Review

Non-alcoholic fatty liver disease: lumen–liver interactions and possible role for probiotics

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1. Introduction

Tremendous progress has been made over the past decade in the understanding of the pathogenesis of non-alcoholic fatty liver disease (NAFLD) and its associated disease burden. Much of this research has re-focused attention on the relationship between intestinal bacteria and liver disease. The concept that gut-derived bacteria and their products injure the liver and cause systemic illness has surfaced and resurged repeatedly. Indeed, one review of this topic written just over a decade ago (subtitled An Idea Whose Time Has Come Again) argued "Given, then, this impressive accumulation of studies in animals and man that suggest a critical role for endotoxins of intestinal origin in a variety of liver injuries and their extrahepatic manifestations, why is this concept so regularly ignored?" [1]

Over the last decade, however, there have been significant advances in our understanding of the relationship between intestinal flora and liver disease; no one would suggest that these issues are being ignored now. While many of these advances have occurred through the study of alcoholic fatty liver disease, the mechanisms discovered have been applied and extended to many areas of liver research, including NAFLD.

2. NAFLD

NAFLD is the most common cause of chronic liver disease in the United States [2]. A recent analysis of the National Health and Nutritional Evaluation Survey (NHANES) III suggests that 10-24% of American adults have NAFLD [3], making NAFLD three times more common than diabetes mellitus and 5–10 times more common than chronic hepatitis C. Other large, population-based surveys in Europe and Japan in agreement regarding the high prevalence of this disorder [4,5]. Further, two major risk factors for NAFLD, obesity and type 2 diabetes, are also rising [6]. Accordingly, it is clear that NAFLD will remain an important issue in hepatology for many years to come.

NAFLD includes a spectrum of hepatic pathology that ranges from fatty liver (steatosis) at the most clinically indolent extreme, to cirrhosis at the opposite extreme where most liver specific morbidity and mortality occurs [7]. While steatosis itself is often clinically silent, this lesion predisposes the liver to serious damage by additional insults, such as exposure to lipopolysaccharide (LPS), certain hepatotoxins or infectious agents, leading to the eventual development of cirrhosis [8]. Non-alcoholic steatohepatitis (NASH) likely represents an intermediate stage characterized by steatosis with lobular inflammation. NASH progresses to clinically significant cirrhosis in a small but significant number of persons [9]. Indeed, NAFLD is now thought to be responsible for most of what was once classified as ‘cryptogenic’ cirrhosis [10]. Cryptogenic cirrhosis accounts for half of the annual liver-related deaths.

The histopathologic changes of NAFLD are similar to alcoholic fatty liver disease (AFLD). Specifically, biopsy features include steatosis, mixed inflammatory cell infiltration, glycogen nuclei, and Mallory’s hyaline. Various degrees of fibrosis are also often present. A system of classification involving progressive ‘grades’ for steatosis and steatohepatitis and ‘stages’ for varying degrees of fibrosis has been proposed [11].

Given the histopathologic similarities between AFLD and NAFLD, there may be a common pathway for liver injury in alcohol-induced and obesity-related fatty liver disease. Since research on pathogenesis of NAFLD is relatively incipient, many clues from the pathogenesis of AFLD are being applied to NAFLD. In particular, research in rodents, as well as observations in humans, support parallels regarding the importance of the intestinal flora to the pathogenesis of NAFLD.
3. NAFLD pathogenesis

The specific etiology of NAFLD remains an area of active research. Present data, however, point to a ‘multi-hit’ hypothesis [12,13]. According to this theory, the initial insults (‘hits’) that promote hepatic steatosis are common (e.g. obesity, sub-clinical insulin resistance) and generally tolerated by the liver, which becomes fatty but induces various protective mechanisms to survive these initial stresses. However, the adaptations also enhance the fatty liver’s vulnerability to subsequent insults (e.g. certain endogenous or exogenous toxins, including ethanol and lipopolysaccharide) that amplify the initial stress by increasing the production of proinflammatory cytokines (e.g. tumor necrosis factor (TNF)-α). This exacerbates insulin resistance and leads to further oxidative stress and organelle dysfunction within liver cells. The latter eventually kills some hepatocytes and promotes the accumulation of inflammatory cells within the liver. This condition is designated as non-alcoholic steatohepatitis (NASH). After years of such chronic hepatic inflammation, some patients with NASH develop a fibrogenic response that leads to cirrhosis as shown in Fig. 1.

This hypothesis is supported by many studies that have documented the association between NAFLD and other insulin resistance syndromes, including type 2 diabetes, obesity, hypertriglyceridermia, and polycystic ovary disease [14,15]. The possibility that insulin resistance per se may play a fundamental role in the pathogenesis of NAFLD is suggested by other work which demonstrates a very high prevalence of insulin resistance among individuals with hepatic steatosis (the earliest stage of NAFLD) and evidence that the severity of insulin-resistance increases in parallel with the severity of NAFLD, such that clinically-overt, type 2 diabetes is most common in NAFLD patients with cirrhosis [16]. Indeed, the diagnosis of type 2 diabetes is strongly predicative of the fact that cirrhosis has developed in older patients with NAFLD [17].

Until very recently, it was unclear how insulin resistance might lead to liver damage. The association between end-stage liver disease and type 2 diabetes is also well established, and suggests that liver damage might cause insulin resistance (rather than vice versa). This paradox was resolved by pharmacological and genetic manipulation of the enzyme, IKKβ, in experimental animals. Recent study demonstrates that chronic activation of IKKβ, which promotes the activation of NF-κB (the transcription factor that induces inflammatory cytokine production) also causes insulin resistance [18]. Thus, it is now evident that stimuli that cause insulin resistance also promote the production of proinflammatory cytokines, such as TNF-α, and thus may incite an inflammatory response that damages the liver. Because TNF-α also activates the IKKβ pathway, hepatic inflammation perpetuates insulin resistance by causing further activation of IKKβ. Therefore, insulin resistance and inflammatory cytokine production are intimately linked because both are consequences of IKKβ activation (Fig. 2). Given this relationship, it becomes important to identify endogenous factors that lead to the activation of the IKKβ pathway.

Studies in experimental animals suggest that diverse factors that increase hepatic oxidative stress promote insulin resistance, proinflammatory cytokine production, and the progression of fatty liver disease. For example, environmental (e.g. high fat diets) or genetic (e.g. β-oxidative defects [19,20]) factors that increase mitochondrial, peroxisomal and/or microsomal oxidation of fatty acids generate oxidants that can activate the IKKβ pathway [21]. This commonly leads to steatosis and, when oxidant generation is extreme, hepatic anti-oxidant defenses are overwhelmed, leading to liver cell death and steatohepatitis. Toxins, such as ethanol [22] or bacterial lipopolysaccharide [23], that promote the production of reactive oxygen species by hepatocytes and liver macrophages, are often exploited as tools to produce fatty liver diseases and insulin resistance in experimental animals. Notably, both factors activate IKKβ and enhance proinflammatory cytokine production [24].

4. Lumen–liver interactions in NAFLD

As noted above, the possibility that luminal bacteria have
a relationship to liver disease has been entertained for many decades. Over the last decade, however, this notion has matured through significant advances in the pathogenesis of AFLD. When ethanol-fed rats are given neomycin (to partially decontaminate the gut) or polymyxin [25] (to modify intestinal flora) together with ethanol containing diets, they are protected from alcohol-induced liver damage. This protective effect is the result of reduced hepatic exposure to intestinally-derived toxins, such as ethanol and bacterial LPS. Both ethanol and LPS activate hepatic macrophages, causing them to release hepatotoxic factors, such as TNF-α, within the liver micro-environment [32] (Fig. 3).

The possibility that increased endogenous production of ethanol by intestinal bacteria might contribute to the pathogenesis of NAFLD was initially suggested by experiments with ob/ob mice [33]. These leptin-deficient mice develop obesity, insulin resistance, dyslipidemia and fatty livers (hepatic steatosis) [34]. Although humans with NAFLD are not usually leptin-deficient, they are often obese, insulin-resistant, and dyslipidemic. Thus, ob/ob mice are considered to be a model for human NAFLD, and studies of these mice have been helpful in clarifying NAFLD pathogenesis and treatment [35]. Treating ob/ob mice with oral neomycin, a poorly absorbed antibiotic, significantly reduces their endogenous ethanol production [21]. This finding demonstrates the critical role of intestinal flora in endogenous ethanol production and suggests that treatment of bacterial overgrowth might reduce potentially harmful levels of intestinally-derived ethanol in humans with NAFLD. Indeed, a subsequent pilot study of patients with NASH demonstrated increased breath ethanol concentrations among obese females with this condition, confirming the suspicion that increased intestinal ethanol production occurs in some humans with NAFLD [36].

Many observations in humans with NAFLD are consistent with such research. Obese individuals with fatty livers are at increased risk for NASH, a progressive, potentially-fatal form of fatty liver disease after undergoing jejuno-ileal (J-I) bypass surgery [37]. This procedure creates a ‘blind loop’ of intestine and, therefore, places the patient at very high risk for IBO. At least two lines of evidence suggest that it is intestinal bacterial overgrowth that exacerbates fatty liver disease after J-I bypass:

1. Treatment with metronidazole, an antibiotic, or surgical removal of the redundant ‘blind loop’ of intestine, reverses J-I bypass-related liver damage [38]
2. NASH recurs quickly after liver transplantation unless the J-I bypass is taken down during the liver transplant surgery [39]

Additional support for the possibility that intestinal bacterial overgrowth exacerbates fatty liver disease progression in

![Image](image_url)
humans is provided by a report that NASH developed in a patient with jejunal diverticulosis and small bowel overgrowth [40]. A more general role for intestinal bacterial overgrowth in the progression of human fatty liver disease to NASH remains speculative at this point, but is supported by evidence that obesity and diabetes (which are major risk factors for NASH) are also associated with intestinal dysmotility [41] and small bowel bacterial overgrowth [42]. Indeed, a recent study demonstrated an increased prevalence of IBO in obese patient with NASH [28]. Further, elevated levels of TNF-α compared to age- and sex-matched controls have recently been reported in NASH patients [43]. Finally, it is worth noting that IBO has frequently been reported in patients with cirrhosis of any cause [44,45], and one study even suggested improvement in liver function in cirrhotics after eradication of IBO with antibiotics [46].

5. Potential therapies and a role for probiotics

Strategies for arresting the progress of NAFLD, or even reversing its changes, are critical to the long-term health of many persons. Ideally, such strategies should interrupt the pathogenesis of NAFLD on multiple levels and be safe, cost-effective, well tolerated, and accepted for long-term use [47]. Presently, however, there are no proven treatments for NAFLD. Indeed, the published literature contains two pilot studies demonstrating efficacy of vitamin E [48,49]. Several other small studies of ursodeoxycholic acid [50–52], triglitazone [53], gemfibrozil [54], betaine [55], N-acetylcysteine [56], or rosiglitazone [57] have been also reported, primarily in abstract form. These studies typically used transaminases or alkaline phosphatase as outcome measures and did not assess the effect of treatment on liver histology. Interestingly, however, all of the aforementioned agents that demonstrated potential for NAFLD are known to have anti-oxidant (and thus, anti-inflammatory and insulin-sensitizing) actions. Therefore, each of these drugs interrupts NAFLD pathogenesis. Because probiotics work at multiple levels to down-regulate endogenous inflammatory mediators and are also inexpensive and safe, they are attractive for the long-term management of NAFLD.

Given the importance of intestinal flora in liver disease, treatments such as antibiotics or probiotics (to alter the intestinal flora) may influence the evolution of NAFLD. (Fig. 4).

The most obvious approach might involve antibiotics to decontaminate the gut flora. Indeed, as noted above, neomycin has demonstrated efficacy in rodent models of AFLD. As further ‘proof of principle,’ antibiotics have been shown to reduce hepatic injury in rats with surgically induced IBO [58]. However, antibiotics may result in unpleasant or unsafe side effects, especially when taken long-term. Further, bacterial resistance can limit antibiotic effectiveness, and predispose patients to life-threatening illnesses. Perhaps most importantly, however, antibiotics could eliminate specific therapeutic benefits of commensal bacteria.

Probiotics, in contrast, are inexpensive, safe, and have no known negative long-term effects. Further, their role may extend beyond the eradication of pathogenic bacteria in the small or large bowel; probiotics have multiple direct beneficial effects that antibiotics lack [59,60]. Further, probiotics are a natural therapy and, as such, are widely accepted by the public. Indeed, they are sometimes considered part of complementary or alternative medicine (CAM). Studies consistently demonstrate extensive use of CAM by patients, including those with liver disease [61].

While pathogenic intestinal flora may cause significant hepatotoxicity via multiple mechanisms described above, evidence is mounting that non-pathogenic strains of bacteria, i.e. commensal bacteria, actually provoke anti-inflammatory or tolerizing responses from surface epithelia.

Fig. 4. NAFLD treatments may be directed against gut bacteria.
Probiotics may impact the development of NAFLD by

1. Competitive inhibition and possible eradication of pathogenic strains of IBO
2. Alteration of the inflammatory effects of pathogenic IBO through changes in cytokine signaling
3. Improved epithelial barrier function
4. Direct decreases in proinflammatory cytokines (e.g. TNF-α)

These mechanisms are not mutually exclusive, and it is likely that probiotics have multiple actions. In fact, researchers reported that a combination probiotic compound, VSL#3, performs all of these functions in a murine colitis model [62]. The molecular mechanisms for these actions are being identified. Further work by the same investigators have found that DNA from VSL#3 can down regulate proinflammatory cytokine secretion and attenuate the NF-κB signal pathway [63]. Such research is in agreement with previous landmark work by other investigators demonstrating that non-pathogenic strains of salmonella downregulate NF-κB activity [64]. Notably, preliminary work on probiotics in ob/ob mice has demonstrated efficacy [65]. In this research, ob/ob mice provided with probiotics had improvement in hepatic histopathology, reduced serum alanine aminotransferase levels, and decreased TNF-α activity (as reflected by reduced Jun N-terminal kinase activity) compared to control ob/ob mice.

Multiple other studies using various probiotic bacteria are expanding our understanding of their multiple mechanisms of action and are directly relevant to liver disease. Previous research, for example, has found high concentrations of endotoxin in portal and systemic circulation in patients with chronic liver disease [66]. This finding is consistent with the pathogenic mechanisms described above. Other research has demonstrated that administration of Bifidobacterium lactis reduces the incidence of bacterial translocation in adult Wistar rats after 80% gut resection [67], and the Journal recently reported that Lactobacillus johnsonii La 1 alone or in combination with antioxidants diminish bacterial translocation and endotoxiaemia [68]. Murine models of acute liver injury have also demonstrated reductions in bacterial translocation and hepatic injury after administration of probiotics using various lactobacillus and bifidobacterium species [69,70]. Other studies have demonstrated that commensals trigger a differential cytokine response in intestinal epithelial cell/leukocyte co-cultures [71].

Meanwhile, probiotics are increasingly being studied in human trials. Probiotics may have multiple beneficial effects on inflammatory bowel and diarrheal diseases [72,73]. VSL#3, for example, has also been shown to reduce the risk of recurrence of pouchitis [74]. In addition to the above mechanisms, some have proposed that probiotics may enhance intestinal epithelial viability by providing essential nutritional support (e.g. medium chain fatty acids) that inhibits apoptosis of luminal epithelial cells [75]. Finally, consistent with the theory that gut inflammatory signaling is relevant not only to the local lumenal environment and mesenteric circulation, oral probiotics have been found to have systemic immunomodulatory effects [76] and are increasingly being successfully investigated as treatments for systemic atopic diseases [77,78].

Preliminary evidence from a single uncontrolled human pilot study suggests that probiotics may have efficacy in NAFLD [79]. Researchers administered a combination probiotic along with vitamins, nutrients, and probiotics to ten patients with biopsy-proven NASH, and found a decrease in alanine transferase and alkaline phosphatase compared to basal values. After withdrawal of the study product, the lab values reverted back toward basal values. More controlled trials are needed to confirm this finding.

6. Summary

NAFLD is a common and serious disease. The pathogenesis of NAFLD continues to be elaborated. Recent progress suggests some commonality with the pathogenesis of AFLD; specifically, the importance of lumen–liver interactions and increased activity of TNF-α. Such understanding furthers interest in the intestinal flora, which may be an appropriate target for therapy. Such therapy should be safe, well-tolerated, inexpensive, appropriate for long term use, and optimally, work at multiple levels to down-regulate inflammatory mediators. Probiotics could be an option.

References


