Treatment options for nonalcoholic fatty liver disease

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***Abstract*:** Nonalcoholic fatty liver disease (NAFLD) has become increasingly recognized as the most common cause of abnormal liver enzymes in the last few decades and is among the most common forms of chronic liver disease in the Western world and across the globe. With the growing epidemic of obesity and diabetes, NAFLD is estimated to affect about one-quarter of the US population. Although most patients with NAFLD have nonprogressive bland steatosis, a minority of patients develop the histological subtype of nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis, hepatocellular carcinoma, and liver-related death. This is especially true when NASH patients have type 2 diabetes. Treatment of NAFLD should therefore be directed towards patients with established NASH. Sustained weight loss seems to improve insulin resistance and associated NASH. In fact, weight loss with bariatric surgery leads to biochemical and histological improvement in morbidly obese patients with NASH. Several pharmacologic agents have been studied in an effort to improve insulin resistance and pro-inflammatory mediators potentially responsible for the development and progression of NASH. While some studies have shown initial promise, none has established long-term efficacy using randomized clinical trials. This paper briefly reviews the epidemiology, natural history, and pathophysiology of NAFLD and NASH and then focuses on the clinical trials of various therapeutic modalities for NAFLD. These include weight loss agents, bariatric surgery, insulin-sensitizing agents, lipid-lowering agents, antioxidants, probiotics, anti-tumor necrosis factor agents, cytoprotective and other novel agents.

***Keywords*:** NAFLD, NASH, treatment

Background

Nonalcoholic fatty liver disease (NAFLD) is one of the most common forms of liver disease across the world. NAFLD represents a spectrum ranging from bland steatosis to nonalcoholic steatohepatitis (NASH), and is closely associated with obesity and metabolic syndrome [Ludwig *et al.* 1980]. Owing to the increasing prevalence of NAFLD and the potential for NASH to progress to cirrhosis and liver-related mortality, more research has been focused on this important liver disease over the last two decades. NAFLD has become a common diagnosis with the increasing prevalence of obesity, diabetes mellitus, and other components of the metabolic syndrome. The Adult Treatment Panel III defines metabolic syndrome as having three of the following: visceral obesity, hypertension, elevated fasting blood glucose, hypertriglyceridemia, or low high-density lipoprotein (HDL) levels. The criterion for visceral obesity is a waist circumference greater than 40 inches in men and greater than 35 inches in women. Hypertension is defined as blood pressure greater than 130/85 mm Hg. Glucose levels above 100 and triglyceride levels of 150 or greater are considered elevated. HDL levels below 40 for men and below 50 for women are considered low [National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), 2002]. Because as many as 90% of NAFLD cases have at least one component of the metabolic syndrome and as many as 33% have three or more components, NAFLD is characterized as the hepatic manifestation of the metabolic syndrome [Almeda-Valdés *et al.* 2009; Rafiq and Younossi, 2009].

While the presence of metabolic syndrome is highly predictive of NAFLD, metabolic syndrome is not always accompanied by NAFLD, nor is

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NAFLD always accompanied by a component of metabolic syndrome. Rarely, patients with NAFLD may have a body mass index (BMI) that is within acceptable limits, and liver function enzymes may also be normal. Nevertheless, the prevalence of NASH and fibrosis increases with increasing number of metabolic conditions (diabetes, hypertension, visceral obesity)[Hossain *et al.* 2009].

It is important to note that NAFLD is a diagnosis of exclusion that is finally reached after evaluation for asymptomatic liver enzyme elevation. Alternatively, a NAFLD diagnosis is reached inadvertently after hepatic steatosis is incidentally noted on abdominal imaging. Nonetheless, several dilemmas accompany attempts to diagnose NAFLD or its subtype of NASH. Serological tests, panels, and biomarkers are not yet validated and routine radiographic modalities have unavoidable limitations. Currently, liver biopsy remains the 'imperfect' gold standard for diagnosis and staging of NAFLD [Wieckowska and Feldstein, 2008; Adams and Angulo, 2007; Charatcharoenwitthaya and Lindor, 2007].

Epidemiology

It is difficult to estimate the true incidence and prevalence of NAFLD and NASH because of the absence of accurate and noninvasive diagnostic measures. Currently no studies have examined the incidence of NAFLD and NASH in North America, and there are no prospective studies evaluating prevalence except in patients undergoing bariatric surgery. Two population-based studies have attempted to estimate the prevalence of NAFLD in general United States population [Szczepaniak *et al.* 2005; Clark *et al.* 2002]. Analysis of the third US National Health and Nutrition Examination Survey (1988–1994) found that 23% of the population had unexplained elevation of alanine transaminase (ALT) [Clark *et al.* 2002]. Another study, which used proton magnetic resonance spectroscopy to measure hepatic steatosis in Dallas County, found the prevalence of NAFLD to be 33.6% [Szczepaniak *et al.* 2005]. Notably, ALT was normal in most (79%) of these patients with hepatic steatosis [Browning *et al.* 2004]. Autopsy studies on reportedly nonalcoholic patients have suggested that steatosis occurs in 36% of lean patients and in 72% of obese patients. Furthermore, steatohepatitis is present in 2.7% of lean patients and in 18.5% of obese patients [Wanless and Lentz, 1990]. Overall, in the general United States population, estimates of NAFLD prevalence range from 23% to 33.6% [Szczepaniak *et al.* 2005; Browning *et al.* 2004; Clark *et al.* 2002], while estimates of NASH prevalence range from 2% to 5.7% [Wanless and Lentz, 1990]. Among patients undergoing bariatric surgery, as many as 96% have NAFLD and up to 25% have NASH [Ong *et al.* 2005; Dixon *et al.* 2001]. Of the 47 million people in the United States with metabolic syndrome, up to 80% may have NAFLD [Ong *et al.* 2008].

NAFLD and NASH have been noted in all ethnic groups, all regions of the world, all ages, and in both genders. In the United States, the prevalence is greater in the Hispanic population when compared with non-Hispanic Whites or non-Hispanic African Americans [Ong *et al.* 2008; McCullough, 2005; Weston *et al.* 2005; Browning *et al.* 2004; Caldwell *et al.* 2002]. The Dallas proton magnetic spectroscopy study reported NAFLD prevalence of 33% in non-Hispanic Whites, 45% in Hispanics, and 24% in non-Hispanic African Americans [Browning *et al.* 2004]. Reports have also suggested that the prevalence of NAFLD among Asian Indians is comparable to that seen in the West [Misra *et al.* 2009; Amarapurkar *et al.* 2007; Duseja *et al.* 2007; Malik *et al.* 2007; Duseja, 2006; Madan *et al.* 2006; Misra and Vikram, 2004; Singh *et al.* 2004]. In East Asia, estimates of NAFLD prevalence vary from 11.5% to 20.8% [Shifflet and Wu, 2009; Chen *et al.* 2006; Park *et al.* 2006; Fan *et al.* 2005; Yiu and Leung, 2004].

As noted previously, NAFLD is seen in all age groups. Prevalence peaks in the fourth decade in men and in the sixth decade in women [Ruhl and Everhart, 2003]. Earlier studies described a greater frequency of NAFLD in women. More recent studies are showing that the prevalence of NAFLD in men is equal to or greater than the prevalence in women [Shifflet and Wu, 2009; Amarapurkar *et al.* 2007; Weston *et al.* 2005; Ruhl and Everhart, 2003; Clark *et al.* 2002]. With the increasing prevalence of obesity in children and adolescents, NAFLD also affects the pediatric population. However, this review focuses primarily on adult the population.

Natural history

The natural history of NAFLD has emerged over the past two decades. Most cases of NAFLD do not progress to advanced liver disease. The risk

of progression seems to be determined by histological subtype. In fact most patients with bland steatosis typically follow a benign course, whereas NASH carries the potential to progress. A few reports suggest that bland steatosis may rarely progress to NASH [Ong and Younossi, 2007; Ong *et al.* 2005, 2008; Dixon *et al.* 2001; Wanless and Lentz, 1990]. Although most reports suggest that the presence of metabolic syndrome or its components is predictive of NASH and NASH-related fibrosis [Ong and Younossi, 2007; Marchesini *et al.* 2003], a few indicate that metabolic syndrome is not predictive [Uslusoy *et al.* 2009; Singh *et al.* 2004]. Nonetheless, a growing consensus points to progression when NASH occurs in the setting of insulin resistance or type 2 diabetes.

Several studies report that all NAFLD patients are at greater risk for overall mortality when compared with non-NAFLD patients [Ong *et al.* 2008; Ong and Younossi, 2007; Ekstedt *et al.* 2006; Adams *et al.* 2005]. This increase in overall mortality stems from the metabolic syndrome that is often comorbid with NAFLD [Ong and Younossi, 2007; Targher and Arcaro, 2007]. In many long-term follow-up studies of NAFLD, coronary artery disease continues to be the most common cause of overall mortality [Rafiq *et al.* 2009; Ong *et al.* 2008; Ong and Younossi, 2007]. Since insulin resistance is a main pathogenic factor, NAFLD patients are also at greater risk for developing diabetes. A recent study by Adams *et al.* found that patients with NAFLD (diagnosed on the basis of unexplained ALT elevation) were at significantly greater risk for developing diabetes (20/106, 18.9% *vs.* 15/246, 6.1%; $p < 0.001$) and metabolic syndrome (27/81, 33.3% *vs.* 51/226, 22.6%; $p = 0.056$) than patients with normal ALT [Adams *et al.* 2005]. Further analysis revealed that the increased risk may have been due to concomitant metabolic risk factors (baseline waist circumference, hypertension, and insulin resistance).

In addition to cardiac mortality, liver-related mortality is also increased in NAFLD patients, especially in those with NASH [Rafiq *et al.* 2009; Ong *et al.* 2008]. One recent study noted findings consistent with NASH in 41.6% ($N = 173$) of patients diagnosed with NAFLD by biopsy [Rafiq *et al.* 2009]. As reported in previous studies, the two most common causes of death in these NASH patients were coronary artery disease and malignancy [Ong *et al.* 2008; Ong and Younossi, 2007; Ekstedt *et al.* 2006; Adams *et al.* 2005]. Liver-related mortality was the third most common cause of death in this cohort of NASH patients. For comparison, liver-related mortality is typically around the thirteenth most common cause of death in the general population [Ong *et al.* 2008; Ong and Younossi, 2007]. Again, patients with NAFLD and type II diabetes seem to be at greater risk for liver related mortality [Rafiq *et al.* 2009]. In another study of biopsy-proven NASH, age and the presence of inflammation on initial biopsy were independent risk factors for progression to advanced fibrosis [Adams *et al.* 2009]. Although the risk factors for progression remain to be clarified, 15–25% of NASH cases progress to cirrhosis over 10 to 20 years [Ong and Younossi, 2007; Matteoni *et al.* 1999; Bacon *et al.* 1994; Powell *et al.* 1990]. NAFLD patients who have progressed to cirrhosis are at risk for hepatocellular carcinoma, similar to patients with cirrhosis due to other etiologies. In addition to potential for progression, NAFLD patients suffer from impaired health-related quality of life [Afendy *et al.* 2009].

Pathophysiology

A brief review of NAFLD pathogenesis will help inform the discussion of potential therapies. Although factors such as medications, toxins, parenteral nutrition, and certain surgical procedures can lead to hepatic steatosis (secondary NAFLD), this article focuses on primary NAFLD, which is associated with insulin resistance, obesity, and the metabolic syndrome.

NAFLD pathogenesis is widely believed to result from a series of liver insults, commonly referred to as the 'multi-hit' hypothesis [Estep *et al.* 2009; Malhi and Gores, 2008; Miele *et al.* 2003; Charlton *et al.* 2002]. The first hit involves the development of hepatic steatosis due to insulin resistance. Insulin resistance leads to increased serum levels of nonesterified or free fatty acids (FFAs). Subsequently, increased FFA transport into hepatocytes and increased hepatic *de novo* lipogenesis exceed hepatic FFA β-oxidation and very low-density lipoprotein (VLDL) export, leading to increased hepatic steatosis [Malhi and Gores, 2008]. Several studies suggest that hepatic steatosis is largely due to the increase in lipogenesis and decreases in lipid export [Miele *et al.* 2003; Charlton *et al.* 2002].

The exact mechanisms which drive the progression from bland steatosis to steatohepatitis are still not fully elucidated. It is clear however that apoptosis can be the pathogenic marker of steatohepatitis in some NAFLD patients [Malhi and Gores, 2008]. For this reason, much attention has been focused on the mechanisms that lead to hepatocellular apoptosis. It has been proposed that accumulating FFAs in steatotic hepatocytes promote apoptosis through a number of pathways, including increased stress of membrane bound organelles such as mitochondria, endoplasmic reticula (ER), and lysosomes [Malhi and Gores, 2008]. Lipotoxicity occurs on a molecular level as circulating FFAs activate a number of complex intracellular pathways, including induction of Bim expression, activation of c-jun N-terminal kinase, the pro-apoptotic protein Bax and Toll-like receptor 4. Sufficient activation of these pathways may then lead to lysozomal and mitochondrial permeabilization, oxidative stress, inflammatory gene expression, ER stress, and hepatocyte apoptosis [Malhi and Gores, 2008].

Visceral obesity also seems to contribute to the development of NASH via the endocrine functions of white adipose tissue (WAT) as well as insulin resistance. Several adipokines secreted by WAT and implicated in the pathogenesis of NAFLD include adiponectin, leptin, resistin, tumor necrosis factor alpha (TNF-α), complement component 3, apelin, vaspin, visfatin, interleukins 1 beta, 6, 8, and 18, and plasminogen activator inhibitor type 1 [Estep *et al.* 2009]. Furthermore, angiotensinogen may also play a role in NASH pathogenesis [Estep *et al.* 2009]. These 'second hits' lead to increased fibrosis and progression to steatohepatitis and cirrhosis. Current treatment modalities target one or more of these components (Figure 1).

Treatment

As the potential for liver disease progression occurs almost exclusively in patients with NASH, our efforts should focus mainly on prevention and treatment of this NAFLD subtype. However, given that NADFLD is associated with cardiovascular mortality, it is important to treat components of metabolic syndrome for all NAFLD patients to potentially improve cardiovascular outcomes.

As of 2009, no pharmacological agents have been approved for the treatment of NAFLD or NASH. Therefore, most clinical efforts have been directed at treating the components of metabolic syndrome, namely obesity, diabetes, hypertension, and dyslipidemia. Other interventions are directed at specific pathways potentially involved in the pathogenesis of NAFLD, such as insulin resistance, oxidative stress, pro-inflammatory cytokines, apoptosis, bacterial overgrowth, and the angiotensin pathway (Table 1).

Obesity

The therapeutic measure with the best potential for treating NAFLD is sustained weight loss by dietary changes with or without exercise, or bariatric surgery. Several studies have investigated the effects of dietary changes upon NAFLD [Tendler *et al.* 2007; Baba *et al.* 2006; Thomas *et al.* 2006; Huang *et al.* 2005; Suzuki *et al.* 2005; Hickman *et al.* 2004; Kugelmas *et al.* 2003; Zhu *et al.* 2003; Okita *et al.* 2001; Knobler *et al.* 1999; Ueno *et al.* 1997; Andersen *et al.* 1991; Palmer and Schaffner, 1990]. Some include exercise because it may promote increased muscle mass in addition to promoting weight loss, thereby increasing peripheral insulin sensitivity [Stewart *et al.* 2005]. Of the studies that have focused on weight loss by dietary measures with or without exercise, all but six are limited by the absence of histologic evaluation. Owing to the invasive nature of liver biopsy, many studies use biochemical improvement as the primary measure of efficacy. One study using histological assessment by Huang and colleagues evaluated the effect of intense dietary counseling on 23 patients with biopsy-proven NASH [Huang *et al.* 2005]. After 1 year, 9 of 15 patients showed histologic improvement associated with greater weight loss. Most studies are also limited by small study size and short duration. The study with the largest cohort and longest duration to date reported that weight loss and exercise were significantly associated with ALT improvement in 136 patients over 36 months. However, this study did not include liver biopsies [Suzuki *et al.* 2005].

Orlistat and sibutramine are two US Food and Drug Administration (FDA)-approved medications for weight loss. Orlistat is an enteric lipase inhibitor. Sibutramine, a serotonin and norepinephrine reuptake inhibitor, promotes satiety and increases energy expenditure by stimulating thermogenesis. At least four studies have examined the effects of these medications on NAFLD [Hussein *et al.* 2007; Harrison *et al.* 2004, 2009; Sabuncu *et al.* 2003]. Sabuncu and colleagues treated patients with sibutramine or orlistat

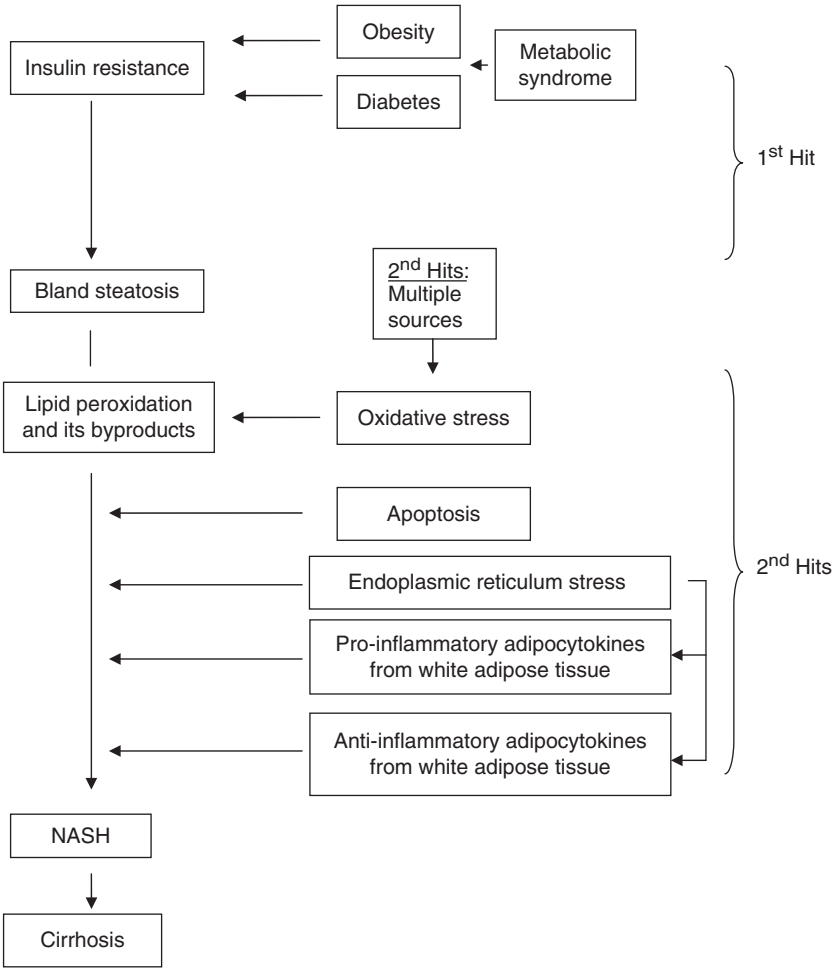

Figure 1. Nonalcoholic steatohepatitis (NASH) pathogenesis.

along with dietary restriction in an open-label study for 6 months [Sabuncu *et al.* 2003]. Improvements were noted in homeostatic model assessment (HOMA) scores, aminotransferases, and sonographic findings, but liver biopsies were not obtained. Three studies have evaluated the effect of treatment with orlistat over 6 months [Hussein *et al.* 2007; Harrison *et al.* 2004, 2009]. Paired biopsies revealed improvements in steatosis, inflammation, and fibrosis in most patients. A recently published study by Harrison and colleagues. randomized 50 overweight patients with biopsy-proven NASH to a 1400 kcal/day diet plus 800 IU vitamin E daily with or without orlistat for 36 weeks. They found that weight loss of 9% or more of body weight, not treatment with orlistat, resulted in significant improvements in insulin sensitivity, adiponectin, and steatosis, ballooning, inflammation and NAFLD score (NAS) on repeat biopsy [Harrison *et al.* 2009]. Thus, it is important to note that the improvements in NAFLD patients treated with these medications are largely attributed to weight loss. Given that medication-assisted weight loss is often difficult to sustain, these therapies may be most appropriate only in patients who are highly motivated.

On the other hand, several studies have shown that weight loss following bariatric surgery leads not only to biochemical improvement but also to histological improvement [Furuya *et al.* 2007; Liu *et al.* 2007; Sjostrom *et al.* 2007; Barker *et al.* 2006; Csendes *et al.* 2006; De Almeida *et al.* 2006; Jaskiewicz *et al.* 2006; Klein *et al.* 2006; Mathurin *et al.* 2006, 2009; O'Brien *et al.* 2006; Clark *et al.* 2005; Mattar *et al.* 2005; Mottin *et al.* 2005; Stratopoulos *et al.* 2005; Dixon *et al.* 2004, 2006; Kral *et al.* 2004; Silverman *et al.* 1995; Grimm *et al.* 1992; Ranlov and Hardt, 1990; Rucker *et al.* 1982]. While no randomized blinded studies can be conducted, an analysis of numerous paired biopsy studies reveals convincing evidence that significant improvement in steatosis and inflammation occurs in NAFLD patients after weight loss surgery.

Table 1. Potential treatments and their targets.

Target	Treatment
Obesity	Weight loss - Diet with or without exercise - Pharmacologic • Orlistat • Sibutramine - Surgical treatment of obesity
Insulin resistance	Insulin sensitizing agents - Thiazolidinediones (TZDs) - Metformin - Meglitinides
Dyslipidemia	Lipid lowering agents - Statins - Fibrates - Omega-3 fatty acids
Oxidative stress	Antioxidants - Vitamin E - Other vitamins - Betaine - N-Acetyl-cysteine - Lecithin - Silymarin - Beta-carotene
Pro-inflammatory cytokines	Anti-tumor necrosis factor agents - Pentoxifylline
Bacterial overgrowth	Probiotics - VSL#3
Apoptosis	Cytoprotective agents - Ursodeoxycholic acid (UDCA) Novel treatments - ACE inhibitors/ARBs - Oligofructose - Incretin analogs

ACE, angiotensin-converting enzyme; ARBs, angiotensin receptor blockers

On the other hand, reports on the progression of fibrosis are mixed. Mild fibrosis progression is reported in some. However, a meta-analysis of bariatric surgery assessing 766 paired liver biopsies from 15 studies reports improvement in steatosis in 91.6% of patients, improvement in steatohepatitis in 81.3%, and improvement in fibrosis in 65.5%, with complete NASH resolution in 69.5% [Mummadi *et al.* 2008]. In most studies, these improvements occurred primarily in patients showing the greatest improvement in components of metabolic syndrome and insulin resistance.

Insulin resistance

Given that insulin resistance is a key contributor in the development of NAFLD and NASH, much attention has been placed on insulin sensitizers. Thiazolidinediones (TZDs) are peroxisomal proliferator activated receptor-γ (PPAR-γ) agonists that promote hepatic fatty acid oxidation, decrease hepatic lipogenesis, and increase peripheral and hepatic insulin sensitivity [Oh *et al.* 2008]. At least 11 studies have evaluated their potential as agents for NAFLD treatment [Argo *et al.* 2009; Chalasani *et al.* 2009; Juurlink *et al.* 2009; Aithal *et al.* 2008; Lutchman *et al.* 2007; Belfort *et al.* 2006; Ratziu *et al.* 2006, 2008; Promrat *et al.* 2004; Sanyal *et al.* 2004; Neuschwander-Tetri *et al.* 2003; Shadid and Jensen, 2003; Caldwell *et al.* 2001]. Troglitazone has been withdrawn from the market due to hepatotoxicity. Rosiglitazone and pioglitazone continue to be used and both improve insulin sensitivity and aminotransferases [Aithal *et al.* 2008; Ratziu *et al.* 2008; Neuschwander-Tetri *et al.* 2003]. Studies have generally shown improvements in steatosis. The effect of TZDs on inflammation, fibrosis, and other histological features of NASH are less uniform [Argo *et al.* 2009; Chalasani *et al.* 2009; Juurlink *et al.* 2009; Aithal *et al.* 2008; Lutchman *et al.* 2007; Belfort *et al.* 2006; Ratziu *et al.* 2006, 2008; Promrat *et al.* 2004; Sanyal *et al.* 2004; Neuschwander-Tetri *et al.* 2003; Shadid and Jensen, 2003; Caldwell *et al.* 2001].

For example, the Fatty Liver Improvement with Rosiglitazone Trial (FLIRT) by Ratziu and colleagues randomized 63 biopsy-proven NASH patients to receive rosiglitazone or placebo for 1 year [Ratziu *et al*. 2008]. They found that treatment with rosiglitazone improved steatosis, transaminase levels, adiponectin, and insulin sensitivity, despite significantly more weight gain than in the placebo cohort. No significant change was noted in fibrosis or NAS score. Adverse effects included painful swollen legs and anemia. No hepatic toxicity was noted.

Many have suggested that NASH often recurs after discontinuation of TZD therapy, suggesting that long-term therapy may be necessary [Argo *et al*. 2009; Lutchman *et al*. 2007]. This may be problematic given the concerns for cardiotoxicity, congestive heart failure, edema, osteoporosis, and weight gain with TZDs, especially with rosiglitazone [Juurlink *et al*. 2009; Aithal *et al*. 2008; Lutchman *et al*. 2007]. It has also been noted that TZD therapy in the absence of lifestyle modification is often not effective [Ratziu *et al*. 2008; Lutchman *et al*. 2007]. Carefully designed studies with larger populations and longer durations are needed to better assess the efficacy and safety of long-term TZD use in patients with NAFLD.

The results of the PIVENS (Pioglitazone *versus* vitamin E *versus* placebo for the treatment of nondiabetic patients with nonalcoholic steatohepatitis) trial by the NIDDK NASH CRN Research Group have recently become available [Chalasani *et al*. 2009]. This large randomized, multicenter, double-blind, placebo-controlled study randomized 247 nondiabetic NASH patients to receive 96 weeks of pioglitazone 30 mg qd, vitamin E 800 IU qd or placebo [Chalasani *et al*. 2009]. Ninety percent of the subjects had biopsies at the end of the study. In this cohort of nondiabetic NASH patients, treatment with pioglitazone did not meet the primary endpoint of the study, which was defined by a decrease in NAS by at least two points, with at least a one-point improvement in ballooning. Nevertheless, treatment with pioglitazone was associated with in significant improvements in steatosis, inflammation, serum ALT, but neither pioglitazone nor vitamin E improved fibrosis scores. Treatment with pioglitazone was also significantly associated with greater weight gain (mean 4.7 kg: $p < 0.01$ versus placebo) when compared to the vitamin E or placebo groups. While the primary endpoint was not met by treatment with pioglitazone in this cohort of nondiabetic adults, it would be beneficial to investigate the effect of pioglitazone in NASH patients with insulin resistance or type 2 diabetes.

Metformin improves insulin resistance by it reducing hepatic gluconeogenesis, lipogenesis and fatty acid oxidation, increasing peripheral and hepatic insulin sensitivity, decreasing intestinal glucose absorption, and lowering serum lipid concentration. Several small trials have shown that metformin is relatively well tolerated in NASH patients and effective in improving aminotransferase levels and insulin resistance [Loomba *et al*. 2008; Bugianesi *et al*. 2005; Schwimmer *et al*. 2005; Duseja *et al*. 2004; Haukeland *et al*. 2004; Nair *et al*. 2004; Uygun *et al*. 2004; Marchesini *et al*. 2001]. Notably, weight loss has been noted with metformin use, in contrast to the weight gain sometimes observed with TZD use. All but three of the trials were open label [Bugianesi *et al*. 2005; Haukeland *et al*. 2004; Uygun *et al*. 2004]. In the study by Uygun and colleagues, metformin treatment resulted in improvement in necroinflammatory activity, although the difference did not reach statistical significance [Uygun *et al*. 2004]. Bugianesi and coworkers reported significant improvement in steatosis, inflammation, and fibrosis [Bugianesi *et al*. 2005]. The recent randomized, double blind, placebo-controlled trial by Haukeland and colleagues. examined the effects of 6 months of metformin ($N = 24$) versus placebo ($N = 24$) in patients with biopsy-proven NAFLD [Haukeland *et al*. 2004]. Treatment with metformin did not significantly improve steatosis (by histological or CT evaluation), NAS score, transaminases, or markers of inflammation or insulin resistance. Significant improvements in body weight, total cholesterol, low-density lipoprotein (LDL), glucose, and HbA1c were noted in the metformin cohort. So while the results regarding histological improvement of NAFLD findings with metformin treatment are mixed at best, metformin may have utility due to its ability to address insulin resistance and the cardiometabolic risk factors which often accompany NAFLD.

One small study evaluated the efficacy of nateglinide, a meglitinide that stimulates pancreatic insulin release and pancreatic beta cell growth [Morita *et al*. 2005]. This study showed that treatment with nateglinide in five biopsy-proven

NASH patients resulted in statistically significant biochemical and histological improvement.

Dyslipidemia

Statins reduce cholesterol production by competitively inhibiting hepatic hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase. While statin use in the setting of hepatic disease should be monitored due to possible hepatotoxicity and muscle toxicity, severe hepatotoxicity due to statins is exceedingly rare and statin use is widely believed to be safe in patients with liver disease [Lewis *et al*. 2007; Browning, 2006; Gómez-Domínguez *et al*. 2006]. Ekstedt *et al*.'s retrospective review of 17 NAFLD patients treated with statins for 10.3–16.3 years showed significant improvement in steatosis and no significant increase in fibrosis [Ekstedt *et al*. 2007]. Only a few small pilot studies have evaluated the efficacy of statins for NASH treatment [Antonopoulos *et al*. 2006; Hatzitolios *et al*. 2004; Rallidis *et al*. 2004; Kiyici *et al*. 2003]. These studies suggest some biochemical improvement, but long-term efficacy and safety remain to be established.

A few studies have also evaluated the therapeutic potential other lipid-lowering agents, such as fibrates and omega-3 fatty acids. Studies evaluating the role of fibrates indicate that fibrates may be efficacious in treating NASH since they activate peroxisome proliferator-activated receptors alpha (PPAR-α), leading to increased HDL-C and decreased triglycerides, LDL, and VLDL [Fernández-Miranda *et al*. 2008; Athyros *et al*. 2006; Nakamuta *et al*. 2005; Basaranoglu *et al*. 1999; Laurin *et al*. 1996]. More recently, Fernández-Miranda and coworkers studied the effects of 48 weeks of fenofibrate in 16 patients with biopsy-proven NAFLD [Fernández-Miranda *et al*. 2008]. They observed significant improvement in triglycerides, glucose, alkaline phosphatase gamma-glutamyl transpeptidase, aspartate transaminase (AST), ALT, apolipoprotein A1 levels, and the grade of hepatocellular ballooning degeneration. Unfortunately, no significant improvement was noted in insulin resistance, hepatic steatosis, necroinflammation, or fibrosis. In addition, an earlier open-label study by Laurin and colleagues that reported an absence of histologic improvement after 12 months of clofibrate therapy [Laurin *et al*. 1996].

Omega-3 fatty acid treatment results in some improvement in biochemical and radiological features of NAFLD, but no one has examined histological changes [Cussons *et al*. 2009; Zhu *et al*. 2008; Capanni *et al*. 2006; Hatzitolios *et al*. 2004]. While there may be a role for these agents in NASH treatment, larger placebo-controlled trials with histological assessment would be helpful.

Oxidative stress

The number of antioxidants with potential beneficial hepatic effects is increasing. On the other hand, only a few have been studied systematically. Antioxidants have therapeutic potential because fatty acid oxidation produces reactive oxygen species, which cause direct cellular damage and activate pro-inflammatory cytokines. Transforming growth factor beta1 is believed to promote fibrosis, and vitamin E has been shown to inhibit its activity. Of the antioxidants, vitamin E has been studied the most, with a few studies showing some positive preliminary results [Argo *et al*. 2009; Lutchman *et al*. 2007; Ersöz *et al*. 2005; Kawanaka *et al*. 2004; Vajro *et al*. 2004; Harrison *et al*. 2003; Kugelmas *et al*. 2003; Hasegawa *et al*. 2001; Lavine, 2000]. Four studies have evaluated histological improvement [Argo *et al*. 2009; Lutchman *et al*. 2007; Harrison *et al*. 2003; Hasegawa *et al*. 2001]. Hawegawa and coworkers report improvement in NASH features after 1 year of vitamin E [Hasegawa *et al*. 2001] treatment. Harrison and coworkers report improved fibrosis after 6 months of vitamin E, but inflammation did not improve [Harrison *et al*. 2003]. Sanyal *et al*. conducted a small pilot study in 2004 showing that vitamin E alone is not as effective as vitamin E and pioglitazone [Sanyal *et al*. 2004]. Significant improvement in steatosis, ballooning, and fibrosis were noted in the vitamin E and pioglitazone cohort.

As detailed above, the PIVENS trial by the NIDDK NASH Clinical Research Network found that 96 weeks of treatment with vitamin E in nondiabetic NASH patients resulted in significant improvement in the NAS score [Chalasani *et al*. 2009]. The primary endpoint of an improvement of at least two points in the NAS score was met, despite no improvement in fibrosis. These results provide encouraging support for further study of vitamin E therapy for NASH.

Several small studies have examined the potential therapeutic benefit of other antioxidants, such as betaine, S-adenosylmethionine (SAM), and N-acetyl-cysteine (NAC). SAM and betaine,

which increases SAM levels, have anti-TNF-α, cytoprotective and anti-apoptotic activity [Purohit *et al.* 2007; Barak *et al.* 1993]. Studies with animal models show that SAM has anti-steatogenic properties [Kwon *et al.* 2009; Abdelmalek *et al.* 2001]. A 1-year open-label study of betaine by Abdelmalek and colleagues resulted in statistically significant aminotransferase improvement [Abdelmalek *et al.* 2001]. Histological improvement was not statistically significant, and betaine was not beneficial in a later randomized control trial [Patrick, 2002].

Finally, N-acetyl-cysteine has beneficial effects in animal models of NAFLD, largely as a result of increasing hepatic glutathione, which decreases oxidative stress and the resultant upregulation of the inflammatory cascade [Baumgardner *et al.* 2008]. Two small studies of short duration show significant improvement in liver-function tests (LFTs), but follow-up biopsies were not performed [Pamuk and Sonsuz, 2003; Gülbahar *et al.* 2000]. Findings regarding these agents and others, such as silymarin, lecithin, and beta-carotene are very preliminary. Although it is unlikely that these antioxidants will become the mainstay of NASH therapy, they may serve as adjunct therapy in combination with other more targeted therapies.

Pro-inflammatory cytokines

TNF-α is a pro-inflammatory cytokine that is activated by the reactive oxygen species created by lipid peroxidation. It promotes necroinflammation, fibrogenesis, hepatic insulin resistance, and apoptosis [Satapathy *et al.* 2004]. Pentoxifylline is a xanthine derivative that is currently approved for treatment of claudication due to its effects on blood viscosity. Pentoxifylline has been shown to inhibit TNF-α and improve histology in animal models of NASH [Yalniz *et al.* 2007] and to significantly decrease TNF-α levels in NASH patients [Duman *et al.* 2007]. Three prospective trials have examined pentoxifylline's efficacy in NASH patients [Adams *et al.* 2004; Satapathy *et al.* 2004, 2007]. In 2007, Satapathy and colleagues treated nine patients with biopsy-proven NASH for 1 year with pentoxifylline 400 mg tid [Satapathy *et al.* 2007]. Significant reductions in AST and ALT were noted, as well as improvements in steatosis and lobular inflammation. Scores were significantly decreased by Brunt staging in six patients at the end of treatment, and four of six patients showed improved fibrosis. Again, larger studies are warranted.

Bacterial overgrowth

One possible source of increased oxidative stress may be bacterial overgrowth in the gastrointestinal tract. Bacterial overgrowth leads to increased hepatic exposure to ethanol and bacterial lipopolysaccharide, which are hepatotoxic and upregulate TNF-α and other pro-inflammatory mediators [Velayudham *et al.* 2008; Lirussi *et al.* 2007]. Two small studies have evaluated the effect of the probiotic VSL#3 in humans. Loguercio and colleagues reported improvement in LFTs, malondialdehyde, 4-hydroxynonenal, and S-nitrosothiol levels after 120 days of treatment [Loguercio *et al.* 2005]. Solga and colleagues reported that steatosis measured by proton magnetic resonance spectroscopy actually increased in three out of four patients treated with VSL#3 for 4 months [Solga *et al.* 2008]. While animal studies have suggested beneficial effects of probiotics, convincing evidence has yet to be demonstrated in humans, and further studies will be necessary before conclusions can be drawn.

Apoptosis

As detailed above, increased hepatocyte apoptosis may play a key role in progression from NAFLD to NASH [Malhi and Gores, 2008]. With the number of complex intracellular pathways in the apoptotic cascade, a large number of possible therapeutic targets exist. Although several studies of caspase inhibitors are underway, the long-term efficacy and safety of these agents are unknown.

On the other hand, several researchers have evaluated the potential efficacy of ursodeoxycholic acid (UDCA) because of its purported cytoprotective and immunomodulatory effects. It is postulated that UDCA may also be anti-apoptotic [Lazaridis *et al.* 2001]. UDCA is a naturally occurring bile acid that is approved for gallstone prevention and treatment of primary sclerosing cholangitis and primary biliary cirrhosis. Initial open-label pilot studies report improvements in aminotransferases and steatosis [Ersöz *et al.* 2005; Kiyici *et al.* 2003; Laurin *et al.* 1996; Guma and Viola, 1992]. However, a randomized placebo-controlled study of NASH patients did not confirm these findings [Dufour *et al.* 2006; Lindor *et al.* 2004; Mendez-Sanchez *et al.* 2004; Vajro *et al.* 2000]. In this study, 166 patients with biopsy-proven NASH were randomized to receive UDCA at 13–15 mg/kg/day or placebo for 2 years [Lindor *et al.* 2004]. Of the cohort

enrolled, 126 patients completed the study and 107 had paired liver biopsies. When compared with placebo, UDCA did not result in statistically significant improvements in aminotransferases, steatosis, inflammation, or fibrosis. UDCA was well tolerated. Nonetheless, these findings may warrant further investigation of UDCA as an adjunct with antioxidants or TZDs or in higher doses.

Other novel treatments

As stated earlier, many factors may contribute to NASH development and progression, and so a number of preliminary studies have been conducted on various other agents. As noted, the renin–angiotensin system (RAS) promotes fibrosis when hepatic stellate cells are activated by angiotensin II (ATII). Animal studies show that inhibiting the RAS, specifically at the ATII type 1a receptor, decreases stellate cell activity and fibrosis [Fujita *et al*. 2007; Ibanez *et al*. 2007; Yoshiji *et al*. 2001]. Angiotensin-receptor blockers are thought to decrease insulin resistance by their activation of PPAR-γ. A small pilot study of seven patients with hypertension and NASH reported improved aminotransferases and significant improvement in serum markers of fibrosis [Yokohama *et al*. 2004]. Necroinflammation improved in five patients and fibrosis improved in four patients.

Oligofructose, which has also been studied in the treatment of NASH, is an oligosaccharide derived from fruits and vegetables such as bananas, onions, chicory root, garlic, and others. It is indigestible and considered a prebiotic, as it may stimulate the growth of beneficial gut bacteria. Animal studies show that oligofructose decreases serum and hepatic triglycerides by decreasing hepatic triglyceride uptake and lipogenesis [Daubioul *et al*. 2000; Kok *et al*. 1996]. A small 8-week crossover study by Daubioul and colleagues randomized seven patients with biopsy-proven NASH to receive oligofructose or maltodextrine, which served as placebo [Daubioul *et al*. 2005]. Improvements in insulin and aminotransferase levels were reported.

Finally, some animal studies and a few case reports in humans suggest that incretin analogs may have therapeutic potential for NASH. This class promotes glucose-dependent insulin secretion, decreases excess glucagon secretion, and promotes satiety by delaying gastric emptying [Ding *et al*. 2006; Tushuizen *et al*. 2006]. There is obviously much room for future research into pathogenic mechanisms and possible therapeutic agents for NAFLD.

Conclusion

While much progress has been made in the epidemiology, natural history, and pathogenesis of NAFLD, as yet no single treatment modality has established efficacy in the treatment for NASH. To date, no pharmacological agent has been approved for treatment of NASH or could be recommended for routine use in clinical practice. While a number of agents targeting insulin resistance and pro-inflammatory factors demonstrate some potential, additional research is warranted to establish their safety and efficacy.

Even so, it is important to attempt to reverse conditions associated with NAFLD. If insulin resistance or type 2 diabetes is present, an insulin sensitizer may be helpful. For patients with dyslipidemia, lipid-lowering agents including statins should be considered. Furthermore, ACE inhibitors may be helpful, especially in hypertensive or diabetic patients. Finally, a multi-disciplinary team of experts may be needed to effectively treat patients with visceral obesity. For patients who are appropriate candidates, bariatric surgery may be helpful. Despite the absence of an established treatment for NAFLD, future investigations of NAFLD will continue to be very active and exciting.

Conflict of interest statement

None declared.

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