Management of Non-alcoholic Fatty Liver Disease and Steatohepatitis

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver enzymes and chronic liver disease in the US with expected rise in incidence paralleling the epidemic of obesity. A subset of patients with NAFLD have the progressive form of NAFLD that is termed non-alcoholic steatohepatitis (NASH), which is characterized by specific features on liver histology including hepatocellular ballooning degeneration, lobular inflammation, and zone-3 steatosis with or without peri-sinusoidal fibrosis. Non-alcoholic steatohepatitis can progress to cirrhosis and result in liver-related death. Insulin resistance is commonly seen in patients with NASH and often co-exists with other features of the metabolic syndrome including hypertension, hyperlipidemia, and obesity. Although weight loss through lifestyle modifications including dietary changes and increased physical exercise remains the backbone of management of NASH, it has proved challenging for patients to achieve and maintain weight loss goals. Thus, it is often necessary to couple lifestyle changes with another pharmacologic treatment for NASH. Insulin sensitizers including the biguanides (metformin), thiazolidinediones (pioglitazone and rosiglitazone), and glucagon-like peptide-1 receptor agonists (exenatide) are large groups of medications that have been studied for the treatment of NASH. Other agents with anti-inflammatory, anti-apoptotic, or anti-fibrotic properties which have been studied in NASH include vitamin E, pentoxifylline, betaine, and ursodeoxycholic acid. This review will provide a detailed summary on the clinical data behind the full spectrum of treatments that exist for NASH and suggest management recommendations. (J Clin Exp Hepatol 2012;2:156–173)

NAFLD

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the US, comprising 20–30% of the population,[1] a number that closely mirrors the US estimated age-adjusted prevalence of obesity of 33%.[2] Liver biopsy remains the diagnostic gold standard and the histologic spectrum of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), which has features of hepatocellular injury, lobular inflammation, and zone 3 steatosis with or without perisinusoidal fibrosis. The prevalence of NASH is estimated to range from 3% to 6% and this subgroup of patients is a focus of concern because as opposed to bland steatosis, histologic findings of NASH confer risks of progression to cirrhosis and development of hepatocellular carcinoma.[3,4] Non-alcoholic steatohepatitis-associated cirrhosis is currently the third leading cause of liver transplantation in the USA but is projected to become the number one indication between the years 2020 and 2025.[5]

The pathogenesis of NASH is currently not well defined but is hypothesized to involve complex interactions of genetics and environmental factors. The early 'two-hit' model of NASH proposed that the 'first hit' involves accumulation of lipids in the form of triglycerides (TG).[6] This lipid-rich environment then provides the optimum setting for oxidative stress constituting the 'second hit' that triggers hepatocellular injury, inflammation, and fibrosis. A recent alternative hypothesis has suggested that the metabolites of fatty acids induce hepatocellular injury in NASH rather than the fatty acids themselves. The 'lipotoxicity' model of NASH suggests that the accumulation of TG in the liver is not a cause of liver injury but rather a parallel event that may be protective. This occurs when excessive or inappropriate peripheral lipolysis, as well as excessive de novo lipogenesis, exceeds the liver's ability to oxidize fat or convert it to TG for secretion.[7] The flux of free fatty acids from the liver forms metabolites that cause hepatocellular injury in the form of stress to the endoplasmic reticulum, inflammation, apoptosis, and necrosis. Other contributors to the pathogenesis of NASH include mitochondrial dysfunction,[8,9] impaired adenosine triphosphate (ATP) production,[10] hypoxia due to impaired blood

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Abbreviations: Alk-phos: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; DEXA: dual-energy X-ray absorptiometry; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; HOMA: homeostatic model assessment; LDL: low-density lipoprotein; NAFLD: non-alcoholic fatty liver disease; NAS: NAFLD activity score; NASH: non-alcoholic steatohepatitis; QUICKI: quantitative insulin sensitivity check index; TG: triglyceride
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Table 1 Treatment in non-alcoholic fatty liver disease: The University of California San Diego approach.

I. Patients with NAFLD—no evidence of NASH or fibrosis on biopsy
a. Lifestyle intervention:
i. Diet assessment: Consider referral to nutritionist to implement calorie-restricted diet
ii. Weight assessment: If patient meets BMI threshold for being overweight or obese, patients should exercise at least 3 times a week for 20–30 minutes per session if it is considered safe by primary care physician. The goal for weight loss is 5–10% over 3 months. Note that type and quantity of exercise has not been well-studied
b. Cardiovascular risk factors modification:
i. Check lipid profile including HDL, LDL, TG, and treat per ATP III of the National Cholesterol Education Program guidelines
ii. Screen for insulin resistance or diabetes and treat per ADA guidelines
c. Review patient's medications to stop or change intake of medications associated with NAFLD if possible. Some of these medications include: Amiodarone, methotrexate, systemic glucocorticoids, tetracyclines, tamoxifen, estrogens, anabolic steroids, valproic acid
II. Patients with NASH on biopsy (evidence of hepatocyte ballooning, inflammation with or without fibrosis)
a. Lifestyle interventions as above
b. Cardiovascular risk factors modification as above
c. Medication list review as above
d. Treatment with medications:
i. Vitamin E 800 IU daily for non-diabetics
ii. Pioglitazone with or without metformin in diabetics
III. Patients with NASH-cirrhosis
a. Screening for esophageal varices with esophagogastroduodenoscopy
b. Screening for hepatocellular carcinoma
c. Management of complications of cirrhosis
d. When appropriate, referral to transplant center for evaluation

ADA: American Diabetes Association; ATP: adult treatment panel; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; TG: triglyceride.

flow,[11,12] gut-derived endotoxin and ethanol,[13,14] and dysregulation of adipokine[15,16] and cytokine production.[17,18]

While the exact etiology of NAFLD and NASH is unclear, insulin resistance appears to be central to the pathogenesis of NASH by allowing inappropriate levels of lipolysis from the adipose tissue and impairing peripheral glucose disposal. Besides insulin resistance, NAFLD is also closely associated with other characteristics of the metabolic syndrome including central obesity, hypertension, and hyperlipidemia.[19] The obesity epidemic and increasing prevalence of the metabolic syndrome is predicted to be paralleled by an increasing prevalence of NAFLD in the future. The anticipated growing burden of NAFLD on our healthcare system has pushed significant research efforts toward finding treatment for this disease. Table 1 outlines the management guidelines that are currently used by the NAFLD clinic at the University of California San Diego stratified by the severity of NAFLD.

This paper will review the full spectrum of pharmacologic treatments that have been studied for NASH. These include the treatment categories of metformin, peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands, other anti-diabetic therapies, vitamin E, pentoxifylline, other antioxidants, ursodeoxycholic acid, statins and ezetimibe, weight loss, and new emerging treatments. The use of bariatric surgery and specific dietary and exercise regimens for weight loss in NASH are beyond the scope of the discussion and will not be included in this review. However, there are several recent reviews that offer insights into the use of bariatric surgery for patients with NASH.[20–22]

The studies chosen for this review are prospective cohort or randomized controlled trial (RCT) in humans involving more than 10 subjects. When available, studies with liver biopsy confirmed NAFLD or NASH as inclusion criteria are favored over those with clinical or radiographic diagnosis. Studies are discussed in chronologic order to provide historic perspective leading to our current understanding of the class of medications.

METFORMIN

Metformin is in the class of medication called the biguanides and is a widely used oral medication for the treatment of type 2 diabetes. The insulin-sensitizing effect of metformin is attributed to its ability to activate the AMP-activated protein kinase (AMPK) pathway which switches cells from anabolic to catabolic pathways. This results in inhibition of gluconeogenesis and lipogenesis in favor of fatty acid β oxidation and fatty acid and glucose uptake in the liver tissue and peripheral skeletal muscle.[23,24] Metformin has also been shown in ob/ob mice, an animal model of hepatic steatosis, to reverse hepatomegaly, steatosis, and aminotransferase abnormalities.[25,26]

There are numerous studies assessing the effects of metformin in NASH (Table 2). The first human study using metformin for NASH was done by Marchesini et al where 14 patients treated with metformin 500 mg thrice daily were compared with six controls over 4 months.[27] Metformin-treated patients had greater decrease in alanine aminotransferase (ALT) ($P<0.05$), as well as a trend

Table 2 Studies of metformin in adult patients with non-alcoholic fatty liver disease.

Reference	Type of study	No. of patient* treatment/ control	Diabetic patients	Drug	Compared with	Duration of treatment	NAFLD vs biopsy-proven NASH	ALT improved†	Histology improved†
Garisnis, 2010	Prospective, randomized	40 Total 15 Met+diet 25 Diet	Excluded	Met 200 mg bid × 1 week, then 500 mg bid	Dietary treatment alone	6 mo	NAFLD (by USG)	No	No
Haukeland, 2009	Double-blind, randomized, placebo-controlled, multi-centered	44 Total 20 Met 24 Pbo	12 4 Met 8 Pbo	Met 500 mg/ day increasing to max dose of 2500 mg or 3000 mg/day (if >90 Kg) by weeks 4–5	Placebo	6 mo	NAFLD (by biopsy within 18 months enrollment)	No	No
Shields, 2009†	Double-blind, randomized, placebo-controlled	16 Total 9 Met 7 Pbo	Excluded	Met 500 mg/ day then 1000 mg/day if no amino-transferase improvement at 3 months+ diet/exercise	Placebo+ diet/exercise	12 mo	NASH	No	No
Loomba, 2009†	Open-label, uncontrolled	26	7	Met 500 mg/ day × 2 weeks → 1000 mg × 4 weeks → 2000 mg	–	12 mo	NASH	No	Yes
Bugianesi, 2005†	Open-label, randomized, controlled, multi-centered	110 Total 55 Met 28 Vit E 27 Diet	Excluded	Met 2000 mg/ day	Vitamin E 800 IU/day Diet (caloric deficit 500 calories/day)	12 mo	NAFLD	Yes	No (no biopsy in control group for comparison)
Uygun, 2004†	Randomized, controlled	34 Total 17 Met+diet 17 Diet	Not stated	Met 850 mg bid+diet	Diet	6 mo	NAFLD	Yes	No
Nair, 2004	Open-label, uncontrolled	15	1	Met 20 mg/Kg/ day (max 2 g/ day)	–	48 wk	NAFLD	No	Yes
Marchesini, 2001†	Open-label, non-randomized, controlled	20 Total 14 Met 6 Controls	Excluded	Met 1500 mg daily	Controls (nutritional counseling)	4 mo	NASH	Yes	–

*Number of patients who completed treatment; †improvements in ALT or histology are reported if significantly better compared to baseline (when there was no comparison group) or better than comparison group; ‡all patients in the study were also given lifestyle interventions (nutrition counseling with or without weight lost instructions).
ALT: alanine aminotransferase; Met: metformin; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; USG: ultrasound; Vit E: vitamin E.

toward greater decrease in liver size. Subsequently, Nair et al published a study of 15 patients treated with metformin 20 mg/Kg/day for 48 weeks.[28] There was a significant decrease in weight, body mass index (BMI), and waist-hip ratio with most occurring in the first 3 months of treatment. The aspartate aminotransferase (AST) and ALT decreased significantly by the third month but a rebound increase in both levels by 6 months cancelled this out so that there was no change by the end of treatment. Insulin resistance parameters mirrored changes of the transaminases and homeostasis model assessment of insulin resistance (HOMR-IR) scores with improvement by 3 months but none seen thereafter when aminotransferases began rising. Paired liver biopsies were available in only 10 patients (67%) and 3 patients had improved steatosis, 2 with improved inflammatory scores, and 1 with improved fibrosis.

The earliest RCT in metformin dates back to the early 2004 when Uygun et al randomized 34 patients to dietary restrictions (1600–1800 calories per day) vs metformin+ diet for 6 months and found significant improvement in

ALT, AST, BMI, index of insulin resistance, insulin level, and C-peptide associated with metformin treatment.[29] There was no histologic improvement but only 23 patients (70%) underwent baseline biopsy. Therefore, it is unclear whether all patients met the diagnosis of NASH. Subsequently, Bugianesi et al conducted RCT comparing metformin 2000 mg daily vs two control groups of vitamin E 800 IU daily or diet restriction alone.[30] All groups had BMI improvement but this was not significantly different between groups. The metformin group had greater reduction in ALT ($P<0.0001$) and by the end of treatment, 31 patients in the metformin group had ALT normalization (<40 U/L) vs 12 patients in the control arm ($P=0.0006$). Multivariate analysis showed that normal ALT was significantly associated with change in BMI ($P=0.0002$) and metformin treatment ($P=0.0011$). Treatment with metformin was also associated with significant reduction in fasting insulin (4.5 μU/mL vs 1.5 μU/mL, $P=0.029$) and HOMA scores (decrease of 1.5 units vs 0.5 units, $P=0.0002$). The levels of high-density lipoprotein (HDL) and TG did not change significantly between groups. When compared with vitamin E alone, a greater percentage of metformin-treated patients had normalized ALT levels (44% vs 14%, $P=0.019$). The study also compared the control arms. While the diet arm patients had greater BMI loss than vitamin-E-treated patients (1.8 Kg/m^2 vs 1 Kg/m^2, $P=0.038$), ALT levels and other metabolic parameters were not different. Only 17 paired biopsies were performed with all taking place in metformin-treated patients. These showed a significant reduction in steatosis ($P=0.003$), necroinflammation ($P=0.012$), and fibrosis ($P=0.012$).

Loomba et al published a study of 26 overweight or obese patients who were treated with metformin 2000 mg/day for 48 weeks.[31] Eight patients (31%) met the primary histologic outcome and the average NAFLD activity score (NAS) improved from 8.2 to 5.9 ($P<0.001$) with most of the changes attributable to improvement in cellular injury (ballooning degeneration) and not parenchymal inflammation or steatosis. All responders had improved aminotransferases. Liver fibrosis scores did not change significantly. There were no significant biochemical changes and no changes in measures of insulin sensitivity including fasting blood glucose, insulin, C-peptide, and free fatty acids. However, HOMA-IR scores did improve by 44% ($P=0.04$). Patients on metformin lost an average of 6 Kg during the 48 weeks of treatment. There was a strong correlation between weight loss and improved NAS ($r=0.76$, $P<0.0001$), as well as positive correlation between weight changes and serum aminotransferase changes (for ALT: $r=0.52$, $P=0.005$; for AST, $r=0.50$, $P=0.009$). Body fat composition was assessed by dual-energy X-ray absorptiometry (DEXA) and showed that weight loss was due to decreases in both lean body and fat mass so that overall, percent body fat did not change significantly. In addition, the total abdominal fat as evaluated by computed tomography (CT) did not change. However, visceral fat decreased while subcutaneous fat increased. Magnetic resonance imaging (MRI)-assessed liver fat also did not change significantly (14.9–13%, $P=0.50$). When comparing histologic responders with non-responders, it was noted that responders had greater decreases in weight (−10.5 Kg vs −4 Kg, $P=0.02$) and BMI (−3.8 Kg/m^2 vs −1.4 Kg/m^2, $P=0.02$) and a trend toward greater decreases in ALT and AST. None of the patients with baseline BMI of 40 or more achieved histologic response. During the 24 weeks of follow-up post-treatment, it was noted that all but two patients had gained weight (average gain 4.1 Kg) and improvements of ALT and HOMA during treatment were rising toward baseline levels.

Shields et al performed RCT in 19 patients with non-diabetic NASH comparing metformin vs placebo.[32] They did not find any significant difference in the effect of metformin on liver histology compared with placebo. Both groups showed improvement in serum ALT as well as mild improvement in liver histology. However, this was a small, specialized group of patients. Military training and its emphasis on physical health may have affected the results.

A more recent study by Garinis et al included 50 patients with BMI >25 Kg/m^2 and findings of liver steatosis by ultrasound (USG). Patients were randomized to metformin 1000 mg daily + hypocaloric diet (1300 kcal/day) vs diet alone for 6 months.[33] This study showed that metformin + diet compared with diet alone was better in improving fasting glucose, HOMA-IR, adiponectin levels, and reducing the risk of developing metabolic syndrome. However, there was no significant improvement in weight loss or hepatic steatosis.

Haukeland et al randomized 36 patients with biopsy-proven NAFLD to either metformin or placebo for 6 months.[34] Paired liver biopsies in all patients (100%) showed a slight reduction in liver steatosis but there were no significant differences between groups in any of the components of the NAS. There were no significant changes in AST or ALT between groups though within-group comparisons showed improved serum ALT in both groups. Compared with placebo, the metformin group had significant decreases in body weight ($P<0.001$), BMI ($P<0.001$), cholesterol ($P<0.004$), low-density lipoprotein (LDL) ($P<0.001$), and fasting glucose ($P=0.032$).

Currently, metformin remains a safe option for the treatment of NASH. However, the paucity of RCTs and the small number of patients with biopsy-proven NASH in these few trials provide weak support for evidence-based recommendations of metformin use. The weight loss and insulin-sensitizing properties of metformin are attractive for use in the overweight, insulin-resistant NAFLD population. In our opinion, metformin is a weaker agent than pioglitazone in improving steatosis but it has been shown to improve ballooning degeneration in both the TONIC trial and the open-label pilot study of metformin. We commonly utilize it for the management of patients

Table 3 Studies of peroxisome proliferator-activated receptor-gamma (PPAR-γ) in adults with non-alcoholic fatty liver disease.

Reference	Type of study	No. of patient* treatment/ control	Diabetic patients	Drug	Compared with	Duration of treatment	NAFLD vs biopsy-proven NASH	ALT improved†	Histology improved†
Aithal, 2008†	Double-blind, randomized, placebo-controlled	61 Total 31 Pio 30 Placebo	Excluded	Pio 30 mg daily	Placebo	1 yr	NASH	Yes	Yes
Belfort, 2006†	Double-blind randomized, placebo-controlled	40 Total 23 Pio 17 Placebo	All had impaired glucose tolerance or DM II	Pio 30 mg/day × 2 months then 45 mg/day + diet (↓calorie intake by 500 kcal/day)	Placebo + diet (reduced calorie intake by 500 kcal/day)	24 wk	NASH	Yes	Yes
Promrat, 2004†	Open-label, uncontrolled	18	Excluded	Pio 30 mg daily	–	48 wk	NASH	Yes	Yes
Ratziu, 2008†	Double-blind, randomized, placebo-controlled	63 Total 32 Rosi 31 Placebo	20 Total 9 Rosi 11 Placebo	Rosi 4 mg/day × 1 month → 8 mg/day	Placebo	1 yr	NASH	Yes	Yes
Neuschwander-Tetri, 2003	Open-label, uncontrolled	25	8 DM II 7 insulin resistance	Rosi 8 mg/day	–	48 wk	NASH	Yes	Yes
Omer, 2010	Open-label, randomized	64 Total 20 Rosi 22 Met 22 Rosi + Met	All had DM II or glucose intolerance	Rosi 4 mg/day	Met 1700 mg daily Met 1700 mg/day + Rosi 4 mg daily	1 yr	NASH	Yes**	Yes**
Idilman, 2008	Prospective, randomized, controlled	74 Total 25 Rosi 24 Met 25 Con	–	Rosi 8 mg/day + diet/exercise	Met 1700 m daily + diet/exercise Diet (25 kcal/Kg) + exercise (10 K steps/day and jogging 20 minutes bid)	48 wk	NASH	No	Yes (in Rosi and Met group compared to diet/exercise)

*Number of patients who completed treatment; †improvements in ALT or histology are reported if significantly better compared to baseline (when there was no comparison group) or better than comparison group; ‡all patients in the study were also given lifestyle interventions (nutrition counseling with or without weight lost instructions); **in Rosi and combination group, but not Met.
ALT: alanine aminotransferase; Con: control; DM II: diabetes mellitus type 2; Met: metformin; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; Pio: pioglitazone; Rosi: rosiglitazone.

with pre-diabetes (or polycystic ovarian syndrome) for prevention of diabetes in our NAFLD clinic. Metformin is contraindicated in patients with renal insufficiency (serum creatinine > 1.5 mg/dL in males or > 1.4 mg/dL in females) due to risk of lactic acidosis.

THIAZOLIDINEDIONES

Thiazolidinediones (TZDs) currently in clinical use include rosiglitazone and pioglitazone. This class of oral medications works by activating the PPAR-γ, a nuclear receptor expressed in the liver, muscle, and adipose tissue that regulates adipocyte differentiation, fat metabolism, and inflammation. Activation of the receptor leads to multiple potential beneficial effects for NASH including inhibiting hepatic fatty acid synthesis,[35] remodeling adipose tissue to sequester fatty acids,[36] promoting an insulin-sensitive profile by increasing adiponectin levels,[37] and decreasing pro-inflammatory cytokines. In rats with early-phase hepatic fibrosis induced by carbon tetrachloride, pioglitazone was shown to prevent pericentral fibrosis, inflammation and necrosis and decrease serum tumor necrosis factor-α level.[38]

There is significant interest in the use of TZDs for the treatment of NASH (Table 3). Promrat et al treated 18 patients with biopsy-proven NASH with pioglitazone 30 mg daily for 48 weeks and found significant improvement in ALT, AST, and liver histology after treatment.[39] Paired

liver biopsy examination showed significant improvement in steatosis, hepatocellular injury, parenchymal inflammation, Mallory bodies, and also fibrosis.

Subsequently, Belfort et al randomized 47 patients with impaired fasting glucose or type 2 diabetes to either pioglitazone or placebo for 6 months.[40] Pioglitazone use was associated with mild weight gain (2.5 Kg) and an increase in body fat (1.5%, $P<0.01$) compared with placebo, but also demonstrated improved insulin sensitivity with 34% ($P<0.001$) reduced insulin concentration and 17% ($P=0.04$) reduced free fatty acid levels. The pioglitazone group had greater reductions in AST (40% vs 21%, $P=0.04$), ALT (58% vs 34%, $P<0.001$), anti-inflammatory cytokines such as tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β levels ($P=0.02$), improved hepatic insulin sensitivity (48% vs 14%, $P=0.008$), and increased adiponectin levels ($P<0.001$). Histologically, pioglitazone use reduced inflammation ($P=0.008$), ballooning necrosis ($P=0.019$), and steatosis ($P=0.003$) but not fibrosis. Aithal et al studied 61 nondiabetic patients with NASH who were randomized to either pioglitazone 30 mg/day or placebo for 12 months.[41] Similar to the Belfort study, pioglitazone-treated patients gained weight compared with placebo ($P=0.02$), but had improved ALT level ($P=0.009$), reduced gamma glutamyltranspeptidase (GGT) ($P=0.002$) and ferritin ($P=0.01$), and improved insulin sensitivity as shown by decreased fasting glucose level ($P=0.02$) and C-peptide level ($P=0.02$). However, fasting insulin and HOMA-IR were not different. Paired liver biopsies showed that pioglitazone reduced hepatocyte injury (ballooning, apoptosis, and necrosis) ($P=0.005$), fibrosis ($P=0.05$), and Mallory bodies ($P=0.004$) compared with placebo but not steatosis, parenchymal inflammation, or portal inflammation. Interestingly, the placebo group also had a modest reduction in ALT ($P=0.02$) and reduced steatosis ($P=0.03$) but this was accompanied by worsening hepatocellular injury (ballooning, apoptosis, and necrosis) ($P=0.02$). This finding supports previous knowledge that advanced histologic changes in NAFLD can exist[42] in the presence of normal liver enzymes and transaminase changes do not correlate with fibrosis progression.[43]

Several studies have evaluated rosiglitazone alone or in combination with other medications for treatment of NASH. Neuschwander-Tetri et al conducted an open-label trial using rosiglitazone 4 mg twice daily for 48 weeks in 26 patients with biopsy-proven NASH.[44] Rosiglitazone use was associated with increased weight of 6.4 Kg ($P<0.001$). Serum levels of ALT, AST, alkaline phosphatase (alk-phos), and GGT all significantly decreased but returned to baseline values 24 weeks post-treatment. There were no changes in TG or cholesterol levels during or post-treatment. Rosiglitazone showed significant improvement in steatosis ($P=0.004$), ballooning ($P=0.003$), and zone 3 fibrosis ($P<0.02$, shifting from dense collagen bands to more delicate deposition) but no significant change in global fibrosis, lobular or portal inflammation. Rosiglitazone use was also associated with improved insulin resistance as shown by decreased HOMR-IR, fasting insulin, and increased QUICKI score.

Ratziu et al conducted a randomized, placebo-controlled trial comparing rosiglitazone vs placebo including 63 patients with biopsy-proven NAFLD.[45] The primary endpoint was >30% reduction in steatosis or disappearance of steatosis at the end of treatment. Compared with placebo, the rosiglitazone group had more subjects reach the primary endpoint (47% vs 16%, $P=0.014$) and normalize transaminase levels (38% vs 7%, $P=0.005$). There was no significant improvement in fibrosis, hepatocyte ballooning, lobular inflammation, or composite NAS. However, a smaller proportion of patients in the rosiglitazone group had progression of ballooning, portal inflammation, and fibrosis compared with placebo. At the end of the trial, 53 patients in this study went on to be enrolled in the open-label trial for 2 additional years to test whether prolonged rosiglitazone treatment may provide greater improvement in NASH.[46] Twenty-five patients who previously received rosiglitazone and 28 patients who previously received placebo were given rosiglitazone 8 mg/day and a total of 44 patients completed the extended study. Rosiglitazone decreased ALT by 25% in this group with no breakthrough during therapy and ALT normalized in 45% of patients. This showed that the efficacy of rosiglitazone was not modified by previous treatment or rebound during the 4 month wash-out period. During the extension trial, there was no significant reduction in steatosis or improvement in insulin sensitivity suggesting that most of rosiglitazone's anti-steatogenic and anti-insulin resistance effect were maximized during the first year of therapy with no further effect thereafter. There was no significant reduction in NAS, ballooning, inflammation, or fibrosis in the open-label phase of this study.

Several studies with more complex design have compared rosiglitazone alone with metformin alone or in combination. One of the earlier studies by Idilman et al randomly assigned 74 patients into three groups—(group 1) 25 patients to diet (25 kcal/Kg × ideal body weight) and exercise alone, (group 2a) 24 patients to diet and exercise + metformin 850 mg bid, and (group 2b) 25 patients to diet and exercise + rosiglitazone 8 mg daily.[47] Patients in the metformin and rosiglitazone groups had significant improvement in AST ($P=0.047$ and $P=0.018$, respectively) and ALT levels ($P=0.017$ and $P=0.001$, respectively) but this was not different from the diet + exercise alone group. Patients treated with metformin or rosiglitazone had significant decreases in plasma glucose, insulin levels, HOMA-IR, and C-reactive protein (CRP). Treatment with rosiglitazone or metformin (group 2) led to a significant improvement in NAS, steatosis, and ballooning compared with diet + exercise alone (group 1) but no change in fibrosis.

Omer et al studied the effects of rosiglitazone vs metformin vs combination of rosiglitazone+metformin in 64 patients with NASH and either diabetes type 2 or glucose intolerance treated over 12 months.[48] As expected by the known weight reduction benefits of metformin, the metformin and metformin+rosiglitazone group had a significant reduction in BMI at the end of treatment ($P<0.05$) while there was no change in the rosiglitazone group. Paired liver biopsies were available in 35 (55%) of the patients and NAFLD score improved significantly in the rosiglitazone (2.6 points decrease, $P=0.012$) and combination group (3.9 points decrease, $P=0.026$) but not in the metformin group. The results of this study suggest that rosiglitazone was more effective than metformin in improving the biochemical and insulin resistance profile, as well as histologic improvement. Interestingly, the typical weight gain effect of rosiglitazone was not observed in this study.

In summary, TZDs appear to have a promising role in the treatment of NASH. Boettcher et al conducted a meta-analysis showing the efficacy of pioglitazone vs placebo in improving histologic parameters including ballooning degeneration, steatosis, lobular inflammation, as well as milder improvements in fibrosis. Compared with placebo, TZD use was associated with a significant reduction in serum AST (mean reduction 9 U/L) and ALT (mean reduction of 19 U/L). However, there was a significant increase in body weight and total body fat.[49] The weight gain associated with TZDs is mainly due to increase of peripheral rather than central fat, which may be less metabolically active.[50] Besides weight gain, other side effects of TZDs include fluid retention, anemia, fracture, and in some instances potentially increased cardiovascular risks. Practitioners have become more cautious with the use of TZDs since 2007 when the Food and Drug Administration (FDA) released a black-box warning for this class of medication based on concerns for increased risk of ischemic myocardial infarction and cardiovascular deaths associated with rosiglitazone.[51] Pioglitazone is preferred over rosiglitazone in the treatment of NASH but this is based upon expert opinion because pioglitazone has a favorable effect on lipid profile and better results in placebo-controlled trials. However, there are no head to head studies comparing these two interventions in the treatment of NASH.

OTHER ANTI-DIABETIC THERAPIES

Alpha-glucosidase Inhibitor

Alpha-glucosidase inhibitor is a class of oral medications used to treat type 2 diabetes. Members of this class are saccharides that work to reduce digestion and absorption of carbohydrates in the gut by competitively binding to enzymes in the brush border of the small intestines. Acarbose, an α-glucosidase inhibitor, was studied in 22 rats fed a high-fat liquid diet with or without acarbose for 3 weeks. All rats developed histologic findings of NASH despite similar food intake, weight gain, and liver weight between groups. Liver tissue in rats fed acarbose had attenuated steatosis, inflammation, hepatic collagen (all $P<0.05$), and a reduction in inflammatory cytokines such as TNF-α ($P=0.009$). Future studies will be needed to explore the efficacy of this class of medication for NASH in humans.

Incretin Analogs

Incretin analogs are a relatively new class of anti-diabetic medications which is being used to augment insulin production in patients with type 2 diabetes. In humans, glucagon-like peptide-1 (GLP-1) is a protein made in the small intestine and proximal colon and acts on β-cells in the pancreas to induce insulin secretion. The GLP-1 also has other effects of enhancing satiety, delaying gastric emptying, and increasing lower gastrointestinal motility which contributes to its beneficial effects on weight loss. Exendin-4 (exenatide) is a long-acting agonist of the GLP-1 receptor and has been found in diabetics to reduce fasting and post-prandial glucose concentrations by promoting insulin secretion and suppressing glucagon secretion, slowing gastric emptying, and inducing weight loss.[52,53] Animal studies showed that exendin-4 reversed hepatic steatosis in obese ob/ob mice by improving insulin sensitivity.[54] Rats with induced NASH after being fed a 1 month high-fat diet had decreased GLP-1 receptors. Additionally, hepatocytes from these animals had activation of genes involved in fatty acid β-oxidation and insulin sensitivity when treated with exendin-4.[55] Recently, GLP-1 receptors have been found on human hepatocytes, and the number of GLP-1 receptors has been found to be decreased in liver biopsy specimens of NASH patients compared with normal patients.[56]

Kenny et al studied exenatide prospectively in 8 patients with diabetes mellitus type 2 who were treated with subcutaneous exenatide for 28 weeks.[57] One patient discontinued treatment at 20 weeks due to persistent nausea and abdominal pain. Paired liver biopsies showed that 3 patients had significant improvement in liver histology. Treatment was associated with weight reduction, improved fasting glucose, decreased hemoglobin A_{1c}, and improved ALT. The most commonly reported side effects were gastrointestinal in nature (i.e., nausea and abdominal pain). Previous studies indicate that nearly 40% of patients have gastrointestinal side effects with exenatide. Some studies suggest that incretin analog therapy may be associated with increased risk of acute pancreatitis. In summary, there are limited data to suggest that incretin analog therapy would be a viable strategy for treatment of NASH.

VITAMIN E

Vitamin E consists of 8 fat-soluble compounds and includes 4 tocopherols and 4 tocotrienols. Alpha-tocopherol is the

Table 4 Studies of vitamin E in non-alcoholic fatty liver disease.

Reference	Type of study	No. of patient* treatment/ control	Diabetic patients	Drug	Compared with	Duration of treatment	NAFLD vs biopsy-proven NASH	ALT improved†	Histology improved†
Lavine, 2011 (TONIC trial) Pediatrics	Double-blind, randomized, placebo-controlled, multicenter	172 Total 58 Vit E 56 Met 58 Pbo	Excluded	Vit E 800 IU/day	Metformin 1000 mg/day Placebo	96 wk	NAFLD	No	Yes ↓ballooning and NAS vit E vs Pbo ↓ballooning Met vs Pbo
Sanyal, 2010	Double-blind, randomized, placebo-controlled, multicenter	247 Total 84 Vit E 80 Pio 83 Pbo	Excluded	Vit E 800 IU/day Pio 30 mg/day	Placebo	96 wk	NASH	Yes	Yes
Nobili, 2008‡	Randomized, placebo-controlled	53 Total 25 Vit E 28 Pbo	1 DM	Vit E 600 IU/day + ascorbic acid 500 mg/day	Placebo	24 mo	NAFLD	No	No

*Number of patients who completed treatment; †improvements in ALT or histology are reported if significantly better compared to baseline (when there was no comparison group) or better than comparison group; ‡all patients in the study were also given lifestyle interventions (nutrition counseling with or without weight lost instructions).
ALT: alanine aminotransferase; DM: diabetes mellitus; Met: metformin; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; Pbo: placebo; Pio: pioglitazone; Vit E: vitamin E.

most biologically active form of vitamin E, as well as the most well-studied form of vitamin E for treatment of NASH. Oxidative stress is thought to be a contributing factor in the pathogenesis of NASH. Studies suggest that α-tocopherol decreases pro-inflammatory cytokine production,[58] reduces profibrogenic signals from stellate cells activated by free radicals,[59] and may help to replete glutathione,[60] a key intracellular antioxidant.

There are several studies evaluating the effects of vitamin E in NAFLD (Table 4). Nobili et al studied 90 children with NAFLD who were given calorie-restricted diet and exercise. Patients were randomized to treatment with α-tocopherol 600 IU/day + ascorbic acid 500 mg daily ($n=45$) or placebo ($n=45$).[61] At the end of 12 months, there was significant weight loss, improvement in liver enzymes, insulin resistance, and lipids (cholesterol and TG) in both groups. However, there was no difference between groups. Patients were asked to continue their assigned treatment in a 12-month open-label extension with the end of study liver biopsy (after 24 months total treatment).[62] Fifty-three patients participated in the study extension with 25 initially randomized to α-tocopherol + ascorbic acid and 28 initially randomized to placebo. The primary endpoint for the study was improvement in NAFLD activity score by ≥2 points. At the end of 24 months, the antioxidant group had greater decrease in cholesterol compared with placebo (−35 vs −21, $P=0.02$), but otherwise there were no significant differences in weight, serum transaminases, HOMA-IR, fasting glucose, or insulin. Histologically, both groups had significant improvement in steatosis, lobular inflammation, hepatocyte ballooning, and overall NAS but there were no differences between groups. This study showed that lifestyle intervention (LI) with individualized diet and exercise was effective in improving steatosis, inflammation, ballooning, and NAS, but failed to demonstrate any efficacy of α-tocopherol + ascorbic acid in improving liver histology.

Subsequently, Lavine et al conducted a large RCT including 173 children aged 8–17 years with NAFLD and elevated ALT (≥60 U/L) who were randomized to 96 weeks of treatment with either metformin 500 mg twice daily, vitamin E 400 IU twice daily (RRR-α-tocopherol from Nature Made) or placebo.[63] The primary outcome was sustained reduction in ALT level (defined as ≤50% baseline or ≤40 U/L from 48 weeks to 96 weeks of treatment). Eighty-seven percent of subjects completed treatment and had paired liver biopsies. The ALT level improved in all 3 groups with no significant differences between the groups. Improved ALT levels were significantly different between vitamin E and placebo at week 24 but this difference did not remain significant at weeks 72 and 96 because the placebo group had continued reduction in ALT. For patients with baseline NASH or borderline NASH, the resolution of NASH was significantly greater in the vitamin E group compared with the placebo group ($P=0.006$). This was attributable to improved hepatocellular ballooning (mean score change −0.5 vs +0.1, $P=0.006$). Treatment with vitamin E was also associated with significantly improved NAS (−1.8 vs −1.2, $P=0.02$) but had no effect on steatosis, inflammation, or fibrosis. A greater percentage of subjects had improved hepatocellular ballooning in the metformin group compared with placebo (44% vs 21%, $P=0.02$) but there were no improvements in steatosis, inflammation, NAS, or resolution of NASH. Truncal fat values measured

by DEXA decreased, but were not different between groups. Quality of life improved in all groups with no difference between groups.

Sanyal and colleagues conducted a pivotal adult NASH treatment trial.[64] Patients with NASH (defined as NAS >5) were randomized to pioglitazone 30 mg daily vs vitamin E 800 IU (RRR-α-tocopherol) vs placebo for 96 weeks. The primary outcome was improvement in liver histology defined as improvement by ≥1 point in ballooning; no increase in fibrosis score, and either decrease of NAS ≤3 or decrease of ≤2 points with at least 1 point decrease in either lobular inflammation or steatosis score. Blinded review of the pre-treatment liver biopsies showed that 17% of subjects in the placebo group, 18% in the vitamin E group, and 28% in the pioglitazone had no hepatocellular ballooning. Ninety percent of subjects had paired liver biopsy. Vitamin E was significantly better than placebo in improving NAS (43% vs 19%, $P=0.001$, NNT 4.2), but pioglitazone did not reach statistical difference (34% vs 19%, $P=0.04$). Further analysis was performed due to the fact that a higher proportion of subjects in the pioglitazone group did not have ballooning degeneration at the baseline biopsy. After central pathology review excluded subjects without initial hepatocellular ballooning, both vitamin E and pioglitazone treatment were associated with significant histologic improvement compared with placebo (placebo vs vitamin E: 23% vs 52%, $P<0.001$; placebo vs pioglitazone: 23% vs 47%, $P=0.002$). Compared with the placebo group, both treatment groups had a significant reduction in steatosis, lobular inflammation, and NAS but the improvement in ballooning was only significant in the vitamin E group ($P=0.01$). The proportion of subjects with resolved steatohepatitis was significantly improved in the pioglitazone group ($P=0.001$). However, there were no significant changes in fibrosis. Treatment with vitamin E and pioglitazone was associated with significant decrease in AST and ALT with most changes occurring in the first 24 weeks and sustaining afterwards. In the pioglitazone group, insulin resistance was significantly improved with therapy compared with placebo despite weight gain (mean increase of 4.7 Kg by week 96, $P<0.001$). However, after completion of pioglitazone treatment, the HOMA-IR returned to baseline while the weight gain persisted.

Based on this single RCT, treatment of NASH in adults with vitamin E appears to be a good option for improvement in both histology and aminotransferases. However, recent studies have also suggested an association between vitamin E use and an increased risk of all-cause mortality in patients with chronic diseases,[65] as well as increased risk of prostate cancer in healthy men.[66] Thus, patients chosen for treatment with vitamin E should be aware of these risks or be considered for alternative treatments. Furthermore, it is unclear how long patients should continue therapy and how different vitamin E formulations and sources may alter efficacy.

OTHER ANTIOXIDANTS

S-adenosylmethionine (SAM) is a co-substrate responsible for methyl group transfers in the body which may be protective for the liver against fatty infiltration. Levels of SAM are postulated to be reduced in patients with NASH.[67] Betaine (trimethylclycine), originally found in sugar beets, has been shown in animal models of ethanol-induced liver injury to increase hepatic SAM levels and reduce TG accumulation and liver injury.[68] Abdelmalek et al conducted a pilot study at the Mayo clinic where 10 patients with NASH were treated with betaine 20 g daily for 12 months.[69] Significant improvement in ALT ($P=0.02$) and AST (=0.007) were seen in the 7 patients completing treatment. There were no changes in BMI or other biochemical markers. Paired liver biopsies in 6 patients showed a trend toward decreased steatosis ($P=0.10$) and necroinflammatory grade ($P=0.08$) with a significant improvement in fibrosis ($P=0.04$).

Subsequently, a multicenter, randomized, double-blind, placebo-controlled trial was conducted to study the effects of 12 g betaine vs placebo treatment over 12 months in patients with NASH.[70] There were no changes in weight, BMI, aminotransferases, or lipids. The study did not show any significant improvement in steatosis, lobular inflammation, ballooning, NAS, or fibrosis. There were significantly more gastrointestinal side effects (i.e., nausea, vomiting, abdominal bloating, and diarrhea) in the betaine group compared with placebo (33% vs 9%, $P<0.05$). In summary, published data do not support the use of betaine for the treatment of NASH.

PENTOXIFYLLINE

Pentoxifylline (PTX) is a phosphodiesterase inhibitor that has been shown to inhibit TNF-α, a pro-inflammatory cytokine elevated in patients with NAFLD that may mediate insulin resistance and contribute to the progression of NASH.[71,72] In animal models of NASH, PTX attenuates steatohepatitis by reducing TNF-α mRNA expression and also mediates a hepatoprotective effect by increasing hepatic glutathione production.[73]

A number of studies have been done to evaluate the effects of PTX in NAFLD (Table 5). Early pilot studies showed benefits in treatment of NASH with decreased ALT[74,75] and histologic improvement in NAS, necroinflammation,[76] steatosis, and fibrosis.[75] Buranawati et al conducted a randomized, placebo-controlled trial in 32 patients with NASH comparing PTX 1200 mg daily vs placebo for 6 months.[77] Treatment with pentoxifylline was associated with significant decreases in AST and ALT compared with placebo (AST: 41% vs 16%, $P=0.008$, ALT: 46.7% vs 17%, $P=0.001$). Subsequently, Lee et al published a smaller study with 20 patients who were initially treated with 3 months of low calorie diet (1200 kcal for women, 1500 kcal for men) and daily exercise before randomization

Table 5 Studies of pentoxifylline in adults with non-alcoholic fatty liver disease.

Reference	Type of study	No. of patients* treatment/ control	Diabetic patients	Drug	Compared with	Duration of treatment	NAFLD vs biopsy-proven NASH	ALT improved†	Histology improved†
Zein, 2011	Double-blind, randomized, placebo-controlled	49 Total 23 PTX 26 Pbo	5	Pentoxifylline 1200 mg daily	Placebo	1 yr	NASH	Yes	Yes
Van Wagner, 2011	Double-blind, randomized, placebo-controlled	26 Total 19 PTX 7 Pbo	3	Pentoxifylline 1200 mg daily	Placebo	1 yr	NASH	No	No
Lee, 2008‡	Double-blind, randomized, placebo-controlled	20 Total 11 PTX 9 Pbo	2	Pentoxifylline 1200 mg daily + low calorie diet and daily exercise	Placebo + low calorie diet and daily exercise	12 wk	NASH	No	–
Buranawati, 2007‡	Double-blind, randomized, placebo-controlled	32 Total 16 PTX 16 Pbo	5	Pentoxifylline 1200mg daily + low calorie diet	Placebo + low calorie diet	24 wk	NASH	Yes	–
Georgescu, 2007 (no intergroup comparisons made)	Open-label, non-randomized, uncontrolled	48 Total 13 PTX 13 UDCA 10 Ator 12 Losartan	17	Pentoxifylline 800 mg daily	UDCA 15 mg/Kg daily Atorvastatin 20mg daily Losartan 50mg daily	30 wk	NASH	Yes (for all 4 groups)	Yes (for PTX, Ator, and Losartan groups)
Satapathy, 2007	Open-label, uncontrolled	9	3	Pentoxifylline 1200 mg daily	–	1 yr	NASH	Yes	Yes
Adams, 2004	Open-label, uncontrolled	20	7	Pentoxifylline 1600 mg daily	–	1 yr	NASH	Yes	–

*Number of patients who completed treatment; †Improvements in ALT or histology are reported if significantly better compared to baseline (when there was no comparison group) or better than comparison group; ‡all patients in the study were also given lifestyle interventions (nutrition counseling with or without weight lost instructions).
ALT: alanine aminotransferase; Ator: atorvastatin; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; Pbo: placebo; PTX: pentoxifylline; UDCA: ursodeoxycholic acid.

to receive either PTX 1200 mg daily or placebo for 12 weeks.[78] Reduction in AST was significantly greater in the pentoxifylline group compared with placebo ($P=0.0380$), but improvement in ALT did not reach statistical significance.

Most recently, two RCTs examined the efficacy of pentoxifylline in the treatment of NASH. Van Wagner et al randomized 30 patients in a 2:1 fashion to PTX 400 mg thrice daily or placebo for 1 year with the primary endpoint of improvement in ALT. While there was a trend toward reduction of AST and ALT in the PTX group, this was not statistically significant compared with placebo. Steatosis and ballooning improved in the PTX group but this was also not significantly different from placebo. There were no improvements in lobular inflammation or fibrosis scores.[79] In the second study, Zein et al randomized 55 patients with biopsy-proven NASH to either PTX 400 mg 3 times a day or placebo.[80] Compared with 13.8% of patients in the placebo arm, 38.5% of patients in the PTX arm showed a 2 or more point improvement in NAS that was statistically significant. The PTX also significantly reduced steatosis ($P<0.001$), lobular inflammation ($P=0.02$), and fibrosis score ($P=0.038$) with no change in ballooning compared with placebo. Of those who had paired biopsies, 25% in the PTX group vs 3.9% in the placebo group had resolution of NASH at the end of the study ($P=0.03$). Furthermore, 57% of patients in the PTX group vs 23% in the placebo group had ALT normalization or improvement of at least 30% compared with baseline. The main side effects were nausea and vomiting and occurred more frequently in the treatment arm.

Several important differences may explain the contradicting results of the two most recent RCTs that have included histology examination. Importantly, the primary endpoints were different between the two studies. Baseline biochemistry values were similar between groups in the Van Wagner study while those in the Zein study had higher baseline AST and ALT levels in the PTX group compared with placebo. It may be easier to show an improvement in

Table 6 Studies of ursodeoxycholic acid in adults with non-alcoholic fatty liver disease.

Reference	Type of study	No. of patients* treatment/ control	Diabetic patients	Drug	Compared with	Duration of treatment	NAFLD vs biopsy-proven NASH	ALT improved†	Histology improved†
Ratziu, 2011	Double-blind, randomized, placebo-controlled, multicenter	124 Total 60 UDCA 64 Pbo	40 24 UDCA 16 Pbo	UDCA 28–35 mg/ Kg/day	Placebo	1 yr	NASH	Yes	–
Leuschner, 2010‡	Double-blind, randomized, placebo-controlled, multicenter	147	21	UDCA 23–28 mg/ Kg/day	Placebo	18 mo	NASH	No	No
Adams, 2010	Open-label, uncontrolled	12	3	UDCA 28–32 mg/ Kg/day	–	24 wk	NASH	No	–
Lindor, 2004‡	Double-blind, randomized, placebo-controlled, multicenter	126	–	UDCA (13–15 mg/ Kg/day)	Placebo	2 yr	NASH	No	No
Laurin, 1996 (no intergroup comparisons)	Open-label, non-randomized, uncontrolled	30 Total 19 UDCA 11 clofibrate	5	UDCA (13–15 mg/ Kg/day)	Clofibrate 2000 mg/ day	1 yr	NASH	Yes	Yes

*Number of patients who completed treatment; †improvements in ALT or histology are reported if significantly better compared to baseline (when there was no comparison group) or better than comparison group; ‡originally, Leuschner's study had 186 patients enrolled (95 UDCA, and 91 Pbo) but 39 patients were removed for protocol violations, Lindor's study had 166 patients enrolled (80 UDCA, 86 Pbo) but 40 patients were withdrawn—final number of patients in each treatment groups were not stated.
ALT: alanine aminotransferase; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; Pbo: placebo; UDCA: ursodeoxycholic acid.

ALT and AST levels if one group begins with higher aminotransferases. In terms of histology, almost a quarter of the patients included in the Van Wagner study had cirrhosis at baseline while the Zein study excluded patients with cirrhosis. It is possible that PTX may not be potent enough to show improvement in liver histology in patients who have already developed cirrhosis.

Based on the 4 RCTs, PTX 1200 mg daily appears to improve aminotransferases and may improve histology via reduction in steatosis, inflammation, and even fibrosis as suggested by the Zein study. Further studies are needed to confirm these findings.

URSODEOXYCHOLIC ACID

Ursodeoxycholic acid (UDCA) is a natural, hydrophilic bile acid that may be useful for the treatment of NASH through its anti-inflammatory, anti-apoptotic,[81] and immunomodulatory properties.[82] A pilot study by Laurin et al using low-dose UDCA in 24 subjects had shown improved ALT (but not AST) and hepatic steatosis in 63% (12/19) of patients but there were no weight or lipid improvements.[83] However, in a RCT, Lindor et al showed that UDCA 13–15 mg/Kg/day was not significantly better than placebo in improving biochemistry or liver histology.[84]

Recently, studies using high doses of UDCA (HD-UDCA) have been performed (Table 6). In a small pilot study, Adams et al followed 12 patients with biopsy-proven NASH who were prescribed UDCA 28–32 mg/Kg/day for 3 months and found that only 2 patients (17%) had normalization of AST levels with no significant changes in ALT, bilirubin, alkaline phosphatase, fasting glucose, or lipids.[85] In a large multicenter trial, Leuschner et al randomized 185 patients to either HD-UDCA 23–38 mg/Kg/day or placebo.[86] At the end of 18 months of treatment, there were no differences in the overall histologic scores between the treatment or placebo groups.

More recently, Ratziu et al studied 126 patients randomized to either HD-UDCA (28–35 mg/Kg/day) or placebo for 1 year with reduction in ALT as the primary endpoint. At the end of treatment, there was a significant reduction in ALT in the HD-UDCA (–28.3 ± 55%) compared with placebo (–1.6 ± 35.4%; $P < 0.001$).[87] Treatment with HD-UDCA was also associated with reduction in AST and GGT, but no significant changes in alk-phos or total bilirubin. Multivariate analysis showed that predictors of ALT response included HD-UDCA treatment, high baseline ALT, increased age, and male gender. The HD-UDCA appeared to be well-tolerated with the most commonly reported adverse effects being diarrhea and abdominal discomfort.

Table 7 Studies of statins in adults with non-alcoholic fatty liver disease.

Reference	Type of study	No. of patients* treatment/ control	Diabetic patients	Drug	Compared with	Duration of treatment (mo)	NAFLD vs biopsy-proven NASH	ALT improved†	Histology improved†
Nelson, 2009	Double-blind, randomized, placebo-controlled	14 Total 10 Simva 4 Pbo	5	Simvastatin 40 mg daily	Placebo	12	NASH	No	No
Hyogo, 2008	Open-label, uncontrolled	31	9	Atorvastatin 10 mg daily	–	24	NASH	Yes	Yes
Gomez-Dominguez, 2006‡	Open-label, uncontrolled	22	5	Atorvastatin 10–80 mg daily	–	6–12	NAFLD	Yes	–
Rallidis, 2004	Open-label, uncontrolled	5	Excluded	Pravastatin 20 mg daily	–	6	NASH	Yes	Yes

*Number of patients who completed treatment; †improvements in ALT or histology are reported if significantly better compared to baseline (when there was no comparison group) or better than comparison group; ‡all patients in the study were also given lifestyle interventions (nutrition counseling with or without weight lost instructions).
ALT: alanine aminotransferase; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; Pbo: placebo; Simva: simvastatin.

Side effects caused two drop-outs and three dose adjustments in the HD-UDCA groups.

The discrepancy of findings in the 2 most recent clinical trials using high-dose UDCA might have been due to differences in the placebo effect, modalities of assessing histology, and having different primary endpoints (histologic vs improved ALT). Data from the Ratziu study showed a short-term 3-month improvement in ALT in the placebo group that rebounded back to baseline by 6 months and persisted through the duration of treatment. This is in contrast to the placebo group in the Leuschner study which showed pre-, post-treatment differences of −38 U/L. Pooled data from prior NASH studies show improvement in both mean serum ALT (20 U/L) and AST (8 U/L) in the placebo arm.[88] This placebo effect was not observed in the Ratziu study. Thus, based on current studies, there is no substantial benefit of ursodiol in the treatment of NASH.

OBETICHOLIC ACID

Obeticholic acid, a synthetic bile acid known to activate farsenoid X receptor, has been proposed to be a potential treatment agent for NASH in animal models. The Non-alcoholic Steatohepatitis Clinical Research Network (NASH-CRN) is conducting a large multicenter, randomized, placebo-controlled clinical trial to examine the efficacy of obeticholic acid at 25 mg orally daily vs placebo for 72 weeks in improving liver histology in patients with biopsy-proven NASH.

STATINS AND EZETIMIBE

Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are a popular class of medication known for their use in reducing LDL and incidence of cardiovascular events. Atorvastatin was shown to reduce hepatic TG contents by increasing PPAR-α expression in animal models of NAFLD using fructose-fed rats.[89] Pitavastatin has been shown to attenuate liver steatosis and fibrosis in choline-deficient diet-fed rats.[90]

There are several studies of statins in patients with NASH (Table 7). An early pilot study in 5 patients treated with pravastatin for 6 months showed improved aminotransferases. Of the 4 patients who had paired liver biopsies, 3 had improved inflammation and 1 had decreased steatosis.[91] A second larger pilot study enrolled 22 patients with high baseline cholesterol and NAFLD diagnosed by ultrasound who were treated with 6–12 months of atorvastatin 10–80 mg daily depending on baseline cholesterol levels.[92] At the end of 6 months, 36.3% had normalization of aminotransferases. Of the remaining patients, an additional 20% had normal transaminase levels at 12 months. No histologic endpoints were examined because biopsies were not performed. Hyogo et al studied 31 patients with NASH and dyslipidemia treated with atorvastatin 10 mg daily for 24 months.[93] As expected, patients had significant improvement in their lipid profile. In addition, patients also had significant reductions in AST, ALT, GGT, TNF-α levels, and increased adiponectin levels. Insulin resistance parameters such as insulin, plasma glucose, and HOMA-IR remained the same. Seventeen patients had follow-up liver biopsies which showed improved steatosis and NAS score (from 4.1 to 2.9, $P<0.001$).

The only randomized, doubled-blind, controlled trial in statins was published by Nelson et al where 16 patients were randomized to 12 months of simvastatin 40 mg daily vs placebo.[94] Only 14 patients completed the study and 10 had paired biopsies (two in placebo group). Though the simvastatin group had a 26% reduction in LDL, this was not statistically different from placebo. Serum

aminotransferases were noted to be increased in the placebo arm compared with the treatment arm but this difference did not reach statistical significance. Histologically, there was no improvement in steatosis, necroinflammation, or fibrosis. The study was limited by small numbers of paired liver biopsies and most patients having mild NASH at baseline (grade 1, stage 1) making it hard to demonstrate improvement. Given the well-established benefit of statins, the insignificant difference in LDL reduction between groups may suggest that the study was underpowered to assess the efficacy of statins in NASH.

Recently, there has been growing interest in the use of ezetimibe (Zetia) in NASH. Ezetimibe is a strong inhibitor of the Niemann-Pick C1 Like 1 (NPC1L1) protein, a critical mediator of cholesterol absorption of enterocytes and hepatocytes.[95] In obese rat models with hepatic steatosis, ezetimibe improved dyslipidemia, liver steatosis, and insulin resistance.[96]

In a small open-label pilot study, Yoneda et al treated 10 patients with NASH and concurrent dyslipidemia with ezetimibe 10 mg daily for 6 months.[97] All patients were given standard calorie diet and exercise counseling for 3 months. All patients showed significant reductions in AST, ALT, GGT, LDL, cholesterol, and TG. Histologically, paired biopsies in all 10 patients showed improved steatosis and NAS with no significant change in necroinflammation, ballooning, or fibrosis.

Overall, most studies of statins in NASH are uncontrolled trials that appear to suggest beneficial effects of improving transaminases. One RCT had a small sample size and was underpowered but suggested that statins are not beneficial monotherapy treatment of NASH. The study by Yoneda et al suggests that ezetimibe may be a promising treatment for NASH; however, larger RCTs are needed to clarify the results. Nevertheless, statins (and ezetimibe) do have well-established benefits of reducing LDL, a major cardiovascular risk factor that often co-exists as part of the metabolic syndrome. Therefore, these agents should be routinely considered for use in patients with NASH.

WEIGHT LOSS

Dietary changes and increase physical activity remain the backbone of treatment regimens for patients with NASH. However, a majority of patients have difficulty achieving and maintaining desirable weight loss. Evidence suggests that a loss of 5–10% from baseline body weight improves insulin resistance, aminotransferases, and histology.

Promrat et al conducted an interesting trial to evaluate effects of LI with goal of 7–10% weight reduction in patients with NASH.[98] Sixty-five patients who had elevated liver enzymes (ALT > 41, AST > 34 U/L) and BMI between 25 Kg/m^2 and 40 Kg/m^2 who had biopsy confirmed NASH were randomized to control group vs LI group. The primary endpoint of the study was an improvement of ≥ 3 points in NAS or post-treatment NAS of ≤ 2 points after 48 weeks of intervention. The mean weight change in the LI group was −8.7 Kg compared with −0.5 Kg in the control group ($P = 0.005$). Histologically, the LI group had a −2.4 points decrease in NAS compared with −1.4 in control group ($P = 0.05$) with most of the changes attributable to decrease steatosis (−1.1 LI vs −0.3 control, $P = 0.02$). There were no significant different in parenchymal inflammation, ballooning injury, and fibrosis scores. The ALT levels improved significantly in the LI group by 42.4 U/L compared with 16.5 in the control group ($P = 0.01$), although there was no difference in AST levels.

In terms of weight loss medications, Orlistat (Xenical) is the most well-studied drug in patients with NAFLD. Orlistat is a reversible inhibitor of gastric and pancreatic lipase that blocks about 30% of dietary TG from being absorbed. A RCT using orlistat in 52 patients with NAFLD showed that though BMI improvement was not statistically different between groups, treatment with orlistat was associated with significant improvement in ALT and steatosis as measured by USG.[99] Subsequently, Harrison and colleagues conducted a larger open-label trial comparing combination of orlistat 120 mg TID + vitamin E (d-α-tocopherol) 800 IU/daily + a multivitamin at night and a standard 1400 calorie/day diet vs vitamin E 800 IU/day + multivitamin and 1400 calorie/day diet for 36 weeks in 50 patients with NASH.[100] The primary endpoint was improvement in NAS and fibrosis score. The orlistat group lost 8.3% body weight which was a significant overall change but was not significant compared with 6.0% in the control group. There were no significant differences in any of the liver histologic parameters including steatosis, ballooning, inflammation, NAS, or fibrosis between the treatment groups.

In summary, weight loss remains a desirable goal for all patients with NASH. Structured education programs on diet, physical exercise, and behavioral strategies to meet established goals may increase the number of patients achieving weight loss that is significant enough to improve aminotransferases and histology.

EMERGING AGENTS

Bile Acid Sequestrants

Bile acid sequestrants (BAS) are a group of medications that disrupt the enterohepatic circulation of bile acids by decreasing their reabsorption from the gut. This mechanism is hypothesized to promote conversion of hepatic cholesterol to bile acids which may reduce hepatic fat. The BAS are approved by the FDA for treatment of hyperlipidemia and type 2 diabetes, conditions which are associated with NASH.[101–103] An early open-label RCT by Taniai et al included 40 patients with NASH and hyperlipidemia of which 21 were controls and 17 were randomized to treatment with 3 g of colestimide for 24 weeks.[104]

The colestimide group had greater decrease in LDL ($P=0.004$), AST ($P=0.042$), and visceral fat but no difference in ALT or HOMA-IR. Hepatic steatosis as measured by CT in a small subset of patients (10 treatment and 17 controls) was not significantly different and histologic assessment was limited as there were only 2 patients who had paired liver biopsies.

Our group recently completed a randomized, double-blind, placebo-controlled trial where 50 patients were randomized to 24 weeks of treatment with colesevelam 3.75 g daily vs placebo.[105] The primary endpoint of the study was to determine if colesevelam reduces liver fat as measured by a novel magnetic resonance imaging technique named proton-density fat fraction (MRI-PDFF) which can provide segment by segment fraction to give whole liver fat mapping. The colesevelam group had significant reduction in LDL compared with placebo. However, the colesevelam group had an unexpected increase in MRI fat fraction in all nine liver segments with overall mean difference of 5.6% when compared with placebo, and this was well-correlated with MR spectroscopy ($r^2=0.96–0.98$ [$P<0.0001$]). Thirty-one patients had paired liver biopsy with no significant difference in steatosis, inflammation, ballooning, fibrosis, or NAS. Thus, the measurable increase in liver fat associated with colesevelam was only detected by MRI-PDFF and not by biopsy. These findings suggest that MRI-PDFF may be a better tool than liver histology to quantify changes in hepatic steatosis in the setting of a clinical trial. Overall, despite its beneficial role in the treatment of dyslipidemia and type 2 diabetes, BAS may not be effective in the treatment of NASH.

Angiotensin-converting Enzyme Inhibitors and Angiotensin-II Receptor Blockers

Recent evidence has shown that the renin–angiotensin system (RAS) might be involved in cell signaling that promotes insulin resistance, differentiation of pre-adipocytes into mature, insulin-resistant adipocytes, and helps modulate cytokine and adipokine production.[106] Previous studies showed that the renin–angiotensin system may be up-regulated during liver injury contributing to recruitment of inflammatory cells and activation of stellate cells, and that the use of angiotensin-receptor inhibitors attenuated these effects and improved liver enzymes and histology.[107–109] Attempts to block RAS overexpression with the use of angiotensin-converting enzyme inhibitors (ACEI) or angiotensin-II receptor blockers (ARBs) thus could be beneficial for patients with NAFLD and NASH.

There are few human trials studying the effects of ACEI or ARBs in NASH. Yokoham et al studied seven patients with both NASH and hypertension treated with losartan 50 mg daily for 48 weeks.[110] As expected, systolic blood pressure improved significantly in all patients but in addition, AST, ALT, and biomarkers of hepatic fibrosis improved as well. Histologically, hepatic necroinflammation improved in 5 patients, fibrosis in 4 patients, ballooning in three patients but there was no change in steatosis.

Combination therapy with an ARB for NASH was evaluated in an open-label study by Torres et al where 135 patients were randomized to rosiglitazone 4 mg twice daily (group 1), rosiglitazone 4 mg + metformin 500 mg twice daily (group 2), or rosiglitazone 4 mg twice daily + losartan 50 mg daily (group 3) for 48 weeks.[111] Baseline characteristics of this study cohort were all well-matched except that the rosiglitazone group had higher baseline NAS. Paired liver biopsies assessment showed no difference in steatosis, hepatocellular inflammation, or fibrosis between the treatment groups. Within-groups analysis showed, however, that all treatment had improvement in steatosis, necroinflammation, ballooning degeneration, and fibrosis ($P\leq0.001$). The NAS also significantly improved with the greater change in those with initial higher NAS. In terms of weight change, group 2 with rosiglitazone + metformin had a mean decrease in weight (−1.2 Kg) while groups 1 and 3 had increase in weight (0.9 Kg and 3.7 Kg). All 3 groups had favorable insulin tolerance profiles with significant improvement in glucose, insulin, and HOMA-IR ($P\leq0.001$) and mean AST and ALT levels ($P\leq0.001$). Overall, this study showed that the addition of metformin or losartan to rosiglitazone did not lead to incremental improvement in liver histology.

Currently, there is an on-going phase III RCT in the United Kingdom to determine whether 24 months treatment with losartan is effective in improving liver fibrosis in patients with NASH. The study will provide better insight into the potential beneficial effects of ARBs in NASH.

CONCLUSION

Non-alcoholic fatty liver disease is the most common liver disease in the western world with anticipated increasing prevalence worldwide paralleling the epidemic of obesity and increasing burden of the metabolic syndrome. Insulin resistance is a common feature among patients and has a central role in the complex interaction of genetics and environmental factors that contribute to the pathogenesis of NAFLD. Weight loss through dietary changes and increased physical exercise is the backbone of treatments for NAFLD although failure of patients to obtain and maintain desirable weight goals has been the main downfall of this approach. Metformin with its weight loss effect as well as safety profile is an attractive option for treatment of NAFLD in patients with pre-diabetes for prevention of future risk of diabetes. The TZDs especially pioglitazone have more robust data and pooled analysis from randomized controlled studies suggesting that TZDs improve both aminotransferases and histology in patients with NASH. However, the weight gain effects must be considered to

help select the appropriate treatment modality in patients with NASH. The PIVENS trial has clearly shown the efficacy of vitamin E in improving liver histology in NASH. However, recent concerns about vitamin E's association with increase mortality and prostate cancer have brought to light the need for long-term studies on its efficacy and safety in patients with NASH. Further studies are needed to define predictors of response to treatment, whether baseline factors can predict differential treatment response, and if combination therapy is more effective than monotherapy for NASH. Statins have not shown conclusive benefits as a treatment of NASH given the small limited studies but should be used in all dyslipidemic patients with NAFLD as well as NASH patients in order to reduce cardiovascular complications. In conclusion, the search for treatments of NASH has shown several promising candidates but therapy remains to be individualized and further refined. However, treatment of metabolic comorbidities should be strongly considered to reduce the cardiovascular risks associated with NAFLD/NASH.

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CONFLICTS OF INTEREST

All authors have none to declare.

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