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Nonalcoholic fatty liver disease: predisposing factors and the role of nutrition☆

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Abstract

More than 20% of Americans have nonalcoholic fatty liver disease (NAFLD), and this is, by far, the leading cause of abnormal liver enzymes in the United States. Nonalcoholic steatohepatitis (NASH), a more serious form of NAFLD, can proceed to cirrhosis and even hepatocellular carcinoma. These liver diseases represent the hepatic component of the metabolic syndrome, and this spectrum of liver disease represents a major health problem both in the United States and worldwide. Hepatic steatosis is closely linked to nutrition, including obesity, possibly high-fructose corn syrup consumption and consumption of certain types of fats. There are a variety of second insults or "hits" that appear to transform simple steatosis into NASH, with some of these second hits including certain proinflammatory cytokines, oxidative stress and possibly industrial toxins. In certain underdeveloped countries, it appears likely that industrial toxins play a role in NASH, and there is increasing interest in the potential interaction of industrial toxins and nutrients. Moreover, optimal therapy for NAFLD appears to include lifestyle modification with exercise, diet and weight loss. Certain nutrients may also be of benefit. Important areas for future research are the effect(s) of nutritional supplements on NAFLD/NASH and the effects of industrial toxins.
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Keywords: Non-alcoholic steatohepatitis; Industrial toxins; Non-alcoholic fatty liver disease

1. Introduction

Obesity-associated fatty liver disease was first described by Westwater and Fainer [1] nearly 50 years ago. However, little progress was made until 1979, when Adler and Schaffner [2] described fatty liver, hepatitis and cirrhosis mimicking alcoholic liver disease in a group of overweight patients often with diabetes and lipid abnormalities. The following year, Ludwig et al. [3] coined the term *nonalcoholic steatohepatitis* to describe similar pathologic findings in a group of obese, often diabetic, female patients with abnormal liver function tests. Since then, the term *nonalcoholic fatty liver disease* (NAFLD), has been used to describe a larger spectrum of steatotic liver disease, generally associated with the metabolic syndrome [4,5].

NAFLD is defined by clinicopathologic criteria [4–6]. Clinically, patients do not consume significant quantities of alcohol (generally defined as no more than two drinks per day). Pathologically, several patterns of disease exist, which resemble alcoholic liver disease. The sine qua non of NAFLD is macrovesicular steatosis or fatty liver. If this condition exists in isolation, the patient is said to have simple steatosis or nonalcoholic fatty liver (NAFL). The majority of patients seem to tolerate this condition well and likely have limited progression to cirrhosis [6,7]. However, some patients with steatosis develop superimposed necroinflammatory activity with a nonspecific inflammatory infiltrate, hepatocyte ballooning with Mallory's hyaline and, sometimes with fibrosis, called nonalcoholic steatohepatitis (NASH) (Table 1). Some of these patients will develop cirrhosis, which may become complicated by hepatocellular carcinoma and die of a liver-related cause. This article will review the epidemiology, clinical presentation, pathogenesis and treatment of NAFLD, with a focus on nutrition and

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Table 1
Classification of NASH

NASH (primary)	NASH (secondary)		
Metabolic syndrome	Nutrition	Gastrointestinal surgery	Drugs/environmental toxins
Obesity	Rapid weight loss	JIB	Tamoxifen
Hyperlipidemia	TPN	Others	Amiodarone
Type 2 diabetes mellitus	Others		Petrochemical exposure

TPN, total parenteral nutrition; JIB, jujeno-ileal bypass.

potential nutritional and environmental interactions contributing to the disease.

2. Epidemiology

NAFLD is now the most common liver disease in the United States and possibly worldwide. Furthermore, the number of affected patients is growing rapidly, and the disease has reached epidemic proportions. NAFLD is the hepatic manifestation of the metabolic syndrome, and it is important to first examine the epidemiology of obesity, the metabolic syndrome and other contributing factors such as high-fructose corn syrup and saturated fat consumption. Occupational exposure to petrochemicals may produce a more aggressive cholestatic NAFLD, even in the absence of the metabolic syndrome. Chemotherapy associated steatohepatitis (CASH) is likely to become a growing area of concern for cancer survivors.

The prevalence in obesity in US adults has more than doubled since the 1960s, with the greatest increase occurring since 1980 [8]. Recently analyzed data from the National Health and Nutrition Examination Survey (NHANES) reveal that in 2003–2004, the prevalence of overweight [body mass index (BMI) 25.0–29.9] in the US adult population was 34.1%, and the prevalence of obesity (BMI 30.0 or higher) was 32.2% [9]. Children and adolescents (age 2–19) were not spared and the prevalence of overweight (BMI for age $\geq$95th percentile) in this group was 17.1% in 2003–2004 [9].

The prevalence of metabolic syndrome has risen along with obesity. The prevalence of Adult Treatment Panel III-defined metabolic syndrome in US adults is estimated at 26.7% using NHANES 1999–2000 data, an increase of 3.6% from NHANES III data (1988–1994) [10].

It is interesting to note that the rising prevalence of obesity and metabolic syndrome closely parallels the increased consumption of high-fructose corn syrup. Before 1970, per capita consumption of high-fructose corn syrup was essentially nil [11]. However, consumption rose dramatically in the 1980s, and by 2000, was 91.6 g per person per day and accounted for 42% of all caloric sweeteners [11]. Furthermore, increased consumption of high-fructose corn syrup in the form of artificially sweetened beverages has been implicated in the obesity epidemic [11]. In bariatric surgery patients with NAFLD, higher carbohydrate consumption, not limited to fructose, has been associated with increased hepatic inflammation on biopsy [12].

Lipotoxicity caused by long-chain fatty acids has been implicated in the development of numerous obesity related diseases including NAFLD. These fatty acids constitute a significant component of dietary fatty acids and are synthesized by the liver via de novo fatty acid biosynthesis. Significant changes have occurred in dietary fat intake in the US over the last 40 years. From 1965 to 1991, the consumption of total and saturated fat decreased, only to begin rising in 1994–1995 [13].

The true prevalence of NAFLD is unknown because it is unethical to perform liver biopsies on unselected asymptomatic patients from the general population. Both the sensitivity and specificity of noninvasive tests are limited, and only liver biopsy can distinguish steatosis from steatohepatitis and fatty cirrhosis. However, various strategies have been used to estimate the prevalence of NAFLD with surprisingly concordant results. These strategies include unexplained alanine aminotransferase (ALT) elevation or fatty liver on ultrasound in nondrinking but otherwise unselected patients and post-mortem histological examination of the livers of random deaths such as traffic fatalities. When these data are examined in total, it appears that, in adults, the prevalence of simple steatosis is 20% to 30%, and NASH, 2% to 3% [14]. The problem is not limited to adults, and nearly 10% of obese children may have NAFLD [15].

In addition to obesity/metabolic syndrome, dietary high-fructose corn syrup and long-chain saturated fat and occupational exposure to petrochemicals have also been implicated in NAFLD. In a group of Bulgarian petrochemical workers, elevated liver enzymes (up to 18% prevalence) and dyslipidemia (up to 25%) were found, often in association with hepatomegaly and decreased serum glutathione (GSH) [16]. In Brazil, the prevalence of elevated liver enzymes was 3.56-fold higher in petrochemical workers than in a control population regardless of the presence of obesity, exercise, smoking and alcohol consumption [17]. Another, rapidly expanding group of NAFLD patients with "toxic exposures" includes cancer patients with CASH, which will not be reviewed here [18].

3. Clinical features and prognosis

Although NAFLD may present at any stage, including cirrhosis with hepatocellular carcinoma, the most common presentation is in the asymptomatic, nondrinking patient with mildly elevated transaminases (ALT usually greater than aspartate aminotransferase [AST]). Patients will generally have associated metabolic comorbidities such as obesity, the metabolic syndrome, diabetes and dyslipidemia. Middle-aged males are most commonly affected, and Hispanics seems to be at particularly high risk, while African Americans are relatively spared [19,20]. The most

common symptoms include vague upper abdominal discomfort or right upper quadrant fullness, while the most common physical exam findings are obesity and hepatomegaly. Liver biopsy is the gold standard diagnostic test and is the only way to differentiate isolated steatosis from steatohepatitis, with or without fibrosis. However, this invasive test is not always required, particularly in patients with the typical presentation, after the serologic exclusion of other liver diseases and especially if imaging shows fatty infiltration of the liver.

The prognosis of NAFLD is variable and seems to depend greatly on the severity of the presenting disease on initial liver biopsy. Simple steatosis appears to progress infrequently, but NASH may progress both histologically and clinically. In a prospective repeat liver biopsy study, 31.8% of patients with NASH had progressive fibrosis occurring at a median of 4.3 years [21]. Furthermore, NASH may progress to histologically confirmed cirrhosis in up to 15% of patients [22]. Although definitive data on hepatocellular carcinoma and NAFLD are lacking, up to 29% of these cancers develop on a background of cryptogenic cirrhosis, the majority of which are probably due to NAFLD [23]. A major criticism concerning the clinical relevance of NAFLD is concern that affected patients will die from metabolic syndrome-associated cardiovascular disease, dying with NAFLD but not from it. However, in a study with up to 18 years of follow up, 11% of patients with histologically confirmed NASH died a liver-related death, which was as many deaths as from coronary artery disease [24]. Moreover, NASH is an inflammatory state, which may predispose the patient to other problems, such as cardiovascular conditions [25].

4. Mechanisms

The mechanisms leading to NASH are likely to be multiple. Certainly, the development of hepatic steatosis in experimental animals can be caused by many factors. In patients with NASH, it is felt that there is a baseline of steatosis plus some other insult, the so-called 2-hit theory [5,26]. Some of the likely second hits include oxidative stress, mitochondrial dysfunction, abnormal methionine metabolism, insulin resistance and industrial toxins (of particular interest for this article).

4.1. Oxidative stress

Oxidative stress is well documented in NASH. Studies from Sanyal et al. [27] demonstrated that immunohistochemical staining for 3-nitrotyrosine, a marker for oxidative stress, was elevated in liver biopsies of subjects with NAFLD, and NASH patients had significantly elevated levels above both normal controls and NAFLD patients. Thioredoxin, an oxidative, stress-inducible thiol-containing protein, which has major antioxidant properties, was significantly elevated in the serum of patients with NASH, compared to those with simple steatosis or healthy volunteers [28]. The potential sources for the reactive oxygen species are multiple, with hepatic cytochrome P450 2E1, liver mitochondria, adipose tissue and iron overload serving as some of the possible sources. Increased CYP2E1 can occur with obesity [29,30]. Recent studies by Emery et al. [30] documented increased CYP2E1 levels in morbidly obese human subjects with NASH. Importantly, levels significantly decreased following gastric bypass surgery and weight loss. Obesity, per se, is a state of increased oxidative stress, with waist circumference correlating with urinary isoprostane levels, a marker for oxidative stress. Indeed, fat is an active metabolic organ, and fat can generate reactive oxygen species such as hydrogen peroxidase [31]. Lastly, similar to data in human beings, animal models of fatty liver also show lipid peroxidation and oxidative stress.

4.2. Mitochondrial dysfunction

Mitochondrial abnormalities are also postulated to be responsible for increases in reactive oxygen species (ROIs) and represent one of the “hits” or insults in most schemes of NASH. Mitochondria generate most of the cells’ adenosine triphosphate (ATP) at the expense of high ROI formation. ROI formation is thus further enhanced when electron flow is impaired. Steatosis induced by multiple diverse mechanisms is associated with lipid peroxidation [32]. Products of increased lipid peroxidation alter mitochondrial DNA and inhibit normal electron flow. Tumor necrosis factor (TNF) also impairs mitochondrial respiration [33]. Conversely, disruption of mitochondrial respiration markedly enhances TNF hepatotoxicity [34]. Thus, there is a close interaction between TNF and mitochondrial dysfunction in the development of many forms of liver injury. Moreover, many forms of oxidative stress lead to antioxidant depletion, which then further enhances oxidative stress and cytokine-mediated hepatoxicity. Mitochondrial abnormalities are noted at many different levels in NASH [35]. Sanyal et al. [27] demonstrated that NASH was associated with enlarged mitochondria and loss of mitochondrial cristae and paracrystalline inclusions in 9 of 10 subjects, compared to zero of six subjects with simple steatosis on evaluation with electron microscopy. NASH patients also had immunohistochemical evidence of lipid peroxidation, again associating oxidative stress and mitchondrial dysfunction. Diehl’s group showed that humans with NASH have impaired ATP regeneration after fructose infusion to deplete ATP [36].

4.3. Dysregulated cytokine metabolism

Abnormal cytokine metabolism is a major feature of both alcoholic and nonalcoholic steatohepatitis. We first reported dysregulated TNF metabolism in alcoholic steatohepatitis over 15 years ago, with the observation that cultured monocytes (which produce the overwhelming majority of systemic circulating TNF and are surrogate markers for Kupffer cells) from alcoholic patients spontaneously produced TNF and produced significantly more TNF in

response to an endotoxin [(lipopolysacchride (LPS)] stimulus [37]. Subsequently, increased TNF concentrations have been well documented in multiple animal studies to play a critical role in the development of alcoholic liver disease (ALD) [38]. The vast majority of cytokine abnormalities observed in alcohol-induced liver diseases are also observed in NASH [39]. Much of what we know of the mechanism(s) of NASH comes from animal models, such the methionine-restricted, choline-deficient diet (MCD) nutritional model of fatty liver or genetically obese mice and rats (ob/ob mice and fa/fa rats exhibit obesity, insulin resistance, hyperglycemia and hyperlipidemia and have fatty livers) [39]. Similar to rats chronically fed alcohol, ob/ob mice are much more sensitive to endotoxin hepatotoxicity [40]. They also have increased TNF production [39,40]. Importantly, fat stores in these mice also serve as a major source of cytokine production. Rats fed MCD develop severe steatosis and increased serum/hepatic TNF levels similar to obese ob/ob mice [41,42]. These MCD rats are highly sensitive to endotoxin hepatotoxicity [41,42]. Isolated Kupffer cells from these rats also are primed to overproduce TNF in response to an LPS stimulus. Activation of the transcription factor, nuclear factor kappa B (NFkB), appears to play an important role in this liver injury [43]. Thus, rodents with genetically and nutritionally induced NASH have dysregulated cytokine metabolism similar to that seen in human NASH and to that seen in ALD. Recent data from our laboratory also demonstrate that obese patients with NASH have increased serum TNF concentrations and increased monocyte production of inflammatory cytokines such as IL-8, similar to alcoholic steatohepatitis [44]. Studies by Crespo et al. [45] showed significantly increased hepatic TNF mRNA and increased hepatic TNF receptor 1 mRNA in NASH patients. A TNF polymorphism has been associated with NASH [46]. Thus, human beings with NASH have increased serum levels of TNF and hepatic TNF and other proinflammatory cytokines as one likely mechanism for liver injury.

Adiponectin, a cytokine secreted from adipose tissue, can act directly on hepatic tissue and inhibit glucose production [47], and it has potent antiinflammatory, anti-TNF effects. Low levels of adiponectin have been associated with high visceral fat and have been implicated in the development of the insulin resistance syndrome. Pioglitazone, an insulin-sensitizing agent, given to subjects with type 2 diabetes, resulted in a threefold increase in plasma adiponectin levels which, in turn, was strongly associated with a decrease in hepatic fat and improvements in muscle and hepatic insulin sensitivity [48]. In a mouse model of NASH, administration of adiponectin was associated with decreases in hepatic lipid contents and serum ALT levels [49]. Furthermore, in a study of 90 morbidly obese Chinese subjects, plasma levels of adiponectin inversely correlated with those of ALT [49]. Research from our laboratory showed that serum adiponectin levels were significantly reduced in obese compared to lean children and were significantly reduced in children with NAFLD compared to obese children without NAFLD [4]. Significantly decreased adiponectin receptor II mRNA in the liver has also recently been documented in NASH, and this correlated with serum transaminase and fibrosis score [50]. Further studies in human beings on the effects on adiponectin in NAFLD/NASH are underway in several laboratories including our own.

4.4. Abnormal Methionine/SAMe/Betaine Metabolism

S-adenosylmethionine (SAMe) is an important metabolic intermediate in the transsulfuration pathway and is formed from methionine and ATP in a reaction catalyzed by methionine adenosyl transferases (MAT) [51–53] (Fig. 1). SAMe deficiency occurs in many forms of liver disease. This deficiency was first identified in ALD in the early 1980s when it was observed that alcoholic subjects had a delayed clearance of an oral bolus of methionine but had no detectable accumulation of any other metabolic intermediates of the transsulfuration pathway (presumably due to a blocked conversion of methionine to SAMe) [51]. Subsequently, Mato et al [52–54] confirmed this postulate and demonstrated that the functional MAT was indeed subnormal in liver biopsies from alcoholic subjects. Because SAMe is a precursor for GSH synthesis, SAMe deficiency may result in GSH deficiency which is observed in many forms of liver disease [51]. In animal studies, exogenous SAMe corrected hepatic deficiencies of both SAMe and GSH. GSH is required for optimal expression of MAT activity in liver, and hepatic deficiency of MAT may, in part, be due to GSH deficiency. Also, hepatic MAT is sensitive to oxidative stress; oxidation of cys 121, the active site of the enzyme located in its flexible loop, results in loss of its activity [55]. Thus, subnormal hepatic MAT activity in NASH patients could occur due to oxidation of this active site. Moreover, MAT1A knockout mice develop steatohepatitis with hepatic SAMe deficiency [56].

In the transmethylation pathway, SAMe is converted to *S*-adenosylhomocysteine (SAH). While SAMe levels tend to be low in most forms of liver disease, SAH levels are often elevated, as are homocysteine levels [57] (Fig. 1) SAH can sensitize hepatocytes to TNF-induced hepatotoxicity, and homocysteine is a major inducer of fatty liver [57]. Elevated homocysteine also is a major risk factor for atherosclerosis and stroke and, thus, of relevance to obesity. Increases in SAH and/or decreased SAMe/SAH ratios inhibit most transmethylation reactions. Thus, it is important to be able to remove excess levels of both SAH and homocysteine from the liver.

One way of removing homocysteine and *S*-adenosylhomocysteine is by providing betaine for the conversion of homocysteine to methionine [58,59]. The other pathway for regeneration of homocysteine to methionine involves the enzyme methyltetrahydrofolate reductase (MTHFR, to produce 5-MTHF) (Fig. 1). However, there is a frequent polymorphism in this gene that leads to decreased activity and may potentially lead to more problems with fatty

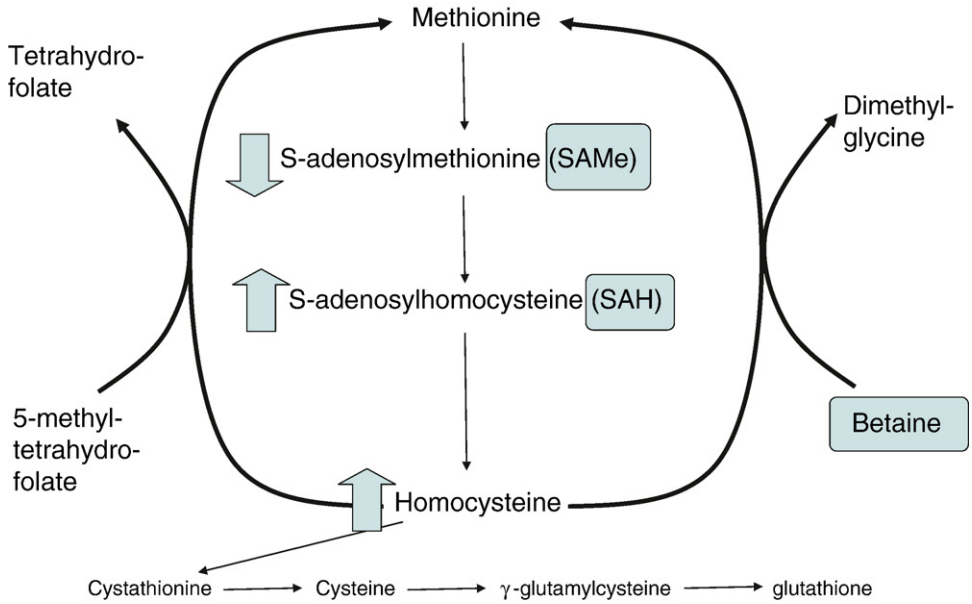

Fig. 1. Diagram of hepatic methionine metabolism. There is impaired conversion of methionine to SAMe because of decreased hepatic methionine adenosyltransferase (MAT1A) activity. Low SAMe may predispose to increased endotoxin stimulated TNF production. In contrast, there are increased levels of both SAH and homocysteine in many forms of fatty liver. Increased homocysteine has been implicated in the etiology of fatty liver, and increased SAH sensitizes to TNF hepatotoxicity. Homocysteine and SAH can be removed either by giving betaine to regenerate methionine or through 5-methyltetrahydrofolate metabolism. Polymorphisms in the 5-MTHFR gene can impair this pathway and potentially exacerbate fatty liver. Moreover, the ratio of SAMe to SAH is critical in controlling methyltransferase reactions. Thus, this metabolic pathway seems to be vital in the genesis of many types of liver disease, and there are molecular targets, such as SAMe and betaine, that may provide novel therapeutic interventions. These potential interventions may have great relevance to prevention/treatment of toxin-induced NASH.

liver [60]. Indeed, MTHFR knockout mice develop fatty liver [60]. Additionally, the presence of this polymorphism has been linked to atherosclerosis and complications of diabetes such as accelerated nephropathy in human beings. Thus, abnormalities in the hepatic transsulfuration/transmethylation pathways on multiple levels may lead to the development of fatty liver and potentially to sensitization to TNF-induced hepatotoxicity.

4.5. Insulin resistance

Several studies have reported the association between insulin resistance, insulin resistance syndrome and NAFLD/NASH. Marceau et al. [61] reported the common presence of features of the insulin resistance syndrome in morbidly obese subjects undergoing gastric bypass surgery who had histological diagnosis of NASH. Marchesini et al. [62] reported hyperinsulinemia and insulin resistance in subjects with ultrasonographic evidence of fatty liver. Insulin resistance was more closely related to hepatic fat than BMI, suggesting an independent role of insulin resistance. Knobler et al. [63] reported 48 subjects with hepatic steatosis and noted the association with features of the insulin resistance syndrome — abdominal obesity, dyslipidemia and glucose intolerance. Cortez-Pinto et al. [64] also noted the association of features of insulin resistance syndrome and NASH. Direct assessment of insulin resistance in nondiabetic subjects with fatty liver and NASH by insulin clamp techniques has further confirmed the association between NASH and insulin resistance. Sanyal et al. [27] demonstrated decreased peripheral insulin sensitivity and increased hepatic oxidative stress in nondiabetic subjects with hepatic steatosis and NASH. Marchesini et al. [65] demonstrated that nondiabetic lean or mildly overweight subjects with NAFLD have both peripheral and hepatic insulin resistance. These defects are similar to those seen in subjects with type 2 diabetes mellitus. The cumulative evidence from these studies shows that insulin resistance represents a reproducible predisposing factor for NASH.

The mechanism(s) that lead to the association between insulin resistance and NASH are not clearly understood. Several possibilities have been explored: (a) insulin resistance leads to fatty liver and NASH, (b) hepatic steatosis leads to insulin resistance, or (c) some common factors lead to both insulin resistance and NASH [5,66]. Insulin resistance favors accumulation of free fatty acids in the liver and predisposes to oxidative stress by stimulating microsomal lipid peroxidases. The direct effect of high insulin levels is to decrease mitochondrial β-oxidation of fatty acids, a recognized mechanism of hepatic fat accumulation. Insulin is an anti-lipolytic hormone. Insulin resistance could contribute to hepatic steatosis by favoring peripheral lipolysis and hepatic uptake of fatty acids. Insulin also inhibits oxidation of free fatty acids, thus increasing toxic free fatty acids in the liver. Free fatty acids, in particular dicarboxylic acid, may themselves be cytotoxic. Fatty acids are both substrates and inducers of cytochrome P450 2E1. Levels of CYP 2E1 are normally suppressed by insulin and invariably increased in the livers of patients with NASH.

Table 2
Selected genes participating in liver detoxification function whose transcriptome was down-regulated 1.5-fold or more in high carbohydrate diet-induced fatty liver of mice

Gene code	Gene name
AF464943.1	GSH *S*-transferase, mu 4
J03952.1	GSH *S*-transferase, mu 1
J03953.1	GSH *S*-transferase, mu 3
NM_010357.1	GSH *S*-transferase, alpha 4
NM_008182	GSH *S*-transferase, alpha 2 (Yc2)
NM_028089.1	Cytochrome P450, family 2, subfamily c, polypeptide 55
AF128849.1	Cytochrome P450, family 2, subfamily b, polypeptide 20
NM_008898.1	Cytochrome P450, family 3, subfamily a, polypeptide 41
NM_009993.1	Cytochrome P450, family 1, subfamily a, polypeptide 2
AK018563.1	Arylformamidase

Liver fat has also been postulated to cause insulin resistance. Banerji et al [67] showed that in African American males with type 2 diabetes mellitus, liver fat was inversely related to insulin sensitivity. The effect of exogenous insulin in improving blood glucose control in subjects with type 2 diabetes mellitus has been shown to correlate inversely with liver fat [68]. The effect of insulin-sensitizing agents, rosiglitazone and pioglitazone, on glycemic control in subjects with type 2 diabetes mellitus also correlates with reduction in liver fat [69,70].

4.6. Industrial exposure

The potential interaction between nutrition and the hepatic response to xenobiotics can be viewed from two perspectives:

4.6.1. The effect of nutrition on hepatic detoxification capability

The first question of how nutrition influences the hepatic response to xenobiotics has begun to receive some investigative attention, especially following the recognition of NAFLD/NASH. Its high prevalence, reaching over 20% of the American population, highlights the question of whether fatty liver disease may affect the hepatic response to industrial pollutant exposure. Unfortunately, no thorough clinical or experimental studies have been performed to assess the impact of fatty liver on its detoxification capability.

As emphasized earlier in this review, a major objective of current research on NASH is identification of factors initiating and supporting the progression of NAFLD to NASH. Thus, the theory of the "second hit" or "second hits" has evolved in search for external or endogenous factors that initiate the NASH [26,71]. Several candidates for "second hits," both endogenous and exogenous, have been considered, with the latter including a number of hepatotoxins, which can be further divided according to their origin (biologic or synthetic/industrial). This second group is of particular interest for this review because the amount and variety of hepatotoxins in the environment (including the food and water), have increased in our industrialized society. An example of a hepatotoxin of biologic origin is the Gram-negative bacterial LPS. This compound is classically known to induce liver inflammation via activation of peripheral blood monocytes and liver-resident macrophages to produce inflammatory agents such as cytokines, leukotrienes and others. Indeed, it has been demonstrated that the fatty liver, whether produced by the lack of leptin (ob/ob mice) [72], by forcing deposition of lipids in the liver through genetic deletion of microsomal triglyceride transfer protein [73] or by feeding animals a methionine-choline deficient diet [41,42] has a higher propensity than the normal liver to undergo transition to necroinflammation in response to LPS. Thus, it is likely that powerful immune system stimuli such as bacteria or their products may at least contribute to, if not initiate, the progression of NAFLD to NASH.

The number and the wide diversity of chemical structures of hepatotoxins from industrial sources (including the pharmaceutical industry) is much larger than the number of biologic agents. Unfortunately, very few studies have approached the issue of fatty liver sensitivity to xenobiotics. In a recent study, we performed a large-scale gene profiling analysis of the liver in a nutrition model of NAFLD (induced by high-carbohydrate diet feeding of mice). We detected changes in a large number of genes involved in biotransformation of xenobiotics (Deaciuc, unpublished data). Some of these genes are presented in Table 2. Because the changes consisted of down-regulation of gene expression, we postulate that the fatty liver in this model of NAFLD may have an impaired detoxification/neutralization capacity, thus being more vulnerable to toxic effects of industrial pollutants, such as those evolving from the petrochemical industry. A closer examination of the data in Table 2 reveals that the down-regulation of enzymatic detoxification capacity was extensive in that it included GSH conjugation enzymes (GSH *S*-transferases), cytochromes P450 and others (not listed). Although the mechanisms underlying these changes remain to be elucidated, we hypothesize that such a background may sensitize the liver to hepatotoxic agents whose action may lead to liver necrosis, an obligatory condition of NASH. Assessment of the impact that changes in gene expression may have on the transition of NAFLD to NASH is ongoing in our laboratory.

Another important aspect of fatty liver response to xenobiotics may be the liver's capacity to store liposoluble xenobiotics. This concept is based on experimental data showing that adipose tissue stores lipophilic xenobiotics, such as organochlorine compounds [74,75]. It is important to note that most of the nonparticulate xenobiotics of petrochemical origin are highly lipophilic. Because most of the xenobiotics absorbed into the bloodstream from the gut first pass through the liver, some may be retained in the fat macrovacuoles of hepatocytes. While this may contribute to the immediate clearance from the blood of such compounds, it may also contribute to their subsequent release into the cytoplasm in higher concentrations once the liver fat deposits diminish. This may take place concomitantly with the loss of body weight.

4.6.2. The effect of xenobiotics on liver function

A second aspect of the hepatic response to xenobiotics is that exposure to industrial petrochemical agents has been documented to induce NAFLD even in the absence of obesity, diabetes, dyslipidemia and insulin resistance [76–78]. Moreover, the liver disease was at least partially reversible with removal from the work environment. In exposed employees, both with and without metabolic comorbidities, NAFLD occurred earlier, featured a cholestatic component, and were more likely to present with more advanced disease such as NASH associated with fibrosis than in patients with NAFLD who had no petrochemical exposure [77].

In a parallel vein, industrial pollutants, particularly those originating from diesel exhausts, have been shown to have a strong vasoconstrictive effect [79] and may serve as a "second hit" in the cardiovascular system in "sensitive" animals. Thus, it has been demonstrated that nonparticulate diesel exhaust pollutants enhance the vasoconstrictive effects of endothelin 1 in the hearts of sensitive ApoE$^{-/-}$ mice [80]. These mice also have more electrocardiographic abnormalities that their wild-type controls after exhaust exposure. ApoE$^{-/-}$ mice have NAFLD and hyperlipidemia, and we are beginning to investigate whether diesel exhaust exposure causes a second hit or insult in the livers of these mice as well as their hearts.

Earlier experimental data [81] have documented that nutritional factors including proteins, carbohydrates, fats, vitamins and minerals may modulate the liver's capacity to process xenobiotics. Unfortunately, this area has not received much investigative attention. We believe that because of the increased prevalence of NAFLD among Americans and others in developed countries, more attention should be paid to potential links between nutrition and biotransformation of industrial pollutants by the liver.

5. Treatment

5.1. Overview

The type of treatment and its aggressiveness depend on the severity of the liver disease, as well as related comorbidities. For example, patients with decompensated cirrhosis may require liver transplantation to prevent death, while patients with simple steatosis may require only lifestyle modifications because they are at low risk for progression. Treatment becomes more complicated for patients with biopsy-proven NASH, particularly those with fibrosis or asymptomatic cirrhosis. Aggressive, multimodality treatment is indicated for these patients because there is the opportunity to slow or even reverse the progression of their liver disease, preventing liver-related morbidity and mortality. As a separate issue, metabolic syndrome-associated conditions such as diabetes and dyslipidemia, must be identified and treated, not only for the sake of the liver, but to reduce cardiovascular mortality as well.

There are insufficient high-quality studies to precisely determine the proper treatment of NASH. To this end, the US Food and Drug Administration has not approved any medications for use specifically in NAFLD, and all medications should be considered experimental. However, based on knowledge of the pathogenesis of the disease, multiple smaller human trials of reasonably good quality have suggested the effectiveness of multiple treatments. These include lifestyle modifications, nutritional supplements and prescription medications; the first two are addressed in this review.

5.2. Lifestyle modification

Lifestyle modifications are the cornerstone of NAFLD therapy. They include weight loss from diet and exercise; tobacco, alcohol and illicit drug cessation and avoiding relevant prescription drugs and occupational exposures [5]. For the overwhelming majority of patients, weight loss is the most important factor. Gradual weight loss of at least a 10% reduction in weight is the goal to improve serum transaminases as well as reduce hepatic steatosis, inflammation and fibrosis on biopsy [82]. Energy expenditure must exceed energy intake, and caloric reduction as well as increased physical activity should be prescribed. Current general recommendation for exercise is at least 30 min of moderately intense physical activity at least 5 days per week [83]. Exercise should probably include both aerobic exercise to expend calories and resistance training to increase muscle mass, which should, in turn, increase insulin sensitivity. In addition to diet and exercise, there is a role for pharmacologic therapy, such as Orlistat, and bariatric surgery in selected obese patients. Not only is bariatric surgery effective in improving steatosis, inflammation and fibrosis, it may even reverse cirrhosis [84]. However, caution is advised in cirrhotic patients with contemplated bariatric surgery because rapid weight loss has been reported to acutely worsen histology [85].

Altered dietary macronutrient composition may be able modulate NAFLD, even in the absence of weight loss. Based on the association of the rise in per capita high-fructose corn syrup consumption with obesity, as well as the hepatic effects of high-fructose diets in animal models, it is reasonable to conclude that NAFLD patients should limit high-fructose corn syrup consumption, although data are lacking. Furthermore, because polyunsaturated fatty acids appear to be protective in NAFLD, and long-chain saturated fatty acids mediate lipotoxicity, it is reasonable to limit consumption of saturated fats while increasing consumption of omega 3 fatty acids found in fish and flaxseed oil supplements as well as canola and safflower cooking oils [86]. Likewise, in an animal model of obesity and insulin resistance, soy protein was protective against the development of fatty liver by modulating nuclear transcription factors, such as SREBP (sterol response element binding protein) [87]. At this time, it seems reasonable to intermittently substitute soy for meats high in saturated fats, although data are lacking. The idea of

using macronutrients to treat obesity and fatty liver is paradoxical but intriguing, and further data from controlled human trials are required.

Hepatoprotection from coffee and caffeine consumption is an emerging diet and lifestyle modification. In the NHANES III population, which featured a significant number of patients with unexplained ALT attributed to NAFLD, coffee consumption was strongly protective of liver disease in a dose dependent fashion [84,88]. These data warrant further investigation, especially in the setting of environmental exposure.

5.3. Nutritional supplements

5.3.1. Vitamin E

Vitamin E is a potent chain-breaking antioxidant that is widely used as a nutritional supplement, and patients with various types of liver disease frequently have low serum vitamin E concentrations. Vitamin E has been shown to attenuate a variety of types of experimental liver injury, to reduce endotoxin (LPS)-stimulated NFkB activation and TNF production and to block stellate cell activation.

One initial study of vitamin E and NASH involved an open-label pilot study of 11 children with NASH treated with vitamin E. Patients were treated with dietary instruction and high-dose vitamin E (between 400 and 1200 IU/day) for 4–10 months. Mean BMI did not change during the course of the study. All patients improved their ALT levels, but histology was not obtained in this trial [89]. Another randomized controlled trial (RCT) studied 28 children with NASH treated with vitamin E. Groups were randomized to dietary instruction or dietary instruction with vitamin E (400 IU/day × 2 months; 100 IU/day × 3 months) and followed up for 5 months. Vitamin E was effective in reducing and normalizing transaminases in children with NASH (no effect on ultrasound liver fat was noted) [90].

A nonrandomized, non-placebo-controlled pilot study in 22 adult Japanese patients examined patients with NAFLD and NASH treated with vitamin E. Patients were treated with dietary instruction for 6 months followed by vitamin E (300 IU/day) for 1 year. Plasma transforming growth factor (TGF)-β1 levels were significantly higher in the patients with NASH when compared to patients with NAFLD or healthy subjects. Elevated TGF-β1 in patients with NASH improved after 1 year of vitamin E treatment and correlated with improvements in ALT levels. In the patients with NASH, five of nine also showed histologic improvement with decreased inflammation and fibrosis after treatment with vitamin E [91]. Our group studied 16 adults with NASH treated with vitamin E. Patients were treated with either dietary instruction alone or dietary instruction and 800 IU/day of vitamin E for 12 weeks. Both groups demonstrated an improvement in BMI. Both groups also demonstrated a significant improvement in ALT and AST; however, subgroup analysis showed no further benefit in the patients treated with vitamin E and dietary instruction when compared to the group receiving dietary instruction alone [44]. Weight loss is known to decrease oxidative stress and appeared to be more important than vitamin E in improving liver enzymes in this small trial. Another RCT evaluated 20 adults with NASH treated with vitamin E or vitamin E plus pioglitazone. Patients were treated with 400 IU/day of vitamin E for 6 months with or without pioglitazone (30 mg/day). Both groups showed a significant improvement in the degree of steatosis, although the vitamin E plus pioglitazone group showed a greater improvement in liver histology [92]. Some of the initial enthusiasm for high-dose vitamin E in NASH has been diminished by a recent meta-analysis suggesting a very small but significant increase in overall mortality in patients receiving high-dose vitamin E [93]. There are many problems with this meta-analysis, and they may have no relevance to patients with NASH, but concerns have been raised. Definitive results of vitamin E therapy in NASH from recently initiated National Institutes of Health trials are eagerly awaited.

5.3.2. S-adenosylmethionine

SAMe is available in the United States as a nutritional supplement. SAMe has been shown to protect against a variety of forms of drug- and toxin-induced experimental liver injury [94]. SAMe treatment decreased indicators of lipid peroxidation, decreased histologic evidence of liver injury, maintained mitochondrial GSH and helped maintain mitochondrial permeability transition in experimental liver injury [95]. SAMe not only protects hepatocytes from injury but also has been shown to down-regulate the pro-inflammatory cytokine TNF and to up-regulate the anti-inflammatory cytokine interleukin 10 after LPS stimulation [96]. SAMe has attenuated inflammation and liver injury in a nutritional deficiency model of steatohepatitis, and we have initiated a clinical trial of SAMe in NASH patients [42]. A large, multicenter trial from Spain of SAMe in alcoholic cirrhosis showed significantly improved outcome in patients with mild/moderate cirrhosis [97].

5.3.3. Betaine

Betaine (trimethylglycine — initially discovered in the juice of sugar beets) acts as an organic osmolyte to protect cells from stress and functions as a major methyl donor (see Fig. 1) [98,99]. SAMe and betaine together are involved in linked vital metabolic processes in the liver. Betaine has been shown to protect against a variety of forms of experimental liver injury and to reduce hepatic fat in experimentally induced fatty liver injury [58]. Betaine facilitates the conversion of homocysteine back to methionine and helps remove both SAH and homocysteine (Fig. 1). Betaine has been used with success in a human pilot study in NASH patients, and larger trials are ongoing in NASH with promising initial results [100].

5.3.4. Zinc

Zinc is an essential trace element required for normal protein metabolism, membrane stability and function of

hundreds of zinc metalloenzymes [101]. Zinc deficiency may complicate many types of liver disease. Severe zinc deficiency can present in multiple diverse ways ranging from skin lesions to cognitive dysfunction [101]. Not only can there be zinc deficiency in liver disease, but there also may be altered zinc metabolism due to stress and inflammation [101]. Much of this altered zinc metabolism is thought to be mediated by stress hormones and proinflammatory cytokines such as TNF [102]. Indeed, injection of endotoxins, a major stimulus for TNF production, or TNF itself causes a decrease in the serum zinc level similar to that seen in liver disease [102].

Zinc supplementation has been shown to protect against a variety of toxin-induced liver injuries in experimental animals [103]. Zinc therapy is the primary treatment of choice for Wilson's disease. In Wilson's disease, zinc increases intestinal metallothionein, which blocks copper absorption, as well as inducing hepatic metallothionein to sequester copper [104]. Zinc has been studied extensively in experimental models of alcohol-induced steatosis and steatohepatitis, and it has been shown to have marked hepatoprotective effects. Zinc induces multiple pathways to protect against alcohol-induced toxicity, including preserving intestinal integrity and preventing endotoxemia, leading to inhibition of endotoxin-stimulated TNF production [103–106]. Zinc also directly inhibited the signaling pathway involved in endotoxin-stimulated TNF production and reduced oxidative stress. Importantly, zinc supplementation suppressed alcohol-mediated elevations of cytochrome P450 2E1 activity. Zinc also inhibited hepatocyte apoptosis at least partially through suppression of the Fas/Fas Ligand pathway [103]. The potential protective effects of zinc have been reviewed in relation to other types of cellular injury, such as endothelial injury, and we suggest that the potential role of zinc therapy should be evaluated in steatosis/steatohepatitis (especially in relation to toxin-induced steatohepatitis).

5.4. Case presentation

A 64-year-old white female was recently diagnosed with cirrhosis after undergoing an exploratory laparatomy and bilateral oophorectomy secondary to ovarian cysts. A computed tomography of the abdomen revealed a nodular liver, splenomegaly and no ascites. Seven years previously, the patient underwent a percutaneous liver biopsy for evaluation of elevated transaminases, and the biopsy showed NASH without fibrosis. However, a recent endoscopy showed grade 2 distal esophageal varices and portal hypertensive gastropathy, documenting progression to cirrhosis. She had no history of blood transfusions or use of illicit drugs, and her history for smoking and drinking was negative. The patient recently retired after 39 years from a furniture company. She worked as a painter and was exposed to lacquers and organic solvents.

The patient's liver enzymes remained mildly elevated, and blood tests revealed no other etiology for her liver disease besides NASH. The patient presented with NASH but no significant fibrosis, and she progressed to cirrhosis with esophageal varies in 7 years. Whether working with organic solvents over a long period of time caused/accelerated her steatohepatitis is unclear and is a critical issue. However, we are recognizing many new patients in whom industrial exposure may have played a role in the development/progression of their disease.

5.5. Conclusions

NAFLD is the leading cause of liver enzyme abnormalities in the United States, and NASH can proceed to cirrhosis and even hepatocellular carcinoma. Thus, this spectrum of liver diseases represents major health problems for the American public. The etiology is likely multifactorial, with a baseline of steatosis required before a second hit. This steatosis may be due to many factors (many related to nutrition), with obesity as a major contributor, and, potentially, other factors such as high amounts of dietary high-fructose corn syrup and certain types of lipids playing a role. Factors acting as a second insult that lead to NASH range from oxidative stress to cytokines. Industrial toxins have recently been implicated as a second hit/insult. Importantly, nutritional factors and nutritional supplementation may attenuate NAFL/NASH. Examples include lifestyle modification and supplements, such as vitamin E, SAMe, betaine and zinc. Further interdisciplinary translational research is required to investigate whether steatosis and industrial toxins act in an additive or synergistic fashion to cause NASH, whether certain nutrients or nutrient deficiencies predispose to toxin-induced liver injury and whether nutritional interventions may play a preventive/therapeutic role in such toxin-related injury.

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