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Risk factors and Mechanisms of Non-alcoholic Steatohepatitis

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Abstract

The worldwide proportion of overweight and obese individuals has increased yearly for more than a decade. Along with rates of obesity, the incidence of co-morbid conditions such as type 2 diabetes, cardiovascular disease and liver disease has also increased. The form of liver disease associated with obesity is termed non-alcoholic steatohepatitis (NASH) due to the histological similarities to livers of chronic alcoholics. NASH has been observed in adult as well as pediatric populations; however, the etiology of this disease is still unknown. This review outlines some of the risk factors commonly associated with NASH and describes molecular mechanisms proposed to underlie disease pathogenesis.

Keywords

cardiovascular disease; diabetes; liver disease; obesity; steatohepatitis

Introduction

Nonalcoholic steatohepatitis (NASH) is a cryptogenic form of liver disease that is often observed in obese patients in the absence of identifiable causes such as alcohol abuse, drug toxicity or viral infection. As the name suggests, NASH is histologically indistinguishable from alcoholic hepatitis (ASH) and is characterized by fat accumulation in hepatocytes, mixed cell type inflammation, focal necrosis and fibrosis. Although NASH is commonly associated with obesity and features of the metabolic syndrome such as insulin resistance, the pathogenic mechanisms leading to disease progression remain poorly understood.

This disease has been observed worldwide and the prevalence of NASH is estimated to be as high as 40–100% in obese adults as reviewed elsewhere (1). Based on the NHANES III survey, 15–25% of obese children have NASH as evidenced by elevated serum transaminase activity (2–4). However, it is now widely recognized that serum transaminase levels may not accurately reflect hepatic injury and that biopsy specimen provide the only clear means of discerning true disease state. Thus, it is likely that the prevalence of NASH is much higher than anticipated, especially for children since biopsies are not routinely performed in pediatric patients.

As of yet, specific non-invasive diagnostic tools for NASH are lacking. In addition, the cause (s) of this disease have not yet been elucidated. Thus, diagnosis and treatment are difficult.

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Herein, we review several risk factors that may be considered useful diagnostic tools and describe recent results from animal models that provide some insight into the molecular mechanisms underlying NASH pathogenesis.

Risk Factors Associated With NASH

Adiposity and adipose tissue

Adiposity is clearly one of the most well-studied risk factors for NASH (5;6). A clinical series of 39 Korean patients revealed that body mass index (BMI) values were useful in distinguishing NASH from simple steatosis, with a BMI of 28.9 suggested to be the threshold for the existence of NASH (7). It has been proposed that abdominal adiposity is particularly related to disease state (8–10). For example, Singh et al (11) reported that waist circumference as well as the waist to hip ratio were independent predictors of the degree of hepatic necroinflammation. Additional studies have related abdominal fat area to NASH and reportedly, a visceral fat area >158 cm² was found to be an independent predictor of disease state (12). Dorsocervical lipohypertrophy has also been strongly associated with the severity of NASH (13). With respect to advanced disease, a significant positive correlation has been identified between advanced fibrosis stage and indices of adiposity (e.g. BMI, blood cholesterol and LDL levels) (5;6;14).

Pre-adipocytes have a phagocytic capacity comparable to macrophages and genetic profiling has revealed a similar pattern of expression of several genes involved in the inflammatory cascade in these two cell types. In fact, Weisberg et al. (15) demonstrated that the majority of genes up-regulated in white adipose tissue of obese mice were related to macrophage function and inflammation. Preadipocytes constitutively express several chemokines, including MIP-1 α and MCP-1; chemokine expression is enhanced in the presence of cytokines such as TNF- α (16) and enhanced expression of TNF- α and IL-6 has been consistently detected in the setting of obesity (17;18). A positive correlation exists between adiposity (BMI and %body fat) and IL-6 protein levels in serum, as well as mRNA levels of both cytokines in abdominal adipose tissue (17;18). These cytokines have been shown to inhibit lipoprotein lipase activity and insulin sensitivity (19;20), most likely via mechanisms involving the stress-activated kinase c-jun (21). These findings clearly implicate adipose tissue in the development of local and systemic inflammatory responses; however, the factors that initiate these inflammatory responses remain unknown.

Leptin is an adipocyte-derived cytokine that controls food intake and energy expenditure. Receptors for leptin are expressed throughout the body and leptin binding to these receptors is known to trigger a pro-inflammatory signaling cascade (22;23). For example, leptin elicits the activation of the transcription factor NF κ B, which initiates the synthesis of adhesion molecules (e.g., ICAM-1) and chemokines (MCP-1). Moreover, there is considerable evidence linking leptin and cytokine production and it appears that serum leptin may represent an independent predictor of the severity of illnesses associated with obesity (24;25).

Although it is primarily secreted by adipocytes, recent reports demonstrated that leptin was also expressed by activated stellate cells (23). Leptin enhanced the expression of cytokines related to the induction of fibrosis in the liver (e.g. transforming growth factor- β , TGF- β) and induced the over-production of matrix proteins that are components of fibrosis scarring such as collagen α I-1 (23;26–28). Moreover, in animal models of NASH spontaneous conversion from simple fat accumulation in hepatocytes to injury and fibrosis does not occur in mutant mice that do not express leptin (29). Taken together, these previous findings suggest that the adipokine leptin may be an important link between adiposity and liver injury in obese people.

Diabetes

Insulin resistance and type 2 diabetes mellitus (T2DM) are common features of the metabolic syndrome associated with obesity and likely contribute to co-morbidities of the liver. Several recent studies indicate an enhanced prevalence and severity of liver disease in diabetic patients (30–32). In fact, data collected from obese subjects undergoing gastric bypass surgery suggested that patients with T2DM were 128 times more likely to have NASH and 75 times more likely to have severe fibrosis compared to non-diabetic obese patients (33). Why diabetic patients appear more susceptible to NASH is not yet clear. Although insulin resistance is commonly associated with NASH, a direct mechanistic link between disease pathogenesis and hyperinsulinemia has not been established.

One potential mechanistic link between diabetes and NASH involves advanced glycation end products (AGE). The formation of these adducts occurs at highly accelerated rates during T2DM. Compared to healthy controls or patients with simple steatosis, serum and hepatic AGE levels are significantly elevated (34). The interaction of AGE with the associated cell surface receptor (RAGE) has been linked to the induction of oxidative stress (35), as well as enhanced fibrogenic potential of cultured human stellate cells (36). Thus, AGE signaling via RAGE and the resulting reactive oxygen intermediates has a potential causal role in enhanced pathology found in diabetic patients.

Sleep apnea

Obstructive sleep apnea (OSA) is a condition commonly associated with obesity that is characterized by chronic, intermittent cessation of breathing during sleep due to airway obstruction. Although only 2% to 4% of the general population is thought to have this condition, it occurs with much higher frequency in the setting of obesity. As reported by Tanné et al, the prevalence and severity of NASH was greatest in patients with pronounced OSA, suggesting that the intermittent hypoxia due to OSA may play a role in NASH pathogenesis (37). In fact, this idea has been examined in murine models of dietary obesity. While intermittent hypoxia did not directly cause liver injury in lean animals, exacerbated necroinflammation and fibrosis were observed in the livers that were already steatotic (38–40), suggesting that hypoxia may serve as an additional insult that aids in the progression from simple steatosis to more advanced disease stages.

Race/ethnicity

Demographic analysis of obese populations has provided striking evidence of ethnic variations in the extent and incidence of NASH. Steatohepatitis is common among Hispanic populations, most likely due to the high degree of obesity among this ethnic group (41). In contrast, despite the prevalence of obesity and other major risk factors such as type 2 diabetes, the existence of NASH in the African-American patient population is quite rare and obese African Americans reportedly had a significantly lower odds of having severe hepatic pathology when compared to Caucasian patients (42). In one series of 159 patients with NASH and 206 with cryptogenic cirrhosis, African-Americans represented only 1% and 0.6% of these patient groups, respectively (43). A study by Solga et al. found that steatohepatitis was absent completely in obese African-Americans (44). It has been speculated that both genetic and environmental factors (e.g. dietary habits) may be related to the decreased incidence of liver disease in African Americans. Further investigations into this apparent ethnic variation in the prevalence of NASH may provide insight into the pathogenesis of this disease.

Gender

Clear differences in the amount and distribution of body fat exist between men and women. Men typically store fat in the upper body, specifically around the organs in the abdominal cavity

(i.e. visceral fat)(45). Women tend to have greater stores of subcutaneous, lower-body fat (9). Gender also influences circulating lipid (triglycerides and fatty acids), lipoprotein and cholesterol levels (46). Reasons for differential fat accumulation in men and women are not well understood; however, evidence suggests that lipid metabolism may play a role in the observed differences. For example, in a study of Japanese men (n= 26) and women (n=23) with evidence of the metabolic syndrome, serum triglycerides and particulate cholesterol were higher in men than women, which was attributed to lower plasma levels of lipoprotein lipase, an enzyme that catalyzes the hydrolysis of triglycerides (47). At the cellular level, there are distinctions between males and females in the activity of lipid handling enzymes such as hepatic lipase, an enzyme responsible for cholesterol remodeling. Hepatic lipase activity in men is twice that of age-matched females, which likely contributes to an enhanced atherogenic lipid profile of lower HDL and increased levels of dense LDL particles in men (48). Gender distinctions in hepatic lipase activity have been attributed to suppression by androgenic steroids (10;49) and visceral adiposity (45;48). Taken together, it seems likely that the male gender would exacerbate symptoms of the metabolic syndrome and hepatic steatosis.

Rodent models have provided clear evidence of gender differences in NASH pathogenesis. Studies in rats and mice have demonstrated significantly worse histopathology and biochemical evidence of injury in male versus female rodents (50;51); however, examinations of NASH directed toward establishing the role of gender in human subjects are scarce and the limited information available is contradictory. A study of 597 men and 556 women in the southwestern region of France revealed that the prevalence of the metabolic syndrome in men (23%) was significantly greater than the observed prevalence in women (12%) (52). In a study of 365 American adults, 90% of men had NASH while only 30.8% of women had NASH. The male gender in that study was also significantly associated with the incidence of diabetes and metabolic syndrome. (53). Studies in American pediatric subjects also identified males to be at a higher risk of having obesity-associated liver disease (54). In contrast to the French and American studies, one series of 193 Japanese patients with biopsy-proven NASH reported that the female gender was predominant, but only among subjects ≥ 55 years of age (55). On the other hand, there are several reports across multiple ethnic groups that fail to identify a gender difference, which precludes the ability to draw any conclusions concerning the role of gender as a risk factor for NASH.

Molecular Mechanisms Underlying NASH

Endotoxemia

There is sufficient evidence to suggest a role for gut-derived endotoxin in the pathogenesis of alcoholic steatohepatitis (ASH). Due to histological similarities between ASH and NASH, it is reasonable to predict that these diseases may share some common underlying mechanisms. Endotoxin, which is derived from the outer cell wall of gram negative bacteria, is believed to play a causal role in ASH and experimental models using rodents have added to our understanding of a role for endotoxemia in NASH. For example, 12–16 weeks of feeding a high fat diet to rats was sufficient to induce hepatic injury, which was associated with diminished gut barrier function and bacterial overgrowth (56). In murine steatohepatitis induced by feeding a methionine/choline deficient diet (MCDD) intestinal permeability and portal vein endotoxemia have been observed (57;58). Moreover, animals with MCDD-induced steatohepatitis were sensitized to the deleterious effects of endotoxin (29;59). Increased susceptibility to injury in endotoxin-treated mice was associated with macrophage dysfunction and altered cytokine regulation (29;60). In further support of the idea that gut-derived bacterial products play a causal role in the pathogenesis of NASH, treatment of mice with probiotic dietary supplements that rid the gut of gram negative bacteria prevented histological alterations and insulin resistance associated with steatohepatitis (61).

In humans, severe steatohepatitis often occurs during situations believed to cause bacterial overgrowth and endotoxemia, such as the jejunoileal bypass surgical procedure for weight reduction. A study by Wigg et al. evaluated NASH patients for small bowel bacterial content and found that bacterial overgrowth was prevalent among NASH patients compared to healthy age-matched controls (62). In more recent studies, protein levels of lipopolysaccharide binding protein (LBP), a class I acute phase protein up-regulated in response to systemic endotoxemia, were found to be highest in individuals with NASH (63). In addition, a positive correlation was found between LBP and hepatic mRNA expression of inflammatory cytokines. Thus, evidence from animals as well as human studies supports the idea that gut-derived bacterial toxins may participate in NASH pathogenesis.

Toll like receptor-4 signaling

The liver is a first line of defense against gut-derived endotoxin and various other bacterial components. Kupffer cells, the resident hepatic macrophages, are primarily responsible for endotoxin clearance. Kupffer cell activation by endotoxin requires the CD14/Toll-like receptor complex (TLR) on the cell surface, and the presence of LBP (64). Ligand interaction with the TLR-4 complex results in the recruitment of multiple adaptor molecules to the cell membrane, such as MyD88, interleukin-1 receptor-associated kinase (IRAK) and tumor necrosis factor receptor-activated factor-6 (TRAF-6). Phosphorylation of IRAK and TRAF-6 further propagates this signaling cascade, eventually resulting in the translocation of NFkB to the nucleus where it facilitates the production of various inflammatory mediators.

In rodent models of ASH, the activation of proinflammatory mechanisms has been linked to TLR-4 signaling. Similar to ASH, previous studies have demonstrated activation of NFkB and enhanced sensitivity to TLR-4 ligands in mice with steatohepatitis induced by feeding MCDD (65). Thus, in an attempt to clarify signals upstream of NFkB activation during NASH, we investigated the role of TLR-4 in the genesis of steatohepatitis. Our results indicated that MCDD-induced NASH was accompanied by a significant induction of the expression of TLR-4, as well as the adaptor molecules MD-2 and CD14 (58). Moreover, injury and inflammation were markedly attenuated in TLR-4 mutant mice. These findings provide strong evidence that TLR-4 signaling is, indeed, important for the pathogenesis of NASH. Thus, it appears likely that targeting the TLR-4 pathway could provide a novel therapeutic approach for the treatment of NASH.

Dietary fatty acids and TLR-4 signaling

Although bacterial overgrowth and significant plasma LBP levels have been found in NASH patients, evidence of overt endotoxemia has not been detected in humans; therefore, a role for other toll like receptor ligands cannot be ruled out. Recent evidence has established a link between dietary fatty acids and TLR-4 signaling, which might provide an alternative to the hypothesis of endotoxin involvement in NASH. In a study by Tsukumo et al. (2007), TLR-4 mutant C3H/HeJ mice fed a high saturated fat diet were protected from excessive adiposity and insulin resistance (66). Further investigations revealed that dietary saturated fatty acids were a likely stimulus for TLR-4 signaling. In fact, ex-vivo exposure of soleus muscle preparations to palmitic, stearic or lauric saturated fatty acids resulted in a significant inhibition in glucose uptake and insulin signaling in wild type mice (66). Consistent with findings in vivo, these effects were blunted significantly in soleus muscle isolated from C3H/HeJ mice or TLR-4 null mice. In addition to insulin sensitivity, saturated fatty acids have been shown to induce a pro-inflammatory signaling cascade involving NFkB activation and the subsequent production of various cytokines and chemokines by cultured macrophages (67;68). In support of the idea that fatty acid-stimulated TLR-4 signaling is involved in NASH pathogenesis, TLR-4 null mice were protected from dietary fatty acid-induced increases in hepatic TNF- α , MCP-1 and IL-6 expression as well as the enhanced accumulation of macrophages (68;69). Taken together,

these studies provide strong evidence that dietary fatty acids are endogenous agonists of TLR-4 signaling, and form the connection between inflammatory disorders and over nutrition. The exact nature of the interaction of free fatty acids with TLR-4 remains to be elucidated.

Summary

In conclusion, we now know that NASH is intimately related to obesity and may be considered one of the common features of the metabolic syndrome. Although the influence of gender is still equivocal, adiposity, race and the presence of T2DM are among the major risk factors for disease severity. As depicted in Figure 1, information gained from animal models strongly suggests the involvement of the innate immune system via toll-like receptor signaling. Yet, how risk factors contribute to this proposed mechanism is not clear. Since appropriate diagnostic tools and treatment strategies for NASH patients are still lacking, intense focus on mechanistic studies in this area of research are greatly needed.

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Figure 1. Overview of steatohepatitis risk factors and proposed mechanism

Over nutrition can lead to enhanced circulating fatty acids and possibly endotoxemia. Current data suggests that these agents stimulate intracellular signaling via interaction with TLR-4, resulting in the production of cytokines and chemokines, which stimulate inflammation, steatosis and ultimately lead to NASH. Several risk factors such as degree of adiposity, race and the presence of T2DM enhance the severity of NASH.