

# Nonalcoholic Fatty Liver Disease: Relationship to Insulin Sensitivity and Oxidative Stress. Treatment Approaches Using Vitamin E, Magnesium, and Betaine

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## Abstract

Nonalcoholic steatotic hepatitis (NASH), the most prevalent form of progressive liver disease in the United States, is considered to be a manifestation of insulin resistance syndrome. There is increasing evidence that steatosis in NASH is a result of the pathology in fat metabolism occurring in obesity and insulin resistance. For steatosis to progress to necroinflammation and fibrosis, however, the theory of mitochondrial oxidative-stress induced cellular damage is receiving wide acceptance. Treatment approaches that address these etiologies are reviewed: betaine, magnesium, and vitamin E.

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## Introduction

Nonalcoholic steatotic hepatitis (NASH) is part of the spectrum of nonalcoholic fatty liver disease (NAFLD), a condition becoming increasingly recognized both in the United States and worldwide due to its prevalence in obesity, diabetes, and insulin resistance syndrome.<sup>1</sup> NAFLD can manifest as simple steatosis (fatty liver), which rarely has any sequelae, or can progress to steatosis with inflammation or fibrosis, in which case it is termed NASH. NASH is the most prevalent form of progressive liver disease in the United States.<sup>2</sup> Due to the fact that approximately 50 percent of NASH patients develop liver fibrosis – 15-30 percent develop cirrhosis, and three percent may progress to liver failure<sup>3,4</sup> – there is an increasing need to recognize and understand the etiology and treatment of this condition.

## Epidemiology

NAFLD is known to affect 10-39 percent of the general global population with an average incidence of 20 percent.<sup>5,6</sup> It is the most common cause of increased liver enzyme levels in adults in the United States.<sup>7</sup> NAFLD occurs commonly in diabetics and the obese: 50 percent of diabetics (ranging between 21 and 78 percent), 57-74 percent of obese persons,<sup>5</sup> and 90 percent of morbidly obese persons (over 200 percent of ideal body weight)<sup>8</sup> are affected.

NAFLD also occurs in children: 2.6 percent of normal weight children and up to 52.8 percent of obese children have been diagnosed with fatty liver disease.<sup>9</sup> Obesity in children is currently an epidemic in the United States; e.g., the National Health and Nutrition Examination Survey from 1988 to 1994 found 20 percent of children aged 12-17 years of age to be overweight and 8-17 percent to be obese.<sup>10</sup> Obesity in children has been directly related to NASH, elevated serum ALT levels, and lower levels of serum antioxidants.<sup>10</sup>

In an urban, hospital-based hepatology practice of 1,226 patients, NASH was the second most common diagnosis after chronic viral hepatitis.<sup>11</sup> In the United States it is estimated that over 30 million adults have NAFLD. Of these, 8.6 million may have NASH.<sup>5</sup> This prevalence far outnumbers that of chronic hepatitis C (4 million

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adults) and is probably an underestimate since NASH is, for the most part, asymptomatic and is becoming increasingly more prevalent in both children and adolescents.

NAFLD characterized only by stable steatosis has a low risk of progressing. Unlike NAFLD, NASH progresses to fibrosis and cirrhosis in up to 50 percent of patients.<sup>3,4</sup> There are few natural history studies on the progression of NAFLD to NASH and risk of mortality. One retrospective study of 30 NASH patients found a 67-percent 5-year survival rate and a 59-percent 10-year survival rate.<sup>12</sup> The only detailed natural history study as of this writing looked at liver biopsies in 132 patients with NAFLD, including NASH and cirrhosis.<sup>13</sup> The study reviewed up to an 18-year period in which 25 percent of those initially diagnosed with evidence of hepatocyte necrosis (with or without fibrosis) had progressed to cirrhosis. Of those initially diagnosed with cirrhosis, 11 percent died of a liver-related cause, and 80 percent of the patients that developed cirrhosis during the study had previously shown evidence of fibrosis on initial biopsy. Current research suggests NASH is a major contributor to the development of cryptogenic cirrhosis, a diagnosis of cirrhosis that has no other identifiable cause.<sup>14</sup>

### Clinical Features

The majority of patients with NASH are asymptomatic, with the exception of discomfort in the right upper quadrant, fatigue, and malaise. Hepatomegaly can occur but does not necessarily accompany symptoms.<sup>15</sup> Acanthosis nigricans (hyperpigmentation) is more commonly found in children with NAFLD.<sup>16</sup> Elevations of aminotransferase levels are common (2-3-fold increases), and recent research identified an AST/ALT ratio of greater than one as a significant predictor of existing fibrosis, unless the patient already has progressed to cirrhosis.<sup>2</sup> Less than 50 percent of patients have elevated alkaline phosphatase levels, and only 10-15 percent have elevated serum conjugated bilirubin levels.<sup>15</sup> Hypoalbuminemia, thrombocytopenia, elevated bilirubin, and prolonged clotting time indicate advanced liver disease.<sup>5</sup> Fibrosis and cirrhosis in newly diagnosed

NASH patients are not rare: fibrosis has been found in 66 percent of patients,<sup>5</sup> and cirrhosis in 7-16 percent on initial biopsy.<sup>14</sup> Histopathology on liver biopsy in NASH is identical to the damage in alcohol abuse. The presence of macrovesicular steatosis, inflammatory cell infiltration, hepatocyte ballooning, and necrosis are the main histological features of NASH.<sup>5,14</sup>

*Table 1. Predictors of Fibrosis in NASH<sup>2,18</sup>*

<b>Body Mass Index over 30</b>
<b>45 or more years of age</b>
<b>AST:ALT ratio greater than 1</b>
<b>Type 2 diabetes mellitus</b>
<b>In those with BMI over 35</b>
<b>ALT&gt;40</b>
<b>Hypertension</b>
<b>Insulin resistance</b>

Although hepatic iron does appear to be a factor increasing risk for fibrosis,<sup>17</sup> elevated ferritin is only found in about 50 percent of patients. Elevated ferritin levels, however, do not always indicate elevated hepatic iron stores. In NASH, hepatic iron levels are usually normal. Elevated ferritin and transferrin levels, when they do occur, may be the result of hepatocyte necrosis.<sup>2</sup>

Several studies have examined signs and symptoms that correlate with presence of fibrosis (Table 1). In a study of 144 patients with NASH,

over age 45, the presence of type 2 diabetes and a body mass index (BMI) of 30 or over were predictive of fibrosis, and indicated the need of a liver biopsy to provide both an accurate diagnosis and to serve as a baseline to determine the efficacy of treatment.<sup>2</sup> In a study of 105 obese patients (BMI over 35), independent predictors of fibrosis were hypertension (140/90 or above), an elevated index of insulin resistance, and a serum ALT level over 40.<sup>18</sup> This population is not uncommon; 26 percent of the American population is considered obese (BMI over 30 kg/m<sup>3</sup>).<sup>19</sup>

**Pathogenesis of NAFLD and NASH**  
*Insulin Resistance and its Relationship to Fatty Liver*

Multiple authors have proposed that NASH be included as a clinical feature in the metabolic disorder of insulin resistance.<sup>20-22</sup> Insulin resistance, estimated to occur in approximately 25 percent of the general population, has been

associated with hyperinsulinemia, abnormal glucose tolerance, type 2 diabetes mellitus, hypertriglyceridemia, decreased high-density lipoprotein levels, hypertension, abnormal fibrinolysis, increased visceral fat accumulation, hyperuricemia, polycystic ovarian syndrome, and other lipid abnormalities.<sup>23</sup> This constellation of signs and symptoms, particularly hypertension, hypertriglyceridemia, and impaired glucose tolerance, has been designated as metabolic syndrome or syndrome X.<sup>24</sup>

NASH has been shown to be strongly associated with the major features of syndrome X: obesity, central fat accumulation, diabetes, dyslipemia (depressed HDL levels, elevated triglycerides), hypertension, and cardiovascular disease.<sup>25</sup> Indications of insulin resistance-type 2 diabetes mellitus or glucose intolerance are present in up to 30 percent of patients with NASH, commonly coexisting with hypertriglyceridemia or hypercholesterolemia.<sup>26</sup> The phenomenon of NASH as simply another manifestation of insulin

*Table 2. Diagnostic Criteria for Insulin Resistance Syndrome<sup>30</sup>*

Clinical Feature	Definition
1. Diabetes mellitus, glucose intolerance, or hyperinsulinemia and two or more of the following:	
2. Hypertension	140/90 or current documented use of antihypertensive medication
3. Elevated triglycerides, and/or decreased HDL lipoprotein cholesterol levels	>1.7 mmol/L <0.9mmol/L for men, <1.0 mmol/L for women
4. Central (truncal obesity)	Waist/hip ratio >0.9 for men and >0.85 for women or a BMI >30kg/m <sup>3</sup>
5. Microalbuminuria	Urinary albumin/creatinine ratio of 20 mg/g or urinary albumin excretion rate of 20 mcg/min.

resistance in type 2 diabetics, who commonly have NAFLD, may be indicated by data that show type 2 diabetics are more likely to die from liver disease than from cardiovascular disease.<sup>27</sup>

The relationship between NASH and obesity also involves insulin resistance. Central (truncal) or visceral obesity is a central feature of syndrome X,<sup>28</sup> and waist-to-hip ratios and BMI are significantly greater in NASH patients than in controls.<sup>21,29</sup> Insulin resistance has also been found to occur in significant numbers of NASH patients. In one population of 66 patients (both lean and obese), 98 percent were insulin resistant, and only 39 percent of those were diabetic.<sup>30</sup> Insulin resistance was determined by fasting levels of serum C-peptide (a measure of insulin production), insulin, and glucose. Insulin resistance was not dependent on increasing body mass index but significantly related to evidence of central obesity; i.e., increased waist-to-hip ratios were present even in lean individuals. This finding has also been seen in other NASH studies.<sup>21</sup>

In another study, 19 NASH patients who were not obese or diabetic and who had normal serum lipids still had significantly lower insulin sensitivity than controls ( $p=0.0003$ ) and significantly higher insulin secretion ( $p=0.001$ ).<sup>31</sup> In this

**Table 3. Signs and Symptoms of Metabolic Syndrome (Syndrome X) in “Healthy” NAFLD Patients<sup>21</sup>**

Sign or Symptom	Prevalence in Study
Overweight (BMI between 25-29.9 kg/m <sup>3</sup> )	67%
Central obesity (waist >102 cm for men and >88 cm for women)	47%
Impaired glucose metabolism: Elevated fasting insulin (>100 pmol/l) Postload hyperinsulinemia (>1000pmol/l) First-degree relative with diabetes	57% 27% 47%
Elevated triglycerides (>2 mmol/l)	47%
Hyperuricemia (>400 micromol/l)	27%
Hypertension (>160/95 or currently on medication) First-degree relative with hypertension	17% 57%
Cardiovascular disease Previous acute MI or angina First-degree relative less than 55 years if male and 60 years if female	0 27%

study 47-percent of the NASH patients met the criteria for insulin resistance required in Europe (Table 2).

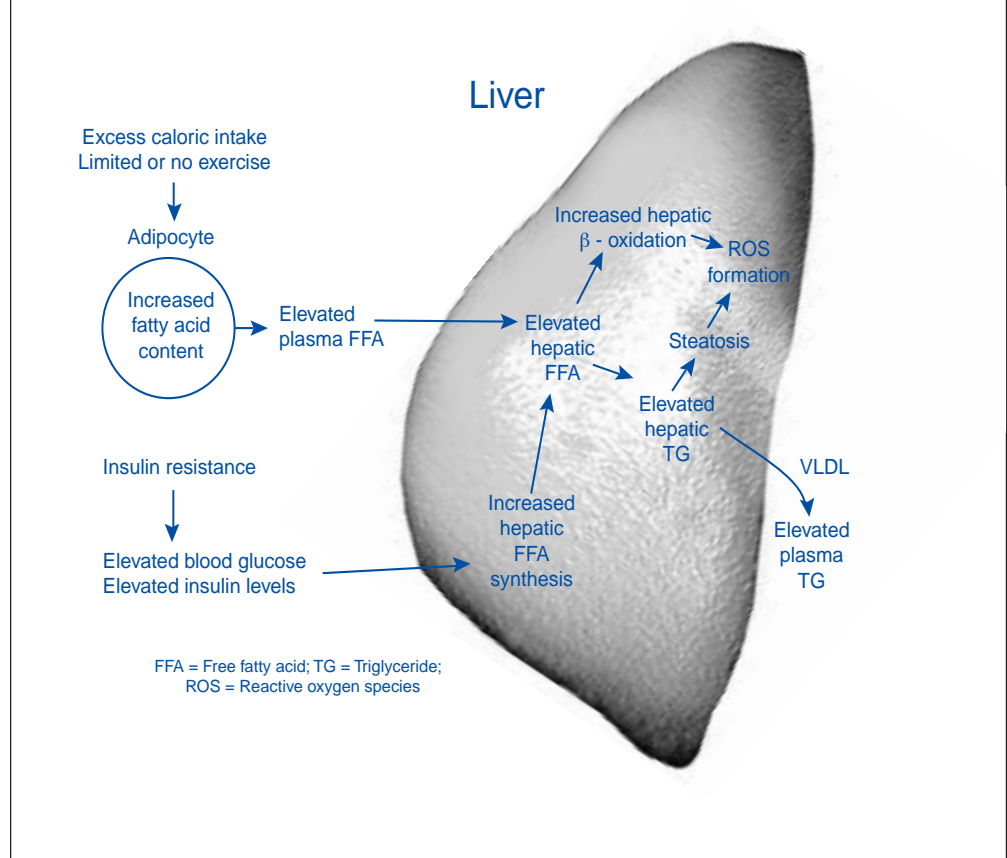
An even more significant study of insulin insensitivity, although smaller, was a group of patients with both NAFLD and NASH who were chosen because they were neither diabetic nor obese and had normal two-hour oral glucose tolerance tests.<sup>21</sup> The study included 30 lean NAFLD patients, 21 with NASH and nine with pure fatty liver. Insulin sensitivity testing revealed all of the patients had at least one clinical sign of metabolic syndrome or syndrome X (Table 3). Their fasting insulin levels, lipid profiles, and waist-to-hip ratios were significantly different from a healthy

control group and similar to a comparison group of type 2 diabetics. These studies indicate that insulin resistance, apparent in NASH, may exist even without, and possibly preceding, apparent glucose intolerance or obesity.

Insulin resistance is the most common and reproducible factor in the pathogenesis of NASH.<sup>32</sup> Insulin resistance contributes to the increased entry of fat into hepatocytes by increasing the intrahepatic production of free fatty acids from glucose not taken up by peripheral adipocytes and myocytes. Obesity also contributes to hepatic steatosis by increasing the amount of free fatty acids entering the hepatocyte. It appears the adipocytes of obese individuals release free fatty acids even in the presence of insulin. In overweight individuals with insulin resistance, both occur.<sup>33</sup>

The usual pathway for free fatty acid metabolism in the liver is through  $\beta$ -mitochondrial-oxidation. Under the stress of increasing free fatty acid influx to the liver in NASH, this pathway is insufficient and excess fatty acids are converted to triglycerides and stored in the cytoplasm, leading to steatosis. Triglycerides are also secreted into the plasma as VLDL, leading to hypertriglyceridemia (Figure 1).<sup>20</sup> An excellent review by Pessayre,<sup>33</sup> details the preceding explanation of hepatic fat metabolism in NAFLD and NASH. Hepatic steatosis is now the leading cause of liver enzyme abnormalities in adolescents and one of the top three causes in adults.<sup>33</sup>

**Figure 1. Free Fatty Acid Metabolism in NASH<sup>33</sup>**



In some individuals, steatosis, the initial stage of NAFLD, does not progress to steatohepatitis and has no sequelae. Steatosis is, however, considered the initial incident necessary in the development of liver cell damage in NASH. It has been considered the “first hit” because it is necessary to predispose the hepatocyte to inflammation and progression to fibrosis and cirrhosis.<sup>34</sup>

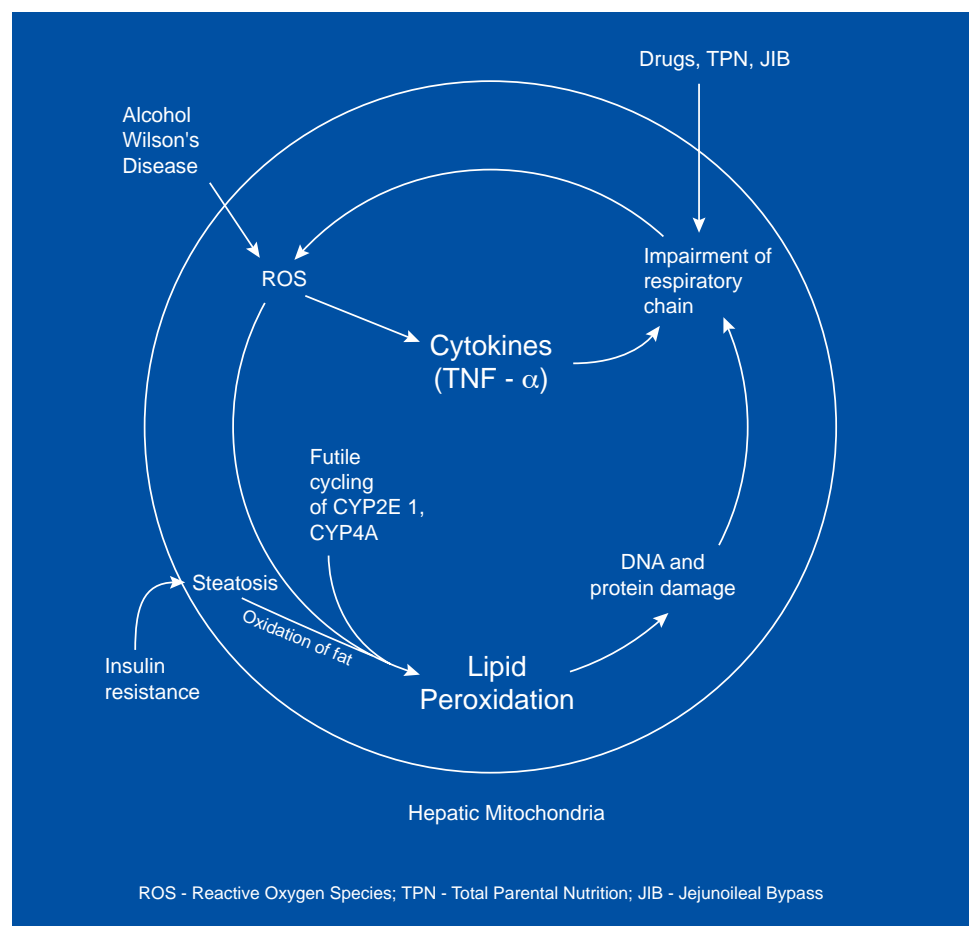
### ***Oxidative Damage and the “Second Hit”***

The histology of NASH is identical to alcoholic hepatitis, with the initial damaging incident in alcoholic hepatitis being lipid peroxidation and oxidative stress.<sup>14</sup> The same mechanism has been proposed as the “second hit” in NASH, the mechanism that generates inflammation and leads

to fibrosis and cirrhosis.<sup>34</sup> In normal liver, mitochondrial metabolism of free fatty acids is a source of free radicals;  $\beta$ -oxidation of free fatty acids produces hydrogen peroxide.<sup>35</sup> Animal models of NASH have shown increased evidence of lipid peroxidation.<sup>36</sup> Liver biopsies of patients with NAFLD and NASH show significantly higher levels of lipid peroxidation compared to controls.<sup>29</sup>

Both in animal models and human studies, hepatic mitochondria are the main source of oxidant stress.<sup>33</sup> Once mitochondrial reactive oxygen species are initiated they can further oxidize fat deposits, cause more lipid peroxidation, mitochondrial DNA damage, inhibit  $\beta$ -oxidation, and create a continuing cycle of damage (Figure 2). This cycle involves mitochondrial damage, the release of pro-inflammatory cytokines, damage to Kupffer cells, and the constant consumption of antioxidant enzymes and vitamins in the liver.<sup>37</sup> The increased demand on antioxidant reserves is evident in the lower levels of plasma  $\alpha$ -tocopherol seen in obese children when compared to non-obese children with similar dietary intakes of vitamin E.<sup>38</sup> Antioxidant levels and lipid peroxide levels have also been assessed in insulin resistance. In a study of 36 nondiabetic individuals, the more insulin resistant the patients were, the higher their levels of hydroperoxides.<sup>39</sup> Inversely, the more insulin resistance, the lower the plasma levels of carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene, and lutein),  $\alpha$ -tocopherol,  $\delta$ -tocopherol. Plasma concentrations

**Figure 2. Proposed Cycle of Damage in Steatohepatitis<sup>33,44</sup>**



of several carotenoids and tocopherols were significantly related to elevated levels of hydroperoxides. The authors of this Stanford University study concluded that tocopherols and carotenoids should be acknowledged as “environmental factors” that modulate insulin effects. Other human studies also indicate that levels of lipophilic antioxidants may control insulin sensitivity.<sup>40-43</sup>

Other sources of oxidant stress in NASH are the cytochrome P450 enzymes CYP2E1 and CYP4A. Both are involved in the hydroxylation of fatty acids and the production of lipid peroxides when they are up-regulated.<sup>44</sup> CYP2E1 has been shown to be persistently up-regulated in type 2 diabetes, insulin resistance, central obesity, and

NASH.<sup>45</sup> CYP2E1 is also up-regulated by a high-fat/low-carbohydrate diet.<sup>44</sup> Research in hepatic cell lines that over-express CYP2E1 have revealed the critical role antioxidants play in preventing hepatocyte injury in NASH. For example, lowering levels of reduced glutathione enhances the toxicity of arachidonic acid in CYP2E1-over-expressing cells; however, that damage can be prevented by adding a range of antioxidants, including tocopherol.<sup>44</sup>

CYP2E1 may also play a role in hepatic fibrosis: oxidative stress resulting from up-regulation of this cytochrome has been shown to also up-regulate collagen I production in rat hepatic stellate cells, one of the initial steps in fibrosis.<sup>46</sup> This process was enhanced by glutathione depletion and reversed by antioxidants.

**Table 4. Medications Known to Cause Fatty Liver<sup>5</sup>**

<b>Glucocorticoids</b>
<b>Synthetic estrogens</b>
<b>Aspirin</b>
<b>Calcium-channel blockers</b>
<b>Tamoxifen</b>
<b>Methotrexate</b>
<b>Tetracycline</b>
<b>Valproic Acid (Depakote)</b>
<b>Cocaine</b>
<b>Zidovudine (AZT)</b>
<b>Didanosine (ddl)</b>

**Other Potential Causes of NASH**

There is evidence that bacterial endotoxins can induce steatohepatitis, through production of the cytokine tumor necrosis factor-alpha (TNF- $\alpha$ ).<sup>47</sup> Bacterial endotoxin stimulates hepatic Kupffer cells and may lead to increased free radical production and hepatic steatosis and fibrosis.<sup>48</sup> Small intestinal bypass surgery is no longer a preferred treatment for morbid obesity due to the high incidence of steatohepatitis and cirrhosis that develop following this surgery.<sup>49</sup> Several patients have required liver transplantation after small intestinal bypass and NASH has returned in some individuals after transplant. The incidence of resulting hepatic failure was due to portal endotoxemia, a fatal complication of the surgery. The incidence of liver failure and steatohepatitis was reversed by the use of metronidazole therapy in these patients. The theory behind the reversal of NASH in these patients is that metronidazole eliminated a bacterial species producing endotoxin. Bacteroides is suspected by Lictman et al as the pathogen responsible for endotoxemia in these patients.<sup>50</sup>

Small intestinal overgrowth, determined by C-D-xylose and lactulose breath tests, has been found in 50 percent of NASH patients as opposed to 22 percent of controls.<sup>50</sup> TNF- $\alpha$  levels were doubled in NASH as compared to controls (p=0.001). Endotoxin levels were not elevated but the authors of this study point out that systemic levels may not reflect portal endotoxemia and that “bound” endotoxin (bound to plasma proteins) was not measured. When measured in alcoholic hepatitis, bound endotoxin levels are 6-10 times higher than healthy controls.<sup>51</sup>

**Drugs and Environmental Hepatotoxins**

Specific prescription medications – amiodarone (Cordarone), perhexiline, and 4,4'-diethylaminoethoxyhexesterol (DEAEH) – are known to cause steatohepatitis in humans. The mechanisms are similar to mechanisms described above for free radical-induced inflammation. The drugs accumulate in the hepatic mitochondria and

alter  $\beta$ -oxidation pathways leading to the formation of reactive oxygen species and resulting in lipid peroxidation.<sup>52</sup> Other drugs shown to cause fatty liver disease are listed in Table 4.

Environmental toxins, specifically petrochemicals and organic solvents, have been shown to cause NASH.<sup>5</sup> Specific petrochemicals implicated in one study included benzene, toluene, styrene, hexane, carbon tetrachloride, chloroform, methanol, and vinyl chloride.<sup>53</sup> The solvents these workers were exposed to are primarily metabolized by CYP2E1, one of the two main cytochrome P450 enzymes up-regulated in the pathology of NASH. These solvents are commonly found in cigarette smoke, paints, automobile exhaust, pesticides, air fresheners, and solvents used for cleaning and dry cleaning.

NASH has also been seen in individuals following intestinal surgeries, prolonged total parenteral nutrition, small intestinal diverticulosis with bacterial overgrowth,<sup>14</sup> protein-calorie malnutrition (kwashiorkor), starvation (anorexia nervosa), rapid weight loss, inflammatory bowel disease, and HIV infection.<sup>5</sup>

## Treatment of NASH

### Weight Loss

Weight loss is the only currently accepted treatment for NASH in both pediatric and adult overweight or obese patients.<sup>54,55</sup> Weight management and good metabolic control of diabetes and hyperlipidemia are always indicated in NAFLD and NASH. Neither, however, guarantee the reversal of NAFLD or NASH.<sup>5</sup> Decreases in weight usually correlate with decreased steatosis. However, rapid weight loss has been associated with increasing steatohepatitis and the degree of inflammation and fibrosis may worsen after weight loss.<sup>56</sup> It appears that with increased amounts of hepatic fatty infiltration, weight loss may actually increase necroinflammation, portal fibrosis, and bile stasis.<sup>5</sup> The rate of weight loss is important in minimizing the influx of free fatty acids to the liver; a rate of 500 grams (1.1 pounds) per week for children and 1600 grams (3.5 pounds) per week for adults has been suggested.<sup>56,57</sup>

### Magnesium

Given the role of insulin resistance in NAFLD and the incidence of obesity and type 2 diabetes in NASH, addressing the “first hit” of insulin resistance and triglyceride storage in hepatocytes is crucial.

Depletion of magnesium from normal cells creates cellular insulin resistance.<sup>58</sup> Magnesium levels are related to insulin resistance in type 1 and 2 diabetics and in nondiabetics. In patients with type 1 diabetes, low serum and plasma magnesium levels have been documented in several trials and are considered a relatively common finding; 25-48 percent of type 2 diabetics have been shown to have low blood magnesium levels.<sup>59-61</sup> Low plasma magnesium is significantly correlated with decreased glucose disposal in both type 1 and type 2 diabetes.<sup>62,63</sup>

Magnesium concentrations also appear to be related to insulin resistance in nondiabetic populations. Eighteen “healthy patients” (nondiabetic) who had lower levels of plasma magnesium (below 0.80 mmol/L) were significantly more likely to have higher fasting insulin levels and insulin resistance than those who had plasma magnesium above 0.80 mmol/L. Insulin resistance was defined by elevated plasma glucose and insulin after an oral glucose challenge.<sup>64</sup> Of note, the magnesium deficiency-related insulin resistance was independent of body mass index or waist-to-hip ratio.

When a nondiabetic group of subjects were fed a low magnesium diet for four weeks, insulin sensitivity decreased by 25 percent.<sup>65</sup> Magnesium supplementation in type 2 diabetics (41.4 mmol) has been shown to lead to a significant lowering of fructosamine levels, indicating an increase in insulin sensitivity.<sup>59</sup>

There is evidence that magnesium may also act as an antioxidant: magnesium increases the rate of production of the free-radical quenching enzyme superoxide dismutase,<sup>66</sup> while magnesium depletion appears to increase cellular sensitivity to oxidative damage<sup>67</sup> and the production of oxygen radicals in cell studies.<sup>68</sup> There are no studies in NASH patients looking at either mag-



**Table 5. NASH and NAFLD Patients' Lab Data Before and After Diet and Vitamin E.<sup>74</sup>**

	Normal Value	Before Treatment		After Diet		After Vitamin E	
		NASH	NAFLD	NASH	NAFLD	NASH	NAFLD
Body wt. (kg)		73 ± 5	75 ± 4	67 ± 3*	69 ± 4*	65 ± 4	70 ± 4
Triglycerides (mg/dL)	30-150	225 ± 8	229 ± 17	100 ± 5*	102 ± 6*	110 ± 6	127 ± 10
AST (IU/L)	8-35	121 ± 8	59 ± 3***	100 ± 11	27 ± 3*	36 ± 4**	28 ± 2
ALT (IU/L)	6-37	171 ± 4	167 ± 8	161 ± 14	43 ± 3*	37 ± 4**	41 ± 3

ALT (alanine transaminase); AST (aspartate transaminase)

\* p < 0.01 compared with before treatment

\*\* p < 0.01 compared with after diet therapy

\*\*\* p < 0.01 compared with NASH

nesium levels or magnesium supplementation on liver enzyme levels or liver histology. There is sufficient evidence, however, that reducing insulin resistance in both diabetics and nondiabetics with both NAFLD and NASH improves steatosis.<sup>33</sup> Considering the evidence for magnesium depletion and its effect on insulin resistance, evaluation of magnesium status and repletion in both NAFLD and NASH is warranted.

**Vitamin E**

Vitamin E has been shown to protect against liver fibrosis in animal models<sup>69,70</sup> and has also been shown to improve insulin sensitivity in type 2 diabetes, nondiabetics, and hypertensives.<sup>71,72</sup> Vitamin E supplementation (600 IU/day for four weeks) has also been able to significantly raise erythrocyte magnesium levels and plasma reduced glutathione levels while increasing insulin sensitivity in hypertensives.<sup>71</sup>

Two small studies using vitamin E revealed some important data in the treatment of NASH. The first study looked at 400-1200 IU of dl-α-tocopherol in children 8-14 years of age.<sup>73</sup> All children in the study were obese and had a history of elevated AST and ALT levels for over three months with evidence of fatty liver on liver ultrasonography. Median serum ALT was approximately 3.9 times the upper limit of normal, AST was 2.3 times the upper limit of normal, and alkaline phosphatase was 1.5 times the upper limit of normal. All of the children were considered to have NASH even though their diagnoses were not confirmed by liver biopsy. Each participant was started on 400 IU vitamin E and had liver enzyme levels repeated monthly. If AST and ALT values were not within normal limits one month after beginning treatment, the dose of vitamin E was raised by 400 IU per month to a maximum of 1200 IU. The children were followed for 5.2 months.

At that time their weight was not demonstrably different but serum ALT and AST had returned to normal by the end of the third month. Alkaline phosphatase was still elevated but had dropped significantly. Four of the children were able to reach normal ALT and AST levels with 400 IU, four needed 800 IU, and two needed 1200 IU. Two children discontinued vitamin E and had a recurrence of elevated ALT and AST levels within two months. An important point made in this study by the authors is that weight loss is difficult in children. These study participants were able to achieve normal ALT and AST levels without losing weight, which they were unable to do although they had been counseled by a physician and had regular meetings with a dietician during the study.

The second study evaluated the use of 300 IU  $\alpha$ -tocopherol and weight reduction in 22 adult overweight patients with liver biopsy-evaluated NAFLD or NASH.<sup>74</sup> Prior to taking vitamin E, all patients were given a six-month treatment of dietary therapy with caloric consumption limited to 30 kcal/kg body weight/day. During the weight reduction period, patients with NAFLD lost an average of 6 kg and had a significant lowering of ALT and AST to near normal limits (Table 5). The NASH patients lost the same amount of weight but did not experience the same drops in AST and ALT. After 12 months on 300 IU  $\alpha$ -tocopherol, however, AST and ALT levels in the NASH patients dropped significantly. AST fell to within normal limits for all NASH patients and ALT values were near normal. ALT and AST values for the NAFLD patients were not appreciably affected by  $\alpha$ -tocopherol. Most importantly, repeat liver biopsy in nine of the 12 NASH patients after 12 months of  $\alpha$ -tocopherol treatment revealed that inflammation and fibrosis were significantly improved in five, and the remaining four had significant improvement in steatosis. Since weight did not change in any of the NASH patients while on  $\alpha$ -tocopherol, this improvement was not due to continued weight loss.

The other important aspect of this study was the evaluation of transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) in these patients. TGF- $\beta$  is a peptide found in many cell types that regulates wound

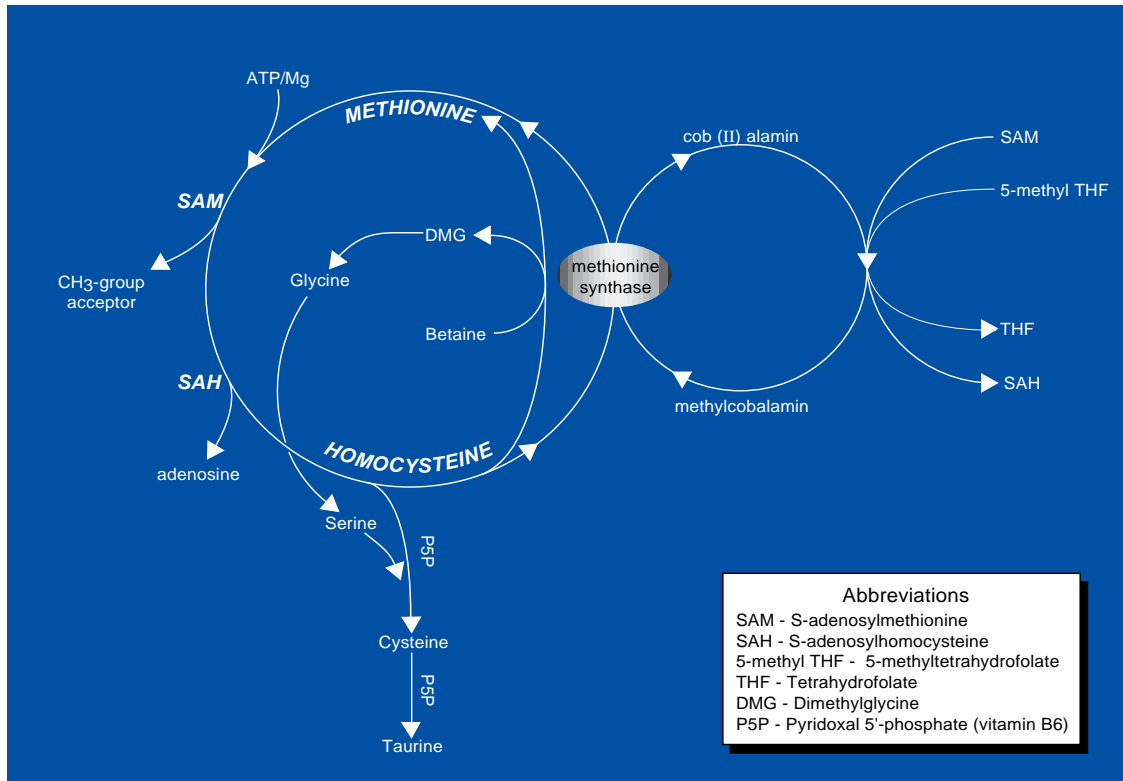
healing and apoptosis.<sup>75</sup> The isoform found in hepatic cells, TGF- $\beta$ 1, has been found in many models of hepatic fibrosis and levels increase in chronic active hepatitis and fibrotic alcoholic liver disease.<sup>76</sup> Kupffer cells and stellate cells, two cell types involved in the inflammatory sequence in NASH, secrete TGF- $\beta$ 1 as part of the process of fibrosis.<sup>75</sup>

Plasma TGF- $\beta$ 1 was measured in the NASH patients at baseline, after the completion of the dietary intervention, and after one year on  $\alpha$ -tocopherol. Baseline levels, which had been significantly higher than NAFLD patients or healthy controls ( $p < 0.01$ ), were unchanged after dietary intervention, but significantly decreased after  $\alpha$ -tocopherol ( $p < 0.01$ ). This study, if repeated with a larger population, may reveal more important information about why dietary therapy and tocopherol did not work in the same populations with NAFLD and NASH. It may also reveal more about TGF- $\beta$ 1 as a possible mechanism for the efficacy of  $\alpha$ -tocopherol. Finally, it may offer a diagnostic tool for NASH and a way to differentiate NASH from NAFLD, since there are currently no non-invasive methods for the accurate diagnosis of NASH.

Another trial of lecithin, antioxidants, and B complex vitamins assessed the ability of nutrients to alter steatosis in NASH.<sup>77</sup> Four patients with liver biopsy-diagnosed NASH were given a daily protocol of 20 grams lecithin, 250 mg vitamin C, 50 IU vitamin E, 2,500 IU beta-carotene, 50 mcg selenium, and a B complex (300% of RDA) for 12 weeks. CT scans were performed at baseline, and a CT scan and liver biopsy were performed at 12 weeks. There was a significant decrease in fatty liver in two of four patients, and no change in the other two. Given the modest levels of nutrient supplementation in the trial, it is not possible to identify whether vitamin E was effective or whether phosphatidylcholine (in the lecithin) was the effective agent.

Phosphatidylcholine has been used in trials with alcoholic hepatitis and chronic hepatitis B and C to slow or reverse steatosis and halt the progression of fibrosis.<sup>78-80</sup>

Figure 3. Homocysteine Recycling via Betaine, Cobalamin, or Folate



**Betaine**

Betaine, along with choline, methionine, vitamin B12, and inositol, were first known for their ability to protect against the development of fatty liver in animals as early as 1954. Choline, the precursor to betaine, was considered the first known hepatoprotective nutrient soon after its discovery in 1932 when it was recognized that a choline deficiency induced almost immediate pathological changes in hepatic cells in animals.<sup>81</sup> Choline deficiency, particularly in those on long-term parenteral nutrition, has been linked to hepatic steatosis. However, plasma choline deficiency does not appear to be a factor in NASH patients who have not been on long-term parenteral nutrition.<sup>82</sup> Although choline may be present in sufficient amounts in the plasma of NASH patients, a study with betaine points to the importance of hepatic methionine metabolism in NASH.<sup>83</sup> To assess whether oral betaine would raise SAME levels and

decrease hepatic steatosis, seven patients with biopsy-proven NASH were given 10 grams of anhydrous betaine solution twice daily for 12 months. By the end of the trial significant decreases in ALT and AST occurred; normalization in three patients, decreases greater than 50 percent in three others, with one remaining unchanged. Three patients who did not complete treatment also had close to 40-percent decreases in ALT and AST levels. In six of the seven patients who completed the study, repeat liver biopsies were performed. Significant improvements occurred in both steatosis and fibrosis, with improvements in staging of disease. Betaine was safe and well tolerated with only transient side effects in four of 10 patients, including nausea, abdominal cramping, loose stools, and body odor. None of the side effects necessitated dose reduction and all four patients completed treatment.

Betaine plays a critical role in one of three pathways that allow for the recycling of methionine in the liver and regeneration of SAME from homocysteine (Figure 3).

Betaine functions as the basis of the enzyme betaine:homocysteine methyltransferase which donates a methyl group to homocysteine, recycling methionine and producing SAME. Approximately 50 percent of the recycling of homocysteine occurs either through this pathway or via 5-methyl tetrahydrofolate.<sup>83</sup> An elevation of homocysteine is seen in patients with NASH when compared to other chronic liver diseases independent of weight or lipid status; an indication that inherent methionine recycling is affected in NASH.<sup>84</sup> In four short-term studies, oral betaine therapy was shown to markedly decrease homocysteine levels in patients with homocystinuria and elevated homocysteine levels.<sup>85</sup> Oral betaine has also been shown to restore SAME levels to normal in the cerebrospinal fluid of patients with congenital methyltetrahydrofolate deficiency.<sup>86</sup> In rats, betaine as 0.5 percent of the diet doubled SAME levels in controls and increased SAME by 400 percent in ethanol-fed animals. Reversal of fatty infiltration due to ethanol-consumption was also evident in the betaine-supplemented animals.<sup>87</sup>

SAME is considered the most important methyl donor in human biochemical reactions and is necessary in the production of carnitine, coenzyme Q, creatine, methylcobalamin, and phosphatidylcholine.<sup>88</sup> SAME is also considered important in gene regulation, since a large number of genes are dependent on SAME methyltransferase enzymes. As much as 85 percent of methylation reactions and 48 percent of methionine metabolism occurs in the liver. Hepatic function is dependent to a large extent on methionine metabolism. It has been proposed that SAME acts as an “intracellular control switch” with the ability to regulate hepatic cellular regeneration, differentiation and susceptibility to injury by oxidative stress, and hepatotoxin exposure.<sup>89</sup> Lowered SAME levels are suspected to lead to steatosis and steatohepatitis.<sup>90</sup> The transsulfuration pathway (Figure 3) has been shown to be impaired in cirrhosis, potentially contributing to hepatic

glutathione deficiency seen in both alcoholic and nonalcoholic liver disease. Oral SAME (1.2 g/day for six months) led to significant increases in hepatic glutathione in a small controlled trial of patients with alcoholic and nonalcoholic liver disease.<sup>91</sup> The ALT levels in the nonalcoholic liver disease patients decreased significantly as did the AST levels in the alcoholic liver disease patients during SAME therapy, although they did not reach normal levels. Considering that three of the seven NAFLD patients had cirrhosis and three had chronic active hepatitis, a significant lowering of the specific liver enzymes in alcohol and non-alcohol-related liver disease is worthy of attention. This study provides evidence that the production of glutathione levels in hepatic tissue could be significantly up-regulated by SAME, and that up-regulation would have a measurable outcome in terms of hepatic function.

## Conclusion

Current trends in the prevalence of obesity indicate that 40 percent of the U.S. population will be obese by the year 2025.<sup>37</sup> The incidence of diabetes mellitus is predicted to extend to 7.2 percent of the population (29 million Americans) by 2050.<sup>22</sup> Given these trends, particularly in children and adolescents, the prevalence of NAFLD may increase significantly in the next 25 years. The identification and treatment of NASH is critical, since 20-30 percent of these patients may progress to cirrhosis.<sup>4</sup> Large-scale clinical trials of vitamin E and betaine are warranted. If NASH is clearly another symptom of insulin resistance, the use of magnesium as an insulin-sensitizing nutrient in a pilot study would be worth investigating.

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