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# Nonalcoholic Steatohepatitis

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## Abstract

Nonalcoholic steatohepatitis (NASH) has become a major cause of cirrhosis and liver-related deaths worldwide. NASH is strongly associated with obesity and the metabolic syndrome, conditions that cause lipid accumulation in hepatocytes (hepatic steatosis). It is not well understood why some, but not other, individuals with hepatic steatosis develop NASH. The factors that determine whether or not NASH progresses to cirrhosis are also unclear. This review summarizes key components of NASH pathogenesis and discusses how inherent and acquired variations in regulation of these processes impact the risk for NASH and NASH cirrhosis.

## INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is a condition of chronic liver injury and inflammation (hepatitis) caused by excess lipid accumulation in the liver (steatosis) (1). By definition, it is not etiologically related to excess alcohol consumption. NASH is defined histologically and diagnosed by liver biopsy findings (i.e., steatosis plus hepatocyte damage and liver inflammation). It occurs in a subset of individuals with nonalcoholic fatty liver disease (NAFLD), a clinically defined disease entity that broadly encompasses simple hepatic steatosis, NASH, NASH with fibrosis, and NASH-related cirrhosis (1). NAFLD is strongly associated with obesity and, like obesity, NAFLD is a rapidly emerging health concern in many industrialized countries (2, 3). In the United States, about a third of the adult population is estimated to have NAFLD (4).

Most individuals with NAFLD have simple steatosis, which is considered a generally benign condition. However, it is estimated that ∼30% of the patients who have fatty liver may have NASH (5). Unlike simple steatosis, NASH is considered a potentially progressive disorder because hepatocyte damage and liver inflammation may prompt collagen synthesis and deposition (i.e., fibrosis). Liver biopsy series indicate that progressive fibrosis develops in 32–53% of NASH cases, and thus, advanced fibrosis/cirrhosis ultimately ensues in ∼10% of the NAFLD population overall (6, 7). Extrapolation of these data suggests that ∼3% of the general US population has NAFLD-related cirrhosis, making it about five times more prevalent than cirrhosis due to chronic viral hepatitis. Liver fibrosis is the only histologic variable that independently predicts liver-related morbidity, liver-related mortality, and all-cause mortality in NAFLD (8). Individuals with NAFLD and mild to moderate liver fibrosis (stage 1–2) are twice as likely to die of any disease than are NAFLD patients without any fibrosis, and the risk of developing life-threatening consequences of liver disease is >80-fold higher in NAFLD patients with advanced hepatic fibrosis (stage 3–4) than in NAFLD patients with no hepatic fibrosis. Differences in the propensity for liver fibrosis explain why the cumulative incidence of liver-related death was reported to be as high as 18% among patients with NASH versus 3% among those with simple steatosis in an 18-year observational study (9).

In general, NAFLD risk increases with the degree of obesity (10). However, NAFLD risk is also modified by other biophysiological attributes, such as the extent of peripheral versus visceral adiposity, and the degree of insulin resistance in adipose depots and other insulin-sensitive tissues, such as muscle and liver (11, 12). Thus, body size [e.g., body mass index (BMI)] per se is an imperfect predictor of NAFLD risk, and NAFLD can occur in the context of a normal or low BMI. The severity of hepatic damage (i.e., NASH) generally correlates with degree of hepatic metabolic stress, but there is significant interindividual variability regarding NASH outcomes: Liver damage seems to remain relatively stable in most individuals but regresses in some and progresses in others (7). The intensity of hepatic fibrosis generally parallels the degree of liver damage and inflammation (i.e., severity of NASH) (13), but again, not all subjects who have NASH develop advanced hepatic fibrosis. We believe that better understanding of the multiphasic nature of NAFLD pathobiology and unexplained variances at each phase is the key to implementing personalized management for NAFLD patients. In this review, we summarize key components of NASH pathogenesis and discuss how inherent and acquired variations in the regulation of these processes affect the risk of NASH and NASH fibrosis. Our discussion focuses on variability in adaptive mechanisms to metabolic stress and tissue repair processes as possible contributors to the heterogeneity of NASH and susceptibility to liver fibrosis. Because recently discovered genetic factors associated with different disease phases of NAFLD have been covered in a recent excellent review (14), they are not covered here.

## HEPATIC STEATOSIS, EXCESS LIPID ACCUMULATION IN THE LIVER

Excessive lipid accumulation in hepatocytes (fatty liver, hepatic steatosis) is the hallmark of NAFLD. Steatosis can occur in both sexes at any point in life. However, the risk factors for fatty liver vary with both sex and age (15, 16), explaining observations that NAFLD prevalence differs in men and women, and in young and old individuals (3, 17). NAFLD is less common in women than in men during their adolescence and reproductive years, but this sex difference is eliminated by menopause, suggesting female sex hormones protect women from steatosis (15). Postmenopausal women treated with hormone replacement therapy have lower liver enzymes than women who do not receive hormone replacement (18), indicating that female sex hormones might also be protective against NASH. Although NAFLD prevalence generally correlates with BMI, this association is weak in older populations regardless of sex (16, 19), demonstrating that aging also impacts susceptibility to steatosis (20). In aggregate, these observations suggest that the amount (and perhaps the types) of lipids that accumulate in hepatocytes are differentially regulated according to sex, reproductive status, and age, and emphasize that these variables need to be considered when devising strategies to treat steatosis and prevent NASH. Interventions must be individualized to correct specific lipid homeostatic processes that have become dysregulated. Success requires better understanding of the mechanisms that control lipid metabolism, as well as improved diagnostic markers to identify subjects who are struggling to maintain lipid homeostasis.

Hepatic steatosis is a common manifestation of very different processes. It can result from excessive hepatocyte uptake of lipids from the systemic and/or portal circulations, increased de novo synthesis of lipids by hepatocytes, reduced hepatocyte degradation of lipids, or decreased export of lipids from hepatocytes. More than one of these mechanisms might be operative concomitantly, with the combined effects dictating the net hepatocyte content of particular lipid moieties at given points in time. Thus, hepatic steatosis is a heterogeneous condition. Current clinical modalities to detect and quantify hepatic steatosis (e.g., liver biopsy and noninvasive imaging) mainly measure hepatic triglyceride content, and hence, these approaches obscure qualitative and quantitative differences in other types of lipids (e.g., fatty acids, diacylglycerols, phospholipids, sphingolipids, cholesterol) that might also be present. Very little is known about how factors that influence NAFLD susceptibility, such as sex, reproductive status, age, ethnicity, diet, and physical activity, impact the hepatic content of these other lipids. This knowledge gap likely has clinical relevance, since the severity of hepatic steatosis (i.e., hepatic triglyceride content) per se does not predict the risk for NASH, liver fibrosis, or liver-related morbidity/mortality in natural history studies of humans with NAFLD (21). Indeed, hepatic triglyceride biosynthesis protects obese mice from NASH (22), and preclinical research demonstrates that certain types of fatty acids are significantly more hepatotoxic than others (23).

Chronic positive energy balance (i.e., calorie intake in excess of expenditure) is thought to play a significant role in the pathogenesis of obesity-related hepatic steatosis. Hepatocytes use surplus calories to generate triglycerides, which are exported from the liver and stored primarily in peripheral white adipose tissues that are localized either subcutaneously or viscerally. The former functions as the primary fat-storing depot and is the safest place to store excess energy (24). When the storage capacity of white adipose depots becomes saturated, excess energy is redistributed, and lipids accumulate in normally lean tissues such as muscle, pancreas, liver, and heart (24). This "lipid overflow" model is currently considered the basis for lipotoxicity and obesity-related comorbidities, i.e., cardiovascular disease, diabetes, insulin resistance, metabolic syndrome, and NAFLD (24, 25). Briefly, the lipid overflow model is based on evidence that both inherited and acquired factors dictate adipose depot capacity to accommodate excess energy. As their storage capacity becomes saturated, adipose depots generate factors to defend themselves against

further energy accumulation. However, this process has potentially detrimental consequences for other tissues because it increases exposure to fatty acids that would otherwise be stored safely in adipocytes as triglyceride, as well as to adipose-derived factors that can negatively affect energy homeostasis in cells other than adipocytes. Hence, lipid overflow associated with obesity-related metabolic syndrome and hepatic steatosis is reminiscent of that which results from generalized or partial lipodystrophy (25). These latter conditions illustrate that it is important to consider not only BMI but also the question of energy surplus, and whether there is sufficient storage capacity to accommodate the energy excess in peripheral adipose depots, when assessing risk for hepatic steatosis. This logic probably explains why hepatic steatosis is absent in some morbidly obese bariatric patients and why other individuals who have a normal BMI demonstrate severe hepatic steatosis (26, 27).

As mentioned, there is now robust and consistent preclinical and clinical evidence that the severity of hepatic steatosis per se does not correlate with (or predict) steatosis-associated liver damage (i.e., lipotoxicity) (21, 22, 28). Rather, processes that determine hepatic lipotoxicity seem to be more nuanced, likely reflecting complex interactions among multiple factors that regulate how hepatocytes respond to stresses that challenge their ability to maintain energy homeostasis when excess energy can no longer be diverted to adipose depots for safe storage. Consequently, there are likely to be diverse proximal mediators of lipotoxicity, including particular lipid moieties and their metabolic precursors/products, as well as various other signaling molecules that are generated to cope with or compensate for changes in energy flux. Present gaps in knowledge about this biology prevent diagnosis of steatotic livers that are at eminent risk for lipotoxicity. Rather, lipotoxicity is only apparent "after the fact," when NASH is evident.

## NASH, STEATOSIS-RELATED LIPOTOXICITY, AND LIVER DAMAGE

Lipotoxicity can be lethal for hepatocytes, and hepatocyte death is the primary factor that distinguishes NASH from simple hepatic steatosis. Although hepatocyte death initiates NASH, the histologic manifestations of NASH largely reflect the liver's efforts to replace hepatocytes that were killed by lipotoxicity. Dying hepatocytes generate and release damage-associated molecular signals that telegraph their impending demise to neighboring cells, triggering a cascade of wound-healing responses. The process initially activates resident stromal cells and recruits bone marrow–derived cells. These cells then interact to clear dead debris and remodel the hepatic microenvironment to support the growth and differentiation of cells that will ultimately replace hepatocytes that died. Hence, hepatic wound healing requires inflammation, remodeling of the hepatic vasculature and matrix, and outgrowth of liver progenitors. NASH is the net histologic manifestation of hepatocyte injury/death and the resultant wound-healing process. In general, the intensity of the wound-healing response parallels the scope of hepatocyte lethality. Because lipotoxicity drives hepatocyte death in fatty livers and dying hepatocytes initiate NASH, interventions that aim to prevent or treat NASH must abrogate lipotoxicity. Thus, research has focused on delineating the mechanisms for, and consequences of, hepatocyte lipotoxicity.

Hepatic lipotoxicity is the end result of overwhelming cellular stress that results when hepatocytes are challenged to cope with energy excess that cannot be accommodated by normal energy storage depots (i.e., adipocytes). Viewed from this perspective, NASH is part of a systemic disease process, and it is unlikely that hepatocyte accumulation of a single molecular species (e.g., one particular type of lipid) can be blamed for hepatic lipotoxicity. Rather, it is more reasonable to presume that lipotoxicity evolves as the entire body unleashes a number of overlapping adaptive responses to compensate for energy excess. Because some of these alternatives

might have adverse consequences for hepatocytes, such as endoplasmic reticulum (ER) stress or oxidative stress, hepatotoxicity ensues unless hepatocytes can mobilize mechanisms that permit them to withstand the new stresses. However, successful adaptation to these threats may inadvertently increase their vulnerability to other challenges, promoting hepatocyte death despite amelioration of the initial stress. Given this complexity, it is not surprising that the safest therapeutic approach for NASH is to eliminate the most common exogenous causes of net energy excess: overeating and sedentary lifestyle. Many previous interventional studies showed that caloric restriction and regular exercise can improve not only hepatic fat content, but also entothelial functions, cardiac diastolic functions, and insulin sensitivity. Further, significant weight reduction (7–10%) can lead to NAFLD/NASH remission and regression of fibrosis (29). However, the success of this seemingly straightforward strategy varies among individuals, and in any given individual across the lifespan, because of inherent and acquired differences in the multiple interacting mechanisms that control the efficiency of energy utilization at the cellular level. Extension of this logic explains why it has proven even more difficult to predict the net impact of pharmacologic interventions that attempt to interrupt or redirect adaptive responses while systemic energy excess is ongoing.

For the purpose of this review, we break down NASH pathobiology into two phases: hepatocyte damage (lipotoxicity) and tissue repair (inflammation/fibrosis). In this section, we discuss three general processes that are particularly relevant to hepatocyte lipotoxicity: cellular metabolic stress, cellular adaptive mechanisms, and cell death.

## Hepatocyte Metabolic Stress

Metabolic stress that develops in the context of hepatic steatosis is mainly driven by chronic positive energy balance, which stimulates adaptive production of cytokines (e.g., tumor necrosis factor alpha) and adipokines (e.g., resistin). These reduce adipocyte sensitivity to adipogenic hormones that normally promote fat storage in adipocytes (e.g., insulin). Cytokines and adipokines also suppress the production of, and reduce sensitivity to, antiadipogenic factors that normally reduce the drive to store fat in adipose depots (e.g., leptin and adiponectin). Like adipocytes, hepatocytes express receptors for these cytokines, adipokines, and adipogenic hormones, and thus, the combined actions of these factors not only increase free fatty acid influx to the liver (thereby increasing hepatic metabolic load) but also globally impact hepatocyte viability by altering hepatic intermediary metabolism and stress responses, particularly ER stress and oxidative stress. Because free fatty acids that are directly delivered to the liver derive more from visceral than from peripheral adipose depots (30), situations that restrict peripheral adiposity and promote visceral adiposity (i.e., lipodystrophy) have been associated with NASH, as well as NASH-related fibrosis (31, 32). Interestingly, the relationship of regional adiposity (visceral versus peripheral) to NASH-related fibrosis is influenced by sex and menopausal status, suggesting heterogeneity of NASH pathobiology among subpopulations (31). As mentioned, the capacity to store lipids in peripheral, as opposed to visceral, adipose tissue is influenced by sex hormones. In general, fat storage is biased toward visceral adipose depots in men and postmenopausal women (33, 34). This results in a somewhat lipodystrophic phenotype and may partly explain why risk for NASH differs between sexes and between pre- and postmenopausal women (31, 35). Prolonged use of protease inhibitor–containing, highly active antiretroviral therapy in HIV-infected patients also promotes lipodystrophy, NASH, and NASH-related fibrosis (36). Preclinical studies (37) and emerging clinical data (38) suggest that inhibitors of Raptor/mTORC1 may enhance visceral adiposity, providing a possible mechanism to explain why NASH commonly recurs after liver transplantation. Acquired lipodystrophy also develops in autoimmune disorders that display

certain complement abnormalities (39). These examples are illustrative of processes that introduce variability into the outcomes of chronic energy surplus, particularly the liver's ability to withstand chronic metabolic stress and escape lipotoxicity to avoid NASH.

Another key variable that determines hepatic lipotoxicity is insulin sensitivity. As mentioned, insulin is the major adipogenic hormone in adulthood, and adipocytes modulate their production of various factors that control their sensitivity to insulin in order to prevent overly exuberant adipogenesis during chronic energy surplus. The resultant insulin resistance extends beyond adipocyte depots and triggers hyperinsulinemia, eventually leading to exhaustion and apoptosis of pancreatic beta cells, causing glucose intolerance and diabetes. While present, hyperinsulinemia also provides a further stimulus for production of the adipogenesis regulators whose "off-target" actions promote hepatocyte lipotoxicity when energy surplus and adipose insulin resistance increase hepatocyte exposure to free fatty acids. Indeed, diabetic individuals are more likely to have NASH and NASH-related fibrosis than nondiabetics. Thus, insulin-sensitizing interventions (e.g., pioglitazone) would seem to be beneficial for preventing both diabetes and NASH (albeit potentially worsening adiposity). Paradoxically, however, preclinical and clinical data suggest that improving systemic and/or hepatic insulin sensitivity does not always improve NASH. In some rodent models of NAFLD, heightened insulin sensitivity associates with NASH exacerbation, worse liver fibrosis, and increased risk of liver cancer (40–42). Natural history studies of human NAFLD demonstrate that the severity of insulin resistance per se does not independently predict NASH or poor liver-related outcomes (8). The inherent and acquired factors that modify the effects of insulin signaling in NAFLD are poorly understood but likely to be very important modulators of NASH susceptibility and progression in humans.

## Cellular Adaptive Mechanisms

As discussed above, influx of fatty acids into hepatocytes increases in obesity and insulin-resistant states because these conditions increase the concentration of free fatty acids in the circulation, and hepatocytes' uptake of fatty acids is strictly dependent on their exposure to fatty acids (43). Thus, obesity and insulin resistance force hepatocytes to adapt to an increased supply of energy substrate. Hepatocytes dispose of fatty acids by esterifying them to generate triglycerides or oxidizing them in mitochondria and peroxisomes (14). Although all of these processes are highly efficient, they may not be able to keep pace with fatty acid influx when influx rates are consistently high and/or the disposal processes themselves become defective. All of the steps in fatty acid esterification and oxidation are regulated by inherited and exogenous factors, and thus, the net content and character of fatty acids that accumulate in hepatocytes are highly variable among individuals and within an individual at different times. Emerging evidence is revealing the significance of qualitative variations in accumulated fatty acids. For example, hepatic accumulation of certain saturated fatty acids has been demonstrated to provoke hepatocyte lipotoxicity by activating caspases that promote hepatocyte apoptosis (28, 44). Conversely, the conversion of saturated into monounsaturated fatty acids not only fuels hepatocyte triglyceride synthesis but also inhibits hepatocyte degradation of endogenous cannabinoids (45). The latter are released into the circulation and ultimately stimulate appetite and promote obesity and insulin resistance (46). In mouse models of NAFLD, genetic approaches or dietary modifications that inhibit the activity of stearoyl coA desaturase (the rate-limiting enzyme for generating monounsaturated fatty acids from saturated fatty acids) promote hepatocyte lipotoxicity, NASH, and NASH-related fibrosis despite inhibiting obesity and improving insulin resistance (47, 48). These data illustrate how the outcomes of increased hepatic fatty acid exposure are significantly impacted by differences in adaptive responses to fatty acid excess.

The roles of fatty acid oxidation in hepatocyte lipotoxicity have been the subject of much research, and that body of work has been beautifully detailed in recent reviews (14, 49). Here, we emphasize the significance of factors that modulate the efficiencies of hepatocyte fatty acid oxidation and mechanisms that cope with by-products of fatty acid oxidation because the combinatorial actions of these factors dictate ultimate susceptibility to hepatic lipotoxicity (i.e., NASH). For example, dietary factors such as carnitine bioavailability influence fatty acid import into mitochondria for beta oxidation (50). Similarly, drugs that expand perioxisomal mass (e.g., fibrates) increase the peroxisomal contribution to net fatty acid clearance and could be beneficial in patients with NASH, although robust clinical data are currently lacking (51). It is noteworthy that the therapeutic action of peroxisome proliferator-activated receptor alpha (PPAR-$\alpha$) agonists on obesity and fatty acid oxidation depends on sex and estrogens (52). The sex- and estrogen- related differences may drive some of the heterogeneity in treatment response to fibrates.

Mitochondrial and peroxisomal oxidation of fatty acids generates reactive oxygen species (ROS), and this stimulates a myriad of adaptive responses to constrain oxidative stress. These antioxidant defenses are hindered by nutritional deficiencies or changes in the intestinal microbiome that limit availability of choline (53); by factors such as aging and dietary cysteine, which influence the production of hepatic glutathione (54); and by sex- and menopause-related factors that influence choline metabolism (55). Diet, medications, and obesity itself also modulate the intestinal microbiome, as well as intestinal barrier function, which in turn influences hepatic exposure to gut-derived bacterial products. These products, such as lipopolysaccharide (LPS) and bacterial DNA, engage innate immune receptors on liver cells to activate the inflammasome and other downstream signaling mechanisms that generate ROS (56, 57). When ROS production exceeds ROS detoxification chronically, organelles that oxidize fatty acids are damaged. This not only impairs further fatty acid oxidation but also globally impairs cellular energy production while increasing exposure to ROS that escape from the damaged organelles. Factors that regulate autophagy (e.g., age, nutritional status, and certain drugs) control clearance of damaged mitochondria and protect cells during adaptive stress responses (58). Thus, factors inhibiting such mechanisms may influence the susceptibility of hepatocytes to lipotoxicity (59). ROS accumulation and related changes in autophagy and cellular energy homeostasis also modulate protein synthesis and degradation in the ER. Such cellular dysadaptation engenders chronic ER stress over time and also promotes lipotoxicity (60).

The inherent interdigitation of the multiple processes that influence, and are impacted by, fatty acid oxidation affords hepatocytes many opportunities to evade lipotoxicity, and hence, NASH is a relatively infrequent sequela of hepatic steatosis. However, the previous discussion also illustrates the heterogeneity of some of the key mechanisms that regulate hepatic lipotoxicity and underscores the challenges that this heterogeneity poses to developing interventions that are uniformly effective for preventing or treating NASH.

## Cell Death

Hepatocytes that are lethally damaged by lipotoxicity variably activate different mechanisms that result in cell death, including apoptosis, necroptosis, pyroptosis, and necrosis (61). Detailed discussion of these pathways exceeds the scope of this review. Here, we merely wish to emphasize emerging evidence that type of hepatocyte death might be an important variable in the development and progression of NASH. It has long been known that dying cells release damage-associated molecular signals that activate neighboring cells to generate local inflammatory responses (57). Further, early work showed that phagocytosis of dead cell debris (e.g., apoptotic bodies) stimulates hepatic stellate cells to transdifferentiate into fibrogenic myofibroblasts and Kupffer cells

to generate death ligands and tumor necrosis factor alpha (62). Both findings are consistent with evidence that the severity of hepatic inflammation and fibrosis generally parallels the extent of hepatocyte death in various chronic liver diseases, including NASH (13). However, the histologic characteristics of NASH are also distinctive, allowing pathologists to differentiate NASH from other types of chronic hepatitis.

In general, demonstration of swollen hepatocytes with cytoskeletal damage, dubbed ballooned hepatocytes (63), is required to diagnose steatohepatitis (64). Biopsy studies of human NASH have established highly significant correlations between the numbers of ballooned hepatocytes and the severity of liver fibrosis (13). Indeed, ballooned hepatocytes are often encircled by a thin rim of fibrous matrix, and such pericellular/perisinusoidal fibrosis (also dubbed "chicken wire" fibrosis) is another histologic feature that distinguishes steatohepatitis from other types of chronic hepatitis (64). Ballooned hepatocytes are relatively resistant to lipoapoptosis because they are deficient in caspase 9 (65), but they exhibit ER stress and have activated caspase 2 (44). ER stress and caspase 2 (66) have been linked to pyroptosis, a type of cell death that is dependent on NLRP3 inflammasome assembly (61, 67). There is new evidence that byproducts of intermediary metabolism control pyroptosis in macrophages (68), and two factors that are known to be increased in livers of patients with NAFLD (LPS and oxidized phospholipids) induce NLRP3 inflammasome-mediated pyroptosis in certain cell types (69).

In aggregate, these observations raise the intriguing possibility that ballooned hepatocytes may be undergoing pyroptosis. Further research is needed to clarify this issue, as well as its implications for the resultant development of steatohepatitis. Pyroptosis has already been shown to cause release of intracellular ATP, a potent inflammatory response mediator (61).

## TISSUE REPAIR, INFLAMMATION, AND FIBROSIS IN NASH

NASH results when lipotoxicity becomes lethal to hepatocytes. In addition to injured and dying hepatocytes, livers with NASH exhibit wound-healing responses (also known as regenerative activity), as evidenced by variable accumulation of repair-related cell types that are relatively inconspicuous in healthy adult livers, including inflammatory cells, myofibroblasts, and liver progenitors. Wound-healing efforts normally persist as long as liver injury lingers, but these repair responses must be downregulated to complete the regenerative process once the injury heals. Persistence or progression of NASH occurs when there is unrelenting lipotoxicity and/or dysregulated wound healing. This section discusses variables that regulate key components of liver repair.

### Hepatic Inflammation

Hepatocyte lipotoxicity evokes a primal sterile inflammatory response that aims to eliminate damaged cells and promote tissue repair. This process is triggered by dying hepatocytes, which release various signals that activate resident immune cells and recruit bone marrow–derived cells. In NAFLD, these new lipotoxicity-initiated demands are introduced after the immune system has already confronted other obesity-related challenges. For example, obesity alters production of/sensitivity to immunomodulatory cytokines and adipokines, modifies the bioavailability of energy substrates for immune cells, and increases immune cell exposure to gut-derived inflammatory mediators (70–72). All of these forces may gradually reconfigure the immune system and bias its responses to secondary challenges imposed by hepatocyte death.

It is not well understood how obesity-related immune dysregulation impacts the pathogenesis of NASH, but studies in ob/ob mice suggest that it might be important. Ob/ob mice are obese,

insulin resistant, and diabetic owing to a genetic deficiency of leptin. They also exhibit thymic hypoplasia, relative hepatic depletion of natural killer T cells (NKT cells), and macrophage dysfunction, as well as an intestinal dysbiosis that increases their systemic exposure to LPS (73–76). Despite longstanding obesity, insulin resistance, type 2 diabetes, and endotoxemia, ob/ob mice have very mild NASH and do not develop progressive liver fibrosis, either spontaneously or when challenged by exogenous fibrogenic agents (77). This resistance to liver fibrosis might be due, in part, to deficient hepatic NKT cell populations, because liver-resident NKT cells are important sources of fibrogenic cytokines (78). Evidence that hepatic NKT cells are relatively depleted in humans with simple steatosis (79) but accumulate in the livers of humans with NASH-related cirrhosis (80), plus the fact that genetic defects that block NKT cell accumulation protect mice with diet-induced NASH from developing liver fibrosis (78), suggests that interindividual variation in immune responses is another key liver disease modifier in NAFLD.

## Liver Fibrosis

Natural history studies have identified liver fibrosis severity as the only independent predictor of liver-related morbidity and mortality in human NAFLD (8). In retrospect, this finding might have been anticipated because organ failure is generally accepted to result when functional parenchyma is replaced by scar. However, the real breakthrough related to this discovery is that it has helped to identify signaling pathways and cell types that merit particular attention from researchers and clinicians who hope to improve NASH outcomes. The mechanisms and cell types that regulate fibrosis are highly conserved among tissues, and this has led to a consensus that myofibroblasts derived from tissue-resident pericytes are the major producers of fibrous matrix in most chronic tissue injuries (81). Further, it is now known that these pericytes generally resemble mesenchymal stem cells and exhibit Hedgehog pathway activity (82). Hepatic stellate cells (HSCs) are liver-resident pericytes and have long been recognized as major producers of collagen matrix in NASH (81). Hedgehog pathway activation stimulates HSCs to become proliferative myofibroblasts (MF-HSCs), and inhibiting Hedgehog activity in MF-HSCs causes them to revert to a more quiescent, less fibrogenic phenotype that is more typical of HSCs in healthy adult livers (83). Although MF-HSCs are critically involved in the pathogenesis of cirrhosis, they are also necessary for injured livers to regenerate effectively because they orchestrate immune, vascular, and progenitor responses that are necessary for liver repair (84). Unlike cirrhotic livers, which accumulate large numbers of MF-HSCs, livers that are regenerating normally exhibit only transient expansion of MF-HSC populations (85). This suggests that Hedgehog ligands and other factors that control fate decisions in HSCs are critical determinants of NASH outcomes. Although much remains to be learned about this issue, studies in NAFLD patients and animal models of NAFLD support the concept. Hepatic expression of Hedgehog ligands and Hedgehog pathway activity are very low in NAFLD patients with simple steatosis, higher in NAFLD patients with NASH, and highest in NASH patients with cirrhosis (86). Ballooned hepatocytes in livers with NASH are major producers of Hedgehog ligands (87), and these cells are surrounded by Hedgehog-responsive myofibroblasts and collagen fibrils (88). Interventions (such as vitamin E) that reduced lipotoxicity and decreased hepatocyte ballooning suppressed Hedgehog ligand production, reduced hepatic accumulation of Hedgehog-responsive myofibroblasts, and improved NASH in a recent clinical trial (89). Enforced overexpression of Hedgehog ligand in hepatocytes was sufficient to induce liver injury and fibrosis in mice (90).

The Hedgehog pathway is strongly regulated by lipids (91), and conversely, Hedgehog signaling is a major regulator of adiposity (92) and glucose utilization (93), suggesting that interindividual variation in Hedgehog signaling might contribute to variability in extrahepatic (as well as liver)

outcomes of the metabolic syndrome. A recent report demonstrating predisposition to visceral adiposity, premature coronary artery disease, and type 2 diabetes in kindreds with an activating polymorphism in a Hedgehog pathway inhibitor supports this concept (94). Interestingly, Hedgehog signaling suppresses PPAR-γ, a master transcriptional regulator of adipocyte differentiation and pharmacologic target for antifibrotic and insulin-sensitizing agents (95). Hedgehog also interacts with the Notch, Wnt, and Hippo/Yap pathways, as well as the insulin-like growth factor axis (96–99), each of which is a well-established regulator of growth and differentiation. Emerging evidence indicates that all of these pathways regulate cell fate decisions, at least in part, by modulating intermediary metabolism (99, 100). More research is needed to clarify how variability in these proximal mediators of cellular energy homeostasis might be exploited to improve prevention and treatment of NASH.

## CONCLUSION

The risk of disease progression and negative outcomes in NASH is multifactorial. The heterogeneity of NASH reflects variability in exposure and response to metabolic stress, susceptibility to hepatocyte lipotoxicity, and differences in repair-response efficacy. Clinicians as well as researchers need to consider this heterogeneity when attempting to stratify NAFLD populations into subgroups with low or high risk of bad liver outcomes and to determine therapeutic plans so that maximally effective management strategies can be implemented. We need a better understanding of the biological variances of these NAFLD modifiers in order to design and implement effective (and more personalized) NASH therapies.

## DISCLOSURE STATEMENT

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