Non-alcoholic steatohepatitis: the therapeutic challenge of a global epidemic

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide. Non-alcoholic steatohepatitis (NASH) reflects severe liver disease within the NAFLD spectrum and can progress to end-stage liver disease. Within this manuscript we review the available evidence for the treatment of NASH as well as the newer therapeutic agents that are currently being investigated.

Keywords NASH, fatty liver, treatment, vitamin E, pioglitazone

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome that has taken epidemic proportions affecting up to 95 million adults in the United States [1,2]. NAFLD encompasses a spectrum of hepatic pathology ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) and end-stage liver disease [3]. NASH is a pathological diagnosis for which various diagnostic scoring systems have been developed [4-6]. Diagnostic criteria requiring the presence of hepatic steatosis with hepatocellular ballooning or Mallory Denk bodies or fibrosis to define NASH, are the most efficient in predicting liver-related mortality [7]. The requirement for liver biopsy to reach the diagnosis of NASH precludes the use of population wide studies to establish its prevalence, which is currently thought to have reached 12% in North American adults, translating into 25 million people in the USA alone [2]. Despite the risk of overestimating its true prevalence, primarily due to the fact that obese patients are more likely to seek medical attention and eventually have a diagnostic liver biopsy performed, NASH is associated with a 15-20% risk of progression to cirrhosis, making it a considerable medical and financial burden [1,8]. Treatment of NASH has proven to be challenging, which is concerning considering the magnitude of the problem.

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NASH is a therapeutic challenge due to various reasons. First, given its definition, liver biopsy is required to accurately establish the diagnosis. Despite the development of various non-invasive serological markers of fibrosis, the latter is diagnosed more accurately with a liver biopsy, which is significant because fibrosis is the most important determinant of liver-related mortality, despite the fact that its presence is not required to diagnose NASH. Liver biopsies however, are impractical, costly and associated with a higher risk of complications in the setting of obesity [9]. A biopsy is also necessary to directly demonstrate the effects of any new treatment being studied. In addition, the pathophysiology of NASH is complex and not entirely elucidated, rendering the identification of successful therapeutic targets difficult [3]. Clinical trials aiming at treating NASH are not uniform as they are characterized by lack of standard diagnostic criteria for NASH, variable duration of treatment, different therapeutic outcomes (e.g. improvements in blood biochemistry vs. hepatic histology etc.) and inclusion of patients with several co-morbidities (e.g. type 2 diabetes mellitus [DM2]). That makes interpretation of such studies difficult and weakens the available evidence. Lastly, certain treatments studied, such as lifestyle modification (diet and exercise), may be proven to be effective but they are quite challenging to implement, difficult to sustain in a non-research setting and hence non-realistic [10,11]. Future investigations should aim at preventing NASH, understanding its pathophysiology and targeting key components of the pathogenetic process.

NASH, once thought to be the result of "two hits", is now viewed as a more complex condition, resulting from multiple and possibly concurrent 'hits' that trigger steatosis, oxidative stress, inflammation and eventually hepatocellular death and fibrosis [3]. Steatosis develops from disequilibrium between hepatic lipid uptake (fatty acids derived from diet or adipose tissue), synthesis (*de novo* lipogenesis), oxidation and secretion (formation and release of VLDL particles from the

liver) [12,13]. Insulin resistance (IR), frequently seen in obese individuals, is tightly linked to this process as it alters nutrient distribution among tissues and their metabolism. Impaired insulin signaling leads to enhanced adipose tissue lipolysis and increased flow of free fatty acids to the liver, contributing to lipid peroxidation and formation of reactive oxygen species [14,15]. Peripheral IR leads to impaired glucose tolerance whilst IR at the hepatic level contributes to the disequilibrium between glucose and lipid synthesis and oxidation. Inflammation is triggered by signals derived from adipocytes (e.g. cytokines such as tumor necrosis factor [TNF]-α and interleukin [IL]-6), immune cells (e.g. macrophages, Kuppfer cells), nutrients (e.g. ω-6 fatty acids) as well as intestinal microbiota (e.g. endotoxins) [3]. Gut bacteria participate in the deranged metabolic and inflammatory process either indirectly (via altered nutrient metabolism and increased caloric extraction) or directly (by entering the circulation via the increased intestinal permeability seen in obese subjects) [16-19]. To date, therapeutic trials for NASH have aimed at decreasing steatosis, IR, oxidative stress, inflammation and even fibrosis, as will be discussed in this review (Fig. 1).

Approaches to the management of NASH can be divided into lifestyle changes (diet and/or exercise), medications and surgical interventions. Medications include antioxidants (e.g. vitamins E and C, betaine), insulin-sensitizing agents (thiazolidinediones and metformin), lipid-lowering drugs (statins, orlistat, probucol), choleretic agents (ursodeoxycholic acid, UDCA) and medications with anti-inflammatory

(pentoxifylline, PTX) or anti-fibrotic (angiotensin-receptor blockers) potential. Bariatric surgery has also been used for the management of NASH as discussed further in this review.

Lifestyle modifications

Diet and/or exercise: Despite adequate evidence supporting the effect of weight loss (achieved either by diet or exercise) in decreasing the hepatic triglyceride content of patients with NAFLD, there are few data on the role of such interventions for the management of NASH [11,20]. Weight loss of 5-10% from baseline has repeatedly been shown to decrease hepatic steatosis by approximately 50% but its effect on inflammation or fibrosis has not been adequately studied [21,22]. In addition, it is not known which dietary intervention or type of exercise is more beneficial for patients with NASH. The majority of clinical trials in this area are characterized by small sample size, short duration and variable outcomes, the majority of which (e.g. transaminases, steatosis on imaging) are not predictive of liver disease progression [23-25]. The highlights of these studies are discussed below.

Huang et al performed a pilot study of nutritional counseling for the management of NASH [25]. The 15 patients that completed the study were advised to receive 40-45% of their calories from carbohydrates (C/H), 35-40% from fat and 15-20% from protein. At 1 year, histological improvement
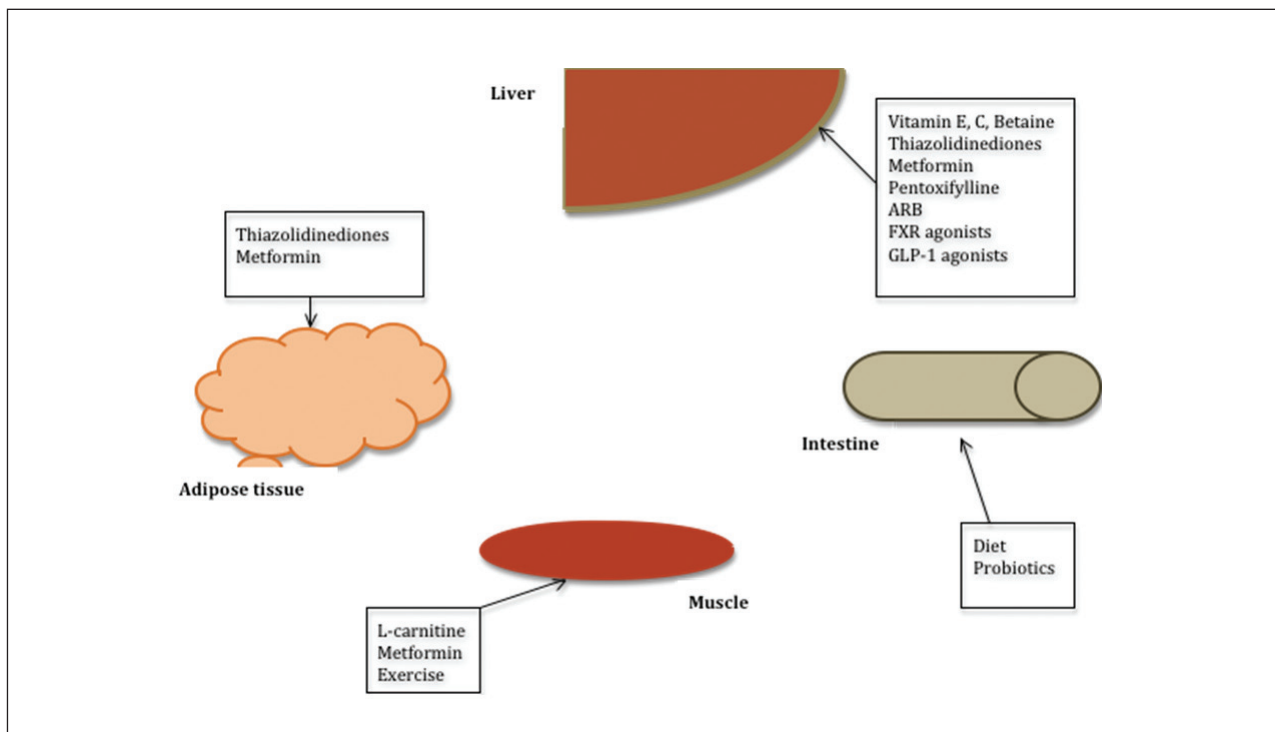

Figure 1 Site of action of treatments studied in non-alcoholic steatohepatitis
ARB, Angiotensin-II Receptor Blockers; FXR, Farnesoid X Receptor; GLP-1, Glucagon-Like Peptide-1

was seen in 9/15 patients; however, there was no statistically significant change in steatosis, hepatitis or fibrosis scores, which may have been secondary to the minimal and not statistically significant weight loss (average 2.9 kg) seen in these patients. Another trial assessed the effect of moderate intensity aerobic exercise (30 min/d, 5 d/wk) and moderate caloric restriction [the latter was used only for patients with high body mass index (BMI)] on transaminase levels in the setting of NASH [24]. Of the 44 patients that were compliant with the exercise program, 20% had normalization in their alanine aminotransferase (ALT) and the majority had significant reductions in both ALT and aspartate aminotransferase (AST). This study did not assess changes in steatosis or hepatic histology. Lastly, Promrat et al randomized 31 patients with NASH to a lifestyle intervention program (diet, exercise and behavior modification) or education (controls) and evaluated the changes in NAFLD activity score (NAS) 48 weeks later [26]. The average weight loss in the intervention group was 9.3% vs. 0.2% in the controls and that correlated with improved NAS. Furthermore, weight loss equal or higher to 7% from baseline was associated with a significant decrease in steatosis, lobular inflammation and ballooning. Fibrosis was not affected by this intervention, which unless it was a matter of low power, indicates that either weight loss cannot improve fibrosis or the reversal of this condition requires longer interventions. Lastly, in a recent meta-analysis, Musso et al showed that weight loss is effective in reducing histological disease severity of patients with NASH, but noted that half of the patients are unable to reach their weight goal with lifestyle interventions [27].

Despite the paucity of data from well-designed, long-term, randomized, intervention studies, there is sufficient evidence to support the use of diet and exercise for the management of other co-morbidities associated with NASH and the metabolic syndrome, such as hypertension and diabetes; hence diet and exercise are considered the first line of treatment for patients with NASH. The use of specific macronutrients, rather than caloric restriction alone, has been trialed for the treatment of NASH as well.

Certain C/H, fatty acids and amino acids that are thought to have metabolically beneficial, anti-inflammatory or anti-oxidant properties have been used in patients with NASH but again, the studies are few, underpowered and their outcomes not consistent. Based on animal data showing anti-steatotic effects of oligofructose, Daubioul et al supplemented 7 NASH patients with 16 g of oligofructose per day for 8 weeks but failed to show decrease in intrahepatic fat as seen on ultrasound [28]. Studies in animals and cell cultures have also revealed an anti-inflammatory role for ω-3 fatty acids, which makes them an attractive option for the management of steatohepatitis. Eicosapentanoic acid (EPA) is a ω-3 fatty acid that was trialed in 23 patients with NASH (2700 mg/d for 1 year) and led to a decrease in transaminase, ferritin, TNF-α receptor and thioredoxin levels [29]. In addition, in 6/7 liver samples obtained in this study, steatosis, inflammation, ballooning and fibrosis were also reduced. Lastly, in an attempt to increase glutathione levels and decrease oxidative stress of patients with steatohepatitis, cysteine-rich, whey protein isolates were given in an open label trial by Chitapanarux et al [30]. This intervention decreased serum transaminases, increased hepatic antioxidant potential and limited macrovesicular steatosis. It is important to note that the both the diagnosis of NASH and the outcomes of this study were determined by imaging and blood biochemistry alone, as no liver tissue was available. To date, there is not enough evidence to support supplementation with specific macronutrients for the treatment of NASH.

Overall, evidence from observational studies linking certain nutrients as well as obesity to the development of NASH has led to certain recommendations regarding the non-medical treatment of this condition, which include slow weight loss (aiming at a decrease of 7-10% from baseline weight over 6 months to 1 year) achieved either by diet and/or exercise, avoidance of high fructose and saturated fatty acids and preference towards complex C/H, fiber and mono- or polyunsaturated fatty acids [31]. New randomized controlled trials (RCTs) with larger sample size and histologic evidence of both NASH and therapeutic response are needed to clarify the role of diet and exercise in the field of NASH.

Medications

Orlistat: Orlistat inhibits the action of gastric and pancreatic lipases and subsequently leads to fat maldigestion, with 30% of dietary triglycerides being passed in the stool rather than being absorbed [32]. It is effective in decreasing the BMI of obese subjects and hence, via its weight-limiting effect, could be useful for patients with NASH. The few studies looking at orlistat for NASH have used it in addition to caloric restriction and/or other medications, making it difficult to delineate its exact contribution [32,33].

Assy et al performed a 6 month-long, open-label trial of orlistat (120 mg TID) and moderate caloric restriction (25 kcal/kg/d) for overweight patients with NASH [32,33]. This intervention led to a mean weight loss of 5.1 kg, decreased steatosis in 71%, inflammation in 78% and fibrosis in 72% of the patients as well as an improvement in serum transaminases, IR index and total cholesterol and triglycerides. Interestingly, these changes did not correlate with weight loss. The lack of a placebo arm and the concomitant use of caloric restriction, preclude us from drawing any conclusions regarding orlistat's effectiveness in NASH. Harrison et al performed a RCT of orlistat plus vitamin E plus dietary restriction (1400 kcal/d) vs. vitamin E and dietary restriction alone, in 41 obese patients with NASH for 36 weeks [32]. Weight loss was similar in the 2 groups (8.3% vs. 6%, non-significant) as were the results at the end of the study that included improvement in serum transaminases, decreased hepatic steatosis, necro-inflammation, ballooning and NAS score. It appears that these changes were secondary to weight loss alone and the use of orlistat did not confer any additional benefit. It would be interesting to assess the role of orlistat monotherapy (i.e. without dietary interventions) for the management of NASH,

Table 1 The effects of vitamin E (mono- or combination therapy) on transaminases and hepatic histology of patients with non-alcoholic steatohepatitis

n, dose (D), duration, study design	Presence of DM2 (yes/no)	ALT, AST	Steatosis	Inflammation	Fibrosis	Ref
n=9, D=800 mg x 24 wk, pilot study	7/9 with IGT or DM2	↓	↓	No change except for ↓in necro-inflammation of patients with IGT or DM2	no effect	[39]
n=12, D=300 mg x 1 yr	100% with IGT	↓only ALT	↓	↓	↓	[40]
n=247, D=800 IU x96 wk, (vs. PIO vs. placebo), RCT	no	↓	↓	↓lobular inflammation	no effect	[35]
n=48, D=800 IU VitE + UDCA x2 years vs. UDCA alone vs. placebo, RCT	27% in VitE+UDCA vs 22% in UDCA vs. 13% in placebo group	↓	↓	no effect	no effect	[41]
n=45, D=1000 IU VitE x6 months (vs. same D VitC vs. placebo; + dietary counseling for all), RCT	61% in vitamin groups vs. 23% in placebo group	no effect	no effect	no effect	↓	[43]

IGT, impaired glucose tolerance; DM2, type 2 diabetes mellitus; RCT, randomized controlled trial; UDCA, ursodeoxycholic acid; PIO, pioglitazone

especially considering that diets requiring significant caloric restriction cannot be sustained long term.

Antioxidant vitamins (Vitamins E and C): Oxidative stress is thought to contribute to the development of NASH and hence the role of antioxidant vitamins has been assessed by several studies. Vitamin E (α-tocopherol) has been trialed more extensively in children and adults [34,35]. Apart from its antioxidant potential, vitamin E also increases peroxisome proliferator-activated receptor (PPAR)-γ-mediated adiponectin expression, subsequently improving glucose control [36,37]. In addition, α-tocopherol is lower in patients with NASH than healthy controls, further justifying its use for the treatment of NASH [38]. The results of clinical trials assessing the effectiveness of vitamin E mono- or combination therapy for the treatment of NASH are conflicting and summarized in Table 1.

In a pilot study, vitamin E supplementation (800 mg/d for 24 weeks) decreased transaminases and hepatic steatosis without altering necro-inflammation or fibrosis [39]. Despite improvements in the IR index, vitamin E failed to improve circulating levels of glucose, triglycerides or cholesterol in this cohort. Lower dose (300 mg) but longer duration of treatment (1 year) with vitamin E not only decreased transaminases and steatosis but also improved inflammation and fibrosis, while decreasing transforming growth factor (TGF)-β levels, in another pilot study of 12 patients with NASH [40]. In a recent RCT, Lavine et al treated children with NAFLD and NASH with vitamin E or metformin or placebo for 96 weeks [34]. Vitamin E failed to achieve the primary outcome of sustained ALT reduction in this cohort, but was successful in reducing NAS as well as hepatocyte ballooning in 58% of children. Interestingly, 28% of patients in the placebo arm had resolution of NASH, which may imply that it is more easily reversible in younger patients. Lastly, the PIVENS trial is the largest RCT assessing the role of vitamin E for the treatment

of NASH, where vitamin E (800 IU/d), pioglitazone (PIO) or placebo were given to 247 non-diabetic adult patients for 96 weeks [35]. Vitamin E was effective in reducing hepatic steatosis and inflammation in 51% of subjects but had no effect on fibrosis in this cohort. Overall, vitamin E monotherapy is successful in decreasing steatosis and inflammation in some patients but fails to reverse the most important predictor of liver-related mortality, hepatic fibrosis.

Apart from monotherapy, few clinical trials have studied the role of vitamin E in combination with other agents in the management of NASH. Dufour et al randomized 48 patients to UDCA (12-15 mg/kg/d) alone, UDCA plus vitamin E (800 IU/d) or placebo for 2 years [41]. The combination with vitamin E was successful in decreasing transaminases and hepatic steatosis but did not improve inflammation or fibrosis. These changes were associated with increased adiponectin levels and decreased hepatocellular apoptosis [42]. In another RCT, vitamin E (1000 IU/d) in combination with vitamin C (1000 IU/d) and dietary counseling for 6 months was successful in decreasing fibrosis, without however affecting steatosis, inflammation, or transaminase levels [43]. Although some patients benefit from vitamin E combined with other agents, the small sample sizes and limited number of these studies preclude us from drawing firm conclusions regarding their effectiveness. In addition, concerns arising from adverse events associated with the chronic use of vitamin E may limit its use.

Recent reports have implicated vitamin E in the development of prostate cancer, hemorrhagic stroke, as well as an overall increase in patient mortality [44-47]. To date, vitamin E is indicated for non-diabetic patients with NASH, however its adverse effects must be taken into consideration prior to prescribing it [48].

Betaine: Choline metabolism leads to the formation of betaine, a methyl donor in the transmethylation pathway that has been shown to decrease steatosis and oxidative stress in

animal models of NAFLD. Impaired transmethylation with increased S-adenosylmethionine (important methyl donor) and decreased S-adenosylhomocysteine and homocysteine levels has been noted in animal with NASH [49]. Betaine can increase homocysteine levels and is hence thought to have beneficial potential in patients with NASH. Abdelmalek et al performed a RCT of betaine (20 g/d) vs. placebo for 12 months in 34 patients with NASH [49]. Betaine was effective in decreasing steatosis without affecting fibrosis or NAS. Interestingly, betaine was unable to decrease S-adenosylhomocysteine levels in this cohort. There is not sufficient evidence to support betaine use for patients with NASH.

Thiazolidinediones (glitazones): PIO and rosiglitazone (RSG) belong to the thiazolidinediones class of peroxisome proliferator-activated receptor (PPAR)-γ agonists. PPAR-γ is a nuclear receptor that directly regulates lipid metabolism and is indirectly involved in glucose homeostasis [50]. Thiazolidinediones have been shown to lower glucose levels, increase adiponectin secretion and re-distribute adipose tissue from the viscera to the periphery [50,51]. Due to their insulin-sensitizing properties, glitazones have been used to control type 2 diabetes mellitus (DM2) and given the importance of IR in the pathogenesis of NASH, they have also been fairly extensively trialed for the management of steatohepatitis [51]. The studies that have looked at the effect of glitazones in the treatment of NASH are discussed below and summarized in Table 2.

A pilot study of 18, non-DM2 patients with NASH, treated with 30 mg/d of PIO for 48 weeks, showed decreased steatosis,

cellular injury, parenchymal inflammation and fibrosis in 2/3 of them, despite a weight gain of 4% on average [52]. These patients also had decreased inflammatory cytokines (TNF-α and IL-6) as well as increased adiponectin levels [53].

Sanyal et al performed the largest study assessing the effect of PIO in the management of NASH in the aforementioned PIVENS trial [35]. In a cohort of 247 non-diabetic adults, 80 were randomized to 30 mg of PIO and the rest to either vitamin E (800 IU) or placebo for 96 weeks. Primary outcome was at least 1-point reduction in ballooning and no worsening of fibrosis in the setting of a NAFLD activity score (NAS) decrease to ≤3 or by 2 points with at least 1-point improvement in steatosis and 1 point in lobular inflammation. The PIO group did not reach the primary outcome but there was a significant reduction in steatosis and lobular inflammation in 48% of subjects. It is important to note that 28% of the patients randomized to the PIO group did not have ballooning at baseline, which could have contributed to the failure to reach the primary outcome. Subsequent sensitivity analyses looking only at patients with ballooning at baseline, showed that PIO was indeed successful in decreasing ballooning as well [48]. As with all thiazolidinediones, an average weight gain of 4.7 kg was seen in those randomized to the PIO arm.

Apart from monotherapy, PIO has been used along with other interventions for the treatment of NASH. The combined effect of diet, exercise and 30 mg/d PIO vs. diet and exercise alone for 12 months, was assessed in a RCT of 61, non-DM2 patients with NASH [54]. The addition of PIO led to a significant decrease in hepatocellular injury and fibrosis, as

Table 2 Effects of pioglitazone and rosiglitazone on transaminases & histology of non-alcoholic steatohepatitis patients with and without type 2 diabetes mellitus

	n, dose (D), duration, study design	Presence of DM2 (yes/no)	ALT, AST	Steatosis	Inflammation	Fibrosis	Ref
PIOGLITAZONE	n=247, D=30 mg/d x96 wk, RCT	no	↓	no effect	no effect	no effect	[35]
	n=61, D=30 mg/d x12 mo, RCT	no	↓	no effect	↓hepatocyte injury	↓	[54]
	n=18, D=30 mg/d x48 wk, pilot study	no	?	↓	↓	↓	[52]
	n=47, D=45 mg/d x6 mo (+500 Kcal/d restriction), RCT	yes	?	↓	↓ necro-inflammation	no effect	[56]
	n=55, D=45 mg/d x6 mo (+500 Kcal/d restriction), RCT	yes	↓	↓	↓ inflammation & ballooning	no effect	[55]
ROSIGLITAZONE	n=53, D=8 mg/d x1+2 yrs, initially RCT and then open-label trial	yes in some patients	↓	↓	no effect	no effect	[59,60]
	n=108, D=8 mg/d x48 wk, RCT (no control arm, RSG vs. RSG+ losartan vs. RSG+ metformin)	yes in some patients	↓	↓	↓	↓	[58]

RCT, Randomized Controlled Trial; DM2, Type 2 Diabetes Mellitus; RSG, Rosiglitazone

well as transaminase and HbA1c levels, but was accompanied by a mean weight gain of 2.77 kg.

Overall the results are not uniform, however, PIO, in the setting of NASH without DM2, has consistently been shown to decrease transaminases, steatosis and inflammation without strong evidence to support an effect on fibrosis.

PIO has also been trialed in diabetic patients with NASH. Belfort et al randomized 55 patients to 45 mg/d of PIO and caloric restriction by 500 kcal/d or caloric restriction alone, for 6 months [55]. Apart from a decrease in transaminases and IR, the PIO group had improved steatosis, inflammation and ballooning on repeat liver biopsies, without any improvement in fibrosis. In a similar study, 47 diabetic patients with NASH treated with the same PIO dose, duration and caloric restriction as above, were found to have decreased steatosis and necro-inflammation, again without any improvement in fibrosis [56]. These findings were associated with improved IR and increased adiponectin levels [57]. Overall, similar to NASH patients without DM2, PIO appears to be effective in reducing IR, transaminases, hepatic steatosis and inflammation but fails to improve fibrosis.

RSG has also been studied in the management of NASH. Torres et al randomly assigned 108 patients with NASH (some of whom had impaired glucose tolerance or DM2) to 8 mg/d RSG alone vs. 8 mg RSG and losartan (50 mg/d) vs. 8mg RSG and metformin (1 g/d) for 48 weeks. There was no control group in this study [58]. All groups had similar improvements in steatosis, inflammation and fibrosis indicating that RSG alone is sufficient. In the absence of a control group, however, it is difficult to draw any conclusions regarding RSG's effectiveness.

In another RCT, RSG (8 mg/d) for one year decreased transaminases and hepatic steatosis without affecting inflammation or fibrosis of 63 patients (some of whom had pre-DM2 or DM2), compared to placebo [59]. Use of RSG was associated with an average weight gain of 1.5 kg during this one year. Fifty three of these patients were followed for another 2 years in an open-label extension trial of RSG and 40 had a repeat liver biopsy at the end [60]. There was no further improvement in hepatic histology with the additional 2 years of treatment; however the beneficial effect on transaminases and insulin sensitivity was still seen 2 years later. To summarize, the limited research on RSG's effectiveness in NASH has shown that it is helpful in improving glucose control, reducing transaminases and potentially limiting steatosis without significant affecting inflammation or fibrosis.

Glitazones have been removed from the market in some countries due to concerns of cardiovascular toxicity (e.g. heart failure) as well as associations with certain malignancies (e.g. PIO and bladder cancer) [61,62]. In addition, use of glitazones has been associated with bone loss and increased fracture risk [63]. These complications have not been described in the NASH trials; however these were not powered to look at that. Lastly, glitazones are associated with weight gain, which is a challenge for overweight patients who are advised to lose weight. The risk of using glitazones in non-diabetic patients with NASH probably outweighs their beneficial effects. On the other hand, for patients with DM2, glitazones may be of more benefit, as they contribute to glucose control in addition to improving hepatic steatosis and inflammation.

Metformin: Metformin belongs to the biguanides class of medications that have been used to control DM2. It acts by decreasing hepatic gluconeogenesis and increasing glucose uptake by muscle tissue [64]. By activating adenosine monophosphate-activated protein kinase (AMPK) it also alters lipid metabolism leading to increased peripheral fatty oxidation and decreased lipogenesis in the liver [65]. Contrary to the glitazones, metformin can lead to weight loss, which is beneficial for patients with NAFLD. Due to its effects on glucose and lipid metabolism, as well as weight, it has been trialed in the management of NAFLD, with fewer studies focusing specifically on NASH [64].

Trials using metformin for the treatment of NASH have had inconsistent results, as described here. In an open-label trial, Loomba et al treated 28 NASH patients with metformin (2 g/d) for 48 weeks [66]. Of the 26 patients that completed the study, 30% had some degree of histological response. Metformin was only able to decrease parenchymal inflammation and cellular injury without affecting the degree of steatosis or fibrosis, despite an average 6 kg weight loss. A RCT of 36 patients with NASH started on metformin and diet (lipid and caloric restriction) vs. diet alone for 6 months showed that metformin was unable to improve hepatic histology, despite beneficial effects on insulin sensitivity and transaminase levels [67]. In another study, 173 children and teenagers with NAFLD (some of whom had NASH) were randomized to metformin (1 g/d) or vitamin E (800 IU/d) or placebo for 96 weeks [34]. Among histological parameters, metformin was associated only with significant decrease in ballooning as it failed to improve NAS or lead to a sustained reduction in ALT. A recent meta-analysis of the available evidence showed that metformin is not successful in improving histological disease severity in patients with NASH and hence should not be used for that purpose [68].

Statins: NAFLD patients are at high risk of cardiovascular complications that are in part secondary to IR and dyslipidemia [31]. Mortality secondary to cardiovascular complications is much higher than liver-related mortality in this group of patients [31,69]. Statins block the hepatic synthesis of cholesterol and are used widely for the management of dyslipidemia. Despite initial concerns regarding hepatotoxicity, they are now considered to be safe for patients with chronic liver diseases, such as NAFLD [70,71]. A few small trials have assessed the use of statins in the management of NASH.

Atorvastatin, along with weight loss counseling, was effective in decreasing steatosis and NAS in 2 open label studies of 1 and 2 years duration (n=22 and 17, respectively) [72,73]. Atorvastatin alone (10 mg/d for 6 months) was also found to be beneficial in decreasing computer tomography-confirmed steatosis in a small cohort (n=27) of hyperlipidemic patients with NASH [74]. Simvastatin assessed in a year-long RCT, failed to alter hepatic steatosis, inflammation or fibrosis, despite a successful decrease in circulating low-density lipoprotein levels in these patients [75]. Based on the current evidence, it is not clear whether statins are helpful in treating or delaying

NASH progression, however, given their documented success in reducing cardiovascular-associated mortality, they can and should be used in patients with NASH and dyslipidemia [76].

UDCA: UDCA has been used in patients with NASH as it is thought to act as an antioxidant, immune-regulatory and anti-apoptotic agent [77]. Initial trials assessed the efficacy of 'normal' dose UDCA (13-15 mg/kg/d) and failed to show any significant histologic improvement apart from some decrease in steatosis [41,78]. Lindor et al performed a large (n=166), 2 year-long RCT of UDCA (13-15 mg/kg/d) vs. placebo in patients with NASH and revealed that UDCA failed to improve any histological component of NASH [79]. Further research was focused on higher doses of UDCA (23-35 mg/kg/d) that were thought to be physiologically more appropriate [80-82].

Leuschner et al randomized 185 patients to UDCA (23-28 mg/kg) or placebo and, apart from decrease in lobular inflammation, failed to show any histological improvement in the 139 patients that proceeded to have a second biopsy [81]. Ratziu et al used slightly higher doses of UDCA (28-35 mg/kg) in a RCT of 126 patients for 1 year [82]. This treatment led to a decrease in ALT and improved fibrotest results. There was no repeat liver biopsy at the end of this study. High-dose UDCA (28-30 mg/kg) has been associated with increased serious adverse events including increased mortality in patients with another chronic liver disease, primary sclerosing cholangitis [83]. The lack of evidence regarding its efficacy in patients with NASH and the potential serious adverse events reported in patients with primary sclerosing cholangitis, make this agent less attractive for the management of NASH.

PTX: PTX is a xanthine with anti-inflammatory, anti-apoptotic, anti-oxidant and potentially anti-fibrogenic properties [84-86]. It has been shown to decrease the synthesis and inhibit the action of cytokines such as TNF-α [87]. In theory, PTX is an ideal agent for the treatment of NASH as it targets multiple steps in its pathogenesis. In animal models it has been shown to reverse steatohepatitis secondary to methionine / choline-deficient diets [88]. A few small trials have used PTX monotherapy in adults with NASH with encouraging results.

In an open-label trial, treatment of 9 patients with 1200 mg PTX for 12 months led to decreased transaminases, hepatic steatosis (in 55%), lobular inflammation (in 67%) and fibrosis (in 67%) [89]. Van Wagner et al randomized 30 patients with NASH to PTX vs. placebo and showed that 1200 mg for 1 year decreased steatosis, ballooning and transaminases compared to baseline but these changes were not significant compared to the placebo group [90]. Lastly, in the largest RCT to date, 55 patients with NASH were randomized to receive PTX vs. placebo; PTX was effective in decreasing hepatic steatosis and inflammation but failed to improve ballooning or fibrosis in this cohort [91]. It is important to note that in this study, subjects were also given dietary and lifestyle modification counseling, which may have accounted for some of the improvement in histology. Also, this cohort consisted primarily of white, non-diabetic males, making the results less generalizable, considering that diabetes is a common co-morbidity of patients with NASH.

Lastly, serum TNF-α levels did not decrease in patients treated with PTX indicating that its beneficial effects in this setting may be driven by other, not yet described pathways. PTX is a fairly safe agent with nausea and vomiting being the most common side effects described [91]. Given its safety profile and its theoretical potential in targeting multiple 'hits' leading to NASH, PTX should be trialed as mono- or combination therapy in larger RCTs in the future.

Angiotensin receptor II blockers (ARB): The renin-angiotensin system (RAS) is involved in various metabolic and inflammatory cascades. Normally, renin cleaves angiotensinogen to angiotensin I, which in turn is converted to angiotensin II with the action of angiotensin converting enzyme (ACE). ARBs and ACE inhibitors have traditionally been used as antihypertensive agents, however, evidence of RAS activation with subsequent inflammation and fibrosis in the setting of chronic liver injury, led to their use in therapeutic trials of NAFLD/NASH [92]. By blocking the RAS, ARBs decrease cytokine production (such as TNF-α), increase adiponectin levels and improve pancreatic insulin secretion as well as insulin signaling at the cellular level, overall decreasing IR [93,94]. They are also shown to have anti-fibrogenic effects by limiting the activation of hepatic stellate cells and decreasing pro-fibrogenic cytokines, such as TGF-β [93,95]. Telmisartan also appears to have a PPAR-γ activating effect, which makes it even more useful in the setting of NAFLD [96]. The few small studies that have targeted NASH with ARBs (e.g. losartan, telmizartan, valsartan) are described here.

Yokohama et al showed that a 48-week course of losartan (50 mg/kg) was successful in decreasing necro-inflammation in 5/7, and fibrosis in 4/7 patients with NASH [97]. This was accompanied by decreased levels of circulating TGF-β and ferritin as well as decreased activation of hepatic stellate cells seen in liver biopsies [93,95,97]. On the other hand, the addition of losartan to RSG had no additional benefit than RSG alone in a RCT performed by Torres et al [58]. The importance of limiting fibrosis development and progression in the natural history of NASH makes ARBs an attractive option for the management of this condition; however, research on their effectiveness for this condition is still in very early stages.

Other agents: There are a few other agents (probucol and L-carnitine) that have been trialed in NASH and will be briefly described here.

Probucol acts by increasing the rate of LDL metabolism and hence has lipid-lowering agent and anti-oxidant properties making it potentially useful for the patients with NASH [98,99]. In an open-label trial, probucol reduced transaminases, steatosis and necro-inflammation in 8 patients treated for 1 year [100]. Another 16 patients with NASH and hyperlipidemia were treated with probucol and pantethine (the active form of vitamin B5) for 48 weeks, in an open-label design [101]. This treatment improved transaminase levels, decreased cholesterol and serum TGF-β and increased adiponectin levels. Liver biopsies performed in 8 patients revealed decreased inflammation in 4/8 and fibrosis in 2/8 patients. No RCT has assessed the histological effect of probucol monotherapy in patients with NASH and hence no conclusions can be drawn at this point.

Carnitine is required for the transport of fatty acids from the cytoplasm into the mitochondria, where β-oxidation occurs. It is also thought to decrease oxidative stress [102]. Impaired oxidation of fatty acids and oxidative stress have been proposed as a pathogenetic factors for the development of hepatic steatosis and L-carnitine supplementation has led to improved steatosis in animal models of NAFLD [103]. In a recent RCT, the addition of 1 g L-carnitine to a diet and exercise program (vs. diet and exercise alone) for 24 weeks resulted in decreased steatosis, hepatocellular injury, parenchymal inflammation and fibrosis in 74 NASH patients [104]. L-carnitine may be a useful tool either as mono- or combination therapy for the management of NASH but further research is needed to solidify these results.

Surgical interventions

Bariatric surgery: Bariatric surgery has been used as a means of weight loss for morbidly obese patients. Currently it is indicated for patients with BMI>40 kg/m^2 or BMI>35 kg/m^2 and other cardio-metabolic risk factors. A recent meta-analysis reviewed the results of studies on this topic [105]. The most common types of bariatric surgery performed in this patient population were Roux-en-Y gastric bypass and adjustable gastric band. Retro- and prospective cohort studies have shown that weight loss following bariatric surgery is beneficial in improving hepatic steatosis and decreasing cytokine levels but may worsen hepatic fibrosis [105]. The lack of RCT assessing the role of bariatric surgery in NASH, the potential biases and methodological errors of the available cohort studies precluded the authors of this meta-analysis from making any evidence-based recommendations regarding the usefulness or safety of bariatric surgery for patients with NASH. RCTs are needed to answer these questions.

Future treatment options for NASH

As it has already been mentioned, there is no type of intervention that allows for complete resolution of the hepatic pathology seen in patients with NASH. The increasing prevalence of this condition and the increasing understanding of its pathophysiology, however, have fueled further studies of newer agents with therapeutic potential, such as incretins, Farnesoid X Receptor (FXR) agonists and probiotics. The results of these studies will be available soon and may provide clinicians with better tools for the treatment of patients with NASH.

Incretins: Glucagon-Like Peptide (GLP)-1 is an insulino-mimetic agent that belongs to the family of incretins; intestinal hormones that have been shown to enhance insulin secretion, decrease glucagon production and delay gastric emptying, leading to improved glucose control [106]. Exenatide is a GLP-1 analogue shown to improve glucose and lipid homeostasis

as well as transaminase levels in obese patients with DM2 [107]. RCTs assessing the role of exenatide or other GLP-1 analogues in improving histology of patients with NASH are required at this stage.

FXR agonists: FXR is a nuclear bile acid receptor with various beneficial metabolic and immune-modulating effects [108]. FXR activation leads to decreased expression of genes involved in *de novo* lipogenesis and increased expression of those involved in β-oxidation, improved insulin sensitivity, decreased gluconeogenesis, as well as decreased synthesis of pro-fibrogenic cytokines, such as TGF-β [108]. The effectiveness and safety of FXR agonists such as obeticholic acid are currently being investigated in phase IIb/III RCT.

Probiotics: Animal studies have provided with preliminary evidence supporting the use of probiotics for the treatment of NASH. Probiotics appear to decrease steatosis, inflammatory signaling and fibrosis is various animal models of NAFLD [109-111]. There are no human studies of probiotic supplementation for NASH but small trials of probiotics for patients with NAFLD have shown them to be effective in decreasing transaminases and markers of lipid peroxidation [112]. RCT assessing the role of probiotics are needed to show whether the results of animal studies can be translated to humans.

Conclusion

The treatment of NASH has been proven to be challenging to date. Many questions regarding the pathogenesis of this condition as well as the type and duration of treatment remain unanswered. The ideal therapeutic strategy should include interventions that are safe, not costly, successful in decreasing mortality and associated co-morbidities and also realistic in terms of application and sustainability over time. Future studies should be more homogeneous regarding disease definitions and modes of assessing treatment effects (e.g. use of liver biopsy). Clinically important outcome measures should be investigated and patient populations studied should be clearly defined (e.g. diabetics or not). Randomized design provides the highest level of evidence and hence should be preferentially used in therapeutic trials for NASH.

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