HHS Public Access

Author manuscript

*Semin Liver Dis*. Author manuscript; available in PMC 2022 November 14.

Published in final edited form as:

*Semin Liver Dis*. 2022 August ; 42(3): 233–249. doi:10.1055/s-0042-1755316.

# Metabolic injury of hepatocytes promotes progression of NAFLD and AALD

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## Abstract

Nonalcoholic liver disease is a component of metabolic syndrome associated with obesity, insulin resistance, and hyperlipidemia. Excessive alcohol consumption may accelerate the progression of steatosis, steatohepatitis, and fibrosis. While simple steatosis is considered a benign condition, nonalcoholic steatohepatitis with inflammation and fibrosis may progress to cirrhosis, liver failure, and hepatocellular cancer. Studies in rodent experimental models and primary cell cultures have demonstrated several common cellular and molecular mechanisms in the pathogenesis and regression of liver fibrosis. Chronic injury and death of hepatocytes cause the recruitment of myeloid cells, secretion of inflammatory and fibrogenic cytokines, and activation of myofibroblasts, resulting in liver fibrosis. In this review, we discuss the role of metabolically-injured hepatocytes in the pathogenesis of nonalcoholic steatohepatitis and alcohol-associated liver disease. Specifically, the role of chemokine production and *de novo* lipogenesis in the development of steatotic hepatocytes and the pathways of steatosis regulation will be discussed.

### Keywords

Liver fibrosis; steatotic hepatocytes; de novo lipogenesis; ER stress; UPR

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**Conflict of interests**: RL serves as a consultant for Aardvark Therapeutics, Altimmune, Anylam/Regeneron, Amgen, Arrowhead Pharmaceuticals, AstraZeneca, Bristol-Myer Squibb, CohBar, Eli Lilly, Galmed, Gilead, Glympse bio, Hightide, Inipharm, Intercept, Inventiva, Ionis, Janssen Inc., Madrigal, Metacrine, Inc., NGM Biopharmaceuticals, Novartis, Novo Nordisk, Merck, Pfizer, Sagimet, Theratechnologies, 89 bio, and Viking Therapeutics. In addition, his institution has received grant support from Allergan, Astrazeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Eli Lilly, Galectin Therapeutics, Galmed Pharmaceuticals, Genfit, Gilead, Intercept, Inventiva, Janssen, Madrigal Pharmaceuticals, Merck, NGM Biopharmaceuticals, Pfizer, and Sonic Incytes. He is also co-founder of Liponexus, Inc. BS has been consulting for Ferring Research Institute, Gelesis, HOST Therabiomics, Intercept Pharmaceuticals, Mabwell Therapeutics, Patara Pharmaceuticals and Takeda. BS is founder of Nterica Bio. UC San Diego has filed several patents with BS as inventor related to this work. BS's institution UC San Diego has received research support from Axial Biotherapeutics, BiomX, CymaBay Therapeutics, NGM Biopharmaceuticals, Prodigy Biotech and Synlogic Operating Company.

## INTRODUCTION

Liver fibrosis results from the excessive deposition of extracellular matrix proteins that form a fibrous scar in response to chronic liver injury.[1] Toxic liver fibrosis is caused by hepatitis B (HBV) or C (HCV) infection, alcohol-associated liver disease (AALD), and nonalcoholic steatohepatitis (NASH).[2] Inflammation plays a key role in the pathogenesis of liver fibrosis.[3] Myeloid cells are the main source of fibrogenic cytokines, including the critical activator of hepatic myofibroblasts TGFβ1, which are not present in normal liver.[3] Hepatic stellate cells (HSCs) are the major source of collagen type I–producing hepatic myofibroblasts in response to toxic liver injury.[4,5]

Until recently, HBV and HCV were the most common causes of liver fibrosis and cirrhosis. With the development of vaccines and highly effective antiviral treatments, the incidence of HBV- and HCV-related liver diseases has declined, while NASH-associated fibrosis and HCC are increasing.[6,7] AALD does not develop in thin or cachectic individuals, occurring most often in obese patients.[2] Increased alcohol intake in patients with high body mass index (BMI> 27) leads to more severe liver disease. Histopathologically, both NASH and AALD can be distinguished from nonalcoholic fatty liver (NAFL) by the development in the latter of steatohepatitis, centrilobular ballooning degeneration of hepatocytes and Mallory–Denk hyaline inclusions,[8,9] neutrophilic infiltration, inflammation, and activation of hepatic myofibroblasts.[10] NASH is driven by ER stress and the associated activation of inflammatory responses that further exacerbate metabolic injury and activate fibroproliferative responses in the liver.[11]

NAFL is characterized by hepatic steatosis and is reversible;[2] however, approximately 20%–24% of NAFL patients develop NASH. Whether steatosis is a benign or pre-condition that makes obese individuals more susceptible to metabolic syndrome, insulin resistance, and inflammation remains controversial.[3] This review summarizes the molecular mechanisms underlying the development of hepatic steatosis and the role of *de novo* lipogenesis in the pathogenesis of NASH- and AALD-induced liver injury.[2]

## 1. The development of NASH and AALD liver fibrosis

### 1.1 NASH-induced metabolic liver injury

The pathogenesis of NASH is often explained by a “two hit” theory: obesity and insulin resistance results in metabolic injury to hepatocytes, activation of *de novo* lipogenesis, lipid accumulation, and lipotoxicity that further exacerbate hepatocyte damage.[2] Adipose tissue contributes to insulin resistance by secreting adipokines and cytokines (e.g., leptin and adiponectin).[12] Endoplasmic reticulum (ER) stress constitutes a potential “second hit” that causes the secretion of inflammatory and fibrogenic cytokines and chemokines (e.g., IL-6, TNFα, IL-1β, TGFβ1).[3] ER stress is associated with changes in the gut microbiota (prevalence of Firmicutes over Bacteroidetes[13]), increased gut permeability, the release of bacterial products such as LPS into the circulation, activation of Toll-like-receptor (TLR)–dependent signaling pathways (specifically TLR4), and the recruitment and activation of inflammatory cells and myofibroblasts in the injured liver.[14]

### 1.2 AALD-associated liver injury

As with NAFLD, alcohol-induced steatosis can progress to alcohol-induced steatohepatitis (ASH) and AALD.[3] AALD results from a chronic imbalance in hepatocyte metabolism due to direct injury by alcohol and alcohol-derived metabolites. Hepatocyte injury occurs via release of acetaldehyde, a toxic ethanol metabolite produced by hepatocytes, or upregulation of cytochrome P450 2E1, a critical enzyme involved in alcohol metabolism.[15,16] Toxic alcohol metabolites, changes in the gut microbiota composition,[17,18] increased intestinal permeability, and the leak of bacterial products into circulation result in inflammation and fibrogenesis.[14]

Despite the etiological differences between NASH and AALD-induced liver injury,[3,14] the mechanisms underlying the pathogenesis of metabolic liver injury are similar, especially at the onset of metabolic injury.[2] The pathogenetic mechanisms in common between these conditions are discussed.

## 2. Pathogenesis of liver fibrosis in NASH and AALD

### 2.1 Inflammation drives NASH and AALD progression

Both NAFL and alcohol-associated fatty liver are considered to be benign and reversible conditions.[3] Fatty liver is characterized by the accumulation of fat droplets (mainly triglycerides and phospholipids) in hepatocytes, and this process is regulated at the level of *de novo* lipid synthesis, lipid secretion (VLDL), and inhibition of β-oxidation.[19,20]

Chronic injury to hepatocytes and hepatocyte apoptosis induce ER stress, reactive oxygen species (ROS) production, and mitochondrial dysfunction, causing the activation of inflammatory responses, including the secretion of the key cytokines/chemokines by myeloid cells.[15,16] Neutrophils are first responders that enter the liver to phagocytose and clear apoptotic cells and cell debris and further facilitate recruitment and activation of other myeloid cells into the damaged liver.[2] Although the specific roles of liver resident Kupffer cells versus bone-marrow–derived macrophages are uncertain, both populations are believed to contribute to liver inflammation; the secretion of IL-6, TNFα, IL-1β, and TGFβ1; and the activation of inflammatory responses that lead to liver fibrosis.[2] ER stress caused by misfolded proteins induces the activation of fatty acid and cholesterol synthesis in metabolically-injured hepatocytes. IL-6 signaling induces inflammatory responses in hepatocytes, including the secretion of IL-6, CXCL1, and CCL2. IL-6, TNFα, and TGFβ1 drive HSC activation into collagen type I–producing myofibroblasts. In addition to neutrophils and macrophages, T and B lymphocytes recruited to the damaged liver mediate the adaptive immune response and contribute to metabolic liver damage, inflammation, and the formation of fibrous scar tissue by activated myofibroblasts (Figure 1).

### 2.2 Contribution of T and B cells to NASH and AALD progression

Macrophage-derived TGFβ1 and IL-6 are critical regulators of naive T-cell differentiation into T helper 17 ($T_H17$) cells, while IL-23 regulates Th17 expansion and proliferation.[21–23] Mouse Th17 cells also produce anti-inflammatory IL-22. In contrast to IL-17, IL-22 acts as a survival factor for hepatocytes,[24] suggesting that the activation of specific T-cell subsets

might reduce liver injury by releasing the hepatoprotective IL-22.[25] In addition, IL-22 can signal through the IL-22 or IL-10 receptors on HSCs to induce their senescence.[26] Overexpression of IL-22 in mice is reported to increase HSC senescence and attenuate liver fibrosis.[26]

Alterations in the intestinal microbiota composition strongly affect the production of IL-17,[27] suggesting a correlation between dysbiosis, the immune response, and liver fibrosis.[14] IL-17A facilitates the activation of myeloid cells and directly activates HSC conversion into fibrogenic myofibroblasts in experimental models of liver fibrosis.[2] IL-17A increases *de novo* lipogenesis and TNFα -TNFRI signaling in metabolically-injured hepatocytes. Unlike IL-17A-secreting T helper 17 (Th17) $CD4^+$ T cells, which exhibit a fibrogenic effect, CD8+ T cells mediate hepatoprotective effects. In support of this observation, ablation of $CD8^+$ T cells in mice was found to exacerbate NASH-induced liver fibrosis, whereas genetic or pharmacological suppression of $IgA^+$ cells attenuated NASH-induced liver fibrosis, perhaps through upregulation of IFN-producing T cells.[28]

### 2.3 Do metabolically-injured hepatocytes contribute to inflammation?

Hepatocytes constitute 60% of the total liver cells and mediate the detoxifying, metabolic, and secretory functions of the liver. Chronic liver injury causes ER stress in damaged hepatocytes, the release of ROS, and hepatocyte apoptosis.[2] Apoptotic hepatocytes release damage-associated molecular patterns (DAMP), TGFβ1, and exosomes containing biologically active factors (such as chemokines/receptors, metabolites, proteases) that can rapidly deliver “stress signals” into the intracellular compartment to mediate intercellular communications.[29–35] Metabolically-injured hepatocytes serve as a source of chemokines, including CXCL1, CCL2, CCL5, TGFβ1/3, IL-6, and TNFα.[2] Although their contribution to inflammation is less than that of inflammatory/myeloid cells, hepatocytes can secrete chemokines and growth factors locally (into the space of Disse) that regulate crosstalk between HSCs and hepatic myeloid cells (liver resident Kupffer cells and bone-marrow–derived inflammatory cells) and endothelial cells. Furthermore, damaged hepatocytes release DAMPs and extracellular vesicles to communicate between hepatocytes and neighboring cells, thereby, promoting liver fibrosis via the activation of HSCs and Kupffer cells.[36] Extracellular vesicles, including microvesicles and exosomes or exosome-like vesicles, transport large quantities of bioactive molecules that are released into the microenvironment and circulation. Hepatocyte-derived extracellular vesicle miRNA (miR-128–3P) contributes to HSC activation and liver fibrosis through downregulation of PPARγ.[37–40]

Damaged hepatocytes are a major source of systemic angiotensinogen, the precursor of angiotensin (Ang) II,[41] which facilitates inflammatory responses in the damaged liver and potentiates TGFβ signaling and fibrosis. AngII drives the release of the cytokines TGF-β, IL-1β, and MCP1 by inflammatory cells and induces the contraction and proliferation of HSCs.[42,43] The release of chemokines (such as MCP-1, MIP-1a, MIP-1b) by steatotic hepatocytes facilitates the recruitment of bone-marrow–derived inflammatory cells into the injured liver.[2] Recent studies have shown cross talk between steatotic hepatocytes and the activation of fibrogenic HSCs/myofibroblasts. Two molecules that are elevated in metabolically-injured hepatocytes are cholesterol, for which the mechanistic link

to NASH remains incompletely understood, and TAZ, a transcriptional regulator that promotes NASH fibrosis.[44] Under physiological conditions, internalization of plasma membrane cholesterol activates soluble adenylyl cyclase (ADCY10), triggering calcium-RhoA–mediated proteasome-mediated TAZ degradation.[44] In response to chronic metabolic injury, elevated hepatocyte cholesterol upregulates TAZ and promotes fibrotic NASH. Increased levels of hepatocyte-derived TAZ result in increased TAZ-TEAD–dependent hepatocyte Indian hedgehog transcription and secretion, leading to the transcription of NASH-specific genes that encode proteins responsible for HSC activation, liver fibrosis, and inflammation.[44,45] TAZ silencing can suppress liver fibrosis and partially reverse NASH.[46]

## 3. The role of *de novo* lipogenesis in hepatocytes in the pathogenesis of NASH- and AALD-associated liver fibrosis

### 3.1 The mechanism underlying hepatic steatosis development in metabolically-injured liver

Many patients with obesity and insulin resistance develop hepatic steatosis. Lipid droplets of steatotic hepatocytes consist mainly of triglycerides and cholesterol.[47,48] Hepatic triglycerides and cholesterol are derived from serum non-esterified fatty acids stored in adipose tissue (59%), *de novo* lipogenesis (26%),[49] and the diet (15%).[50] *De novo* lipid biosynthesis occurs when excessive carbohydrates are consumed or when circulating insulin levels are high[51] (Figure 2). Carbohydrates undergo glycolysis to generate acetyl-CoA molecules which serve as a substrate to fuel fatty acid and cholesterol synthesis.[52] Under fasting conditions, wherein insulin levels are low and glucagon levels are high, metabolic processes are shifted to fatty acid oxidation or lipolysis that allows fatty acid/cholesterol mobilization from adipose tissues into circulation, followed by uptake by the liver.[47] The degree of hepatic steatosis fluctuates in both lean and healthy obese individuals depending on the circadian rhythm, diet and food composition, age, pattern of alcohol consumption (binge drinking vs social drinking), and use of specific medications.[53] Until recently, chronic steatosis (NAFL) was considered to be a benign, reversible condition, and the progression of steatohepatitis to NASH was thought to be driven by inflammatory responses.[54] The critical role of lipotoxicity in the pathogenesis of NASH has been recognized recently. Here we summarize evidence implicating *de novo* lipogenesis in the development of metabolic injury and NASH (Figure 2).

### 3.2 Progression of NAFL to NASH

Approximately 20% of patients with NAFL progress to NASH.[55] To distinguish NAFL from NASH, a scoring system was developed and published in Hepatology, 2005.[56] Now in use worldwide, this score includes the degree of steatosis, chronic steatohepatitis, inflammation, and fibrosis. The grading of biopsies ≥5 was found to correlate with a diagnosis of NASH (see Table 1). Biopsies/tissues with scores <3 are diagnosed as “not NASH.” A brief summary of the criteria used to diagnose NAFL versus NASH in the human liver is shown in Table 1.

### 3.3 Contribution of *de novo* lipogenesis to the progression of NAFL to NASH and AALD

Obesity and insulin resistance lead to the development of metabolic syndrome. Excessive hepatic fatty acid synthesis, inhibition of hepatic lipid β-oxidation, and accumulation of lipid droplets (mainly triglycerides and phospholipids) in hepatocytes results. One key metabolic process implicated in triggering NASH progression is *de novo* lipogenesis of cholesterol and fatty acids.[51,55] The rate of *de novo* lipogenesis in NASH patients is elevated 3-fold over that of NAFL patients,[51,57] underscoring the importance of *de novo* lipogenesis in the metabolically-injured liver.[11,48] The rapid synthesis and excessive accumulation of fatty acids and cholesterol has a lipotoxic effect on hepatocytes.[58,59] Most lipid synthesis takes place in the ER. *De novo* lipogenesis is critical for triggering the ER stress responses under physiological conditions and in response to chronic liver injury.[55]

### 3.4 Lipogenesis is mediated by sterol regulatory element-binding proteins.

*De novo* lipogenesis is regulated by sterol regulatory element-binding proteins (SREBP) 1 and 2,[60] transcription factors that control production of the key enzymes that regulate fatty acid and cholesterol synthesis, respectively. Two conditions that mediate the transcription of SREBP1/2-dependent lipogenic genes have been identified: the energy-depleted state and energy-abundant state. In the energy-depleted state, the regulation of SREBP1/2 function is attributed to activation of an autonomous feedback system that senses the lack of sterol products in the microenvironment. In the “energy-abundant state,” the transcriptional activity of SREBP1/2 is driven by protein accumulation and protein misfolding in the ER, leading to the development of endoplasmic reticulum (ER) stress and subsequent induction of the adaptive unfolded protein response (UPR) system.[61] The UPR is activated to restore homeostasis. If the UPR system fails to repair the underlying problem, prolonged UPR activity increases the transcriptional capability of SREBP1/2, increasing *de novo* lipogenesis, thereby driving steatosis, inflammation, and fibrosis (Figure 2).[62]

## 4 The pathways of SREBP1/2 activation in hepatocytes

### 4.1 De novo lipogenesis in metabolically-injured hepatocytes

The progression of steatosis to steatohepatitis is associated with the activation of inflammatory responses and ROS production.[2] The ER is the major site of lipid synthesis in hepatocytes. Cholesterol synthesis and uptake pathways are regulated through transcriptional regulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme for cholesterol biosynthesis, via LDLR. Promoters of these genes contain the (5′-ATCACCCCAC-3′) sterol regulatory element (SRE).[63] SRE sequences are recognized by the ER membrane-localized transcription factors SRE binding protein (SREBP)1 and 2. SREBP1 plays a critical role in triglyceride synthesis via transcriptional regulation of fatty acid synthase, stearoyl-CoA desaturase, and ATP citrate lyase, while SREBP2 is mainly responsible for mediating cholesterol metabolism by regulating genes such as HMG-CoA reductase and low-density lipoprotein receptor.[64] SREBP2 mediates sterol regulation in all tissues.[64] Two isoforms, SREBP1a and SREBP1b, are arise from transcription of the *SREBF1* gene from different promoters. SREBP1c is expressed in most tissues and regulates homeostasis of fatty acids and triglycerides in lipogenic organs such as the liver.[60] Compared to SREBP1a, SREBP1c lacks 24 amino acid residues in the N-

terminal CREB1-binding transactivation domain and exhibits low transcriptional activity.[65] SREBP1a is highly expressed only in specific tissues and cells[66] and stimulates expression of lipogenic and cholesterogenic genes needed to construct membrane lipids in growing cells (Figure 2).[65,67]

### 4.2 Regulation of SREBP1/2-dependent transcription of lipogenic genes

SREBPs are produced as inactive ER membrane-bound proteins that require post-translational modifications to function as transcription factors that translocate to the nucleus and initiate lipogenic gene transcription. Synthesized as intrinsic ER membrane proteins, SREBP1/2 are transported from the ER to the Golgi for proteolytic cleavage and processing.[68] Sterol levels regulate SREBP1/2 activity by controlling SREBP1/2 transport from the ER to the Golgi, where they undergo proteolytic cleavage before translocating to the nucleus. Several independent mechanisms release SREBP1/2 from the ER. The INSIG:SCAP-S1P/S2P pathway is preferentially activated during the energy-depleted state (activated when low levels of sterols and other lipid levels are detected by specific sensor proteins in the ER), while caspase 2-S1P/S2P are primarily induced during the energy-abundant state (associated with ER stress and insulin resistance activated by TNF signaling),[60] thereby regulating fatty acid and cholesterol synthesis (Figure 4).[60]

### 4.3 INSIG:SCAP-mediated regulation of SREBP1/2

Inactive ER-anchored SREBP1/2 proteins remain in the ER and are processed by Golgi enzymes by binding to the INSIG (precursor bound by insulin-induced gene 1): SCAP (SREBP cleavage-activating protein) complex.[69,70] The specific mechanism that controls release of SREBP1/2 from the INSIG:SCAP is complex and is regulated by sensor proteins responding to the changing levels of insulin, oxysterols, unsaturated FA, and food intake composition.[71–74] SCAP is an ER-sterol–sensing protein that binds to SREBP1 and SREBP2 via a WD40 repeat domain and chaperones both proteins from the ER to the Golgi.[68] Under sterol-rich conditions, SREBPs are held in the ER through their interaction with SCAP, an anchoring molecule, and INSIG, an ER transmembrane protein. Specifically, when cholesterol in ER membranes exceeds a threshold, the sterol binds to SCAP, triggering several conformational changes[75] that prevent the SCAP-SREBP complex from leaving the ER. INSIGs bind SCAP, thereby preventing SCAP-SREBP movement from the ER.[76]

When sterols are depleted, INSIG1 dissociates from SCAP, thereby allowing SCAP to move to the Golgi. Activation of the ER membrane-bound INSIG:SCAP chaperone drives SREBP maturation and activity. The SCAP protein forms a homotetramer with its membrane region to form a stable complex with SREBP1 or SREBP2 through its C-terminal cytoplasmic domain.[77] The SREBP-SCAP complex is released from INSIG upon depletion of sterol in the environment. SCAP assists in the transport of SREBP in coat protein II (COPII ) vesicles from the ER to the Golgi.[68]

Translocation of the SCAP-SREBP complex from the ER to the Golgi leads to sequential proteolytic cleavage of SREBPs by active forms of site-1 membrane-bound serine proteases S1P and S2P.[64] The first cleavage occurs within the 50-amino acid luminal loop, separating the SREBP into two halves. The NH2-terminal half remains attached to the membrane by

its single transmembrane helix. The second cleavage occurs within this helix, releasing the bHLH-Zip domain so that it can enter the nucleus. These sequential proteolytic cleavages activate S1P and S2P.[78,79] S1P cleaves the ER luminal loop of SREBPs only in cholesterol-depleted cells, and site-1 cleavage requires previous cleavage at site-2.[68] The N-terminal region of SREBPs then is cleaved off by S2P. A model has been proposed in which cleavage by S1P allows the first transmembrane segment to unwind, thereby pushing the S2P cleavage site to the membrane surface, where it becomes accessible.[80] Following cleavage, SREBP1/2 $NH_2$-terminal fragments are released from the Golgi, dimerize with importin β via the SREBP helix–loop–helix leucine zipper domain,[81,82] and translocate to the nucleus, where they initiate target gene transcription.[83] SREBPs are responsible for the transcription of more than 30 genes needed for the uptake and synthesis of cholesterol, fatty acids, triglycerides, and phospholipids. A cell-penetrating nuclear transport modifier cSN50.1 interacts with importin β and reduces nuclear translocation of SREBP1/2 induced by lipid depletion in cells.[84] The nuclear concentration of SREBP1c is regulated by circulating insulin via the PI3K–AKT–mTOR– SREBP pathway. Mammalian target of rapamycin complex 1 (mTORC1) regulates SREBP by controlling the nuclear entry of lipin 1, a phosphatidic acid phosphatase. Dephosphorylated, nuclear, catalytically active lipin 1 promotes nuclear remodeling and mediates the effects of mTORC1 on SREBP target genes, SREBP promoter activity, and nuclear SREBP protein abundance. Specifically, the lack of mTORC1-mediated lipin 1 phosphorylation promotes nuclear entry of lipin 1 and promotes the downregulation of nuclear SREBP protein. Whether lipin 1 can directly interact with SERBPs remains unknown.[85]

Nuclear SREBPs are rapidly degraded by the ubiquitin and proteasome pathways.[86] Proteasome degradation of SCAP precedes SREBP degradation. Increased SCAP degradation is linked to downregulation of its chaperon, heat shock protein 90 (Hsp90),[87] which stabilizes SCAP in the ER and Golgi. After dissociating from SCAP, INSIG1 is ubiquitinated and degraded[88]. SCAP is either recycled or proteolytically degraded.[89] SREBP2 directly regulates transcription of the *INSIG1* gene. INSIG1 protein is rapidly degraded unless needed. Feedback regulation of cholesterol synthesis requires a sufficient amount of nuclear SREBP2 for INSIG1 transcription and restoration of ER cholesterol, a regulatory mechanism known as “convergent feedback inhibition.”[88,90] An additional ER-retention membrane protein, the INSIG2 isoform, was identified.[91] Both isoforms can simultaneously interact with SCAP to mediate retention of the SCAP-SREBP complex in the ER membrane.[92]

### 4.4 Regulation of INSIG:SCAP-dependent SREBP1/2 activation

Chronic hyperinsulinemia produces overactive hepatic SREBP1 and lipogenesis despite insulin resistance, often referred to as “selective insulin resistance.”[60,93] Proteolytic cleavage and activation of SREBP1/2 can be stimulated by insulin[94] via signaling through insulin receptor substrate-1 (IRS1) and its downstream targets protein kinase B (PKB/Akt) and mTORC1. mTORC1 regulates activation of hepatic p70 S6 kinase (S6K), the major downstream effector of mTORC1, which in turn can cleave and activate SREBP1/2 and stimulate lipogenesis under conditions of insulin resistance,[95,96] suggesting that selective insulin resistance depends upon mTORC1– S6K1 interaction.[60] SREBP1c activity may

also be induced through the nuclear hormone receptor peroxisome-proliferator-activated receptor-γ (PPARγ)[97] as well as liver X receptor (LXR) activity,[98] each of which plays a critical role in lipogenesis. The LXRs are members of the nuclear hormone receptor superfamily that are bound and activated by oxysterols. These receptors serve as sterol sensors to regulate the transcription of gene products that control intracellular cholesterol homeostasis through catabolism and transport. Ligand-activated nuclear PPARγ heterodimerizes with retinoid X receptors (RXRs) resulting in expression of its target genes such as CD36, a fatty acid transport protein involved in the transport and metabolism of intracellular FA.[99] SREBP1c expression was shown to be upregulated in mouse tissues in an LXR-dependent manner by dietary cholesterol and synthetic agonists for both LXR and its heterodimer partner, the retinoid X receptor (RXR),[98] which did not increase expression of the related gene products SREBP1a and SREBP2.[98] SREBP1a and SREBP2 but not SREBP1c bind to and are stabilized by CBP and P300 as co-activators to recruit the Mediator complex (Figure 3).[100,101]

### 4.5 Non-canonical (SCAP-independent) caspase 2-mediated activation of SREBP1/2

Despite the existence of several negative feedback loops associated with sterol/insulin-INSIG:SCAP-dependent SREBP1/2 regulation, chronic metabolic injury causes constitutive SREBP activation[102,103] via (SCAP)-independent SREBP activation.[11] NASH progression is associated with the lipotoxic effects of excessive accumulation of free fatty acids and free cholesterol[58] on mitochondrial dysfunction,[104] and the induction of TNF signaling in metabolically-injured hepatocytes, resulting in ER stress and insulin resistance. ER stress, defined as a chronic perturbation affecting ER homeostasis, is characterized by the accumulation of aberrant proteins, which disturbs the balance of the protein folding capacity of the ER to keep up with cellular demand.[105] The hepatic ER plays a critical role in the maintenance of lipid membrane composition and regulation of the intrahepatic and plasma lipids (Figure 3).

Specifically, binding of macrophage-derived TNFα to the hepatic TNF receptor 1 (TNFR1) and ER stress cause persistent activation of SREBP1/2 in metabolically-injured hepatocytes via non-apoptotic caspase 2-dependent constitutive activation of S1), which initiates SREBP-activating cleavage.[11] In turn, the development of ER stress inhibits INSIG expression via the PERK-mediated eIF2α signaling pathway,[106,107] shifting toward TNF/TNFR1-caspase 2-S1P/S2P-driven cholesterol synthesis.[108] Recent studies suggest that IL-17 signaling in fatty hepatocytes also regulates TNF-TNFRI-S1P-caspase 2-SREBP1/2 activation.[48] Consistent with this finding, the inhibition of TNF- or IL-17 signaling suppresses caspase 2-dependent SREBP1/2 maturation.[11,48] Although caspase activity is usually increased in apoptotic cells, caspase 2 does not exhibit apoptotic functions in metabolically damaged hepatocytes but instead acts as an enzyme-cleaving protease. Despite its ability to cleave S1P/S2P, caspase 2 cannot cleave SREBP1/2.[11] These observations are consistent with the notion that progression of NAFL to NASH may depend on a second hit, such as ER stress.[11] caspase 2 activates SREBP1/2 through a mechanism that, although not fully understood, is not regulated by feedback inhibition by sterols or unsaturated FA, as observed in normal SCAP-dependent SREBP activation.[11,63] Although caspase 2 does not trigger hepatocyte apoptosis, caspaseexcessive caspase 2-

dependent cholesterol accumulation can increase hepatocyte susceptibility to TNF-induced mitochondrial dysfunction and death.[104]

### 4.6 Alternative activation of SREBP1/2

Apoptotic responses to TNFα in hepatocytes activate pro-apoptotic caspase-3,[109] which mediates the release of SREBP from the ER membrane in an S1P-independent manner, leading to nuclear translocation of SREBP1/2 and transcriptional activation of multiple lipogenic genes. In addition, non-specific SREBP1/2 cleavage by caspases-4 and -12 was observed in alcohol-exposed cells.[110]

## 5. ER stress critically regulates *de novo* lipogenesis in hepatocytes.

Although the pathogenesis of NASH and ALD differs, the metabolic injury of hepatocytes is quite similar. ER stress and UPR activation play a critical role in the development of hepatic steatosis, inflammation, and fibrosis. The role of ER stress in *de novo* lipogenesis is discussed below.

The ER in hepatocytes has a remarkable capacity to adapt to extracellular and intracellular changes, ensuring that vital hepatic metabolic functions are preserved. However, hyperlipidemia and inflammation (specifically high levels of circulating TNFα) can perturb hepatocyte ER homeostasis, contributing to the dysregulation of hepatic lipid metabolism via activation of non-canonical TNF/TNFRI-caspase 2-S1P-dependent pathway of SERBP1/2 activation. ER stress leads to constitutive activation of SREBP1/2 and increased production of toxic lipids, including cholesterol, triglycerides, and fatty acids (Figure 4).

### 5.1 Role of the ER in cellular homeostasis

**5.1.1 ER Functions.—**The ER is a cellular organelle consisting of a continuous membrane system, tubules, sheets, and a nuclear envelope with enclosed sacs. The ER mediates many essential cell functions, including protein synthesis and processing, protein transport, lipid synthesis, and calcium storage.[111] The ER is enriched in hepatocytes due to their unique metabolic functions such as lipogenesis and production of secretory proteins including albumin, alpha-1 antitrypsin, and lipoproteins.[112] ER stress is caused by glucose starvation, depletion of calcium in the ER lumen, inhibition of glycosylation, reduction of disulfide bonds, or excessive accumulation of unfolded and misfolded proteins.[113]

**5.1.2 Chaperones that regulate ER folding.—**Chronic metabolic injury affects proper protein folding in the ER, leading to the accumulation of protein aggregates, cellular dysfunction, and programmed cell death. Inflammatory mediators, including free radicals such as nitric oxide (NO) and ROS, TNFα and other cytokines, and metabolic dysregulation can contribute to protein misfolding. In turn, improper protein folding can cause improper degradation, mislocalization, dominant-negative mutations, and structural alterations that establish novel toxic functions, which can cause disease. The UPR is an evolutionary conserved system that coordinates cellular responses to stress or injury to limit the accumulation of misfolded proteins in the ER and prevent cell death.[113] Proper

protein folding in the ER[114] is controlled by a high concentration of chaperones.[113,115] The first chaperone uses the ability of UDPglucose/glycoprotein glucosyltransferase (UGGT) to add a single glucose to misfolded proteins, making them accessible for binding to the lectin-like chaperones CNX and CRT that repair protein folding.[116] The second ER chaperone system, GRP78/BiP, binds to hydrophobic residues of unfolded proteins and mediates their retrograde translocation and proteasomal degradation.[113,117–119]

### 5.2 UPR signaling is activated to reduce ER stress

The ER engages the UPR to control hepatic protein and lipid homeostasis.[55,120] Although the initial UPR activation maintains tissue homeostasis and regulates lipogenesis, chronic UPR activation leads to dysregulation of the ER regulatory system, often resulting in increased production of misfolded proteins and uncontrolled lipogenesis. Recent studies report that chronic exhaustion of the UPR plays a critical role the pathogenesis of NASH.

Activation of the UPR signaling system restores ER homeostasis via: (a) increasing ER protein folding capacity through expansion of the ER and increased expression of chaperones (such as GRP78/BiP), (b) inhibition of protein translation to limit production of misfolded proteins[121], and (c) activation of autophagy and/or ER-associated protein degradation (ERAD) system that reduces ER stress[122] by re-directing misfolded proteins from the ER back into the cytosol for degradation by the 26S proteasome.[114,123] ER stress and UPR activation regulate cellular processes beyond ER protein folding and play crucial roles in lipid metabolism.[51,106,124,125]

## 6. The UPR signaling pathways

The UPR is adaptive response to ER stress.[112,126] UPR activation comprises 3 arms, each regulated by one of three transmembrane ER-located stress sensors: a) inositol-requiring enzyme 1 alpha (IRE1α), b) double-stranded RNA-activated protein kinase-like (PKR)-like ER kinase (PERK), and c) activating transcription factor (ATF6).[55] While IRE1α and ATF6 are transcription factors, PERK is a global suppresser of protein synthesis. The N-terminus of these proteins is positioned in the ER lumen and the C-terminus in the cytosol, thus connecting the two cellular compartments. Each of these proteins controls their specific downstream signaling cascades through the transcription of UPR target genes, including SREBP. Under physiological conditions, the UPR is inactive (due to inhibitory binding of GRP78/BiP to IRE1α, PERK, and ATF6) to maintain normal proteostasis in healthy hepatocytes. Upon GRP78 dissociation, all branches of the UPR are activated.[127,128] Protein disulfide isomerases (PDIs) regulate UPR stress sensors. In turn, transient UPR activation prevents sudden hepatotoxic injury and promotes cell survival.[129] Thus, when the threshold of misfolded protein accumulation reaches a critical point, GRP78 dissociates from the ER stress sensors, leading to activation of UPR-specific sensors. Transient activation of UPR stress sensors is also controlled by PDIs,[127,128,130] suggesting that multiple factors regulate transient stress sensor activation.[55]In contrast, chronic UPR activation[131–133] leads to protein misfolding, imbalance of calcium homeostasis, and lipid biogenesis (Figure 5),[55]

### 6.1 The IRE1a-XBP1 arm

IRE1, the most conserved ER stress sensor, has two isoforms: IRE1α and IRE1b.[134] IREα is the most abundant and biologically important Type I ER transmembrane protein and exhibits dual enzymatic activities: serine/threonine kinase activity and endoribonuclease (RNase) activity on its cytosolic tail.[62] IRE1α activation is triggered by the binding of ER chaperone Hsp47 or by the direct binding of unfolded proteins to IRE1α,[135,136] triggering IRE1α dimerization through its luminal N-terminal domain and oligomerization and trans-autophosphorylation. Subsequent conformational changes in IRE1αinitiate activation of its RNase domain.[137]

The endoribonuclease (RNase) activity of IRE1α degrades many ER-bound mRNAs, including mIRE1a itself via the regulated IRE1α -dependent decay (RIDD) pathway (in collaboration with RTCB RNA ligases), and acts as an RNA splicing/repair enzyme.[138,139] Upon activation of the tRNA ligase RTCB pathway, IRE1α RNase mediates unconventional splicing of Xbox binding protein 1 (*XBP1)* messenger RNA (by removing a 26-nucleotide sequence from *XBP1* unspliced (*XBP1u*) mRNA, causing a translational frameshift to produce transcriptionally active *XBP1* spliced (*XBP1s*). IRE1α phosphorylates and activates the XBP1 transcription factor XBP1 via its kinase activity. XBP1 translocates to the nucleus and induces transcription of its downstream target genes, including ER chaperones and genes involved in ERAD.[140]

IRE1α recruits TNF receptor associated factor 2 (TRAF2) and apoptosis signal-regulating kinase 1 (ASK1) to mediate the phosphorylation of c-jun N-terminal kinase and nuclear factor kappa B (NF-kB) pathways that transcriptionally activate inflammatory and apoptotic pathways. XBP1 directly regulates transcription of specific genes responsible for the regulation of lipid metabolism (such as farnesyl diphosphate synthase, hydroxysteroid 17-beta dehydrogenase 7,[141] and fibroblast growth factor 21) to protect from ER stress-induced hepatic steatosis.[142] Depletion of XBP1 results in rapid feedback activation of IREα.[112] The IRE1α -XBP1 arm of the UPR plays a critical role in hepatic lipid metabolism through regulation of VLDL secretion and lipogenesis (Figure 5).[143–145]PERK-eIF2a-ATF4 arm

PERK is a Type I ER-resident transmembrane serine/threonine protein kinase consisting of an ER luminal stress-sensing domain and a cytosolic kinase domain. Upon oligomerization, PERK phosphorylates the subunit of eukaryotic translation initiation factor 2 (eIF2a) in response to ER stress. eIF2α serves as a major substrate of PERK[146] and functions to relieve the protein overload in the ER by suppressing the formation of translation initiation complexes to prevent protein translation. Phospho-eIF2α facilitates translation and expression of transcription factor ATF4, which positively regulates transcription of UPR target genes involved in protein folding and autophagy. ATF4 also transcriptionally activates CCAAT-enhancer binding protein (C/EBP) homologous protein (CHOP), which is critical for ER stress mediated apoptosis, DNA damage-inducible protein GADD34, and ATF3.[147–149] The PERK-eIF2α -ATF4 arm of the UPR regulates lipogenesis and steatosis. Phosphorylation of eIF2α is regulated on several levels. In a negative feedback mechanism, ATF4 induces expression of *GADD34* and constitutive repressor of eIF2α phosphorylation (*CReP*), which interact with protein phosphatase 1 (PP1) to promote PP1-mediated de-phosphorylation of eIF2α.[150] Consequently, ATF4 translation resumes,

and ATF4 transactivates UPR target genes involved in protein folding, autophagy, redox homeostasis, amino acid metabolism, and apoptosis (Figure 5).[147–149]

### 6.2 ATF6 arm

ATF6, a type II transmembrane protein, contains a cytosolic bZip domain and possesses leucine zipper transcription factor activity.[112] Full and truncated forms of ATF6 have been identified (ATF6a and ATF6b, respectively).[151,152] Upon ER stress-induced activation, ATF6a (p90) is released from the inhibitory BiP protein and transported from the ER to the Golgi where it is cleaved by S1P and S2P proteases. Proteolytic cleavage of the full length ATF6 results in release of the N-terminal cytosolic transcription factor ATF6b (p50), which translocates to the nucleus and initiates the transcription of genes involved in protein folding and ERAD. ATF6 was also shown to activate the transcription of *XBP1*, *CHOP,* and *BiP*.[153] ATF6 also forms heterodimers with XBP1 to induce transcription of multiple genes involved in ERAD.[154] The ATF6a arm may provide responses that prevent excessive lipogenesis (Figure 5).

### 6.3 UPR proteins differentially regulate de novo lipogenesis

**6.4.1 IRE1a-XBP1 pathway.—**The IRE1α -XBP1 pathway directly drives hepatic steatosis, metabolic liver damage, and hypercholesterolemia (Table 2). XBP1 is a critical pro-lipogenic transcription factor[155] that targets Lipin genes (*LPIN1* and *LPIN3*), *OSBP*, *LSS*, and *GPAT4*. *OSBP* encodes a sterol–sensing protein that modulates SREBP activity in response to sterol PECR, an enzyme involved in fatty acid elongation.[156,157] LSS catalyzes the formation of lanosterol from squalene, and GPAT4 adds a fatty acid to glycerol during lipogenesis.[154,158,159] XBP1 ablation leads to a compensatory upregulation of its upstream enzyme IRE1α (but not PERK, ATF6, or other UPR proteins). These findings further support the proposed role of IRE1α in lipid metabolism and indicate that IRE1α activity is regulated by a feedback mechanism activated by low abundance of XBP1 (IRE1α substrate).[154,160,161] Moreover, the IRE1α-regulated XBP1 and RIDD pathways have opposing effects on the expression of lipogenic genes, with the RIDD pathway promoting lipid hydrolysis and preventing lipid storage by reducing the expression of lipogenic genes.[160] The silencing of lipid metabolism genes through the IRE1α -regulated mRNA decay RIDD system lowers plasma lipid concentrations.[155,160] In addition, IRE1α RNase activity (but not kinase activity) increases the decay of select microRNAs (miR-17, -34a, -96, -125b) that repress translation of caspase 2 mRNA, thereby promoting caspase 2 expression. Targeting of either IRE1α or XBP1 might become a strategy for blocking *de novo* lipogenesis.[162]

**6. 4.2 PERK-eIF2a-ATF4 pathway.—**PERK positively regulates lipid synthesis via its downstream targets eIF2α, ATF4, and CHOP. The absence of PERK is associated with the downregulation of triglyceride and fatty acid production. In response to ER stress, PERK phosphorylates eIF2α, causing subsequent caspase 2-dependent SREBP1/2 cleavage/maturation in immune cells.[163] Phosphorylated eIF2α facilitates translation of the transcription factor ATF4, which also increases *de novo* lipogenesis. The genes encoding the lipogenic enzymes *Acac*, *Scd1*, *Fas,* and *Gpat* are ATF4 targets.[164,165] These findings

indicate that the IREα -XBP1 and PERK-eIF2α -ATF4 pathways can be targeted to suppress caspase for NASH therapy.

### 2-SREBP1/2-dependent cholesterol and fatty acid synthesis

**6.4.3 ATF6 pathway.—**The role of ATF6 in *de novo* lipogenesis is poorly understood. The ATF6 pathway was originally implicated in the suppression of lipid metabolism because of its ability to induce ER expansion in an XBP1-independent manner.[154,166,167] Further, ATF6 was reported to inhibit cholesterol synthesis via interaction with cleaved/activated SREBP2 and transcription inhibitor HDAC1, leading to the downregulation of *HMGCR*, *HMGCS*, *FDFT1* (squalene synthase), and LDLR expression.[168] ATF6 can also suppress hepatic triglyceride accumulation via regulation of transcriptional activity of PPARα/RXRα (retinoid X receptor alpha) heterodimers and activation of fatty acid oxidation (*Cpt1*, *Cpt2*, *Acox1* and *Ppara*) and VLDL formation (*Mttp*, *PDI* and *Apob*).[154,155] In another study, ATF6 activation was shown to upregulate the transcription and expression of *XBP1* as well as genes involved in protein folding that support ERAD machinery, mediate ER homeostasis, and stabilize ER and Golgi biogenesis.[153,169] ATF6 and XBP1s can form heterodimers that promote the expression of select genes involved in ERAD biologic functions.[154,170,171]

## 7. *De novo* lipogenesis in inflammatory cells and fibrogenic myofibroblasts facilitates NASH progression

### 7.1 Lipogenesis contributes to activation of inflammatory cells and hepatic myofibroblasts

*De novo* lipogenesis plays a similar role in other cells, including immune, inflammatory, and mesenchymal cells.[2] Steatosis–inflammation–fibrosis mediated by lipid accumulation in different cell types and lipotoxicity is a final common pathway to the organ pathologies of immunometabolic disorders such as obesity, atherosclerosis, diabetes mellitus, NASH, chronic kidney disease, and neurological disorders. Severe cell stressors induce apoptosis through a terminal UPR. In energy-depleted states, lipids in lipid droplets are degraded via lipophagy to restore energy levels.

### 7.2 Lipogenesis promotes myeloid cell activation

Lipid metabolism is critical for the activation of myeloid cells, induction of inflammatory responses, the host defense mechanism, phagocytosis, and autophagy.[2] Thus, macrophages internalize oxidized low-density lipoproteins and lipids from the environment, leading to the formation of foam cells with an inflammatory phenotype.[172] Metabolic-sensing pathways coordinate shifts in lipid metabolism and regulate macrophage activation.[173] LDLR expression is regulated by LXR, which acts as a cellular free-cholesterol–concentration sensor and mediates the expression of SREBP1c in myeloid cells[174]. SREBP1c was also shown to activate genes encoding inflammasome subunits in macrophages.[66] SREBP2 is an important regulator of LDLR and SREBP1c expression. In addition, SREBP2 is necessary to produce an LXR ligand required for normal SREBP1c expression.[175 174]

### 7.3 Hepatic stellate cells

Lipid metabolism in myofibroblasts and HSCs is not fully understood.[2] Activation of the vitamin A– retinoic acid signaling pathway, the presence of lipid droplets, and downregulation of PPARγ are involved in maintenance of the quiescent HSC phenotype.[176,177]. Quiescence-associated transcription factor ETS1 regulates PPARγ expression levels in qHSCs.[178] In contrast, binding of methyl-CpG binding protein 2 (MeCP2) represses PPARγ transcription, leading to HSC activation into myofibroblasts.[177] Emerging evidence indicates that excessive lipid accumulation facilitates fibrogenic activation of hepatic myofibroblasts. HSCs are sensitive to intracellular cholesterol levels, which causes their activation. Increased SREBP2 and microRNA-33a signaling was observed in activated HSCs and was linked to PPARγ suppression in activated HSCs. In turn, cholesterol accumulation in HSCs increases Toll-like receptor 4 protein (TLR4) levels through suppression of TLR4 endosomal-lysosomal degradation, thereby facilitating LPS and TGFβ signaling in HSCs.[179] Curcumin suppresses LDLR and SREBP expression in activated HSCs by activating PPARγ, reducing cellular cholesterol, and attenuating HSC activation.[180] In addition, curcumin directly regulates SREBP2 expression by suppressing specificity protein 1 (SP-1) transcription factor. The *SREBP2* promoter contains an SP-1 binding GC-box, and SP-1 is implicated in elevated *SREBP* gene transcription.[181] PPARγ and LXR play critical roles in the regulation of *de novo* lipogenesis and cholesterol homeostasis in HSCs. Crosstalk between PPARγ and LXR is modulated by expression of a mutant *PNPLA3* allele (linked to accelerated NASH progression). HSCs carrying I148M *PNPLA3* show impaired LXR signaling, leading to cholesterol accumulation and HSC activation[182].

## 8. Concluding remarks

Lipids play a critical role in the maintenance of body homeostasis, as they serve as a source of energy and provide building blocks for cell membranes.[2] The production of cholesterol and fatty acids also plays a role in the development of hepatic steatosis in response to metabolic injury. D*e novo* lipogenesis contributes to the NASH pathogenesis and is triggered by obesity, insulin resistance, ROS production, ER stress, and TNFα -induced signaling. The synthesis of cholesterol and fatty acids is controlled on multiple levels, including the activation of non-canonical caspase 2-dependent S1P/S2P-induced processing and activation of SREBP1/2, the transcription factors that play a key role in triggering expression of the major lipogenic genes. Therefore, caspase 2 and S1P/S2P proteins are targets for the therapeutic suppression of *de novo* lipogenesis.[2] Blocking of the upstream activators TNFα and IL-17 effectively suppresses the caspase 2-S1P/S2P-DHCR7 pathways, preventing cholesterol and fatty acid production.[2] Other components of the UPR system directly or indirectly affect cholesterol synthesis. Blocking of the IRE1α -XBP1 and PERK-eIF2α -ATF4 pathways may suppress steatosis, while stimulation of ATF6 and the ERAD system can reduce steatosis by decreasing ER stress.

## Grant support:

Supported by the National Institutes of Health *R01DK101737, U01AA022614, R01DK099205, R01DK111866, R01AA028550, P50AA011999, U01AA018663, P30 DK120515, 5U01AA029019, R01DK091183, R01DK09920*

(TK), *P42ES010337 and R44DK115242* (DB). R01 AA24726, R37 AA020703, U01 AA026939, U01 AA026939–04S1, P30 DK120515 and P50 AA011999 (BS). RL receives funding support from *NIEHS (5P42ES010337), NCATS (5UL1TR001442), DOD PRCRP (W81XWH-18–2-0026), NIDDK (U01DK061734, R01DK106419, R01DK121378, R01DK124318, P30DK120515), NHLBI (P01HL147835), and NIAAA (U01AA029019)*.

## Abbreviations:

| | |
|---|---|
| **NASH** | nonalcoholic steatohepatitis |
| **AALD** | alcohol-associated liver disease |
| **aHSCs** | activated Hepatic Stellate Cells |
| **DHCR7** | 7-dehydrocholesterol reductase |
| **HMGCS1** | cytoplasmic hydroxymethylglutaryl-CoA synthase |
| **SREBP** | sterol regulatory element-binding protein |

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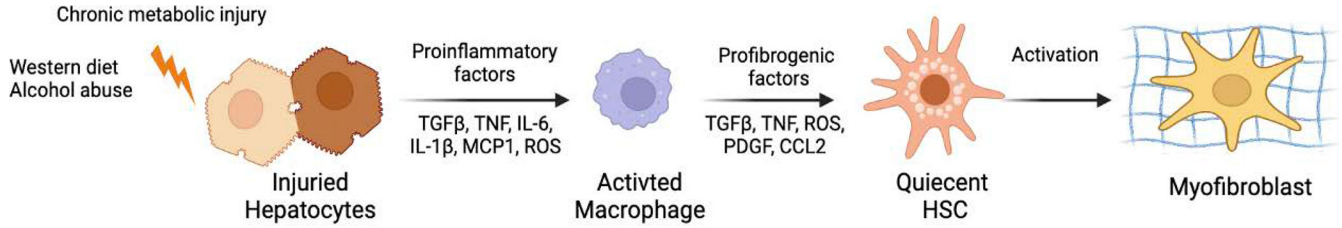


**Figure 1. Pathogenesis of toxic liver fibrosis and therapeutic implications.**

Hepatocyte damage triggers the inflammatory response, leading to activation of macrophages, release of ROS and TGFβ1, and activation of quiescent HSCs into activated HSCs/myofibroblasts that produce collagen type I resulting in liver fibrosis.

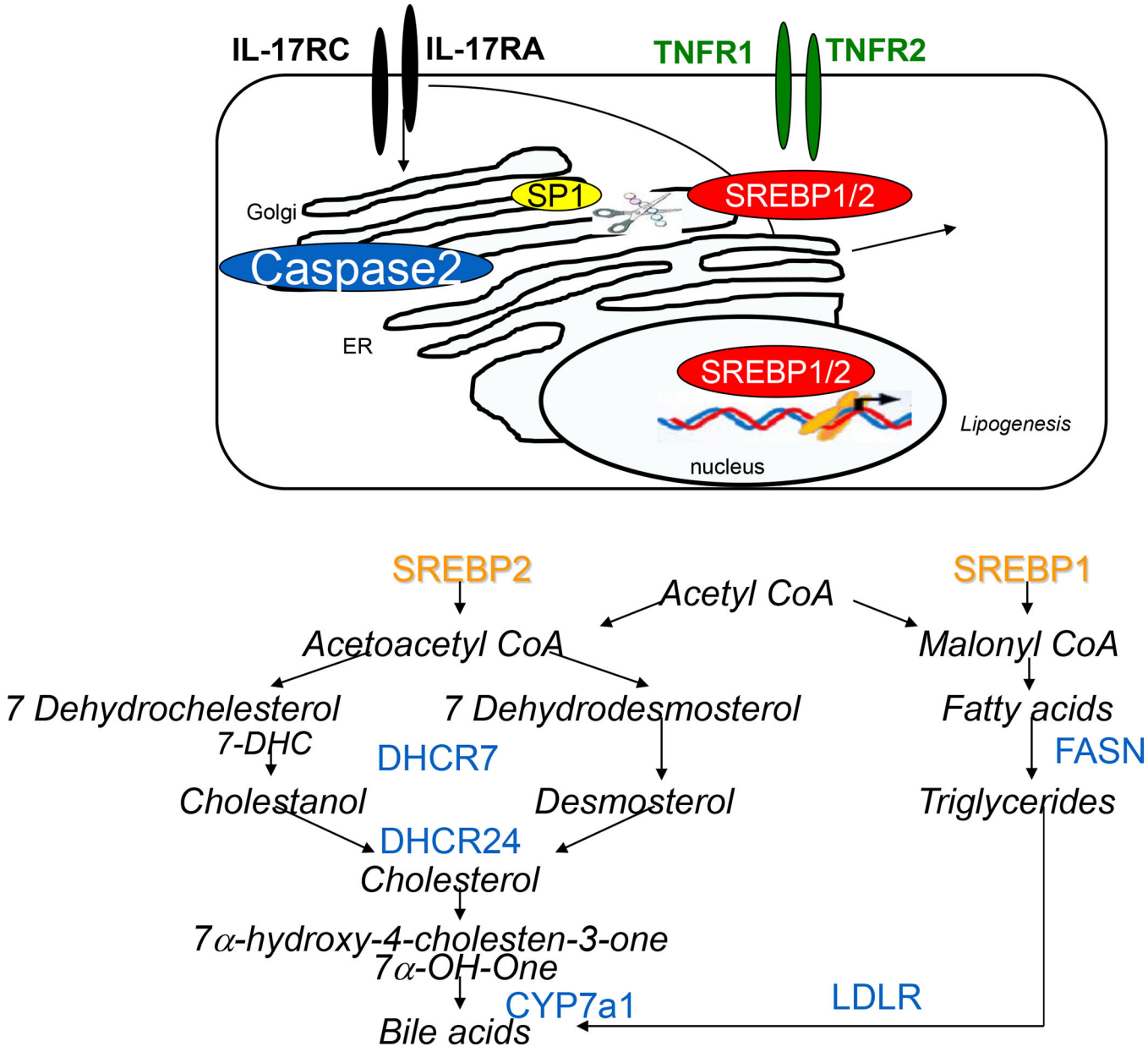


**Figure 2. The caspase-2–dependent activation of *de novo* lipogenesis.**

ER and IL-17A facilitate TNFα/TNFR-mediated lipogenesis in alcohol-damaged hepatocytes via activation of the caspase 2-SP1-SREBP1/2-DHCR7 pathway. Schematic representation of cholesterol and fatty acid synthesis pathways.

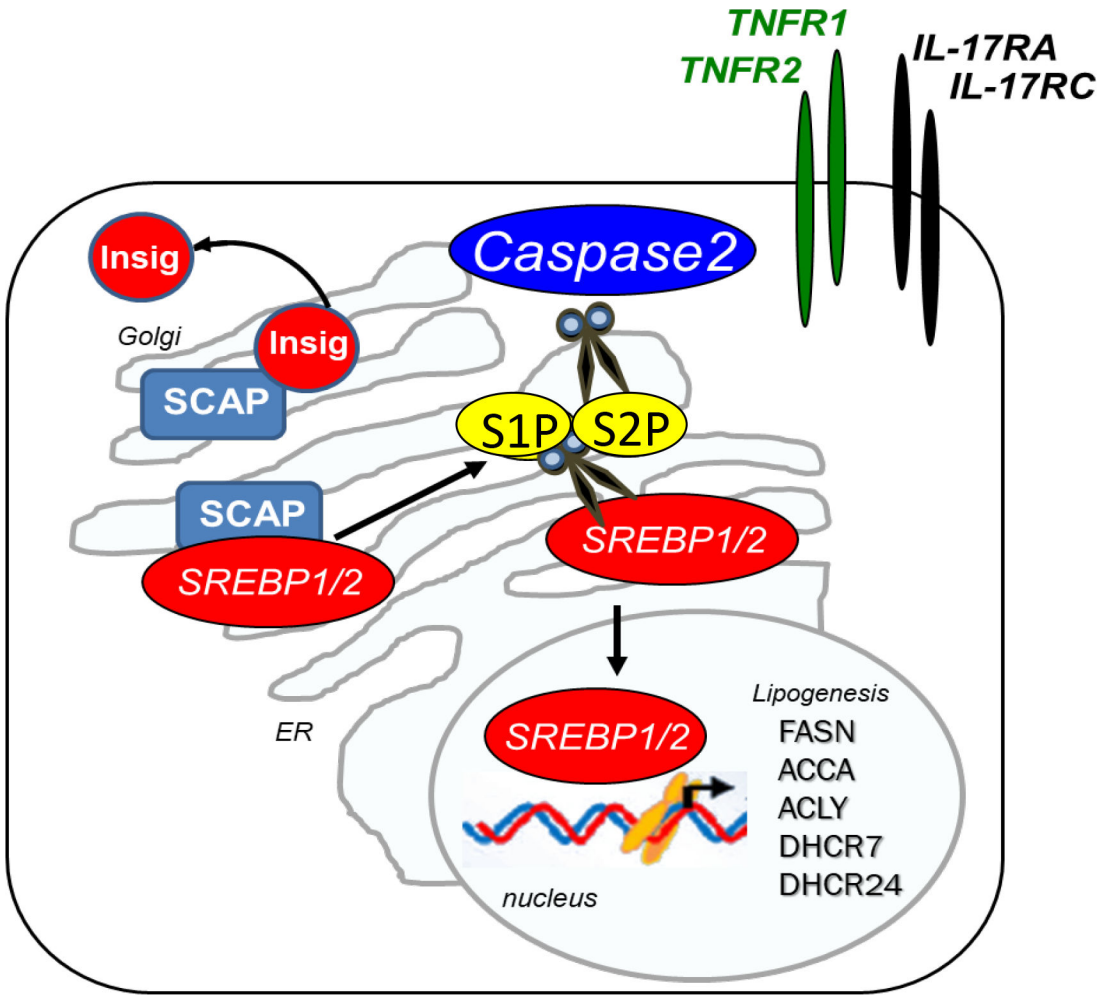


**Figure 3. Canonical and non-canonical activation of SREBP1/2 transcription factors that drive *de novo* lipogenesis in steatotic hepatocytes.**

SCAP-dependent lipogenesis is subject to negative feedback. Cholesterol buildup in ER membranes causes sterol binding to SCAP, which triggers a conformational change that causes SCAP to bind to insulin-induced gene, prohibiting SCAP binding to SREBPs. Conversely, when cells are sterol-deprived, SCAP escorts SREBPs from the ER to the Golgi, where S1P and S2P proteases cleave SREBPs, allowing their translocation to the nucleus to activate *de novo* lipogenesis and cholesterol target gene transcription. During ER stress, lipogenesis is driven in a SCAP-independent manner. The simultaneous activation of TNFα and IL-17 pathways increases caspase 2 gene expression, and its activation is dependent on IRE1$\alpha$. Caspase 2 cleaves S1P into a soluble form that reaches the ER to cleave SREBPs, allowing their translocation to the Golgi, where they are further cleaved by S2P and translocate to the nucleus to activate the lipogenic gene transcription. SCAP-dependent lipogenesis is prevented by cholesterol increase, while SCAP-independent lipogenesis is a non-homeostatic mechanism subject to a positive regulatory loop. The activation of SREBP2 also increases caspase 2 levels, serving as an additional input.

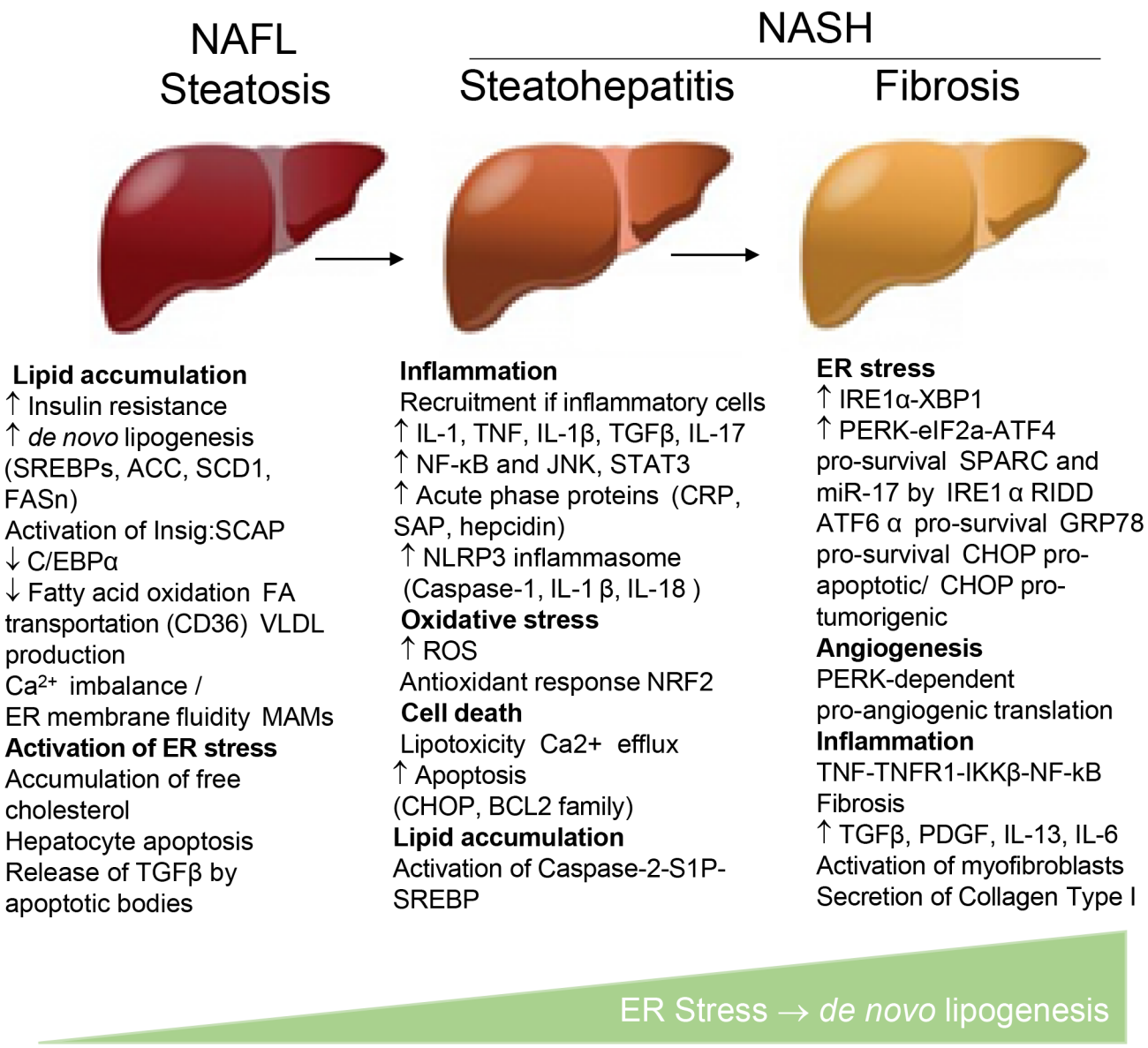


**Figure 4. ER stress drives NAFLD progression.**

Progression of steatosis (NAFL) to steatohepatitis and fibrosis (NASH) is associated with the development of ER stress, lipid accumulation, inflammation, and activation of hepatic myofibroblasts.

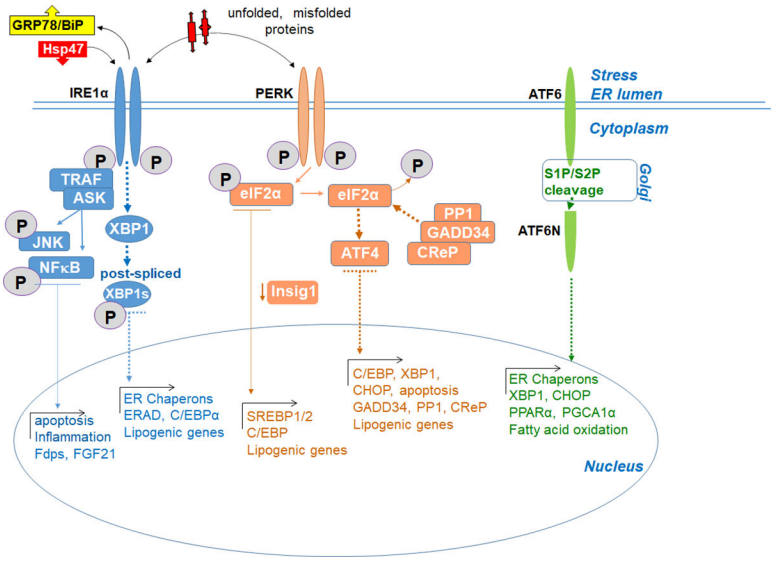


**Figure 5. ER stress activates three UPR arms.**

Progression of steatosis (NAFLD) to steatohepatitis and fibrosis (NASH) is associated with development of ER stress and activation of the unfolded protein response (UPR) to control hepatic protein and lipid homeostasis. Under physiological conditions, UPR is inactive (due to inhibitory binding of GRP78/BiP to IRE1, PERK, and ATF6) to maintain normal proteostasis in healthy hepatocytes. Upon GRP78 dissociation, all 3 arms of the UPR are activated. In response to chronic injury, all arms of the UPR contribute to NASH and, to different extents, support *de novo* lipogenesis.

**Table 1.**

**NASC/CRN grading criteria.**

NASH diagnosis correlates with steatosis, related chronic steatohepatitis, and fibrosis. Biopsies of grade ≥5 were diagnosed as NASH; those of grade <3 were diagnosed as “not NASH.”

| Grade: | Criteria: |
|---|---|
| Steatosis grade: | **0** <5%;<br>**1** - 5–33%;<br>**2** - 34–66%;<br>**3** >66% |
| Steatosis distribution: | centrilobular vs diffuse |
| Lobular inflammation: | **0** - none;<br>**1** < 2 foci/20x field;<br>**2** - 2–4 foci/20x field;<br>**3** > 4 foci/20x field |
| Hepatocellular ballooning: | **0** - none;<br>**1** - mild, few;<br>**2** - moderate-marked, many |
| Portal inflammation: | **0** - none;<br>**1** - mild,<br>**2** > mild,<br>**3** - severe |
| Fibrosis (Trichrome stain): | **0** - none<br>**1a** - mild zone 3 perisinusoidal fibrosis, requires trichrome stain to identify<br>**1b** - moderate zone 3 perisinusoidal fibrosis, also noticeable by H&E<br>**1c** - portal fibrosis only;<br>**2** - zone 3 perisinusoidal fibrosis and periportal fibrosis<br>**3** - bridging fibrosis<br>**4** - cirrhosis |

**Table 2:**

**ER stress pathways are activated in response to metabolic injury induced by NASH or AALD.**

The role of UPR-activated proteins in NASH and AALD is demonstrated.

| Liver Disease | UPR Component | Functions |
|---|---|---|
| NAFL/NASH | IRE1α | Regulates Hepatic lipogenesis through RIDD |
| | | Facilitates diet induced steatosis |
| | | Promotes the progression of NAFL to NASH |
| | XBP1 | Promotes steatosis in metabolically injured hepatocytes |
| | | Promotes diet-induced liver injury |
| | | Regulates Hepatic lipogenesis |
| | | Contributes to diet induced liver injury |
| | ATF4 | Contributes to diet-induced steatosis |
| | GADD34 | Protects from diet-induced steatosis |
| | ATF6 | Protects from diet-induced steatosis |
| AALD | BiP | Protects from alcohol-induced liver injury |
| | ATF4 | Responsible for alcohol-induced steatosis |
| | CHOP | Promotes alcohol-induced liver injury |
| | ATF6 | Promotes alcohol-induced steatosis |

Abbreviations: NAFLD, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; IRE1α, inositol-requiring enzyme 1α; XBP1, Xbox binding protein 1; PERK, double-stranded RNA-dependent protein kinase (PKR)-like ER kinase; ATF4, activating transcription factor 4; eIF2α, eukaryotic initiation factor 2a; GADD34, growth arrest and DNA damage-inducible protein 34; ATF6, activating transcription factor 6; RIDD, regulated IRE1a-dependent decay of mRNA; ALD, alcoholic liver disease; BiP, binding immunoglobulin protein; CHOP, CCAAT-enhancer-binding protein (C/EBP) homologous protein