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Mechanisms of Liver Injury in Non-Alcoholic Steatohepatitis

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Abstract

Non-alcoholic steatohepatitis (NASH) is a disorder marked by alterations in hepatic lipid homeostasis as well as liver injury in the form of cell death, inflammation and fibrosis. Research into the pathophysiology of NASH is dynamic. New concepts from the fields of cell biology, microbiology, immunology and genetics are being tested for their applicability to NASH; discoveries in each of these areas are enriching our understanding of this complex disease. This review summarizes how recent developments from the bench and bedside are merging with more traditional concepts to reshape our view of NASH pathogenesis. Highlights include human studies that emphasize the role of *de novo* lipogenesis in NASH and experimental work uncovering a role for the inflammasome in NASH. Genetic predispositions to NASH are being clarified, and intestinal microbiome is emerging as a determinant of fatty liver. These unique ideas are now taking their place within an integrated picture of NASH pathogenesis.

Keywords

steatosis; lipogenesis; lipolysis; lipotoxicity; ER stress; oxidative stress; inflammasome; Kupffer cell; genetics; adiponutrin; microbiome

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a complex condition that involves derangements of lipid metabolism, cellular integrity, immune homeostasis and tissue repair. The pathophysiologic processes that result in NASH take place both within and outside the liver, integrating intrahepatic events with signals emanating from the intestine and adipose tissue. The purpose of this review is to provide a perspective on disease mechanisms that play a role in the development of NASH. Individual sections cover events that cause hepatic fat accumulation (hepatic steatosis or non-alcoholic fatty liver (NAFL)), followed by events that cause liver injury in the form of cell death, inflammation and fibrosis. Acknowledging

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that genetic and environmental factors influence NASH, we include information about gene polymorphisms and dietary nutrients that increase the risk of fatty liver disease. Finally, we mention the emerging role of the intestinal microbiome in the pathogenesis of hepatic steatosis and NASH.

MECHANISMS OF HEPATIC STEATOSIS

Hepatic steatosis must be present to make a histologic diagnosis of NASH [1]. Although in some instances steatosis may precede NASH and in others it may develop simultaneously with other features of NASH [2, 3], hepatic steatosis represents the cornerstone of NASH. Thus, understanding the events that lead to hepatic fat accumulation is critical for gaining full insight into this complex disease process (Figure 1A).

Role of adipose tissue inflammation

Under normal circumstances, hepatic lipid homeostasis is maintained by balancing fatty acid input from three sources (dietary fat, adipose tissue and *de novo* lipogenesis) with output in the forms of metabolism (oxidation) and export (as VLDL). In states of energy excess, fatty acids not acutely needed for metabolism are converted to triglyceride and transported to adipose tissue for storage. In obesity, adipose tissue becomes dysfunctional, which results in the diversion of excess triglycerides to other organs such as muscle and liver. A principal driver of this ectopic fat accumulation is adipose tissue inflammation [4]. Precisely what initiates adipose tissue inflammation in obesity is uncertain; hypoxia and death of rapidly expanding adipocytes are believed to play a role [5].

Dysfunctional adipocytes secrete cytokines and chemokines, particularly TNF and CC-chemokine ligand-2 (CCL2) [5, 6]. CCL2 recruits macrophages to the adipose tissue, resulting in even more local cytokine production and perpetuating the inflammatory cycle; TNF induces a state of insulin resistance in adipocytes, which stimulates triglyceride lipolysis and fatty acid release into the circulation. At the same time, compromised adipocytes lose their natural ability to secrete adiponectin, an adipokine that facilitates the normal partitioning of lipid to adipocytes for storage [7]. Together these abnormalities accentuate fat loss from adipocytes and promote ectopic fat accumulation. Finally, adipose tissue-derived TNF and CCL2 presumably reach the liver through the circulation, where they can act on hepatocytes to stimulate steatosis [8, 9]. This series of events originating in the adipose tissue sets the stage for excess fat delivery to, and storage in the liver.

Role of specific fatty acid pools in hepatic triglyceride synthesis

From the liver perspective, adipose tissue lipolysis represents the dominant pathway through which fatty acids enter the organ. In lean individuals, 77% of hepatic fatty acids originate from adipose tissue, whereas 19% come from dietary fat and 4% are produced in the liver from dietary carbohydrate via DNL [10]. In obese individuals with enhanced adipose tissue lipolysis, one might expect the adipose tissue proportion to increase; instead it decreases to 60%, and the proportion from DNL increases to 26% [11]. Indeed, NASH subjects with high levels of hepatic fat, accompanied by hyperinsulinemia and high circulating levels of adipose tissue-derived fatty acids, display no sign that circulating fatty acids are

proportionally incorporated into hepatic lipid stores. Instead, DNL is prominent in these individuals -- 3 times higher than in subjects with lower levels of hepatic fat [12]. This suggests that although obesity stimulates adipose tissue lipolysis, adipose tissue-derived fatty acids are not the principal substrate for excess triglyceride synthesis in the liver. Instead, it appears adipose tissue-derived fatty acids are being channeled to oxidation, to provide the energy to drive DNL [13] This is in keeping with the concept that the molecular machinery driving lipogenesis in the liver is impervious to insulin resistance [14].

Molecular regulation of *de novo* lipogenesis

Hepatic DNL is driven in large part by the transcription factor sterol regulatory element binding protein-1 (SREBP-1). SREBP-1 induces several genes involved in hepatic lipogenesis [15, 16], and its overexpression in the liver in vivo is sufficient to provoke hepatic steatosis [17]. SREBP-1 is positively regulated by insulin, which explains its high activity in hyperinsulinemic states such as obesity. Notably, however, SREBP-1 activity can also be up-regulated independently of insulin under conditions of endoplasmic reticulum (ER) stress. Excessive energy intake places stress on the ER, possibly due to heightened demand for the synthesis of proteins such as apolipoprotein B that help transport lipids out of the liver [18]. ER stress prompts an unfolded protein response (UPR), which involves the three UPR transducers inositol-requiring enzyme 1 (IRE1), activating transcription factor-6 (ATF6) and RNA-dependent protein kinase-like ER kinase (PERK). Among these, ATF6 has proteolytic activity capable of processing SREBP-1 from an inactive to a transcriptionally active form [19]. The ability of ER stress to activate SREBP-1 and drive hepatic lipogenesis independent of insulin has recently been demonstrated in a mouse model of obesity and fatty liver [19]; thus, SREBP-1 can be activated by two separate mechanisms during obesity, linking this transcription factor closely to an increased risk of hepatic steatosis.

Role of dietary nutrients in the regulation of DNL

Inasmuch as carbohydrates are substrates for DNL, the amount of carbohydrate in the diet will positively influence the amount of DNL in the liver. The type of carbohydrate in the diet also affects DNL: for example, simple sugars are converted to fatty acids more readily than complex carbohydrates [20, 21]. Even among the simple sugars there is some variation in their ability to drive DNL, with, fructose being a more potent inducer of DNL than glucose [22, 23]. This is in keeping with epidemiologic evidence linking dietary fructose to hepatic steatosis and NASH [24, 25]. It is worth noting that dietary fat can also stimulate DNL by up-regulating SREBP-1. This is particularly true of saturated fats [26, 27], and may explain why diets enriched in both saturated fat and simple sugar carry a higher risk of hepatic steatosis.

The degree to which dietary carbohydrate influences hepatic steatosis has been demonstrated quite dramatically in human subjects. Indeed, in one study where individuals with established steatosis were placed on isocaloric low-carbohydrate or high-carbohydrate diets, those eating the low-carbohydrate diet lost 30% of their hepatic lipid within 2 days [28]. Although DNL was not measured directly in this experiment, one can infer it was reduced

due to substrate restriction. These data and others [12] imply that limiting intake of dietary sugar is an effective means of controlling hepatic DNL and hepatic steatosis.

MECHANISMS OF LIVER INJURY IN NASH

Pathologically, a diagnosis of NASH requires not only hepatic steatosis, but also liver cell damage and accompanying inflammation and/or fibrosis. This section reviews how liver cells can be damaged by lipids, and how resident liver cells react to dead cells and other signals to cause hepatic inflammation and fibrosis (Figure 1B).

A. Mechanisms of Hepatocyte Death in NASH

Lipotoxicity—Although natural history studies indicate hepatic steatosis is a benign condition, certain lipids can be harmful to hepatocytes. This is particularly true of the long-chain saturated fatty acids (SFA) palmitate (C16:0) and stearate (C18:0), which are abundant in animal fat and dairy products and produced in the liver from dietary sugar. SFA can kill hepatocytes directly by activating Jun N-terminal kinase (JNK) and a mitochondrial death pathway [29]; the critical role of JNK in hepatocyte death has been demonstrated in animal models of fatty liver disease [30, 31], and is supported by evidence that JNK is also activated in the livers of NASH patients [32, 33]. SFA have the potential to activate JNK by multiple mechanisms. One is by inducing ER stress, with JNK being activated downstream of IRE1 and PERK [34, 35]. SFA can also activate JNK independently of ER stress through cyclin-dependent kinases [36] or c-Src [37]. Very recently, investigators have proposed a new mechanism by which SFA can induce hepatocyte death involving the inflammasome. This multimolecular complex is responsible for the activation of caspase-1 [38]. Experiments show that SFA stimulate hepatocytes to express inflammasome components [39]. Independent studies indicate that overexpression of these same inflammasome components induce hepatocyte death through a process called pyroptosis [40], which is dependent upon caspase-1 [41]. Caspase-1 activation occurs in the liver in experimental NASH [39, 42]; however, it remains uncertain whether activation of the inflammasome is a direct cause of liver cell death in NASH [42].

Although some investigators implicate SFA themselves as mediators of lipotoxicity [37], others believe cell death is precipitated by non-oxidative metabolites of SFA. One such metabolite is lysophosphatidylcholine (LPC), which induces much the same toxicity as primary SFA in hepatocytes [43, 44]. Ceramides, which can be produced *de novo* from palmitate or generated via hydrolysis of sphingomyelin, can also kill hepatocytes (reviewed in [45]). To date, however, excess ceramides have not been directly linked to hepatocellular injury in experimental or human NASH.

Just as SFA can be toxic to hepatocytes, excess cholesterol can also promote hepatocyte injury. Cholesterol does not kill hepatocytes outright, but rather sensitizes them to death in response to other noxious stimuli. Diets enriched in cholesterol promote accumulation of free cholesterol in hepatocyte mitochondrial membranes [46]; the excess cholesterol disrupts membrane fluidity, which permits the loss of glutathione from mitochondria. Reduced levels of mitochondrial glutathione render hepatocytes sensitive to TNF-induced cytotoxicity. Since obesity is associated with increased circulating levels of TNF [47, 48], the

combination of free mitochondrial cholesterol, glutathione depletion and TNF is believed to contribute to NASH [46]. Notably in humans with NAFL and NASH, free cholesterol levels are increased in the liver [32] and genes regulating cholesterol synthesis are induced [49]. Accordingly, drugs that reduce cholesterol synthesis and those that restore mitochondrial glutathione levels should theoretically ameliorate NASH. This has been demonstrated in experimental animals [46], but awaits confirmation in human subjects.

Mitochondrial dysfunction—The progression from hepatic steatosis to NASH is characterized by a decline in hepatocyte mitochondrial function (reviewed in [50]). One of the principal drivers of mitochondrial deterioration in NAFL and NASH is enhanced fatty acid oxidation that accompanies these conditions [51, 52]. Fatty acid oxidation is up-regulated in NAFL and NASH in part because of increased demand, due to the amplified influx of fatty acids from adipose tissue. Increased oxidation may also provide the energy necessary to drive hepatic lipogenesis and gluconeogenesis, also augmented in these conditions [53]. Unfortunately, a constant flux of fatty acids through mitochondria, with attendant high-level activity of the tricarboxylic acid (TCA) cycle, leads to enhanced delivery of electrons to the mitochondrial respiratory chain (MRC) and electron leakage within the MRC [54, 55]. This generates harmful reactive oxygen species (ROS), which in turn damage the components of the MRC [56], leading to further electron leakage and more ROS production. Over time, mitochondria become progressively more dysfunctional, with oxidative stress, ATP depletion and loss of mitochondrial integrity all contributing to hepatocyte death.

Of note, the mitochondrion is not the only organelle involved in fatty acid oxidation. Microsomes and peroxisomes also metabolize fatty acids [57], and thus they can contribute to ROS production in NASH. Importantly, cytochrome P450 2E1 (CYP2E1), a microsomal enzyme that oxidizes fatty acids, is significantly induced in experimental and human NASH [58, 59]. Some hypothesize that CYP2E1 induction in fatty liver disease is an adaptive response to support the inefficient mitochondrial oxidation of fatty acids, but it may also be a consequence of insulin resistance [60]. CYP2E1 is noteworthy because it is a major producer of ROS, and therefore is in a position to promote oxidative injury to liver cells [60].

B. Mechanisms of Hepatic Inflammation in NASH

Activation of the innate immune system is an important event in the evolution of NASH. In the setting of a fatty liver, innate immunity is triggered in part by “danger signals” generated by hepatocytes as they become dysfunctional and die [61]. Fatty acids themselves can also serve as danger signals [62]; in addition, bacterial endotoxin, which can leak into the circulation from the intestine in the setting of obesity [63], can trigger an inflammatory response. These compounds typically target macrophages, but recent data suggest a role for hepatocytes as well in the generation of immune responses in NASH [39].

Kupffer cells as the initiators of inflammation—As the resident macrophages of the liver, Kupffer cells are uniquely positioned to respond to danger signals from nearby cells as well as those reaching the liver through the portal circulation. Kupffer cells sense and

respond to these molecules through two separate but integrated mechanisms: the Toll-like receptors (TLRs) and the inflammasome. TLRs are a large family of molecules that collectively recognize the gamut of NASH-related compounds (saturated fatty acids, bacterial endotoxins, and DNA from damaged cells). Engagement of these receptors triggers a signaling cascade that activates NF-κB and culminates in the transcription of genes encoding pro-inflammatory cytokines. Studies in experimental animals have demonstrated that TLRs -2, -4 and -9 are all involved in the pathogenesis of NASH [64-67]. Notably, blockade or elimination of these TLRs reduces hepatic inflammation, and in many cases also suppresses hepatic steatosis and fibrosis. This underscores the ability of Kupffer cells to drive hepatic fibrosis as well as feed backward to hepatocytes to enhance steatosis.

Among the cytokines induced by TLR activation in Kupffer cells are TNF and IL-1β. TNF can be readily secreted by Kupffer cells, but IL-1β cannot be secreted until it is first processed by caspase-1. Caspase-1 must itself be converted from an inactive proenzyme to an active form within Kupffer cells; this latter event requires inflammasomes. Interestingly, mice deficient in caspase-1 are protected from experimental NASH [42], in much the same fashion as mice deficient in TLRs. This suggests that for a complete inflammatory response to be mounted in a fatty liver, there must be cooperation among TLRs and inflammasomes.

Inflammasome complexes are typically centered around nucleotide-binding oligomerization domain (NOD)-like receptor proteins (NLRPs) [38]. The one with the greatest relevance to NASH is NLRP3. In Kupffer cells, inflammasome activation and IL-1β secretion can be achieved by treating cells with a SFA plus a TLR-2 or TLR-9 agonist [67]; the same is true if Kupffer cells are exposed to bacterial endotoxin [68] or products released from fatty acid-treated hepatocytes [39]. Overall, the available data indicate that the complex milieu surrounding Kupffer cells in the fatty liver *in vivo* is sufficient to promote the production and processing of cytokines necessary for a robust hepatic inflammatory response, through the integrated actions of TLRs and the inflammasome, enhancing liver injury during NASH.

Downstream consequences of Kupffer cell activation—Once IL-1 and TNF are released by Kupffer cells in the fatty liver, these cytokines stimulate even more fat accumulation within hepatocytes [66, 69]. They can also kill hepatocytes that have been sensitized by steatosis to cytokine-induced injury [46, 66, 70]. In addition, activated Kupffer cells produce chemokines such as CCL2 and RANTES (regulated on activation, normal T cell expressed and secreted) [71]; these compounds amplify hepatic inflammation by recruiting inflammatory monocytes to the liver. Studies indicate that Kupffer cell activation is critical to the initiation of steatohepatitis. Indeed, if Kupffer cells are eliminated from the liver in the early phase of experimental fatty liver disease, monocyte recruitment to the liver is markedly reduced, which limits insulin resistance and overall liver injury [72, 73]. Once circulating monocytes have been recruited to the liver, efforts to curb NASH by depleting Kupffer cells are no longer effective.

Mononuclear cells that invade the injured liver tend to have a more inflammatory phenotype than resident Kupffer cells [74]. These infiltrating mononuclear cells are often described as M1, or pro-inflammatory, as opposed to M2, which are anti-inflammatory [75]. Kupffer cells themselves, however, can also adopt an M1 phenotype upon activation; indeed, the

degree to which this occurs in steatohepatitis may be an important determinant of the ultimate severity of liver injury. In mice, some of the strain-dependent variation in liver damage in alcoholic and non-alcoholic models of steatohepatitis has been attributed to differences in Kupffer cell M1/M2 balance [76, 77]. This has generated interest in factors that govern M1/M2 balance, particularly those that enhance M2 polarization and thus may reduce liver injury in NASH. Candidate molecules include peroxisome proliferator activated receptor-δ (PPARδ) and adiponectin, as well as the cannabinoid receptor CB2 [78-80].

C. Mechanisms of Hepatic Fibrosis in NASH

Liver fibrosis develops in NASH following a sustained period of hepatocyte injury and organ inflammation. This scenario is typical of many chronic liver diseases, and thus it is not surprising that some of the mechanisms promoting liver fibrosis in NASH are shared with other disease processes. The hallmark of hepatic fibrosis is the priming and activation of hepatic stellate cells (HSC) to a myofibroblastic phenotype. One potent stimulus to stellate cell activation is hepatocyte death. HSC are activated by ingestion of fragments of apoptotic cells [81]. This process is believed to occur in fatty livers, as pan-caspase inhibitors, which inhibit hepatocyte apoptosis, suppress hepatic fibrosis in experimental fatty liver disease [82]. Cell death may also trigger fibrosis indirectly in a fatty liver, by first stimulating hepatic inflammation as described above [83]. Indeed, the cytokines TNF and IL-1β, which figure prominently in NASH-related hepatic inflammation, are able to promote liver fibrosis. Notably, TNF and IL-1β do not induce fibrosis by enhancing HSC activation, but instead facilitate HSC survival through the activation of NF-κB [84]. Finally, HSC express TLRs, which makes them a potential target for activation by endotoxin, fatty acids and products of dead cells in the setting of NAFL. Endotoxin and synthetic TLR2 ligands have both been implicated as HSC activators, but saturated fatty acids do not directly stimulate these cells [85, 67]. Overall, many of the same stimuli that promote inflammation in NASH also promote fibrosis, which may explain why fibrosis tends to improve in parallel with inflammation with manipulations designed to reduce experimental NASH [66, 67, 86-88].

It is important to note that some compounds with relevance to obesity and the metabolic syndrome have unique influences on HSC. Leptin, for example, which is abundant in obesity, interacts directly with HSC to stimulate collagen production [89]. Adiponectin, by contrast, which is scarce in individuals with the metabolic syndrome [90], suppresses HSC activation [91]. Thus, the adipokine profile of patients with NAFL/NASH is one that favors hepatic fibrogenesis. Other adipokines including resistin, visfatin and apelin, correlate positively with NASH and liver fibrosis but their roles in HSC activation are less well defined (reviewed in [92]).

GENETIC AND ENVIRONMENTAL FACTORS THAT INFLUENCE NASH

Genetics and the environment add to the complexity of NAFL and NASH. These factors may explain the phenotypic variability of liver disease among populations with seemingly similar behavioral and biological risk profiles for steatosis and steatohepatitis.

Genetic links to hepatic steatosis and NASH

In recent years it has become evident that there is a hereditary predisposition to hepatic steatosis and NASH [93, 94]. Several gene polymorphisms have been linked to NASH, the most notable of which is a C→G polymorphism leading to an I148M substitution in the gene PNPLA3 (patatin-like phospholipase domain-containing 3) [95-97]. PNPLA3 encodes adiponutrin, a protein located in the membrane fraction of hepatocytes and adipocytes. The protein has recently been shown to function as a lysophosphatidic acid acyltransferase (LPAAT), catalyzing the conversion of lysophosphatidic acid to phosphatidic acid in the pathway leading to triacylglycerols [98]. The I148M variant of adiponutrin has enhanced LPAAT activity, which may explain its ability to cause hepatic steatosis. In addition, the variant protein inhibits lipid hydrolysis [99], and in the liver *in vivo*, it enhances fatty acid synthesis, indicating the C→G polymorphism may disrupt hepatic lipid metabolism in several ways [100]. The genetic link between I148M PNPLA3 and hepatic steatosis is so strong, it persists across disparate ethnic groups, even those having dissimilar risk of other metabolic diseases such as diabetes [95]. More importantly, the I148M variant of PNPLA3 is a risk factor not only for steatosis, but for NASH and its complications, including cirrhosis and hepatocellular carcinoma [97, 101-103]. Other genes under study for a connection to hepatic steatosis and NASH are NCAN (neurocan), LYPAL1 (lysophospholipase-like 1), GCKR (glucokinase regulator) and PPP1R3B (protein phosphatase 1, regulatory subunit 3B) [96, 104] (reviewed in [105]).

The gut microbiome as a determinant of hepatic steatosis and NASH

Studies in the last decade have revealed that intestinal microorganisms play an important role in the nutritional status of the host. Gut bacteria, because of their ability to process complex carbohydrates, increase the availability of nutrients for absorption by the host. They can also alter host gene expression, creating a profile that favors fat storage [106, 107]. The gut microbiome is dominated by two bacterial phyla: Bacteroidetes and Firmicutes [108]. Between these two groups, Firmicutes have a greater ability to extract nutrients from the diet [109]. Overeating stimulates the overgrowth of Firmicutes, creating a situation in which dietary excess increases absorptive efficiency and enhances the potential for obesity and its complications [110, 111]. A relative overabundance of intestinal Firmicutes has been documented not only in obese animals [112] but also in overweight humans [110, 113]. Although the gut microbiome definitely influences body weight, its impact on the pathogenesis of NASH is less clear. The microbiome induced by high-energy feeding has been reported to degrade intestinal tight junction proteins [114] and enhance the absorption of endotoxin from the gut (so-called “metabolic endotoxemia”), which could promote fatty liver disease. Furthermore, a research group recently reported that colonization of mice with segmented filamentous bacteria, a strain of Firmicutes that stimulates the production of the pro-inflammatory cytokine IL-17, augments diet-induced NASH [115]. Thirdly, gut bacteria can produce ethanol, which can be absorbed by the intestine and result in potentially hepatotoxic blood ethanol levels even in the absence of ethanol consumption [116]. To date, endotoxin and ethanol have both been detected in the plasma of non-alcoholic subjects with fatty liver disease [117-119]. Few groups, however, have explored whether the gut microbiome differs in humans with NAFL vs. NASH. One report indicates that NASH

patients have a more profound reduction in intestinal Bacteroidetes than subjects with NAFL [120].

Given the putative influence of the gut microbiome on the development of fatty liver disease, researchers have tested probiotics as a treatment for NASH. Studies in animals have been promising, but clinical trials are few in number, and to date they do not uniformly support a benefit of treatment [121, 122].

CONCLUSION

NASH is a disease with roots in obesity and dysfunctional adipose tissue. These initial derangements, fueled by diet and influenced by genetic background, create an environment conducive to the development of hepatic steatosis. Once hepatic steatosis is present, oxidative stress and lipotoxicity can ensue, with further damage arising from gut endotoxin and cytokines. Kupffer cells are master regulators of the process, sensing and reacting to danger signals from hepatocytes, regulating steatosis, recruiting inflammatory cells and signaling stellate cell survival.

Recent research has uncovered exciting new information about NASH pathogenesis, including the importance of the inflammasome and the potential for the gut microbiome to provoke fatty liver disease. Other studies confirm prior suspicions about the role of DNL in the production of excess hepatic triglyceride, and as a result, the importance of dietary carbohydrate in the pathogenesis of NASH. Even genetic studies, specifically those of variant adiponutrin, point to derangements in hepatic lipogenesis as a key risk factor for NASH. These new concepts are reshaping our overall view of the pathogenesis of fatty liver disease and providing new opportunities for disease prevention and treatment.

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ABBREVIATIONS

ApoB	apolipoprotein B
ATF6	activating transcription factor 6
CB2	cannabinoid receptor 2
CCL2	C-C chemokine ligand 2
c-Src	proto-oncogene tyrosine-protein kinase
CYP2E1	cytochrome P450 2E1
DNL	de novo lipogenesis
ER	endoplasmic reticulum
GCKR	glucokinase regulator
HSC	hepatic stellate cells
IL-1β	interleukin-1β
IRE1	inositol-requiring protein 1

JNK	Jun N-terminal kinase
LPAAT	lysophosphatidic acid acyltransferase
LPC	Lysophosphatidylcholine
LYPAL1	lysophospholipase-like 1
MRC	mitochondrial respiratory chain
NAFL	non-alcoholic fatty liver
NASH	non-alcoholic steatohepatitis
NCAN	Neurocan
NLRP	NOD-like receptor proteins
NOD	nucleotide-binding oligomerization domain
PERK	RNA-dependent protein kinase-like ER kinase
PNPLA3	patatin-like phospholipase domain-containing 3
PPARδ	peroxisome proliferator-activated receptor-δ
PPP1R3B	protein phosphatase 1, regulatory subunit 3B
RANTES	regulated on activation, normal T cell expressed and secreted (CCL5)
ROS	reactive oxygen species
SFA	saturated fatty acid
SREBP-1	sterol regulatory element binding protein-1
TCA	tricarboxylic acid cycle
TLR	Toll-like receptor
UPR	unfolded protein response

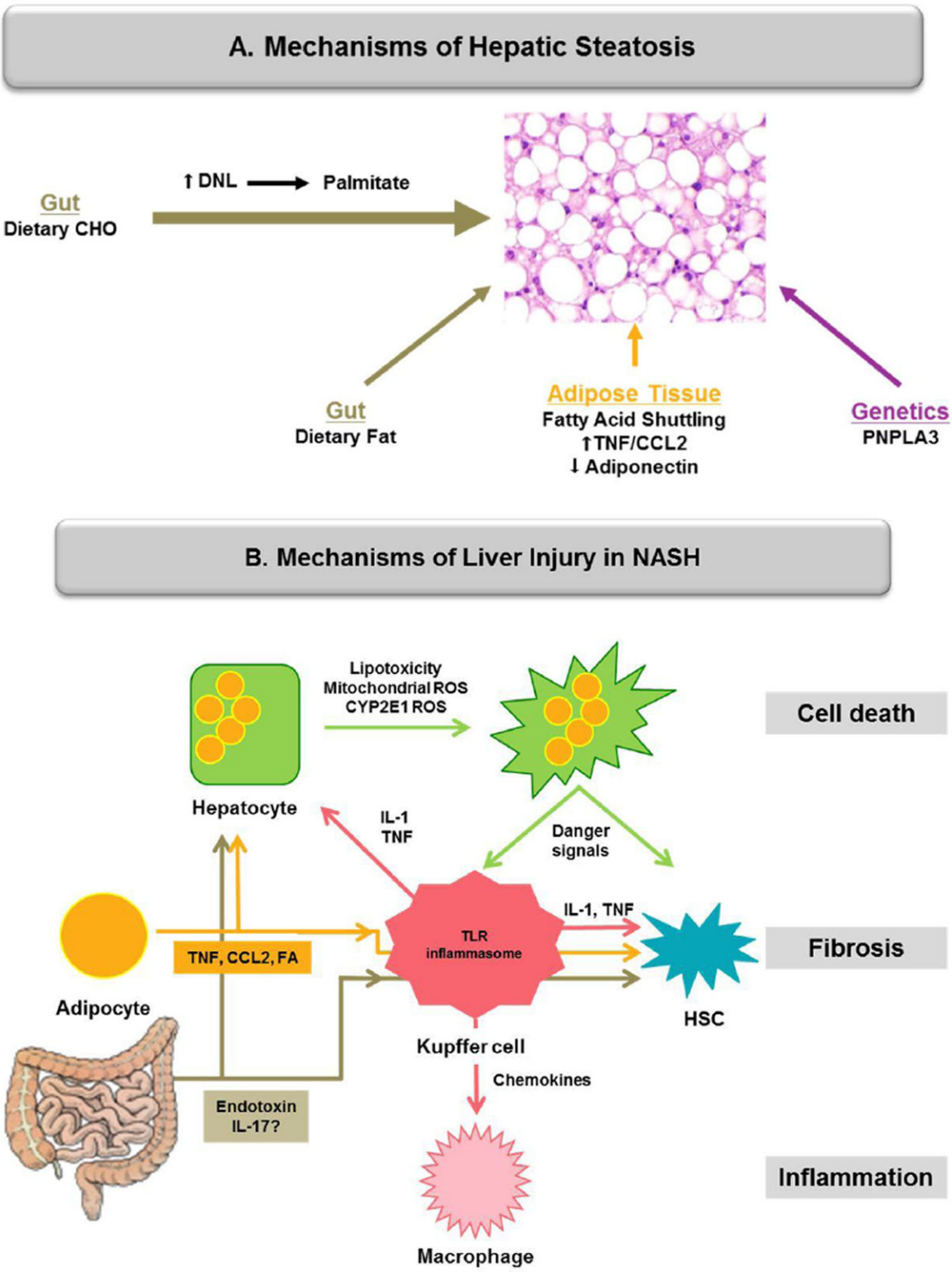

Figure 1. Mechanisms of NASH pathogenesis

A. Mechanisms of Hepatic Steatosis. The fatty acids necessary for triglyceride synthesis in the liver come from three sources: (1) dietary carbohydrate (CHO), which reaches the liver

from the intestine and is converted to fatty acid via *de novo* lipogenesis; (2) dietary fat, which reaches the liver from the intestine; and (3) fatty acids released from adipose tissue, which reach the liver through the circulation. *De novo* lipogenesis is emphasized in the schematic because this pathway is disproportionately up-regulated in persons with NASH [11, 12]. Inflammatory signals from adipose tissue are important regulators of hepatic steatosis: TNF and CCL2, which are induced during obesity, stimulate fat accumulation in hepatocytes, whereas adiponectin, through its down-regulation in obesity, permits fat to accumulation in “ectopic” organs such as liver. Finally, the genetic background of the host plays an important role in the predisposition to and severity of steatosis. Persons inheriting the I148M polymorphism in the PNPLA3 gene (adiponutrin) are at increased risk of steatosis.

B. Mechanisms of Liver Injury in NASH. Liver injury in NASH involves cell death, inflammation and fibrosis. The major events leading to cell death are (a) lipotoxicity, resulting from intracellular accumulation of saturated fatty acids, cholesterol, or other toxic lipids; and (b) oxidative injury, which is the result of ROS production by dysfunctional mitochondria or overactive CYP2E1. Once hepatocytes die they release danger signals, which activate Kupffer cells and HSC resulting in inflammation and fibrosis, respectively; TLRs and the inflammasome are important mediators of these processes. Activated Kupffer cells secrete IL-1 and TNF. These cytokines act on hepatocytes by accentuating steatosis and promoting further cytotoxicity [46, 66, 69, 87]. They can also stimulate hepatic fibrosis by promoting HSC survival [84]. In addition, activated Kupffer cells release chemokines that recruit inflammatory macrophages, continuing the inflammatory cycle [71]. Notably, adipose tissue and the intestine continue to be important to the liver as hepatic steatosis progresses to NASH. Adipocyte-derived fatty acids can activate TLRs and inflammasomes in hepatocytes, Kupffer cells and stellate cells; adipose tissue-derived cytokines can enhance hepatocyte steatosis, accentuate hepatocyte death, and contribute to the inflammatory and fibrotic milieu of the liver. The intestine is a source of bacterial endotoxin, which also signals through TLRs on liver cells promoting a pro-inflammatory, pro-fibrotic state. New data implicate gut bacteria-derived IL-17 as an important mediator of NASH [115].