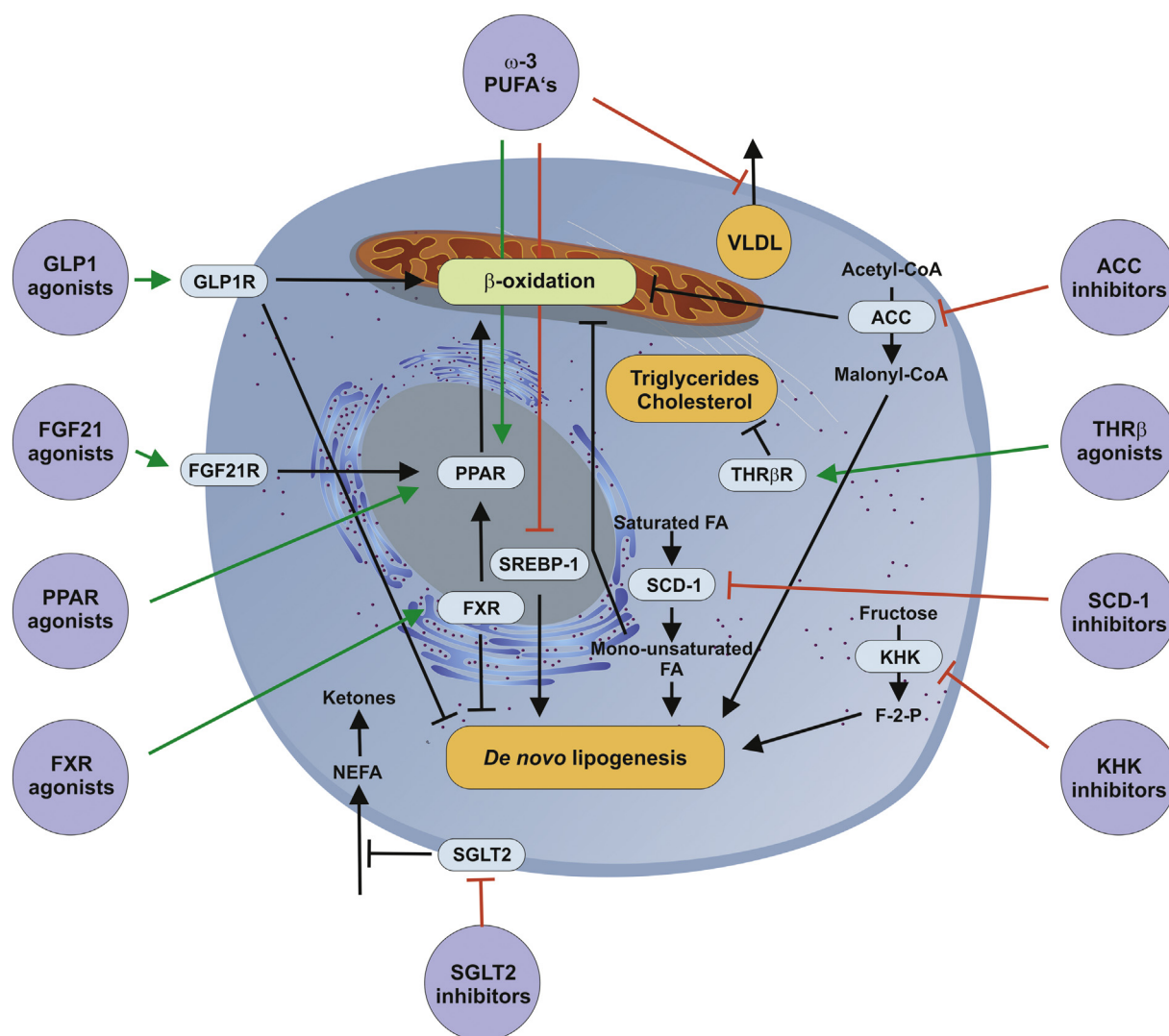


Fig. 4. Therapeutics targeting the hepatic lipidome. Different therapies have varying effects targeting the hepatic lipidome to reduce steatosis. Therapies include omega-3 polyunsaturated fatty acids (ω -3 PUFA's), glucagon-like peptide-1 (GLP-1) agonists, fibroblast growth factor 21 (FGF21) agonists, peroxisome proliferator-activated receptor (PPAR) agonists, farnesoid X receptor (FXR) agonists, sodium/glucose transport protein 2 (SGLT2) inhibitors, acetyl coenzyme A carboxylase (ACC) inhibitors, thyroid hormone receptor beta (TR β) agonists, sterol-CoA desaturase-1 (SCD-1) inhibitors, and ketohexokinase (KHK) inhibitors. Therapies lead to increased mitochondrial β -oxidation and/or decreased de novo lipogenesis.

with NASH, and observed NASH resolution and reduction in liver fibrosis [142].

6.2.9. Fibroblast growth factor 21 (FGF21) agonists

FGF21 is mainly produced by liver, adipose tissue and pancreas, and regulates metabolism. In the liver, FGF21 receptors are expressed by hepatocytes, where FGF21 stimulates mitochondrial β -oxidation. Sanyal et al. examined the therapeutic efficacy of Pegbelfermin, an FGF21 agonist, in a clinical trial (NCT02413372) in patients with NASH and found significant reduction in hepatic fat [128,143].

6.2.10. Acetyl-coenzyme A carboxylase (ACC) inhibitors

ACC converts acetyl-CoA to malonyl-CoA, which is the main substrate for fatty acid biosynthesis, and inhibits carnitine palmitoyl-transferase 1 (CPT1), a carrier protein of fatty acids for mitochondrial β -oxidation. ACC inhibition reduces malonyl-CoA thereby decreases fatty acid biosynthesis and increases mitochondrial β -oxidation. Loomba et al. evaluated GS-0976, an ACC inhibitor, in a clinical trial (NCT02856555) in patients with NAFLD and showed attenuation of hepatic steatosis [144].

6.3. Liposomes

The delivery of therapeutics using nanocarriers/nanoparticles is a promising strategy to improve several possible limitations such as rapid drug metabolism, poor drug solubility, limited drug stability, and undesired side effects [145]. Liposomes or lipid-based nanoparticles have been extensively explored as carriers for delivering drugs. Liposomes provide tremendous opportunities including site-specific drug delivery via passive or active drug targeting, delivery of hydrophobic and hydrophilic drug molecules, very low or no toxicity, and prolonged half-life and a controlled release of the drug [146,147]. Although the liver serves as central metabolic organ for most substances entering the body, liposomal formulations are helpful by maximizing the drug concentration in the target organ such as a diseased liver and enhancing stability and pharmacokinetics of drugs. Moreover, liposomes are the only combinatorial drug delivery platform that have been used in clinical trials to deliver multiple drugs, which will be highly useful for fatty liver disorders with multiple pathologies [146,147].

Pro-resolving lipids are fat-soluble and therefore need to be emulsified to be dispersible in water. This emulsion might be difficult to be absorbed by the intestine due to its large size. Liposomes or

lipid-based drug delivery nanocarriers can be a promising strategy to deliver pro-resolving lipids without using emulsifiers, resulting in their enhanced uptake, protection against degradation, and prolonged functioning. Chang et al. developed liposomal EPA and DHA, which resulted in alleviation of abnormal hepatic lipid accumulation and amelioration of NAFLD in rats. Liposomal EPA protected the hepatic function and inhibited lipid biogenesis while liposomal DHA stimulated lipolysis [124]. Although lifestyle modifications might be useful for NAFLD patients, the use of (liposomal) pro-resolving lipids seems to be an attractive approach to combat hepatic disorders in a target-oriented and safe manner.

Besides Lipo-EPA and Lipo-DHA, Fenofibrate (FNB) containing nanoliposomes (FNB-Nanolipo) were developed to deliver FNB, a potential PPAR α agonist with poor solubility and oral absorption. Coa et al. demonstrated that FNB liposomes enhanced oral absorption and evidenced improved inhibitory effects, as compared to free FNB, in methionine and choline deficient (MCD) diet-induced NAFLD in mice [148].

In another study, Pollock et al. synthesized polyunsaturated ER-targeting liposomes (PERLs), without encapsulation of drugs, which led to a down-regulation of cellular cholesterol levels, including HCV-associated cholesterol. Reduced cholesterol levels led to the inhibited secretion and infectivity of HCV. PERL treatment showed improved therapeutic efficacy than lovastatin, a cholesterol-lowering statin, therefore suggested as a potential antiviral therapy [149].

Taken together, therapeutics remodeling disrupted hepatic lipidome demonstrate a promising approach, and therefore should be explored further as monotherapy or combination therapy for the treatment of chronic (fatty) liver diseases [128].

7. Conclusions and future perspectives

Liver diseases represent a major health-related burden affecting millions of people worldwide. Among others, alcoholic and non-alcoholic liver diseases are emerging liver diseases with high morbidity and mortality, requiring liver transplantation. Recent studies have highlighted that abnormalities in the hepatic lipid metabolism are involved in the initiation and progression of hepatic steatosis during alcoholic and non-alcoholic liver diseases. Increasing efforts have been made in unraveling the underlying mechanisms associating hepatic lipidome with disease pathogenesis. Lipidomic analyses with unique disease-related lipid signatures provide unique opportunities for the diagnosis of liver diseases. The new insights will aid in discovering the novel therapeutic targets for designing new interventions. Furthermore, in recent years, drug targeting (nano)technologies have demonstrated great potential with enhanced therapeutic drug efficacy due to tissue-/site-/cell-/organelle-specific targeting and reduced off-target adverse effects. Although highly promising, lipidomic profiling and targeting lipidomics possess several challenges that are currently been tackled by the coherent efforts from biologists, and pharmaceutical as well as drug targeting scientists. Such challenges include lack of a fully-deciphered hepatic lipidome due to current technical limitations, and diverse disease mechanisms affecting fatty liver diseases that necessitate multimodal treatment strategies or combinatorial approaches affecting hallmarks of the disease i.e. steatosis, inflammation and fibrosis. With the advancement of technologies, the hepatic lipidome fingerprint will be soon revealed, which will provide insights into the liver (patho)physiology and tremendous opportunity for the drug development.

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