22 Adipokines in Non-Alcoholic Fatty Liver Disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of clinicopathological conditions in patients who do not consume excessive amounts of alcohol; these conditions are characterized by hepatic steatosis with or without other pathological changes observed in liver biopsy. The pathogenesis of NAFLD and its progressive form (non-alcoholic steatohepatitis [NASH]) appears to be multifactorial and is the subject of intense investigation. Increasing evidence indicates that the pathogenesis of NAFLD and NASH is hastened by a disturbance in adipokine production. Decreased serum adiponectin and increased tumor necrosis factor-α, which are characteristic of obesity, appear to contribute to the development and progression of NASH. The role of leptin in the pathogenesis of NASH remains controversial and the involvement of serum resistin is primarily documented only in animal models, which may or may not be applicable to the human form of NAFLD. Finally, other adipokines such as vaspin, visfatin, and apelin may play important roles in the pathogenesis of NASH and require further investigation.

Key Words: Non-alcoholic fatty liver disease (NAFLD); non-alcoholic steatohepatitis (NASH); oxidative stress; insulin resistance; adiponectin; leptin; resistin; TNF-α; vaspin; visfatin; apelin.

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of clinicopathological conditions characterized by significant lipid deposition in the liver parenchyma of patients who do not consume excessive amounts of alcohol *(1,2)*. At one end of the NAFLD spectrum is steatosis alone ("simple steatosis"), and at the other end are non-alcoholic steatohepatitis (NASH), NASH-related cirrhosis, and hepatocellular carcinoma. The distinction between steatosis alone and NASH can be made only by liver biopsy. NASH is characterized by hepatic steatosis and by evidence for hepatocyte ballooning degeneration, lobular inflammation, and occasionally, Mallory hyaline or sinusoidal fibrosis *(3)*. NASH and steatosis alone have differential risk for progression *(3)*.

Estimates of the prevalence of NAFLD are high and are expected to increase with the global epidemic of obesity. Recent studies suggest that up to 10 to 24% of the general population and 50 to 90% of obese individuals are affected by NAFLD *(2)*. The prevalence of histologically confirmed NASH is estimated as 1.2 to 4%; in morbidly obese patients it is much more common, with estimates ranging from 20 to 47% *(4)*. NAFLD patients have higher-than-average mortality rates (standardized mortality ratio = 1.34) *(5)*.

From: *Nutrition and Health: Adipose Tissue and Adipokines in Health and Disease*
Edited by: G. Fantuzzi and T. Mazzone © Humana Press Inc., Totowa, NJ

Patients with steatosis alone rarely progress to cirrhosis, whereas 10 to 25% of those with biopsy-proven NASH can progress to cirrhosis *(1,3,4)*. In fact, most patients with cryptogenic cirrhosis seem to have "burned-out NASH" that might also cause hepatocellular carcinoma (HCC) *(6)*. The major risk factors for progression in NASH are the presence of type 2 diabetes, obesity, metabolic syndrome, and elevated aminotransferase, and histological features of ballooning degeneration of hepatocytes and Mallory's hyalines *(4,7,8)*. Ultrasound and other noninvasive modalities can only detect steatosis, and are unable to distinguish NASH from steatosis alone or detect hepatic fibrosis *(9)*.

Of the many treatment strategies currently in use, none is proven to be effective for NASH *(10)*. Treatment strategies include modifying the clinical conditions associated with NASH, such as type 2 diabetes mellitus, hyperlipidemia, and obesity *(2)*. Pharmacological interventions for NASH include the use of ursodeoxycholic acid (UDCA), clofibrate, betaine, N-acetylcysteine, gemfibrozil, atorvastatin, thiazolidinedione, pentoxyfillin, and vitamin E *(2,11,12)*. None of these treatments is capable of preventing NASH progression.

2. PATHOGENESIS OF NASH

The pathogenesis of NASH appears to be multifactorial and is the subject of intense investigation. Suggested theories include the influences of abnormal lipid metabolism and the production of reactive oxygen species (ROS), increased hepatic lipid peroxidation, stellate cell activation, and abnormal patterns of cytokine production, promoting liver injury and fibrosis *(13)*.

The "two-hit hypothesis" of NASH pathogenesis suggests that the first "hit" is the accumulation of excessive fat in the hepatic parenchyma *(4,14)*. This first step has been linked to insulin resistance (IR), which is consistently observed in patients with NAFLD *(13)*. Clinical features of metabolic syndrome (obesity, diabetes mellitus, or hypertrigyceridemia) are commonly observed in patients with NAFLD *(1,17)*. Furthermore, unexplained elevations in alanine aminotransferase (ALT) levels in individuals with metabolic syndrome suggest that NAFLD is the hepatic manifestation of this syndrome *(18)*. Additionally, patients with NAFLD with more "severe" forms of IR are at even greater risk of progressive liver disease *(4,19)*. Animal models of NAFLD also have IR, and the use of the insulin-sensitizing agent, metformin, reverses hepatic steatosis *(16)*.

The second "hit" leading to the development of the progressive form of NAFLD involves oxidative stress. In steatotic livers, an imbalance between pro-oxidant and antioxidant processes results from the induction of microsomal CYP2E1, peroxisomal β-oxidation of fatty acids (FA), release of cytokines from activated inflammatory cells, changes in adipokine levels, or other unknown factors *(4,13,20)*. NAFLD-related oxidative stress may be linked to mitochondrial dysfunction, as mitochondria are the major source of ROS in living cells *(19)*. ROS, in turn, increases the peroxidation of membrane lipids that induce the production of proinflammatory cytokines and activate stellate cells, leading to hepatic fibrogenesis *(4,13)*. Both the first and second hits may involve changes in circulating levels of various pro- and anti-inflammatory cytokines and adipokines.

3. ADIPOSE TISSUE, ADIPOKINES, AND NAFLD

White adipose tissue produces and releases a variety of proinflammatory and anti-inflammatory factors, including adipokines (leptin, adiponectin, resistin, apelin, vaspin,

visfatin, and zinc-α2-glycoprotein), cytokines (such as tumor necrosis factor [TNF]-α and interleukin [IL]-6), and chemokines *(21)*. In addition to adipocytes, white adipose tissue contains several other cell types, including macrophages and monocytes. It is likely that macrophages are retained within adipose tissue in response to both monocyte chemoattrative protein (MCP)-1 and macrophage migration inhibitory factors released by adipocytes in amounts proportional to body mass index (BMI) *(22)*. Cytokines produced by adipose tissue contribute to the increased systemic inflammation associated with obesity *(23)*. The exact contribution of each component of white adipose tissue in the "proinflammatory" state of obesity is not entirely clear. Some studies indicate that more than 90% of the adipokines released from adipose tissue (except for adiponectin and leptin) originate from the nonfat cells embedded in the extracellular matrix *(24)*. In addition, some adipokines (e.g., resistin and adiponectin) are also produced elsewhere in the body *(25)*. Together, these findings suggest that serum adipokine concentrations represent secretions by various cells, including adipocytes. It is increasingly clear that adipokines play an important role in the pathogenesis of NAFLD.

4. ADIPOKINES IN THE EXPERIMENTAL MODELS OF NAFLD

Common experimental models of NAFLD include mice or rats fed high-fat or high-carbohydrate diets, or mice that exhibit a genetic deficiency in leptin, a satiety factor *(15)*. These animal models spontaneously develop steatosis, and some progress to steatohepatitis.

Animal models of NAFLD point to adipokine and cytokine abnormalities in the pathogenesis of NAFLD. For example, leptin-deficient *ob*/*ob* mice are important animal models of NAFLD because they are obese, insulin-resistant, hyperglycemic, and hyperlipidemic. Similarly, leptin receptor-deficient *fa*/*fa* rats and *db*/*db* mice are phenotypically similar to *ob*/*ob* mice, with the addition of hyperleptinemia. It is noteworthy that NAFLD occurs in both leptin-deficient and hyperleptinemic animals with impaired leptin signaling. However, leptin restoration leads to NAFLD reversal in leptin-deficient animals *(15)*.

TNF-α is another important cytokine involved in the pathogenesis of NAFLD; serum levels are high in all animal models of NAFLD. Nonetheless, its origin (i.e., adipocytes themselves or monocytes and macrophages) is not entirely clear. In NAFLD, the lipotoxic effects of excess fat may enhance TNF-α release. Once initiated, this vicious cycle of NFκB/TNF-α becomes self-perpetuating *(15)*. It seems that chronic exposure to TNF-α promotes the accumulation of inflammatory cells in the liver, thereby exposing hepotocytes to damaging factors released by activated monocytes *(15)*. Indeed, anti-TNF-α treatment in *ob*/*ob* mice can improve liver histology and reduce total hepatic FA content *(16,26)*. On the contrary, two recent investigations have suggested a "hepatoprotective" role for TNF-α. Leptin-deficient mice with elevated basal TNF-α expression are protected against acute liver damage, as their ability to induce IL-18 is diminished, and T-cell-mediated hepatotoxicity is reduced *(27)*. Because most other studies indicate that TNF-α enhances liver injury in NAFLD, this work has generated some controversy.

Resistin is another important adipokine, but our understanding of its role in NAFLD is complicated by substantial differences between resistin-encoding genes in humans and animal models. The spectrum of resistin-like molecules in humans and mice is different because the resistin-α encoding gene is absent in humans. Resistin serum content is also

substantially lower in humans (1/250) than in the rodent model. This is a major consideration when interpreting the applicability of animal studies to humans *(28)*. At present, only one study has described the relationship between resistin and NAFLD *(29)*. This study focused on RELM-β, a resistin-like molecule expressed by intestinal goblet cells. Strictly speaking, RELM-β cannot be considered an adipokine, but its effects might be similar to white-adipose-specific RELM-α. Among other changes, fatty liver results from the overexpression of the RELM-β encoding gene in the liver of transgenic mice maintained on a high-fat diet *(29)*. This intriguing finding requires further investigation.

Finally, adiponectin's potential role in the pathogenesis of NAFLD has generated much interest. Adiponectin reduces IR by decreasing triglyceride (TG) content in the muscle and liver tissue of obese mice. It also increases the ability of subphysiological levels of insulin to suppress glucose production by inhibiting hepatic gluconeogenic enzymes *(30)*. Moreover, in lipoatrophic mice, leptin and adiponectin act synergistically *(31)*. Obese mice produce diminished amounts of adiponectin. When replenished, adiponectin dramatically alleviates steatosis in these animals, and attenuates inflammation by suppressing the hepatic production of TNF-α *(32)*. Adiponectin also attenuates CCl_4-induced hepatic fibrosis *(33)*. Together this work points to the multilevel involvement of this adipokine in the pathogenesis of NAFLD and its progression.

5. ADIPOKINES IN PATIENTS WITH NAFLD

The following sections review current clinical work on the potential role of specific adipokines and cytokines in the development of NAFLD. These include adiponectin, resistin, leptin, TNF-α, and other cytokines.

5.1. Adiponectin

Adiponectin is the most frequently studied adipokine in patients with NASH. Over the past few years, several authors have suggested that hypoadiponectinemia may contribute to the development of NASH in obese individuals *(32,34–37)*. Plasma adiponectin levels are significantly lower in patients with NAFLD than in their matched controls. However, there are no differences in adiponectin levels in patients with simple steatosis versus those with NASH *(35)*. Another study of 68 obese patients shows an independent association of hypoadiponectinaemia with steatosis and markers of liver injury *(34)*. Similar studies confirmed the protective effect of adiponectin against the development of radiologically proven steatosis in adult *(36)* and pediatric *(37)* populations.

The role of adiponectin in distinguishing NASH from simple steatosis remains controversial. One study has shown that a reduction in circulating adiponectin levels in NAFLD is related to hepatic insulin sensitivity and the amount of the hepatic fat, but not to the severity of necroinflammation and fibrosis *(38)*. On the contrary, other studies report that hypoadiponectinemia is associated with increased grades of hepatic necroinflammation, independent of IR *(39)*. Musso and coauthors have also suggested that adiponectin could be protective against NASH *(40)*, as its levels correlate negatively with the presence of necroinflammation and fibrosis *(39,40)*. The same study demonstrates that the changes in adiponectin levels probably precede overt manifestation of diabetes *(40,41)*.

Circulating levels of adiponectin reflect a strong genetic component with an additive genetic heritability of 46% *(42)* that is linked to regions on chromosomes 5p, 14q, and

9p. Individuals homozygous for the +276T allele of the adiponectin-encoding APM1 locus have higher adiponectin levels than other subjects *(43)*. Individuals with an allelic combination of +45T and +276G ("TG" haplotype) are more likely to have various components of metabolic syndrome *(44,45)*. These findings are suggestive, but a correlation between these genetic findings and NAFLD has not yet been reported.

It is also important to mention two recent publications focusing on the role of the adipokine receptors in NAFLD and NASH that report contradictory results. Kaser and colleagues report a significant reduction in the immunostaining of the adiponectin receptor AdipoRII as well as its mRNA expression levels in liver biopsies of patients with NASH as compared with patients with simple steatosis *(46)*. On the other hand, Vuppalanchi and colleagues report an increase in the mRNA expression levels of the same receptor in NASH livers *(47)*. These investigators report several other contradictory findings regarding endogenous adiponectin production in the hepatic sinusoids *(46,47)*. Further clarification of the adiponectin receptors status in NASH is warranted because some common haplotypes of Adip-R1 alleles are associated with hepatic steatosis *(48)*.

5.2. Resistin

Resistin has been the focus of much attention because it is implicated in the pathogenesis of obesity-mediated IR and type 2 diabetes mellitus. In addition, resistin appears to be a proinflammatory cytokine stimulating NFκB-dependent macrophage secretion of TNF-α and IL-12 to the same extent as lipopolysaccharide *(49)*. One recent study shows that plasma resistin concentrations are positively correlated with hepatic fat content *(50)*. Others show that plasma resistin concentrations are similar in NASH patients compared with BMI-matched, non-NASH controls *(37,40)*. Despite these suggestive findings, the role of resistin in the pathogenesis of NAFLD remains speculative and requires further clarification.

5.3. Leptin

It is unclear whether serum leptin elevation is associated with the development of steatosis or NASH. Higher-than-normal leptin concentrations are found in various types of NAFLD and NASH, but not in chronic viral hepatitis without cirrhosis *(36,51)*. Most advanced stages of NASH usually correspond to higher leptin levels *(36,51)*. One recent study shows a correlation between serum leptin and serum ALT *(52)*. Because NAFLD is the most common cause of elevated ALT, this study provides indirect evidence connecting leptin with NAFLD.

One mechanism by which leptin may contribute to the development of NASH is to influence IR as well as FA influx into hepatocytes *(53)*. In the later stages of NASH, leptin may also augment systemic, low-grade inflammation, thus providing the "second hit" responsible for advancing simple steatosis to steatohepatitis *(53)*. Additionally, leptin acts as a profibrogenic adipokine, acting both on endothelial cells and Kupffer cells *(54,55)*. However, despite early enthusiasm, the role of leptin in NAFLD remains controversial. Leptin levels have been associated with NAFLD, but this association becomes insignificant after controlling for important confounders *(56)*. Furthermore, a longitudinal study showed no differences in leptin levels between patients with NAFLD who had fibrosis progression and those who did not *(56)*.

In this context, it is important to remember that NAFLD is commonly seen in conjunction with lipodystrophy, a condition characterized by the partial or complete absence of adipose tissue and hypoleptinemia. In such patients, leptin administration improves IR and corrects hepatic steatosis and hepatocellular ballooning injury, whereas the degree of liver fibrosis remains unchanged *(57)*. Clearly, the exact nature of leptin involvement in the development of NASH and its progression requires further investigation.

5.4. TNF-α

TNF-α is of interest because its levels increase with obesity and NAFLD, and pharmacological interventions decreasing TNF-α appear to be therapeutic in patients with NASH. TNF-α is a proinflammatory cytokine capable of orchestrating the synthesis, secretion, and activity of other proinflammatory molecules. In humans, the majority of TNF-α is produced by macrophages. Other tissues also produce TNF-α in response to infection, ischemia, and trauma *(58)*. TNF-α mRNA is found in very low quantities compared with other proteins in human white adipocytes, but an overall increase in the adipose mass usually leads to substantial, cumulative production of this cytokine *(59)*, potentially contributing to the development of obesity-related NAFLD. Several studies have demonstrated that serum TNF-α levels are significantly higher in patients with NASH than in healthy controls *(39,60)*.

The most comprehensive study of TNF-α in patients with NASH shows remarkable increases in the expression of mRNA encoding TNF-α in both hepatic and adipose tissues *(78)*. Similar mRNA increases have been observed for the p55 receptor, but not for the p75 receptor of TNF-α *(61)*. Additional indirect evidence of TNF-α involvement comes from a 12-mo trial of pentoxifylline (1600 mg/d) in patients with NASH *(62)*. Pentoxifylline is a methylxanthine that can suppress both the accumulation of TNF-α mRNA and the activity of its secreted form. In patients with NASH, both alanine aminotransferase and aspartate aminotransferase levels were significantly lower after 12 mo of therapy compared to the baseline ($p = 0.003$), indicating significant improvement in treated patients *(62)*. The treatment of NASH with 400 mg pentoxifylline three times per day had similar results *(63)*. These findings warrant further investigation.

5.5. Other Cytokines

Two potent proinflammatory cytokines, IL-6 and IL-8, are released by both visceral and subcutaneous adipose tissues of obese subjects *(24)*. In fact, human adipose tissue can release more IL-6 and IL-8 than adiponectin, especially in morbidly obese individuals *(64)*. Two studies show that serum IL-8 and IL-6 levels in patients with NASH are significantly higher than in healthy controls *(60)*. On the other hand, IL-6 seems to induce hepatoprotection both in normal and steatotic liver grafts after liver transplantation *(65)*. These contradictory findings emphasize our incomplete understanding of the role of these cytokines in NAFLD.

6. ROLE OF ADIPOKINES IN PROMOTING HEPATIC STEATOSIS, IR, OXIDATIVE STRESS, AND HEPATIC FIBROSIS IN NAFLD

6.1. Adipokines and Steatosis

As previously noted, hepatic steatosis may result from an increase in the delivery of free FA to the liver, increased FA synthesis, decreased FA degradation, impaired TG

release from the liver, or a combination of these factors. Adiponectin exerts a beneficial effect on the accumulation of TGs and on the concentration of FA in skeletal muscle *(68)*. It also enhances FA oxidation both in liver and muscle tissue through activation of acetyl CoA oxidase, carnitine palmitoyltransferase-1 (CPT1), and 5′-AMP activated protein kinase (AMPK) *(69)*, and stimulates lipoprotein lipase activity in animal models *(70)*. Decreased serum adiponectin is associated with lipoprotein lipase (LPL) deficiency in humans, independent of the effects of systemic inflammation and/or IR *(71)*. Therefore, hypoadiponectinemia may stimulate the accumulation of fat in the liver by promoting LPL deficiency, leading to an influx of free FA.

Alternatively, adiponectin may promote hepatic steatosis by increasing FA synthesis or decreasing FA degradation within the liver, or both. For instance, adiponectin treatment normalizes hepatic lipid content in steatotic mice by restoring the activity of CPT1, a rate-limiting enzyme involved in the transport of long-chain FA into mitochondrial matrix *(32)*. Thus, high adiponectin concentrations stimulate β-oxidation of FA in the liver and therefore decreases the intrahepatic lipid load. At the same time, adiponectin downregulates the hepatic lipogenesis pathway *(45)*.

A third mechanism potentially linking hypoadiponectinemia to the development of NAFLD is an increase in hepatic lipid retention owing to adiponectin-dependent suppression of very-low-density lipoprotein (VLDL) synthesis, the chief route of hepatic lipid export *(67)*. The effects of adiponectin on VLDL metabolism are independent of both IR and the size of the adipose tissue compartments *(68)*. Unfortunately, patients included in these studies were not assessed for the presence of NAFLD. Therefore, an important link among adiponectin, VLDL, and the pathogenesis of NAFLD remains uncertain.

Leptin protects against lipotoxicity in nonadipose tissues *(68)*, possibly by a peripheral mechanism. Studies of pair-fed controls receiving the exact amount of food ingested by leptin-treated animals show that controls remain steatotic despite caloric restriction *(73,74)*. In cultured pancreatic islets, leptin lowers TG content by increasing FA oxidation and preventing its esterification *(73)*. A similar mechanism may be at work in the liver, because liver tissue expresses leptin receptors. Indeed, tissue-specific overexpression of wild-type leptin receptors in steatotic livers reduces TG accumulation in the liver but nowhere else *(75)*.

Furthermore, leptin dramatically suppresses the expression of the hepatic stearoyl-CoA desaturase (SCD)-1, the rate-limiting enzyme in the biosynthesis of monounsaturated fats *(74)*. SCD-1 suppression, in turn, supports resistance to both hepatic steatosis and obesity owing to a marked increase in energy expenditure. Two proposed mechanisms for these leptin effects include blocking TG synthesis and exporting VLDL *(74,75)*. These mechanisms lead to a concomitant increase in the pool of saturated fatty acyl CoAs, which allosterically inhibits ACC and reduces the amount of malonyl CoA. Inhibition of the mitochondrial carnityl palmitoyl shuttle system is relieved as a consequence, stimulating the import and oxidation of FA in mitochondria. Thus, leptin administration de-represses FA oxidation, leading to increased fat burning *(74)*. Other proposed mechanisms of antisteatotic effects of the leptin involve increases in a peroxisome proliferator-activated receptor (PPAR) α signaling *(76)* or AMPK activation, or both *(77)*.

Leptin also seems to promote the elimination of the plasma cholesterol through stimulation of its catabolism to bile salts in the setting of decreased cholesterol biosynthesis. Cholesterol elimination is achieved by suppressing the hepatic activity of HMG-CoA

Table 1
Summary of Positive and Negative Effects of Adipokines on Cellular Processes Contributing to Pathogenesis of NASH

	Cellular processes contributing to NASH				
Adipokine	*Lipid accumulation in liver (steatosis)*	*IR*	*Oxidative damage*	*Fibrotic responses*	*Role in NASH progression*
Adiponectin	Suppressed	Suppressed	Suppressed	Suppressed	Prevents NASH
Leptin	Suppression effects are low due to leptin resistance	Suppression effects are low due to leptin resistance	Pro-oxidant	Fibrogenic action	Suppresses initiation of steatosis; stimulates progression of existing steatosis to NASH
Resistin	Possibly steatogenic	Possibly involved in IR; difficult to study in humans	Possibly pro-oxidant	Possibly fibrogenic	Unclear
TNF-α	Steatogenic	Impairs insulin signaling	Pro-oxidant	Fibrogenic action	Augments NASH
Visfatin	Unknown	Mimicking insulin	Unknown	Unknown	Unclear
Vaspin	Unknown	Suppressed	Unknown	Unknown	Unclear
Apelin	Unknown	Inhibits insulin production	Unknown	Unknown	Unclear

reductase, upregulating the activities of both sterol 27-hydroxylase and cholesterol 7α-hydroxylase, and diminishing the cholesterol fraction bound to VLDL by limiting TG supply *(78)*. Lowered leptin signaling might be responsible for the increase in the prevalence of cholesterol gallstones in obese patients compared with the general population *(79)*.

It is important to remember that obesity is associated with leptin resistance and hyperleptinemia. Therefore, exogenous leptin administration does not alleviate lipid accumulation in the liver or improve NAFLD. On the other hand, the development of central and peripheral leptin resistance critically depends on the liver. In animal models, chronic leptin treatment in leptin-naïve animals induces shedding in the soluble leptin receptor protein (SLR). SLR sequesters leptin and prevents productive interactions with its signaling receptor *(80)*, making peripheral leptin activity self-limiting.

It would be interesting to know whether the rate of the SLR synthesis is altered in steatotic livers, or whether the manipulation of SLR production could alter leptin resistance in obesity.

Our understanding of the role of resistin in the development of steatosis is quite preliminary. Resistin is capable of influencing lipid metabolism in rodents. Resistin overexpression in mice and rats leads to plasma TG increases and to significant dyslipidemia *(81)*. Serum resistin levels correlate negatively with HDL cholesterol levels in healthy men *(82)*, suggesting that higher-than-normal resistin levels typically seen in obesity and type 2 diabetes might contribute to the development of fatty liver through its dyslipidemic effects.

Finally, TNF-α's pleiotropic effects can influence lipid metabolism in the liver, as it stimulates *de novo* synthesis of FA, suppresses FA oxidation, and enhances the turnover of VLDL *(83)*. Mice that express T-cell-targeted human TNF-α transgenes provide an animal model for persistent low-grade exposure to TNF-α typical of morbid obesity *(84)*. These mice are dyslipidemic *(84)*. Both mitochondrial and peroxisomal β-oxidations are inhibited in their livers *(84)* with no concomitant increase in the *de novo* FA synthesis *(84)*. Therefore, TNF-α-dependent steatogenesis in the liver is predominantly caused by the suppression of FA decomposition. TNF-α also stimulates VLDL production in the liver and inhibits the activity of lipoprotein lipase in adipocytes *(85)*; these processes favor lipolysis in fat depots and contribute to the development of the TNF-α-dependent hypertriglyceridemia and associated NAFLD.

6.2. Adipokines and IR

Because of the striking association between NASH and IR *(2)*, any factor promoting a vicious cycle of insulin signaling can be steatogenic, and factors counteracting IR can be protective against the development of NAFLD.

Hyperinsulinemia caused by IR increases FA synthesis and impairs both mitochondrial β-oxidation and the export of TGs in multiple ways. Early studies have indicated that adiponectin decreases IR by increasing FA oxidation, which reduces the TG content in nonadipocytes *(31)*, suppresses glucose production in the liver *(69)*, and enhances the hepatic action of insulin *(30)*. These glucose-lowering effects of adiponectin require liver-specific AMPK activation *(69)* and play a key role in the regulation of energy control. AMPK is activated in response to a variety of external signals, including adipokines *(86)*. It is tempting to speculate that AMPK-mediated antiglycemic effects may play a role in the prevention of NAFLD, but this seems unlikely. Recent work indicates that short-term overexpression of a constutively active form of AMPK in the liver can lead to the development of fatty liver in the presence of lowered hepatic glycogen synthesis and circulating lipid levels *(86)*. Most likely, the NAFLD-like disorder in animal models develops from the hepatic accumulation of lipids released from adipose tissue in response to the relative scarcity of glucose. Therefore, additional stimulation of AMPK provided by a sudden increase of adiponectin (e.g., owing to thiazolidinedione [TZD] treatment) may aggravate early stages of the hepatic steatosis. This may also explain the infrequent but potentially serious hepatotoxic side effects of chronic TZD administration *(87)* and the pronounced exacerbation of hepatic steatosis in mice with polygenic obesity treated by rosiglitazone *(88)*.

Leptin exerts a systemic insulin-sensitizing effect *(89)*. An interaction between the insulin and leptin signaling cascades has been studied both in vitro and in vivo *(90)*, but the complete mechanism remains unclear and the results are inconsistent. Most likely, cross-cascade interactions involve insulin receptor substrate (IRS) molecules, PI 3-kinase, Akt, and GSK3 *(90)*. The liver is probably central to the adiposity-independent role of leptin in controlling IR, as some studies have suggested that leptin selectively improves insulin receptor activation only in the liver, but not in skeletal muscle or fat *(91)*. Unfortunately, the insulin-related branch of the leptin-dependent signaling pathway in obese livers is profoundly suppressed *(92)*. Therefore, it is unlikely that therapeutic administration of leptin would alleviate liver steatosis through improved insulin sensitivity.

Resistin reduces glucose tolerance and insulin action, thereby inducing IR. Hyperresistinemia certainly contributes to IR in obese rodents because of decreased gluconeogenic enzyme expression in the liver and to the activation of AMPK *(93)*. In humans, the situation is much more difficult to trace, because serum resistin levels are related to sex, age, and testosterone and estradiol levels *(94)*. These fluctuations in resistin levels and the relatively low homology between resistin and resistin-like molecules in humans and rodents complicate the study of resistin in the development of IR in the liver and NAFLD.

TNF-α alters systemic energy homeostasis in a way that closely resembles the IR phenotype. Mice with a complete knockout of TNF-α signaling show significantly improved insulin sensitivity in both diet-induced and leptin-deficient obesity *(95)*. Long-term exposure to TNF-α completely abolishes insulin-induced glycogen synthesis in hepatocytes *(96)*. Therefore, abnormal production of TNF-α may predispose obese individuals to the development of the IR and NAFLD.

Visfatin is produced both in visceral and subcutaneous adipose tissue and exerts insulin-like effects in various tissues by binding and activation of the insulin receptor *(98)*. Visfatin is upregulated in obesity *(97)* either as a simple reflection of visfatin resistance that parallels the IR in metabolic syndrome, or represents an important compensatory pathway leading to lowered glucose levels. Vaspin, visceral adipose tissue-derived member of the serine protease inhibitor (serpin) family, normalizes serum glucose levels by reversing altered gene expression related to IR, including all other adipokines discussed above *(99)*. In humans, vaspin mRNA expression is not detectable in lean subjects, but is a frequent finding in type 2 diabetes *(100)*. This secreted molecule might be an important insulin sensitizer of adipocytic origin and may play an important role in NAFLD. Finally, apelin is an adipokine that is probably related to peripheral IR. It inhibits glucose-stimulated insulin secretion both in vivo and in vitro by acting on its receptor, which is expressed in β-cells of pancreatic islands *(101)*. Apelin plasma levels are largely increased in all the hyperinsulinemia-associated obese states in mice, independently of diet composition *(102)*, and in obese humans *(102,103)*. In summary, the interplay between insulin-like visfatin, insulin-sensitizing vaspin, and the suppression of insulin production by apelin may represent important avenues for future studies of the pathogenesis of NAFLD and NASH.

6.3. Adipokines and Oxidative Stress

Changes in serum adipokine concentrations augment oxidative stress in patients with NASH. Most studies converge on CYP2E1, peroxisomal release of ROS, and mitochondrial dysfunction. ROS and reactive nitrogen species (RNS) are generated by the

parenchymal cells of the liver, Kupffer cells, and inflammatory cells, which further mobilize cellular defense mechanisms and contribute to liver injury and necrosis.

The potential role of adiponectin as an antioxidant is mostly indirect. One study suggests that serum adiponectin levels negatively correlate with urinary levels of isoprostane, an oxidative stress marker *(104)*. A different line of evidence suggests that the production of adiponectin may be suppressed in the pro-oxidative conditions. For example, inhibited adiponectin mRNA expression is observed in differentiated murine adipocytes after exposure to increasing concentrations of glucose oxidase, H_2O_2, and byproducts of lipid peroxidation *(105)*. Adiponectin synthesis may also be suppressed by an excess of angiotensin II (AngII), a vasoactive peptide *(106)*. AngII indirectly activates NAD(P)H oxidase, which favors the production of ROS. It is noteworthy that administration of the angII type 1 receptor antagonist losartan significantly improves liver biochemical indices as well as hepatic necroinflammation in patients with NASH *(52)*.

Leptin increases markers of lipoperoxidation in the liver while decreasing antioxidant GSH levels and the activities of glutathione-S-transferases (GSTs), superoxide dismutase (SOD), and catalase *(107)*. Similar observations have been made in non-NAFLD patients with other chronic liver diseases *(108)*. Intravenous leptin injections induce the release of nitric oxide (NO) *(109)* by both endothelial and inducible nitric oxide synthases (eNOS and iNOS). As uncoupled eNOS changes from a protective enzyme to a contributor to oxidative stress, leptin-induced stimulation of eNOS and iNOS is a pro-oxidative event *(110)*. Leptin also stimulates cytochrome CYP2E1 expression, responsible for the oxidation of alcohol and the production of ROS. Paradoxically, CYP2E1-dependent production of ROS inhibits apoptosis but accelerates necrosis stimulated by polyunsaturated FA *(111)*. This latter observation is consistent with the necroinflammatory features seen in patients with NASH. Finally, CYP2E1 activity is elevated in patients with NASH as assessed by the rates of oral clearance of chlorzoxazone *(112)*.

Additional observations supporting the role of resistin in promoting oxidative stress include a study of resistin's effects in porcine coronary arteries, which shows increased superoxide radical production, and decreased eNOS activity *(113)*. In humans with normal body weight, serum resistin concentrations are negatively correlated with the concentrations of a marker of oxidative stress, nitrotyrosine *(61)*. Conversely, oxidative stress itself can suppress resistin production in adipocytes, similar to suppressed production of adiponectin. The efficiency of such suppression might depend on a particular genotype at a resistin locus *(115)*.

TNF-α certainly plays an important role in the enhancement of ROS production observed in steatotic livers. Key components of TNF-α signaling include ceramide, which influences the mitochondrial electron transport chain and evokes hydrogen peroxide overproduction *(116)*. In addition, ceramide induces mitochondrial membrane permeability transition (MMPT) and subsequent necrosis *(117)*. Another potential sensitizer to TNF-α-induced cell death is the uncoupling of mitochondrial respiration *(118)*. TNF-α enhances the expression of UCP2, a mitochondrial regulator that increases a proton leak across the inner membrane to dissociate respiration from ATP synthesis and reduce ROS generation. TNF-α-dependent UCP2 stimulation is especially pronounced in steatotic *(119)* and regenerating *(120)* livers. Upregulated UCP2 may compromise cellular ATP levels and worsen liver damage by augmenting cell death, or it may be protective by reducing ROS levels. It is also possible that these two effects cancel each

other *(121)*. It is important to note that the state of UCP2 activity in patients with NAFLD and patients with NASH is not entirely clear. As mitochondrial uncoupling sensitizes the cells to TNF-α-induced death, this effect might outweigh the simultaneous decrease in the ROS production in human subjects.

6.4. Adipokines in Hepatic Fibrosis

Hepatic fibrosis is a wound-healing response characterized by inflammation, activation of matrix-producing cells, extracellular matrix (ECM) deposition and remodeling, and epithelial cell regeneration *(122)*. Major matrix-producing cells in the liver are hepatic stellate cells (HSCs) that may undergo a phenotypic transition to myofibroblast-like cells that synthesize various ECM components and contribute to fibrogenesis.

Adiponectin suppresses the proliferation and migration of HSCs *(123)* and attenuates CH_4-dependent liver fibrosis through suppression of platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β1-induced migration and proliferation *(33)*. Adiponectin can also induce apoptosis in activated HSCs, but not in quiescent HSCs *(124)*. Both AdipoR1 and AdipoR2 receptors are present in both quiescent and activated HSCs; however, AdipoR1 mRNA expression is reduced by 50% in activated HSCs *(124)*. These findings indicate that adiponectin is either essential to maintaining the quiescent phenotype of HSCs or it is capable of reversing hepatic fibrosis by hampering the proliferation of activated HSCs and by inducing HSC apoptosis.

Leptin enhances liver inflammation and fibrogenesis, in part, by upregulating TGF-β. Leptin has a profound positive influence on α(2)(I) collagen mRNA expression in HSCs *(125)*. In addition, leptin augments PDGF-dependent HSC proliferation. Taken together, these studies indicate that leptin is a potent promoter of hepatic fibrosis. Observations in lipodystrophic patients treated with recombinant leptin support this conclusion *(57)*.

Resistin has no known connection with hepatic fibrosis, but this molecule has been implicated in pulmonary fibrosis induced by bleomycin. Cocultures of RELM-α-expressing epithelial cells and fibroblasts stimulate α-smooth muscle actin and type I collagen expression independently of TGF-β *(126)*. Similar resistin-dependent responses might be produced in HSCs, if resistin contributes to the development of NASH.

Recent experiments provide direct evidence of the involvement of TNF-α in fibrogenic responses. When double knockout mice lacking both TNF receptors (TNFRDKO mice) are fed methionine- and choline-deficient (MCD) diets, they develop less pronounced liver steatosis than their wild-type counterparts *(127)*. Similar findings in TNFRp55 knockout mice indicate that even partial suppression of TNF-α signaling can alleviate hepatic fibrosis *(128)*. It seems that TNF-α increases the recruitment of Kupffer cells that can, in turn, produce extra TNF-α and hasten fibrosis in either an autocrine or a paracrine manner. Both these processes can contribute to the development of NASH progression to cirrhosis.

7. CONCLUSIONS

We have learned a great deal about the epidemiology and pathogenesis of NAFLD and NASH over the past decade (Table 1). It is increasingly clear that the development of NASH is a complex process involving multiple mechanisms including IR, oxidative stress, abnormal FA metabolism, and disturbances in the production of inflammatory cytokines and adipokines. Decreased production of adiponectin and increased production

of TNF-α, which are characteristic of obesity, seem to contribute to all major NASH-related cellular processes. Leptin, on the other hand, behaves as a "wolf in sheep's clothing." Its NASH-suppressive effects are diminished by the widespread effects of leptin resistance, and it becomes potentially pro-oxidant and fibrogenic. Resistin's involvement in NASH is documented in rodent models, but may not be applicable to NAFLD in humans. In addition, other adipokines, such as vaspin, visfatin, and apelin require further study in patients with NAFLD and NASH.

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