Therapeutic Perspective

CLINICAL INVESTIGATION

Novel treatment strategies for patients with nonalcoholic fatty liver disease

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Nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome, affects up to 30% of the population. As with the obesity and diabetes epidemic, NAFLD is a significant public health concern. Compared to other chronic liver diseases, management options for NAFLD are limited. While current treatment focuses on weight loss and management of cardiometabolic risk factors, significant weight loss is difficult to achieve and maintain. Two decades of research have produced numerous potential treatments, all hindered by various limitations. Vitamin E may be effective for some patients with nonalcoholic steatohepatitis, but more treatment options are needed, and several promising agents are under investigation. We review treatment strategies previously studied and briefly survey potential treatments on the horizon.

Keywords: insulin sensitizers • metabolic syndrome • nonalcoholic fatty liver disease • nonalcoholic steatohepatitis • pentoxifylline • statin • ursodeoxycholic acid • vitamin E

Nonalcoholic fatty liver disease (NAFLD) is now recognized as one of the most common causes of liver disease worldwide. Over the last decade, the natural history and public health impact of NAFLD has become more clearly understood, especially given the growing obesity and diabetes epidemic. NAFLD is closely associated with metabolic conditions, such as visceral obesity and insulin resistance. The presence of NAFLD is strongly associated with Type 2 diabetes and the other components of metabolic syndrome. Insulin resistance, which underlies metabolic syndrome, is also at the heart of NAFLD pathogenesis.

The prevalence of NAFLD in the USA is estimated at 20–30%. Nonalcoholic steatohepatitis (NASH), which is the progressive subtype of NAFLD, affects an estimated 3.5–5% of the American population [1]. In the morbidly obese, the estimated prevalence of NAFLD and NASH are 72–96% and 18.5–25%, respectively [1,2]. NAFLD and NASH occur in people of all ages, including children, as well as all ethnicities and both genders. In the USA, NAFLD is most common among Hispanics and least common among non-Hispanic blacks, with non-Hispanic whites falling inbetween [1].

Clinically, the diagnosis of NAFLD requires the exclusion of other types of fatty liver, especially alcoholic fatty liver. Pathologically, NAFLD encompasses a spectrum ranging from simple steatosis at one end to nonalcoholic steatohepatitis at the other. Research demonstrates that simple steatosis by itself does not seem to progress to advanced liver disease, whereas 10–15% of NASH patients progress to cirrhosis, with its attendant risks of decompensation and hepatocellular carcinoma [1]. NASH is also the cause of most cases of cryptogenic cirrhosis, which accounts for 10% of liver transplants in the USA on average [101]. The presence of NASH is also associated with increased liver-related mortality [1,3]. For these reasons, it is important

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to differentiate between simple steatosis and NASH, because patients with NASH should be the focus of treatment and intervention.

While steatosis can be noted on ultrasonography and other imaging modalities, histological evaluation is needed to differentiate simple steatosis from steatohepatitis. Although liver biopsy has its limitations, such as procedural risks, cost, sampling variability and inadequate specimen size, it is still considered the 'gold standard' for diagnosis and staging. Other modalities have been studied thoroughly, such as liver stiffness measured by sonography or magnetic resonance, and biochemical or specific biomarker panels and genomics and proteomics analysis; however, these modalities have limitations as well, and to date, none can replace liver biopsy for diagnosis [4]. Nevertheless, the utility of liver biopsy for the evaluation of progression is limited by sampling issues and variability. Therefore, noninvasive serum panels are becoming more and more useful for the the evaluation of progression.

Histologically, steatohepatitis is marked by three criteria: steatosis, lobular inflammation and ballooning degeneration. Findings of pericellular fibrosis and Mallory bodies also help establish the diagnosis, but these are not always noted. With histological findings indistinguishable from those of alcoholic liver disease, the diagnosis of NAFLD and NASH requires a history of limited alcohol consumption (≤10 g/day for females and ≤20 g/day for males). Given that NAFLD and NASH are diagnoses of exclusion, viral, autoimmune, genetic and metabolic hepatitidies need to be ruled out. NAFLD and NASH patients occasionally complain of fatigue or vague abdominal pain, but most patients present asymptomatically. NAFLD is most commonly diagnosed incidentally during an abdominal imaging study or as the conclusion to the investigation of asymptomatic elevation of liver enzymes noted on routine laboratory evaluation. While transaminases may be elevated up to five-times the upper limit of normal, it should be noted that NAFLD and even advanced NASH can present with normal liver enzymes, as with other liver diseases.

Pathogenesis

Several factors contribute to the development of NAFLD. In fact, NAFLD and NASH pathogenesis is thought to be a multihit process, developing in the setting of insulin resistance, obesity and the metabolic syndrome. The first insult occurs when macrovesicular steatosis develops in hepatocytes due to one or more of the following mechanisms: increased *de novo* lipogenesis in the liver, decreased free fatty acid (FFA) oxidation in the liver, and/or decreased export of lipid out of the liver. Lipid accumulation within hepatocytes results, in part, from underlying insulin resistance. In addition, hepatic steatosis contributes to further insulin resistance in the liver, contributing to a vicious cycle [5,6].

Many mechanisms have been proposed for the progression from simple steatosis to steatohepatitis, but the most important 'second hits' have to do with oxidative stress and increased inflammation. Numerous sources of oxidative stress have been described, including hyperinsulinemia, iron overload, mitochondrial reactive oxygen species, cytochome P-450 enzymes, cytokines, various toxins and bacterial overgrowth in the gut [5,6]. Apoptotic pathways and their link to oxidative stress have also been implicated [7]. All of these can lead to lipid peroxidation in the hepatocyte membrane, which then leads to stellate cell activation, generation of proinflammatory cytokines, and ultimately fibrogenesis [5].

Inflammation and the secretion of proinflammatory cytokines also participate in the second hit. White adipose tissue associated with visceral obesity displays endocrine function, secreting adipokines and cytokines, which either promote or suppress inflammation: adiponectin, leptin, resistin, visfatin, apelin, TNF-α, complement component 3, plasminogen activator inhibitor type 1, angiotensinogen and IL-1β, -6, -8 and -18 [8]. The details and interactions between the various mediators of oxidative stress and inflammation are beyond the scope of this article. For further details, the reader is referred to Edmison and McCullough's review of NASH pathogenesis [5]. Treatment measures target individual or multiple steps in this multihit process.

Strategies for treating patients with NASH

Although several therapies for NAFLD and NASH have been examined, no one modality is definitively effective or superior. As NASH is the subtype of NAFLD with the potential for progression to cirrhosis, hepatocellular carcinoma and liver-related death, it is generally accepted that patients with NASH should be the focus of treatment and clinical trials. That said, although the improvement of liver-related outcomes is an important goal in NASH management, it is also vital to address the increased cardiovascular mortality risk in NAFLD patients [1,3]. Accordingly, due attention must be paid to aggressive management of the underlying components of metabolic syndrome and the insulin resistance, which are associated with NAFLD and NASH.

The following sections summarize strategies for treating obesity, insulin resistance, hyperlipidemia, oxidative stress and other mechanisms responsible for pathogenesis of NASH.

■ Medical weight loss as a treatment strategy for NASH

Weight loss is the first step in managing metabolic syndrome, insulin resistance, NAFLD and NASH. Lifestyle modification (diet and exercise) should always be the initial recommendation, although pharmacologic and surgical means of weight loss have been studied in morbidly obese NAFLD and NASH patients. Weight loss around the belt line aimed at reducing visceral fat is especially important, as this may diminish the proinflammatory and other important contributions of white adipose tissue. Exercise to increase muscle bulk may not necessarily decrease absolute weight, but it can increase peripheral insulin sensitivity and decrease hyperinsulinemia [9].

Several studies have examined the effect of diet with or without exercise in NAFLD and NASH, demonstrating that calorie-restricted diet with or without exercise induces weight loss and improvement in transaminases [9–11]. Only a handful of clinical trials have included histological measures of efficacy [10]. A small pilot study by Huang and colleagues provided 12 months of nutritional counseling aimed at weight loss and reducing insulin resistance to 23 patients with biopsy-proven NASH [11]. Of the 15 patients with repeat liver biopsy after 12 months of dieting, nine had histological response, defined as improvement in total NASH score of at least two points. No patient demonstrated worsening of histology [11]. This study demonstrates that dietary intervention can successfully produce histological improvement in patients with NASH, so lifestyle modification should be encouraged. The dilemma with weight loss as a treatment modality is that it is often difficult to achieve and maintain in the less regimented setting of routine clinical practice. Additional methods for effecting weight loss have therefore been examined.

Several trials have studied the efficacy of weight loss medications, notably sibutramine and orlistat, for treatment of NAFLD and NASH. Sibutramine is a satiety-inducing serotonin- and norepinephrine-reuptake inhibitor and orlistat is an enteric lipase inhibitor. Most of the trials with these medications have not included histological evaluation of the liver. A 36-week trial of diet and vitamin E with or without orlistat in patients with biopsy-proven NASH by Harrison and colleagues demonstrated improvements in liver histology. The authors note, however, that histological improvement was correlated with degree of weight loss, not with treatment arm [12]. This trial demonstrates that NASH is improved by weight loss, not necessarily the particular means of weight loss. Following the May 2010 US FDA drug safety communication regarding 13 postmarketing reports of severe liver injury with orlistat, caution should be exercised before considering orlistat for use in patients with known liver disease [102].

■ Surgical weight loss as a treatment strategy for NASH

Bariatric surgery in the morbidly obese effectively reduces excess weight by at least 50%. Improvement in insulin resistance and all components of the metabolic syndrome has been noted after substantial weight loss. Although no placebo-controlled trials have been performed, data from over 20 paired biopsy studies indicate that improvement in steatosis and inflammation is typically seen in NASH patients after bariatric surgery [9,13,14]. The impact of bariatric surgery upon fibrosis is not clear, as some studies report improvement while a few even report mild worsening [9,13,14]. Weight loss of greater than 1.6 kg per week is a known risk factor for worsening inflammation and portal fibrosis, so patients with moderate to advanced fibrosis may be better off with less invasive and drastic procedures. For example, laparoscopic adjustable gastric banding may be ideal in this setting because the rate of weight loss can be managed with the adjustable band [9,14].

■ Insulin sensitizers as a treatment strategy for patients with NASH

Given that insulin resistance plays a major role in the pathogenesis and progression of NAFLD, research has focused on insulin sensitizers in NAFLD and NASH treatment, such as metformin and the thiazolidinediones (TZDs). Metformin is a biguanide with three main effects: decreased hepatic gluconeogenesis, decreased absorption of glucose from the gut, and improved hepatic and peripheral insulin sensitivity. Metformin has also been demonstrated to improve lipid profile. To date, the trials of metformin for treatment of NAFLD and NASH have been small (no more than 55 patients) **(Table 1)** [15–27]. Of the nine trials that included histological evaluation, four reported histological improvement with metformin [15–18], while the other five reported no improvement in histology [19–22,27]. So while it is still unclear whether metformin improves histology in NAFLD patients, several studies have shown improvement in transaminases, lipid and glucose levels, insulin resistance and weight. In fact, some studies suggest that these improvements should be attributed to the weight loss that accompanies metformin use [28]. Although the direct benefit of metformin upon NAFLD and NASH is debatable, it is clear that metformin safely and effectively addresses the weight and other underlying cardiometabolic risk factors associated with NAFLD.

Troglitazone, rosiglitazone and pioglitazone are thiazolidinediones that act as selective agonists of peroxisome proliferator-activated receptor-γ (PPAR-γ). Troglitazone has been withdrawn from the market due to hepatotoxicity. PPAR-γ receptors are found in the liver, adipose tissue and skeletal muscle; activation

Table 1. Insulin sensitizers: metformin.

Study (year)	N†	Design	Intervention (duration)	ALT improvement	Histological improvement	Ref.
Nair *et al.* (2004)	15	Open-label	Metformin (1 year)	Transient	Modest	[15]
Bugianesi *et al.* (2005)	55	RCT	Metformin (6 months)	Yes	Yes	[16]
de Oliveira *et al.* (2008)	20	Open-label	Metformin + NAC (6 months)	Yes	Yes	[17]
Loomba *et al.* (2008)	28	Open-label	Metformin (48 weeks)	Yes	Yes	[18]
Uygun *et al.* (2004)	17	RCT	Metformin (6 months)	Yes	No	[19]
Akyuz *et al.* (2007)	12	Open-label	Metformin (12 months)	No	No	[20]
Idilman *et al.* (2008)	24	Open-label	Metformin (48 weeks)	No	No	[21]
Haukeland *et al.* (2009)	24	RCT	Metformin (6 months)	No	No	[27]
Omer *et al.* (2010)	44	Open-label	Metformin vs rosiglitazone vs metformin + rosiglitazone (12 months)	Only with metformin + rosiglitazone	Only with metformin + rosiglitazone	[22]
Marchesini *et al.* (2001)	20	Open-label	Metformin (4 months)	Yes	N/A	[23]
Duseja *et al.* (2007)	25	Open-label	Metformin (6 months)	Yes	N/A	[24]
Nar *et al.* (2009)	19	Open-label	Metformin (6 months)	No	N/A	[25]
Garinis *et al.* (2010)	25	Open-label	Metformin (6 months)	N/A	N/A	[26]

†The number of patients treated with metformin.

ALT: Alanine aminotransferase; N/A: Not available; NAC: *N*-acetyl-cysteine; RCT: Randomized controlled trial.

of PPAR-γ decreases hepatic and peripheral insulin resistance. TZDs also increase hepatic fatty acid oxidation and decrease hepatic lipogenesis, and thus address two of the mechanisms behind the 'first hit' of hepatic fat accumulation. Several studies demonstrate that TZDs effectively improve biochemical indices and some histological findings of NAFLD and NASH (Table 2) [29–39]. All but one of the trials demonstrate that TZD treatment improves transaminase levels, and others show improved steatosis [30,32,34,37–39] and inflammation [30,32,34,36,39]. On the other hand, fibrosis typically does not improve [30,34,37–39]. All but four of these trials are open-label [34,36–39] and all but three enroll less than 33 patients [35,38,39].

Ratziu and colleagues recently published the results of the 2-year extension of their initial 12-month randomized placebo-controlled trial of rosiglitazone therapy in NASH patients (FLIRT) trial [38]. After the initial year of rosiglitazone or placebo, 53 patients were rolled-over into an open-label extension of rosiglitazone 8 mg/day for an additional 2 years. Although significant improvements were noted in alanine aminotransferase (ALT) and insulin resistance, significant improvement was not noted in mean NAFLD activity score (NAS), ballooning, fibrosis or micromorphometrically determined area of fibrosis. In the group that received rosiglitazone in the initial placebo-controlled trial, an additional 2 years of rosiglitazone also produced no improvement in steatosis. Therefore, the long-term benefit of TZD treatment remains in question until further studies demonstrate otherwise.

Most recently, a large multicenter, double-blind, placebo-controlled trial by the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) compared pioglitazone (30 mg/day; n = 80) to vitamin E (800 IU/day; n = 84) and placebo (n = 83) [39]. Subjects were nondiabetic patients with biopsy-proven NASH. Treatment duration was 96 weeks. Compared to the placebo, treatment with pioglitazone was associated with significant improvement in secondary outcomes, such as improvement in transaminases ($p < 0.001$), hepatic steatosis ($p < 0.001$), lobular inflammation ($p = 0.004$) and mean change in overall NAS ($p < 0.001$). A significant improvement in insulin resistance was also observed with pioglitazone ($p = 0.03$), but significant weight gain was also noted (average 4.7 kg; $p < 0.001$). Fibrosis scores did not show significant improvement ($p = 0.12$).

The primary outcome – improved histology as measured by NAS – was defined as a ballooning score decreased by at least one point, no worsening of fibrosis, and a final NAS of three or less, or a decrease in NAS of two or more points. Improvements were only considered statistically significant for p-values of 0.025 or below. According to these specifications, improvement occurred in 34% in the pioglitazone cohort versus 19% in the placebo group. The p-value of 0.04 did not reach the predetermined value of 0.025.

While these results reinforce earlier findings of improved biochemical indices, steatosis and inflammation, they also demonstrate that pioglitazone has

little effect on fibrosis and is associated with significant weight gain. Perhaps more disturbing is the finding that after pioglitazone is discontinued, insulin resistance returns to baseline and the weight gain persists. Given that the primary outcome was not met and taking into account the possible side effects of weight gain, increased fracture risk, fluid retention and the potential increase in cardiovascular events, further study will be needed before TZDs can be routinely recommended for NASH patients [40]. What remains to be clarified is whether TZDs are more effective than other insulin-sensitizing agents in the population of NASH patients with concurrent diabetes mellitus [34]. In addition, it is still unclear whether improvements in insulin resistance, steatosis and inflammation alone (without fibrosis or overall NAS improvement), is sufficient to alter the natural history of NASH. Again, the possible long-term side effects associated with TZDs must be weighed against possible benefits in the design of future trials.

A number of other insulin-sensitizing agents may also have utility in NASH management (Table 3). One such agent is exenatide, a glucagon-like peptide-1 receptor agonist. The naturally occurring form of this incretin analog, exendin-4, was originally isolated from Gila monster saliva. Although exenatide primarily increases glucose-dependent insulin secretion, it also increases satiety and induces weight loss, which may lead to improved insulin sensitivity. One open-label study of exenatide safety over a period of 3.5 years demonstrated improved insulin sensitivity and transaminases in diabetics [41]. At least three trials are currently examining the effect of exenatide in NAFLD and NASH patients [103–105]. Two of these include pre- and post-treatment histological examination [103,104].

Liraglutide is an acylated glucagon-like peptide-1 receptor agonist, and thus acts in a fashion similar to exenatide. Like exenatide, liraglutide treatment is associated with significant weight loss. In 2009, a double-blind, placebo-controlled study randomized 564 obese, nondiabetic subjects to receive placebo or one of four doses of liraglutide (1.2, 1.8, 2.4 or 3.0 mg) along with diet and exercise [42]. These arms were also compared with open-label orlistat given at 19 sites. Subjects treated with liraglutide lost 2.1–4.4 kg more weight than the placebo group and significantly more weight than the orlistat groups. Blood pressure and prediabetes were also improved in patients receiving liraglutide. A Phase III, multicenter, double-blind, placebo-controlled trial is currently studying the efficacy of 52 weeks of liraglutide for the maintenance of weight loss induced by a 12-week low-calorie diet in obese, nondiabetic patients [106]. Primary outcome measures include the percentage of subjects with maintained weight loss and the mean percentage of bodyweight change. Secondary outcome measures also include changes in cardiovascular risk factors, lipid profile, metabolic syndrome status and insulin resistance measured by homeostatic model assessment (HOMA). If liraglutide can safely and effectively maintain weight loss in nondiabetic patients, it will be worthwhile to examine its utility for management of NASH.

Table 2. Insulin sensitizers: thiazolidinediones.

Study (year)	N†	Design	Intervention (duration)	ALT improvement	Histological improvement	Ref.
Caldwell *et al.* (2001)	10	Open-label	Troglitazone (6 months)	Yes	No	[29]
Neuschwander-Tetri *et al.* (2003)	30	Open-label	Rosiglitazone (48 weeks)	Yes	Yes	[30]
Shadid *et al.* (2003)	20	Open-label	Pioglitazone (18 weeks)	Yes	N/A	[31]
Promrat *et al.* (2004)	18	Open-label	Pioglitazone (48 weeks)	Yes	Yes	[32]
Sanyal *et al.* (2004)	10	Open-label	Pioglitazone + vitamin E (6 months)	No	Yes	[33]
Belfort *et al.* (2006)	26	RCT	Pioglitazone (6 months)	Yes	Yes	[34]
Wang *et al.* (2006)	68	Open-label	Rosiglitazone (6 months)	Yes	N/A	[35]
Aithal *et al.* (2008)	31	RCT	Pioglitazone (12 months)	Yes	Yes	[36]
Ratziu *et al.* (2008)	32	RCT	Rosiglitazone (12 months)	Yes	Yes	[37]
Ratziu *et al.* (2010)	53	Open-label extension	Rosiglitazone (2 years)	Yes	No	[38]
Sanyal *et al.* (2010)	80	RCT	Pioglitazone (96 weeks)	Yes	Some improvement	[39]

†The number of patients treated with thiazolidinediones.

ALT: Alanine aminotransferase; N/A: Not available; RCT: Randomized controlled trial.

Table 3. Insulin sensitizers: other.

Study (year)	N†	Design	Intervention (duration)	ALT improvement	Histological improvement	Ref.
Klonoff *et al.* (2008)	217	Open-label	Exenatide ± MF ± SU (3 years)	Yes	N/A	[41]
Astrup *et al.* (2009)	564	RCT Open-label extension	Liraglutide (RCT: 20 weeks; open-label extension: 84 weeks)	N/A	N/A	[42]
Morita *et al.* (2005)	5	Open-label	Nateglinide (20 weeks)	Yes	Yes	[43]

†The number of patients treated with other insulin sensitizers.
ALT: Alanine aminotransferase; MF: Metformin; N/A: Not available; RCT: Randomized controlled trial; SU: Sulfonylurea.

Nateglinide is a second-generation sulfonylurea insulin secretagog. In a small pilot study, 10 diabetic patients with biopsy-proven NASH were randomized to treatment with nateglinide 270 mg/day or no treatment for 20 weeks [43]. In the treatment group, improvements were noted in HbA_{1c}, liver functions tests, imaging by sonogram and computed tomography, and liver histology. No trials of nateglinide for NAFLD or NASH treatment are currently ongoing, but larger studies of these insulin secretagogs may yield useful findings regarding another possible agent for NASH treatment.

■ Treating oxidative stress with antioxidants in patients with NASH

Of all the agents studied thus far, vitamin E is has the most robust evidence-based support. The potential benefits of using α-tocopherol to target the oxidative stress components of NASH were first demonstrated in adults by Hasegawa and colleagues in 2001 [44]. A total of 12 adults treated with vitamin E 300 mg for 1 year demonstrated improved transaminases. Five out of nine patients with end-of-treatment biopsy demonstrated improved steatosis, and five demonstrated improved inflammation and fibrosis. Subsequent trials of vitamin E were small, and while transaminase improvement was noted more often then not, histological findings were varied [1,10]. The recent trial by NASH CRN that compared vitamin E (800 IU/day; n = 84) to pioglitazone and placebo for 96 weeks in nondiabetic patients showed that the primary outcome measure, improved NAS, occurred more often with vitamin E than with placebo (43 vs 19%; p = 0.001) [39]. Transaminases, steatosis and lobular inflammation were significantly improved as well, although fibrosis scores did not improve significantly. This relatively large, multicenter, double-blind, placebo-controlled study demonstrated that vitamin E is safe, well-tolerated and effective for improving NASH overall. While some concern has been raised about potential increases in bleeding risk and all-cause mortality with vitamin E treatment in other conditions, it is unclear whether these findings are attributable solely to vitamin E intake [107]. Given the safety profile observed with vitamin E over a treatment duration of 96 weeks in the previously mentioned protocol, it would be helpful to examine its safety and efficacy in larger numbers of patients, for longer treatment durations, and perhaps in higher doses or in combination with other agents.

Other treatment strategies for patients with NASH

Given the involvement of hyperlipidemia, apoptosis, TNF-α and a number of other factors, a few additional strategies will be summarized.

■ Lipid-lowering agents used for treating NASH

The potential utility of the statins for NASH treatment has been examined in a few small trials because derangements in lipid metabolism have been implicated in NASH pathogenesis [1,10,45]. Statins decrease cholesterol production by competitively inhibiting hepatic hydroxymethyl-glutaryl coenzyme A. While statins are known to cause mild-to-moderate transaminase elevations, the incidence of severe and permanent hepatotoxicity with statin use is very low, and it is generally agreed that statins can be safely used in patients with compensated liver disease.

The most recent double-blind, placebo-controlled trial of statins for NASH treatment examined 12 months of simvistatin monotherapy in 16 biopsy-proven NASH patients [45]. Results demonstrated that treatment did not improve aminotransferases, steatosis, inflammation or fibrosis. Hyogo and colleagues conducted a prospective trial of 24 months of open-label atorvastatin in 31 biopsy-proven NASH patients with hyperlipidemia and found normalization of transaminases in 23 (74.2%) patients [46]. Significant increases in adiponectin and significant decreases in TNF-α were also noted. As for histological end points, steatosis and NAS were significantly improved in the 17 patients who had follow-up biopsies. However, fibrosis

progressed in four out of the 17 patients, with three progressing to stage three fibrosis. Interestingly, these findings echo those from a previous retrospective review by Ekstedt *et al.*, which noted fibrosis progression in four out of 17 NAFLD patients treated with a statin over a range of 10.3–16.3 years [47]. Certainly, these trials were not designed to assign fibrosis progression to statin therapy. Since some biochemical and histological improvements are observed with statin use, larger trials are needed before conclusions can be made regarding the safety and efficacy of statin therapy for NASH. Currently, no statin trials are ongoing.

By contrast, there is considerable interest in treating NASH with N-3 polyunsaturated fatty acids (PUFAs), as demonstrated by the six clinical trials currently examining the efficacy of PUFAs exclusively in NASH patients [108–113]; five of these include histological examination. PUFAs decrease hypertriglyceridemia and improve insulin sensitivity in humans, and animal studies demonstrate that PUFAs decrease hepatic triglyceride deposition, oxidative stress, inflammation and fibrosis [48,49]. Pilot studies also show improvements in transaminases and imaging characteristics as well as histological improvements [50]. A 12-month trial of eicosapentaenoic acid (EPA), one of the main PUFAs, in 23 biopsy-proven NASH patients, shows that EPA is well tolerated and significantly improves transaminases and serum FFAs. Steatosis, lobular inflammation, ballooning and fibrosis improved in six out of the seven patients who had follow-up biopsy [50]. In addition, PUFAs are currently approved for treatment of hypertriglyceridemia and arteriosclerosis. One study found that the addition of EPA to statin therapy decreased major coronary events by 19% over 5 years [51], highlighting the fact that PUFAs may address both the hepatic and cardiovascular risks associated with NASH. In light of these positive findings and the tolerability of PUFAs, the results of these ongoing, larger Phase II and III trials are eagerly anticipated.

■ Ursodeoxycholic acid as a cytoprotective agent for NASH

Ursodeoxycholic acid (UDCA) is a bile acid produced naturally by the liver. It has been safely used for many years to treat primary biliary cirrhosis and for the prevention of gallstones, acting mainly by thinning out the bile pool. Some reports also note that UDCA may also have antiapoptotic and cytoprotective properties [52]. Initial studies, which were small and open-label, show improvement in transaminases and steatosis. Initial enthusiasm regarding UDCA's use in NAFLD and NASH, especially given its long track record of safety [1,53], has been dampened by a larger multicenter placebo-controlled trial of UDCA at 13–15 mg/kg/day in 107 patients over 2 years, demonstrating no difference between UDCA at these doses and placebo [54]. A more recent trial examined UDCA at a higher dose of 23–28 mg/kg/day [55]. Over 130 patients were treated for 18 months, and over 100 had follow-up liver biopsies. Overall histology and fibrosis scores did not improve in the treatment group. Of the individual histological parameters, only lobular inflammation improved significantly. The only biochemical marker to improve was γ-glutamyl transferase. These two large studies of UDCA at increasingly higher doses indicate that UDCA monotherapy has limited efficacy for the treatment of NASH. Although some agents have demonstrated initial promise, larger placebo-controlled trials indicate that NASH is rather resistant to pharmacologic intervention, at least by monotherapy. Given the number of pathways involved in NASH pathogenesis and side-effect profile, UDCA treatment in conjunction with another agent might be explored more rigorously in the future.

■ Other agents for NASH

A few initial studies have investigated the utility of L-carnitine, which improves mitochondrial FFA transport and β-oxidation [56,57]. In a recent well-designed, placebo-controlled study, Malaguarnera and colleagues randomized 74 biopsy-proven NASH patients to receive L-carnitine 1 g twice daily with diet or placebo plus diet for 24 weeks [56]. The primary measure was histological response, defined by at least a three-point improvement in NASH activity index, which scores steatosis, parenchymal inflammation and hepatocellular injury. The NASH activity index was significantly improved in the treatment group compared with placebo, and 35 of the 36 patients randomized to L-carnitine demonstrated this improvement. Significant improvement was also noted in individual measures of steatosis, parenchymal inflammation, hepatocellular injury, fibrosis and Mallory bodies. Of note, improvement in fibrosis was observed in 31 patients (86%) treated with L-carnitine. Significant improvements were also noted in transaminases, lipid profile, HOMA, C-reactive protein and TNF-α. No trials of L-carnitine in humans are currently in progress.

Pentoxifylline, which is FDA-approved for treatment of intermittent claudication, has been the subject of a few studies owing to its anti-TNF-α properties [58–60]. A small pilot study by Satapathy *et al.* in 2007 demonstrated that treatment with pentoxifylline 400 mg three-times daily for 1 year was associated with significant improvement in transaminases, steatosis and lobular inflammation [58]. Subsequent randomized controlled trials confirm improved transaminases with pentoxifylline treatment [59,60]. Significant improvement in

ballooning and NAS resulted from a 12-month controlled trial by Rinella and colleagues [60]. Two double-blind, placebo-controlled studies of pentoxifylline in NASH patients are ongoing [114,115]. Both include histological evaluation and one measures changes in TNF-α, IL-6 and IL-10, leptin, adiponectin, FFAs, hyaluronic acid, thiobarbituric acid reactive substances and procollagen III N-peptide [115].

Intervention with angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers has some benefit in animal and human NASH studies [61,62]. Angiotensin-receptor blockers have been shown to improve insulin sensitivity via activation of PPAR-γ and possibly via other pathways. A recent double-blind, placebo-controlled trial by Georgescu and colleagues randomized 54 patients with biopsy-proven NASH to receive telmisartan or valsartan daily for 20 months [62]. After treatment, transaminase levels decreased significantly, as did HOMA scores and NAS. The improvement in HOMA scores and NAS were greater with telmisartan than with valsartan. Currently, a Phase III trial has been developed to evaluate the efficacy of losartan in NASH patients [116]. Outcome measures include changes in NAS as well as changes in noninvasive radiological and serological measurements of NASH. Another double-blind, parallel-assignment, Phase IV trial has been designed to examine the effect of valsartan versus hydrochlorothiazide on hepatic triglyceride levels, insulin resistance and abdominal fat mass [117].

Phytotherapeutic approaches, also known as complementary alternative or herbal medicine, have also long been used for the treatment of various hepatic diseases. Milk thistle has a long history of safe and widespread usage, dating back to ancient Greek, Roman and Eastern civilizations [63]. Milk thistle's active compound, silymarin, decreases transaminases, and it has antioxidant, anti-inflammatory, antifibrotic and toxin-blocking effects [63]. It has also been shown to improve insulin resistance in liver disease patients with diabetes [63]. Currently, two double-blind, placebo-controlled studies are examining the safety and efficacy of silymarin treatment in patients with biopsy-proven NASH [118,119]. One trial will measure change in NAS, transaminase levels, HOMA score and biomarkers [118], while the other will measure change in NAS, TNF-α levels and markers of oxidative stress [119].

Future perspective

Several other agents, such as probiotics, betaine and *N*-acetyl-cysteine, have shown some benefit in NAFLD and NASH treatment, but these results are preliminary. In addition, many clinical trials are currently investigating other agents, such as bovine colostrum, phosphatidylcholine, recombinant leptin, phosphodiesterase inhibitors, fatty acid bile acid conjugates and numerous other experimental compounds. With the growing worldwide epidemic of obesity, diabetes and metabolic syndrome, NAFLD and NASH will continue to be a large public health concern. While current treatment still focuses on weight loss, managing cardiometabolic risk factors, and selective use of vitamin E, much progress is yet to be made. Within the next 5 years, noninvasive imaging modalities and biochemistry or biomarker panels will likely be validated and become the preferred means of diagnosis, staging and progression, thereby limiting the use of liver biopsy to select cases. Therapeutic options will grow to include several safe and effective oral agents. Progression due to NASH is preventable, and with the number of agents currently under study, the public health impact and economic burden of NAFLD and NASH can foreseeably be reduced within the next decade.

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Executive summary

- Nonalcoholic fatty liver disease, which is closely associated with the metabolic syndrome (truncal obesity, hypertension, diabetes, hypertriglyceridemia and low high-density lipoproteins), is a significant worldwide public health concern.
- Nonalcoholic steatohepatitis, the progressive subtype of nonalcoholic fatty liver disease, is the focus of treatment.
- Many potential therapeutic agents have been investigated, but thus far, only vitamin E has been shown to be safe and effective.
- Further elucidation of nonalcoholic steatohepatitis pathogenesis and identification and validation of safe and effective therapeutic agents are urgently needed.

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