

# Journal Pre-proof

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PII: S2212-8778(20)30123-X

DOI: <https://doi.org/10.1016/j.molmet.2020.101049>

Reference: MOLMET 101049

To appear in: *Molecular Metabolism*

Received Date: 12 May 2020

Revised Date: 19 June 2020

Accepted Date: 26 June 2020

Please cite this article as: Mahjoubin-Tehran M, De Vincentis A, Mikhailidis DP, Atkin SL, Mantzoros CS, Jamialahmadi T, Sahebkar A, Non-alcoholic fatty liver disease and steatohepatitis: state-of-the-art on effective therapeutics based on the gold standard method for diagnosis, *Molecular Metabolism*, <https://doi.org/10.1016/j.molmet.2020.101049>.

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**Non-alcoholic fatty liver disease and steatohepatitis: state-of-the-art on effective therapeutics based on the gold standard method for diagnosis**

**Short Title:** Histological improvement of NAFLD/NASH

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**Conflict of interest statement:** DPM has given talks and attended conferences sponsored by Amgen, Libytec and Novo Nordisk. Dr Mantzoros has been a shareholder of and reports grants through his institution and personal consulting fees from Coherus Inc and Pangea inc, reports grants through his institution and personal consulting fees from Esai and Novo Nordisk, reports personal consulting fees and in kind support with research reagents from Ansh inc, reports personal consulting fees from Genfit, P.E.S., Astra Zeneca, Aegerion and Regeneron, reports in kind support (educational activity meals at and through his institution) from Amarin, Jansen, Boehringer Ingelheim and in kind support and consulting fees from the California Walnut Commission.

**Abstract**

**Background:** The prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NAFLD/NASH) is increasing. NAFLD/NASH may progress to cirrhosis and hepatocellular carcinoma. However, most patients with NAFLD/NASH will die from a vascular cause. There are no currently approved pharmacological treatments for NASH/NAFLD. A large number of clinical trials have been, or are being, undertaken; however, the challenge is the assessment of the clinical endpoint.

**Scope of review:** The main objective of this narrative review was to evaluate the efficacy of drugs used in clinical trials for the treatment of NAFLD/NASH that included a liver biopsy as the gold standard. A literature search was carried out using 3 databases (PubMed, Scopus, and Google Scholar) to identify the clinical trials that included liver biopsy assessment before and after treatment. Interventional clinical trials (n=33) involving 18 different agents, alone and in combination, were identified.

**Major conclusions:** Pioglitazone is the only agent that has shown consistent benefit and efficacy in a number of clinical trials. Pentoxifylline, rosiglitazone and ursodeoxycholic acid had both positive and negative results from clinical trials. There is also evidence for vitamin E and metformin. Other drugs including bicyclol, cysteamine bitartrate, L-carnitine, liraglutide, obeticholic acid, oligofructose, selonsertib, silymarin, and statins each had a single clinical study. There is an urgent need for further research in this field.

**Key words:** non-alcoholic fatty liver disease; liver biopsy; non-alcoholic steatohepatitis;

## Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasing global public health problem and a common cause of chronic liver disease [1] (the worldwide prevalence of NAFLD is estimated at approximately 25%) [2]. The prevalence of NAFLD is rising in parallel with the increase in type 2 diabetes mellitus (T2DM) and obesity and NAFLD is predicted affect >30% of the US adult population [3].

The classical definition of NAFLD is hepatic steatosis in the absence of other liver disease [4]. In NAFLD, fat accumulates in the liver as a result of increased free fatty acid delivery to the liver, increasing triglyceride synthesis, decreasing triglyceride export and reducing beta-oxidation [5]. NAFLD patients commonly have insulin resistance (IR) that enhances lipolysis from adipose tissue [5]. Liver biopsy in NAFLD shows hepatic steatosis without inflammation or hepatocellular injury (hepatocyte ballooning) [6]; however, between 10-25% of NAFLD patients show inflammatory infiltration leading to non-alcoholic steatohepatitis (NASH). Approximately 25% of patients with simple steatosis, may progress to NASH in 3 years [4]. NASH is characterized by hepatic steatosis and lobular inflammation accompanied by hepatocyte injury (e.g. in the form of ballooning) in the presence or absence of fibrosis [6]. The exact prevalence of NASH is currently unknown due to the need for a liver biopsy for a definitive diagnosis [4]. NAFLD/NASH can progress to cirrhosis, HCC (hepatocellular carcinoma), and can be an indication for liver transplantation [7]. NASH increases the risk of both liver-related morbidity and mortality as well as chronic kidney disease, cardiovascular disease and cancer [2; 3]. Of concern is that the progression from NAFLD to NASH is more aggressive and rapid in children [2].

Despite the increasing numbers of patients, there are limited therapeutic approaches and currently there are no approved drug treatments for NAFLD and NASH [7]. In recent years, an increasing number of emerging therapies have undergone clinical evaluation [8]. In this context, an important challenge in the field of assessing NASH/NAFLD therapeutics is to accurately assess the response to treatment [7]. The gold standard to determine the progression or regression is a liver biopsy [9]. Non-invasive tests such as hepatic enzymes, imaging, NAFLD fibrosis score, Fibroscan and FibroMeter may help NASH diagnosis by identifying fibrosis [2; 5; 10] but remain surrogate markers.

### Search strategy

For this narrative review, randomized controlled trials for the treatment of NAFLD and/or NASH that used liver biopsy assessment before and after treatment were identified using 3 databases PubMed, Scopus and Google Scholar. The key words were (“non-alcoholic fatty liver disease” OR “nonalcoholic fatty liver disease” OR “non-alcoholic steatohepatitis” OR “nonalcoholic fatty liver disease” OR NAFLD OR NASH OR “fatty liver”) AND (biopsy OR histology OR histopathology OR histopathologic OR histological OR histopathologic OR histopathological) AND (trial OR “clinical trial” OR “randomized controlled trial”) (**Table 1**). We excluded studies based on a liver biopsy performed only at the beginning of the trial and not as an endpoint evaluation. We only included agents with evidence of efficacy based on histological outcome (**Table 2**). Clinical trials on agents showing lack of histological efficacy, were excluded. Only articles written in English language were included.

In the included studies, the NAFLD activity score (NAS) was defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2); this score ranged from 0 to 8. A decrease in HOMA-IR (log homeostasis assessment model analysis for IR) score represented improved insulin sensitivity. The NASH activity index represented the sum of scores for parenchymal inflammation (0-4), cellular injury (0-4) and steatosis. Liver tests reported included alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), albumin and bilirubin (**Table 3**).

### Bicyclol

Bicyclol is a derivative of dimethyl-4, 4'-dimethoxy-5, 6, 5', 6'-dimethylene dioxybiphenyl-2, 2'-dicarboxylate (DDB) which is a synthesized analogue of a traditional Chinese medicine from the herb *Fructus Schizandrae*. Bicyclol may be effective for treating chronic hepatitis B and C viral infections (in China) as well as protection against lipid injury and oxidation [11].

Patients (n=31) with NAFLD and impaired fasting glucose (IFG) were enrolled in a randomized open label controlled trial of bicyclol vs vitamin E. After lifestyle changes and a daily dose of 1500 mg/day metformin, the treatment groups received either bicyclol 25 mg three times daily or vitamin E (α-tocopherol) 100 mg three times daily for 24 weeks. Steatosis, inflammation, hepatocellular ballooning, and NAFLD activity scores (NAS) decreased in both groups after treatment. However, decreases in histopathological inflammation (-1.25 vs 0.6) and NAS (-2.68

vs 1.94) in the bicyclol group were significantly improved compared with vitamin E. In addition, bicyclol significantly reduced serum ALT activity (62.6 vs 51.87 U/L) compared with the vitamin E group. In this study, 1.79% of the patients who received bicyclol reported abdominal distension and mild diarrhea during the study while 1.8% of patients in control group reported mild abdominal distension and dizziness. There were no abnormal laboratory results related to either study drugs [12].

### **Cysteamine Bitartrate (CB)**

Cysteamine ( $\beta$ -mercapto-ethylamine) bitartrate (CB) is an approved drug in the USA and EU for nephropathic cystinosis in adults and children [13]. Cysteamine is a sulphhydryl compound that can prevent paracetamol-induced hepatic necrosis and liver damage in paracetamol poisoning [14; 15].

In a randomized placebo-controlled double-blinded trial, 169 children with NAFLD activity scores  $\geq 4$  received either cysteamine bitartrate delayed release (CBDR) or placebo twice daily (300 mg for those weighing  $\leq 65$  kg, 375 mg for those weighing  $>65$ -80 kg, and 450 mg for those weighing  $>80$  kg) for 52 weeks. The primary outcome was a decrease in the NAS of  $\geq 2$  points without worsening fibrosis; the secondary outcome was any decrease in histological features. There were significantly more patients showing an improvement in lobular inflammation in the CBDR group compared with the placebo (36 vs 21%). In a *post hoc* analysis of children weighing  $\leq 65$  kg, those taking CBDR had a 4-fold better chance of histological improvement. While there was no significant difference between groups in the primary outcome measure, patients receiving CBDR had significant changes in the secondary outcomes with a reduction in the mean activities of serum ALT (-53 vs -8 U/L), AST (-31 vs -4 U/L) and GGT (-10 vs -1) compared with placebo. Other biochemical parameters did not differ between groups [16].

### **Docosahexaenoic Acid (DHA)**

DHA acid may be effective in liver steatohepatitis since it can decrease liver triglycerides in NAFLD [17,18].

In a randomized, double-blind placebo-controlled trial, 43 obese NAFLD children with vitamin D deficiency received daily 500 mg DHA plus 800 IU vitamin D or placebo for 12 months. The major limitation of this study was that only the treatment group had a liver biopsy the end of

study due to ethical reasons. DHA plus vitamin D treatment reduced the NAS (from 5.40 to 1.92), steatosis (from 2.25 to 1.0), ballooning (from 1.6 to 0.46), lobular inflammation (from 1.5 to 0.88) and portal inflammation (from 1.6 to 1.0). In addition, DHA and vitamin D improved AST (-8.55 vs 0 U/L) and ALT (-15.75 vs 7.75 U/L) compared with the placebo group. Moreover, triglycerides, low-density lipoprotein cholesterol (LDL-C) and body mass index (BMI) decreased in the treatment group together with a persistent and significant increase in vitamin D levels. None of treated patients developed hypercalcemia and/or nephrotoxicity and no adverse events were reported [19].

In another randomized placebo-controlled clinical trial involving children with NASH, 40 participants received lifestyle modification plus placebo, or lifestyle modification plus a mix containing 250 mg of DHA, 39 UI of vitamin E and 201 mg of choline every day for 6 months. All patients were recommended to follow a hypocaloric diet (25-30 kcal/kg/day) and to engage in twice-weekly 1-h physical activity during the treatment, and for a further 6 months of follow-up. The limitation of this trial was that the end of study, liver biopsy was only performed in the active treatment group for ethical reasons. Significant improvements in steatosis (1.05 vs 1.85), ballooning (1.35 vs 0.60) and NAS (4.35 vs 2.65) were found at the end of study liver biopsy compared with baseline. Severe steatosis (grade 3) was significantly decreased from 50% to 5% of patients. Significant improvements in ALT (from 53.5 to 35.3 IU/L) and fasting glucose levels were seen only in the treatment group. No adverse events were reported [20].

### **L-carnitine**

L-carnitine is a quaternary amine that may prevent the development of NASH [21]. It has been shown that L-carnitine limits oxidative stress, reduces lipid levels and controls inflammatory responses [22]. Furthermore, it mediates the transport of long-chain fatty acids across the mitochondrial membrane. Thereby, L-carnitine facilitates the removal of fatty acids accumulating in mitochondria that lead to unbalanced hepatic fat turnover resulting in steatosis [23; 24].

In a randomized and controlled clinical trial, 74 NASH patients received 2 L-carnitine 1g tablets plus diet or placebo plus diet per day at the same dosage and regimen for 24 weeks. L-carnitine caused a reduction in steatosis (-2.28 vs -1.11), hepatocellular injury (-1.95 vs -1.19), portal inflammation (-1.49 vs -1.07), fibrosis (-1.31 vs -0.85) and NASH activity index (6.23 vs -3.63)



compared with placebo. Each of the component features of the NASH-activity index (steatosis, parenchymal inflammation, and hepatocellular injury) improved significantly. The mean NASH-activity score decreased from 9.42 to 3.19. Overall, 86% had improvement in fibrosis scores and 97% of patients had a histological response. The biochemical parameters AST (-71.7 vs -46.1 IU/L), ALT (-58.4 vs -37.4 IU/L), GGT (-37.6 vs 20.4 IU/L) were also significantly improved, compared with placebo. In addition, compared with placebo, the patients in the L-carnitine group showed significant improvements in total cholesterol, LDL-C, plasma glucose, HOMA-IR, C-reactive protein (CRP), and tumour necrosis factor (TNF)- $\alpha$  [23].

### **Liraglutide**

Liraglutide, a FDA (Food and Drug Administration)-approved medication for treating T2DM, is a long-acting analogue of human glucagon-like peptide-1 (GLP-1) [25; 26]. GLP-1 is an incretin hormone that induces insulin secretion and decreases glucagon secretion [26]. In addition, GLP-1 decreases energy intake and body weight by prolonging gastric emptying and inducing satiety [26]. There is an association between NAFLD and metabolic syndrome that increases the risk of T2DM, dyslipidemia and obesity [27]. Furthermore, liraglutide was shown to have anti-inflammation activity [28]. Therefore, GLP-1 receptor analogue therapy may have potential for the treatment of NAFLD and NASH patients. GLP-1 receptors are present in hepatocytes and it was shown that liraglutide may directly reduce liver fibrosis and steatosis in an *in vivo* study [26].

In a multicentre, double-blinded, randomized, placebo-controlled phase 2 trial of subcutaneous injections of liraglutide, 52 patients who were overweight with histological evidence of NASH received 1.8 mg daily liraglutide or placebo for 48 weeks. There were significantly more patients showing an improvement in hepatocyte ballooning (61 vs 32%,  $p=0.05$ ) and steatosis (83 vs 45%,  $p=0.009$ ) in the liraglutide compared with the placebo group. Indeed, 39% of patients in the liraglutide group vs 9% of patients in placebo group ( $p=0.019$ ) had a resolution of histologically defined NASH. Fewer patients in the liraglutide group showed progression of fibrosis compared with placebo (9 vs 36%,  $p=0.04$ ). Serum GGT activity was significantly reduced in the liraglutide group compared with the placebo group (-33.7 and -7.2 U/L,  $p=0.010$ ). Most adverse events were grade 1 (mild) to grade 2 (moderate) in severity and were transient.



They were similar in the 2 treatment groups, with the exception of gastrointestinal disorders that were more frequent with liraglutide including nausea, diarrhea and abdominal pain [29].

### **Metformin**

Metformin, an insulin sensitizer used to treat DM, may be a promising option for NAFLD [30]. The action of metformin as an antidiabetic agent is through decreasing gluconeogenesis in the liver, increasing the uptake of glucose in the muscle, enhancing oxidation of fatty acids in adipose tissue, and improving insulin sensitivity [31].

In an open label, randomized trial, nondiabetic 55 NAFLD patients received 2 g/day metformin for 12 months. The control group received 800 IU vitamin E (n=28) or a weight-reducing diet. The important limitation of this study was that at the end of study liver biopsy was carried out only in the metformin group for ethical reasons. Histological assessment showed a significant decrease (compared with baseline) in the necroinflammation score (from 1.88 to 1.23,  $p=0.012$ ), fibrosis score (from 2.88 to 2.18,  $p=0.012$ ), and NASH index (from 6.53 to 4.47,  $p<0.0001$ ). Treatment with metformin significantly improved serum ALT and AST activities (compared with vitamin E and placebo). The number of cases with a normal ALT at the end of the study in the metformin group were greater than in the diet group and in the vitamin E group. In the metformin group (vs the diet group) fasting glucose, insulin, HOMA were significantly reduced. No side effects were reported [32].

In a controlled trial, 48 patients with NAFLD were randomly assigned to either metformin or placebo for a period of 6 months. Individuals received 1 tablet (500 mg metformin or placebo) per day, followed by weekly titrations until a maximal daily dose of 2500 mg or 3000 mg (if bodyweight was  $\geq 90$  kg) was reached after 4 or 5 weeks. No differences between metformin and placebo groups were observed for liver steatosis, NAS-score, liver transaminases or markers of insulin resistance or inflammation. Changes in serum activities of ALT and AST did not differ between the groups. In contrast, beneficial effects of metformin were observed on changes in body-weight, serum levels of cholesterol, LDL-C, glucose and HbA<sub>1c</sub>. Two patients in the metformin group dropped out of the study due to gastrointestinal complications and incidence of exanthema [30].

A prospective randomized placebo-controlled trial, evaluated the effects of diet, exercise and placebo compared with diet, exercise and metformin for 12 months in 19 nondiabetic patients

with IR and NASH. Both groups received dietary recommendations for weight loss and exercise 4 times/week. The treatment group received long-acting metformin 500 mg/day (titrated to 1000 mg/day). There were no differences between the 2 groups for steatosis, ballooning, intra-acinar/portal tract inflammation, fibrosis and NAS. There were, however, significant improvements in steatosis and NAS across all study subjects. ALT activities decreased by 40.7 IU/L in the placebo group, 21.5 IU/L in the treatment group and 31.6 IU/L overall. AST activities decreased by 20.1 IU/L, 5.7 IU/L and 13.2 IU/L, respectively. However, the differences between the 2 groups did not differ for ALT, AST, ALP or other biochemical parameters [33].

In a randomized, double-blind, double-dummy, placebo controlled trial conducted in 173 NAFLD children and adolescents; participants received 800 IU of vitamin E or 1000 mg of metformin or placebo daily for 96 weeks. Ballooning degeneration score was significantly improved in both the metformin group (-0.3 vs 0.1) and in the vitamin E group (-0.5 vs 0.1) compared with placebo. NAS (-1.8 vs 0.7) was significantly reduced and resolution of NASH (58 vs 28%) was significantly increased in the vitamin E group compared with placebo. Serum biochemistry parameters did not differ between the metformin group and vitamin E groups compared with placebo. For those taking metformin, adverse effects included dose-dependent nausea, vomiting, and diarrhea, although the reported severity or frequency of adverse events between treatment groups were not significant. Five children in the placebo group, 1 in the metformin group and none in the vitamin E group developed diabetes, though this difference was not statistically significant [34].

### **Obeticholic acid (OCA)**

Obeticholic acid (OCA; 6 $\alpha$ -ethyl-chenodeoxycholic acid) is a bile acid analogue of CDCA (chenodeoxycholic acid) with a 100-fold higher affinity, compared to CDCA, for the farnesoid X receptor (FXR) [35]. FXR is a promising target for NAFLD therapy because it is a nuclear receptor that plays several roles including regulation of lipid metabolism and modulation of liver growth [35]. OCA has anti-cholestatic and hepato-protective properties [36].

In a phase 2, multicenter, double-blind, placebo-controlled, parallel group, randomized clinical trial in 283 non-cirrhotic NASH participants received 25 mg daily OCA orally or placebo for 72 weeks. Histological assessment showed significant improvement (treatment vs placebo group) in fibrosis (35 vs 19%,  $p=0.03$ ), hepatocellular ballooning (46 vs 31%,  $p=0.030$ ), steatosis (61 vs

38%,  $p=0.001$ ) and lobular inflammation (53 vs 35%,  $p=0.006$ ). The scores for fibrosis ( $-0.2$  vs  $0.1$ ,  $p=0.010$ ), hepatocellular ballooning ( $-0.5$  vs  $-0.2$ ,  $p=0.030$ ), steatosis ( $-0.8$  vs  $-0.4$ ,  $p=0.0004$ ), lobular inflammation ( $-0.5$  vs  $-0.2$ ,  $p=0.0006$ ), and NAS ( $-1.7$  vs  $-0.7$ ,  $p<0.0001$ ) were significantly decreased by OCA compared with placebo. Furthermore, compared with placebo, treatment with OCA significantly improved ALT ( $-38$  vs  $-18$  U/L,  $p<0.0001$ ), AST ( $-27$  vs  $-10$  U/L,  $p=0.0001$ ), GGT ( $-37$  vs  $-6$  U/L,  $p<0.0001$ ) activities, and bilirubin ( $-1$  vs  $0.6$   $\mu\text{mol/L}$ ,  $p=0.002$ ). However, OCA treatment increased total cholesterol and LDL-C, and decreased high-density lipoprotein cholesterol (HDL-C) compared with placebo. Clinical adverse events were generally mild to moderate in severity and were similar in the 2 groups for all symptoms except pruritus. Pruritus was reported in 23% of OCA-treated patients and 6% of placebo-treated patients [37].

In a phase 3 randomized placebo-controlled trial 931 patients with NASH and severe fibrosis received OCA 10 mg/day, OCA 25 mg/day, or placebo for 18 months. The primary endpoints were either fibrosis improvement with no worsening of NASH or NASH resolution with no worsening of liver fibrosis. Results showed that once daily OCA 25 mg achieved one primary endpoint (fibrosis improvement with no worsening of NASH) in 23% of participants ( $p=0.0002$ ) while the other primary endpoint was not met. Patients in this group showed improvements in hepatocellular ballooning (35% compared with placebo,  $p=0.001$ ) and lobular inflammation (44% compared with placebo,  $p=0.032$ ). Pruritus, was the most common adverse event that affected 51% of the patients in OCA 25 mg/day group, 28% of the OCA 10 mg/day treatment group, and 19% of the placebo group [38].

### **Resmetirom**

Resmetirom is a liver-targeted agent that binds thyroid hormone receptor- $\beta$  to counteract the toxicities associated with thyroid hormone excess (largely mediated through thyroid hormone receptor- $\alpha$ ) [39]. Resmetirom could improve NASH *via* enhancing hepatic fat metabolism and attenuating lipotoxicity [39].

In a randomized, double-blind, placebo-controlled study NASH patient, fibrosis stage 1-3, (N=125) received resmetirom (MGL-3196) or placebo 80 mg/day for 36 weeks. Results showed that resmetirom reduced hepatic fat compared with placebo ( $-37.3$  vs  $-8.5\%$ ,  $p<0.0001$ ). NAS was significantly reduced in treatment group compared with placebo group. The proportion of

patients with a  $\geq 2$ -point reduction in NAS with at least a 1-point reduction in ballooning or inflammation was significantly greater in the treatment group compared with placebo (46% vs 19%,  $p=0.017$ ). Furthermore, resmetirom significantly reduced ALT, AST, and GGT compared with placebo [39].

### **Pentoxifylline (PTX)**

PTX, a methylxanthine derivative, is a non-selective phosphodiesterase inhibitor which causes vasodilatory effects [40]. PTX is reported to decrease inflammation by inhibiting the production of TNF $\alpha$  that is recognized to promote inflammatory reactions in the development of NAFLD [41]. PTX was initially used in the treatment of intermittent claudication and then for treatment of peripheral artery disease and liver injuries such as alcoholic hepatitis and NASH [42].

In a randomized controlled trial, 30 NASH patients received 1,200 mg PTX or placebo for 12 months. Both histological and biochemical features did not differ between groups. Adverse events were mild, most frequently headache and abdominal cramps, and did not differ between groups [43].

In another randomized placebo-controlled trial 55 biopsy-confirmed NASH patient received 400 mg PTX 3 times/day or placebo for 1 year. Treatment significantly improved steatosis score (-0.85 vs -0.04,  $p<0.001$ ), lobular inflammation (-0.45 vs 0.08,  $p=0.023$ ), fibrosis (-0.2 vs 0.4,  $p=0.038$ ), and NAS (-1.6 vs -0.1,  $p<0.001$ ) compared with placebo. An improvement of 30% or more in ALT activity from baseline was observed in the treatment (57%) compared with placebo group (23%),  $p=0.016$ . Adverse effects were similar in both groups with common adverse events being nausea and vomiting [44].

### **Pioglitazone**

Pioglitazone is currently used as an antidiabetic agent [45]. Pioglitazone is a thiazolidinedione which targets insulin resistance and adipose tissue dysfunction that cause liver lipotoxicity in fatty liver disease [3]. Pioglitazone acts by binding to the PPAR $\gamma$  (peroxisome proliferator-activated receptor gamma) that plays a key role in lipid metabolism and glucose regulation [45].

In a placebo-controlled trial, 55 patients with impaired glucose tolerance or T2DM and NASH received a hypocaloric diet (a reduction of 500 kcal/day) plus 45 mg pioglitazone daily or a hypocaloric diet plus placebo for 6 months. Histological improvement in the pioglitazone group

was significantly more than placebo group; steatosis (65 vs 38%,  $p= 0.003$ ), ballooning (54 vs 24%,  $p= 0.02$ ), lobular inflammation (65 vs 29%,  $p= 0.008$ ) and necroinflammation (85 vs 38%,  $p= 0.001$ ). Treatment (compared with placebo) significantly decreased AST activity (-9 vs -19 U/L,  $p= 0.04$ ) and ALT activity (-39 vs -21 U/L,  $p < 0.001$ ). Furthermore, pioglitazone lowered triglycerides, fasting plasma glucose and insulin levels. Mild oedema and fatigue developed in 1 subject who received pioglitazone, but no other adverse effect were observed [46].

In another randomized, placebo-controlled trial, 74 nondiabetic patients received standard diet, exercise, and either 30 mg/day pioglitazone or placebo for 12 months. Histological features including hepatocellular injury ( $p= 0.005$ ), Mallory-Denk bodies ( $p=0.004$ ), and fibrosis ( $p= 0.05$ ) were reduced in the pioglitazone group compared with placebo. ALT (-37.7 vs -6.9 U/L,  $p= 0.009$ ) and GGT (-121.7 vs -6 U/L,  $p= 0.002$ ) activities were reduced in the treatment compared with the placebo group. No adverse events were observed in the pioglitazone treatment group compared with the placebo group [47].

In a randomized placebo controlled trial, 247 NASH patients without DM received 30 mg pioglitazone daily, 800 IU vitamin E, or placebo for 96 weeks. Compared with placebo, treatment with pioglitazone and vitamin E significantly improved steatosis (69 and 54 vs 31%,  $p < 0.001$ ,  $p=0.005$ ), lobular inflammation (60 and 54 vs 35%,  $p=0.004$ ,  $p=0.02$ ), hepatocellular ballooning (NS and 50 vs 29%,  $p=0.08$ ,  $p=0.01$ ), NAS (-1.9 and -1.9 vs -0.5,  $p < 0.001$ ,  $p < 0.001$ ), with a resolution of NASH (47 and 36 vs 21%,  $p= 0.001$ ,  $p= 0.05$ ) in pioglitazone and vitamin E compared with placebo,  $p$  value for pioglitazone vs placebo and  $p$  value for vitamin E vs placebo, respectively. Furthermore, serum biochemical features significantly improved in the pioglitazone and vitamin E vs the placebo group, respectively: ALT (-40.8 and -37.0 vs -20.1 U/l,  $p < 0.001$ ,  $p=0.001$ ), AST (-20.4 and -21.3 vs -3.8 U/l,  $p < 0.001$ ,  $p < 0.001$ ), GGT (-21.1 and -14.0 vs -4.0 U/l,  $p < 0.001$ ,  $p=0.003$ ), and ALP (-12.0 and -9.3 vs -3.8,  $p=0.004$ ,  $p=0.008$ ) activity ( $p$  value for pioglitazone vs placebo and  $p$  value for vitamin E vs placebo, respectively) [48].

In another randomized, double-blind, placebo-controlled trial, 101 patients with prediabetes or T2DM and NASH received either 45 mg/d pioglitazone or placebo for 18 months. Treatment improved the steatosis score (-1.1 vs -0.2,  $p < 0.001$ ), inflammation (-0.6 vs -0.1,  $p < 0.001$ ), ballooning (-0.6 vs -0.2,  $p=0.001$ ) and fibrosis (-0.5 vs 0,  $p=0.039$ ) compared with placebo. Resolution of NASH in the pioglitazone group (51%) was significantly greater than with placebo (19%),  $p < 0.001$ . The percentage of patients that had  $\geq 2$ -point reduction in NAS without

worsening of fibrosis was significantly greater in the pioglitazone group compared with the placebo group,  $p < 0.001$ . Compared with placebo, pioglitazone treatment significantly decreased AST ( $p = 0.001$ ) and ALT ( $p < 0.001$ ) activities, decreased triglyceride ( $p = 0.018$ ), HDL-C levels ( $p < 0.001$ ) and liver fat content ( $p < 0.001$ ). Common adverse events were musculoskeletal, respiratory/otolaryngologic, and gastrointestinal but there was no difference between the pioglitazone and placebo groups [49].

### **Prebiotics**

Gut microbiota dysbiosis is considered to be a contributing factor to NASH development. Prebiotics are substrates which are selectively used by the host microorganisms. Prebiotics alter the gut microbiota by increasing the growth and activity of health-promoting bacteria [50]. Oligofructose is a prebiotic which enhance *Bifidobacterium* and reduce *Clostridium* cluster XI and I [51]. In addition, oligofructose can lower serum triglycerides, cholesterol and very low density lipoproteins [50].

A randomized trial evaluated the effects of *Bifidobacterium longum* with fructo-oligosaccharides in the treatment of NASH. Patients ( $n = 66$ ) received *Bifidobacterium longum* with fructo-oligosaccharides 2.5 g and lifestyle modification (i.e. diet and exercise) or placebo and lifestyle modification for 24 weeks. There was significant reduction in steatosis (2.22 vs 1.5,  $p < 0.05$ ) and the NAS (6.22 vs 4.29,  $p < 0.05$ ) compared with placebo. Moreover, treatment significantly reduced AST (-69.6 vs -45.9 IU/mL - The authors use IU/mL within the abstract and text and IU/L within the table but the correct units are probably IU/dL -  $p < 0.05$ ), LDL-C (-0.84 vs -0.18 mmol/L,  $p < 0.001$ ), CRP (-2.9 vs -0.7 mg/L,  $p < 0.05$ ), TNF- $\alpha$  (-0.45 vs -0.12 ng/mL,  $p < 0.001$ ), HOMA-IR (-1.1 vs -0.6,  $p < 0.001$ ), and serum endotoxin (-45.2 vs -30.6 pg/mL,  $p < 0.001$ ) [52].

In a placebo-controlled, randomized pilot trial 14 patients with NASH (NAS  $\geq 5$ ) received oligofructose (8 g/day for 12 weeks followed by 16 g/day for 24 weeks) or placebo. Prebiotic therapy significantly decreased steatosis and NAS compared with placebo. Treatment did not alter ALT, ALP and GGT activities. There were no adverse events from consuming oligofructose [51].

### **Rosiglitazone**

Rosiglitazone, an antidiabetic drug, improves insulin sensitivity [53]. IR leads to fat accumulation in the liver and to the development and progression of steatohepatitis. As such, rosiglitazone may be useful in the treatment of NASH by reversing IR [54]. However, evidence on its effect in increasing the risk of cardiovascular events has caused its withdrawal in many countries and limited its use.

In a placebo-controlled trial, 63 patients with biopsy-proven NASH were randomly assigned to either rosiglitazone (4 mg/day for the first month followed by 8 mg/day thereafter) or placebo for a period of one year. More patients treated with rosiglitazone than receiving placebo had significantly improved steatosis (47 vs 16%) and normalized transaminase levels (38 vs 7%). There were no improvements in other histological parameters. The main adverse effect was weight gain (mean gain of 1.5 kg in the rosiglitazone group vs 1 kg in the placebo group;  $p < 0.01$ ), while the main reason for dose reduction/discontinuation was the incidence of painful swollen legs [54].

In a randomized trial, 53 NASH patients received 8 mg/day rosiglitazone or placebo for 2 years. There was no difference in the biochemical parameters or histological features [55].

In another randomized controlled-trial, 137 NASH patients received rosiglitazone 4 mg twice-daily, rosiglitazone 4 mg and 500 mg metformin twice-daily, or rosiglitazone 4 mg twice-daily and losartan 50 mg once-daily for 48 weeks. Serum aminotransferases were reduced in all 3 groups but did not differ between groups. There was no difference between treatment groups for all of the histological parameters. No difference between treatment groups for adverse events was detected [56].

### **Selonsertib**

Activation of apoptosis signal-regulating kinase 1 (ASK1), in the setting of oxidative stress, can lead to activation of stress response pathways that worsens hepatic apoptosis, inflammation, and fibrosis. Therefore, selonsertib, a selective inhibitor of ASK1, could be useful for the treatment of NASH [57].

In a multicenter randomized control trial, 72 NASH patients received either 6 or 18 mg of selonsertib orally once daily with or without once-weekly injections of 125 mg of simtuzumab or simtuzumab alone for 24 weeks. Simtuzumab is a humanized monoclonal antibody against lysyl oxidase-like molecule 2, an enzyme involved in the extracellular matrix remodeling through



crosslinkage of collagen and elastin. Due to the lack of effect of simtuzumab on histological parameters, selonsertib groups with and without simtuzumab were pooled. Treatment significantly improved histological parameters with a reduction in fibrosis (43, 30 and 20%), patients with progression to cirrhosis (3, 7 and 20%), patients with  $\geq 1$  point reduction in NAS (52, 41 and 60%), patients with  $\geq 2$  point reduction in NAS (23, 19 and 20%), steatosis  $\geq 1$  point reduction (32, 30 and 20%), lobular inflammation  $\geq 1$  point reduction (32, 22 and 20%), and ballooning  $\geq 1$  point reduction (16, 33 and 30%) on selonsertib 18 mg  $\pm$  simtuzumab group, selonsertib 6 mg  $\pm$  simtuzumab group, and simtuzumab group, respectively. Compared with baseline, treatment reduced serum ALT (-8, -6 and -3 U/L), AST (-5, -4, and -3 U/L), and GGT (-7, -2 and -2 U/L) activities in the selonsertib 18 mg  $\pm$  simtuzumab group, selonsertib 6 mg  $\pm$  simtuzumab group, and simtuzumab group, respectively. Moreover, triglyceride (-21, 12 and -30 mg/dL), total cholesterol (-10, -5 and -13 mg/dL), HDL-C (-2, 1, and 2 mg/dL), LDL-C (-10, -5 and -25 mg/dL), and HOMA-IR (0.98, 2.17, and -0.22) levels were significantly changed in the selonsertib 18 mg  $\pm$  simtuzumab group, selonsertib 6 mg  $\pm$  simtuzumab group, and simtuzumab group, respectively. The highest number of adverse events in the selonsertib groups were headache and nausea [57].

### **Silymarin**

Silymarin is a mixture of flavonolignans and polyphenolic compounds derived from the milk thistle plant *Silybum marianum* that were used for the treatment of liver disease. Silymarin has anti-inflammatory, antifibrotic and antioxidant properties that may be beneficial in NAFLD patients [58].

In a randomized, double-blind, placebo-controlled trial, 99 biopsy-proven NASH and NAS  $\geq 4$  received 700 mg silymarin or placebo 3 times/day for 48 weeks. Compared with placebo, treatment with silymarin significantly improved fibrosis in patients (fibrosis change: -0.184 in silymarin group vs +0.100 placebo group,  $p=0.026$ ). Triglyceride levels were significantly improved in the silymarin group (-0.20 vs +0.04 mmol/L,  $p=0.017$ ). There were no significant differences in adverse events and discontinuations in the silymarin and placebo groups [58].

In a multicenter double-blind placebo controlled trial, the effect of standardized silymarin preparation (Legalon<sup>®</sup>) was tested. Legalon<sup>®</sup> is a proprietary milk thistle seed extract standardized to a silymarin content of 140 mg/capsule. NASH without cirrhosis patients with

NAS  $\geq 4$  (n=78) receive Legalon<sup>®</sup> 420 mg, 700 mg, or placebo three times per day for 48 weeks. The histological improvement between groups was not significantly different. However, improved steatosis and lobular inflammation in the Legalon<sup>®</sup> group was more than for the placebo group but failed to show a statistically significant histological improvement. There were no significant differences in adverse events among the treatment groups [59].

### **Ursodeoxycholic Acid (UDCA)**

UDCA is a natural bile acid with several hepatoprotective activities [60]. UDCA reduces oxidative stress and has antiapoptotic effects that may be of benefit in NAFLD/NASH [61].

In a randomized clinical trial, 166 NASH patients received between 13 and 15 mg/kg/day of UDCA or placebo for 2 years. There was no difference between UDCA and placebo groups in biochemical or histological features. A trend towards higher incidence of gastrointestinal adverse events in the UDCA compared with the placebo group was noted; however, the rate of clinical adverse events was similar in both groups [60].

In a double-blind, randomized, placebo-controlled trial, 185 NASH patients received 23-28 mg/kg/day UDCA or placebo for 18 months. Treatment with UDCA significantly improved lobular inflammation (-0.51 vs -0.19 in placebo). However, other histopathological features did not differ between groups. Compared with placebo, GGT activity significantly improved in the UDCA group (-52.42 vs -16.84 U/L). Diarrhea was the side effect in the UDCA group (11 in UDCA group vs 1 in placebo group). No patient dropped out due to adverse effects of UDCA [61].

In a randomized placebo-controlled trial, 48 NASH patients received UDCA (12-15 mg/kg/day) plus vitamin E (400 IU twice a day) (UDCA/Vit E), UDCA with placebo (UDCA/P), or placebo/placebo (P/P) for a period of two years. Steatosis was improved in the UDCA/Vit E group ( $p < 0.05$ ). None of the histological parameters altered in the UDCA/P group. There were significant decreases in the ALT and AST activities in the UDCA/Vit E group ( $p < 0.05$ ), and the ALT activity in UDCA/P group ( $p < 0.05$ ). Vitamin E and UDCA appeared safe and their combination was well-tolerated with no patient dropouts as a result of side effects [62].

### **Vitamin E**

Oxidative stress is implicated in NASH pathogenesis. Therefore, vitamin E as an antioxidant may be effective for the treatment of NASH [63].

Twenty nondiabetic and non-cirrhotic subjects with NASH received vitamin E alone (400 IU/day) vs vitamin E (400 IU/day) and pioglitazone (30 mg/day). Combination therapy produced a significant decrease in steatosis cytological ballooning, Mallory's hyaline, and inflammation, compared with vitamin E alone. Both groups were similar with respect to AST, ALT, and ALP activities. Combination therapy of pioglitazone and vitamin D significantly increased the metabolic clearance of glucose and decreased circulating fasting free fatty acid (FFA) and insulin levels [63].

### **Cenicriviroc (CVC)**

CVC is dual antagonist of chemokine receptor (CCR) types 2 and 5. Its anti-antifibrotic and inflammatory effects are mediated by CCR2 and CCR5 blockade. CVC has demonstrated antifibrotic activity in animal models of liver and renal fibrosis [64].

A randomized, double-blind, placebo-controlled trial assessed CVC for the treatment of NASH with liver fibrosis. Patients with NASH, NAS  $\geq 4$ , and liver fibrosis stages of 1-3 received CVC 150 mg or placebo orally for 1 year. The primary outcome was defined as a NAS improvement (2 points) with no worsening fibrosis. Secondary outcomes were defined as a resolution of steatohepatitis with no worsening of fibrosis; fibrosis improvement by 1 stage with no worsening of steatohepatitis. The primary endpoint did not differ between the CVC and placebo groups; however, the fibrosis improvement and no worsening of steatohepatitis (% subjects who achieved improvement in fibrosis) was significantly greater in the CVC group compared with placebo (20 vs 10%,  $p=0.023$ ). Tolerability and safety of CVC was comparable with placebo [64].

### **Elafibranor**

Elafibranor a peroxisome proliferator activated receptor- $\alpha$  (PPAR $\alpha$ ) and peroxisome proliferator activated receptor- $\delta$  (PPAR $\delta$ ) dual agonist. PPAR $\delta$  agonists have shown efficacy in improving liver histology in NASH. Elafibranor improves lipid metabolism, insulin sensitivity, and reduces inflammation [65].

Ratziu et al. evaluated the safety and efficacy of elafibranor in a randomized, double-blind placebo-controlled trial; 276 patients with NASH without cirrhosis received elafibranor 80 mg,

elafibranor 120 mg, or placebo daily for 52 weeks. The primary outcome was no fibrosis worsening; however, this did not differ between the elafibranor and placebo groups. A greater proportion of subjects with a resolution of NASH without worsening fibrosis was observed in the 120 mg elafibranor group compared with the placebo group (19 vs 12%). Liver enzymes, lipids, and markers of systemic inflammation were reduced in the elafibranor 120 mg group. Elafibranor was well tolerated but produced a mild increase in serum creatinine levels [65].

### **Statins**

Besides the well-known cholesterol-lowering effect, statins are also reputed for the lipid-independent pleiotropic effects that justify their use in different patient populations not necessarily having hypercholesterolemia [66-72]. Statins may be recommended in patients with NAFLD/NASH for their lipid-lowering, antioxidant and anti-inflammatory effects as well as a decrease in the associated increased cardiovascular risk [73]. Well-conducted clinical trials aimed at verifying their effect on liver inflammation and fibrosis have not been conducted. However, a large observational cross-sectional multicenter study showed statins to be safe in NAFLD subjects, with no hepatotoxic effect, and with beneficial effects on steatosis, NASH and fibrosis [74]. A small prospective study with no control arm on 20 subjects with NASH, metabolic syndrome and dyslipidemia treated with rosuvastatin monotherapy for 12 months also showed a benefit [75]. This effect may be partially explained by a reduction in Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) levels; TNF- $\alpha$  is known to play a role in the pathogenesis of NASH [68]. In conclusion, in line with current guidelines [76], statins may be prescribed in NAFLD subjects to treat dyslipidemia, prevent cardiovascular risk, and beneficial effects on the liver [77; 78]. No specific indications are available on which statin or dose should be prescribed.

### **Ezetimibe**

Ezetimibe is a LDL-C lowering agent, which can be considered as a safe option for lipid lowering in NAFLD patients [79]. In a randomized controlled trial, the effect of ezetimibe (10 mg/day) in combination with a standard energy diet and exercise was tested in 32 NAFLD patients for a period of 6 months. Fibrosis stage and ballooning score were improved with ezetimibe treatment. However, ezetimibe increased hepatic long-chain fatty acids and HbA<sub>1c</sub>, which calls for further evaluation [80].

## Conclusions

NAFLD has become a growing public health problem with no licensed therapeutic agents to date. The cornerstone of current management is dietary and lifestyle intervention to achieve weight loss, along with the optimization of metabolic risk factors, such as diabetes mellitus and dyslipidemia. However, these goals are difficult to implement mainly due to poor adherence. Therefore, in selected cases the off-label use of medications with demonstrated effects on NASH histological features could be considered. Insulin sensitizers, such as pioglitazone and liraglutide, and hepatoprotective agents, such as vitamin E, may be the preferred options in clinical practice up to now. The optimal duration of these therapeutic trials has not been established yet, and no firm recommendations are available, so that the current management of the more severe patients (i.e. those with NASH and advanced liver fibrosis) is mainly left to the individual experience of treating physicians and local practice [6; 76; 81]. With the aim of bridging this gap, many clinical trials have been conducted with different therapeutic agents and promising results in some cases. The different pathophysiological pathways involved in NAFLD/NASH improvement are presented in **Figure 1**. Of these drugs, those with evidence of efficacy based on liver biopsy are of particular importance (**Table 1**). Pioglitazone and vitamin E have shown benefits on NASH histological features and are currently the only recommended agents in current clinical guidelines [6; 76]. Pentoxifylline and ursodeoxycholic acid have both positive and negative results from clinical trials and need further clarification. Similar results also for rosiglitazone, whose prescription is however hampered by its withdrawal in many countries. Despite its effect in improving IR, two meta-analysis concluded against the any effect of metformin on liver histology of patients with NAFLD and NASH [82; 83].

Bicyclol, cysteamine bitartrate, L-carnitine, liraglutide, obeticholic acid, oligofructose, selonsertib, silymarin, and statins each just have a single clinical study each; there is a need for further confirmation of their efficacy. The same level of evidence also applies to viusid, a nutritional supplement consisting of glycyrrhizic acid, ascorbic acid and zinc, which has been shown to improve histological indices of NAFLD in a single randomized, controlled trial with a follow-up of 6 months [84].

The stages of clinical development of the mentioned drugs are as follows: rosiglitazone (NCT00492700, NCT00492700), ursodeoxycholic acid (NCT00470171), cysteamine bitartrate

(NCT00799578), elafibranor (NCT01694849), cenicriviroc (NCT02217475) and L-carnitine (NCT01617772) are in phase 2. Pentoxifylline (NCT00267670), vitamin E (NCT00655018), metformin (NCT00303537), obeticholic acid (NCT02548351, NCT03439254), and selonsertib (NCT03053063, NCT03053050) are in phase 3. Pioglitazone and silymarin (NCT02973295) are in phase 4 of clinical trials (NCT00994682, NCT00227110). While some drugs such as metformin and pioglitazone previously received their approval (for other diseases), other agents need to be evaluated for their safety additional to the efficacy for NAFLD/NASH. Therefore, in the near future, we may have new approved therapeutic agents for the treatment of NAFLD/NASH.

However, appraising the available literature, some critical points deserve consideration. First of all, the bulk of presented drugs are supported by a single clinical trial. As such, further investigations will be needed to confirm their effect on NAFLD/NASH, and to date many of them cannot be considered with optimism regarding their early introduction into clinical practice. Second, even when multiple trials have been conducted, the sample of enrolled subjects is generally limited and short treatment periods have often been tested. Greater (>300-400 subjects) and more prolonged (>36-48 months) trials should be designed. They should include repeated liver biopsies during treatment, in order to retain adequate statistical power to determine histological outcomes. They should also accurately record long-term adverse effects. In this regard, excessive concerns on the ethical impracticability of liver biopsies for assessing inclusion and efficacy criteria should be considered in relation to the projected burden of NAFLD/NASH. Finally, given available data, the most promising drug on the scene seems to be OCA, which showed a significant improvement of liver fibrosis in 18% (10 mg dose group) and 23% (25 mg dose group) of subjects in the interim analysis of its phase 3 trial. However, the relevant proportion of subjects experiencing moderate to severe pruritus (28% and 51% for the 10 mg and 25 mg dose groups, respectively) leaves concerns on its real practice tolerability. All in all, it should be observed that, also in the best scenarios, the currently available molecules demonstrated a significant improvement of NASH and/or liver fibrosis in only a minority of patients, as such other drugs should be identified, possibly acting on alternative pathophysiological pathways, and tested for their safety and efficacy. The interesting field of long noncoding RNAs should be regarded with attention given the novel insights into their role in NASH and liver fibrosis development [85].

The focus of the present review was to introduce those agents that have documented efficacy based on the currently diagnostic gold standard *i.e.* liver biopsy. While biopsy represents the most valid results, it is as an expensive method that exposes the patients to particular risks due to its invasive nature. Besides, biopsy cannot represent the status of entire liver tissue [86].

These limitations have made the use of biopsy for drug screening trials and large-scale studies a less preferred option, thereby blunting the pace of reliable discovery of new drugs for NAFLD/NASH through clinical studies. Inevitably, a less invasive, low-cost and noninvasive diagnostic method is needed. Considering NAFLD and NASH as multifactorial diseases, no sole alternative indicator could reliably predict clinical outcome or therapeutic beneficial effects [87]. Recently, developments in multi-omics analyses have opened new windows to the pathogenesis of diseases such as NAFLD and NASH. Particularly, advanced integrated analysis of serum/liver cellular lipids in NAFLD patients have revealed substantial metabolic pathways implicated in disease progression. These surrogate markers have considerable potential to identify risk factors and contribute to the monitoring of treatment for NAFLD/NASH [87; 88].

### **Legend to Figure 1**

Possible mode of action of agents used in clinical trials for non-alcoholic fatty liver disease and steatohepatitis that used pre- and post-treatment liver biopsy as the endpoint.



## References

- [1] Hagström, H., Nasr, P., Ekstedt, M., Hammar, U., Stål, P., Hultcrantz, R., et al., 2018. Risk for development of severe liver disease in lean patients with nonalcoholic fatty liver disease: A long-term follow-up study. *Hepatology Communications* 2:48-57.
- [2] Araújo, A.R., Rosso, N., Bedogni, G., Tiribelli, C., Bellentani, S., 2018. Global epidemiology of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: What we need in the future. *Liver International* 38:47-51.
- [3] Wise, J., 2016. Pioglitazone seems safe and effective for patients with fatty liver disease and diabetes. *British Medical Journal* 353:i3435.
- [4] Wong, V.W.S., Chan, W.K., Chitturi, S., Chawla, Y., Dan, Y.Y., Duseja, A., et al., 2018. Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017—Part 1: Definition, risk factors and assessment. *Journal of Gastroenterology and Hepatology* 33:70-85.
- [5] Schwenger, K.J.P., 2018. Non-Alcoholic Fatty Liver Disease: Investigating the Impact of Bariatric Care and the Role of Immune Function. <http://hdl.handle.net/1807/91964>
- [6] Chalasani, N., Younossi, Z., Lavine, J.E., Charlton, M., Cusi, K., Rinella, M., et al., 2018. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 67:328-357.
- [7] Younossi, Z.M., Loomba, R., Rinella, M.E., Bugianesi, E., Marchesini, G., Neuschwander-Tetri, B.A., et al., 2018. Current and future therapeutic regimens for nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology* 68:361-371.
- [8] Cicero, A.F.G., Colletti, A., Bellentani, S., 2018. Nutraceutical Approach to Non-Alcoholic Fatty Liver Disease (NAFLD): The Available Clinical Evidence. *Nutrients* 10:1153.
- [9] Hays, S.M., McGinnis, C., 2018. Nonalcoholic Fatty Liver Disease in Children: Beyond Metabolic Syndrome. *The Journal for Nurse Practitioners* 14:725-731.
- [10] Younossi, Z.M., Loomba, R., Anstee, Q.M., Rinella, M.E., Bugianesi, E., Marchesini, G., et al., 2018. Diagnostic modalities for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. *Hepatology* 68:349-360.
- [11] Liu, G.T., 2009. Bicyclol: A novel drug for treating chronic viral hepatitis B and C. *Medicinal Chemistry* 5:29-43.

- [12] Han, Y., Shi, J.P., Ma, A.L., Xu, Y., Ding, X.D., Fan, J.G., 2014. Randomized, vitamin E-controlled trial of bicyclol plus metformin in non-alcoholic fatty liver disease patients with impaired fasting glucose. *Clinical Drug Investigation* 34:1-7.
- [13] Armas, D., Holt, R.J., Confer, N.F., Checani, G.C., Obaidi, M., Xie, Y., et al., 2018. A Phase 1 Pharmacokinetic Study of Cysteamine Bitartrate Delayed-Release Capsules Following Oral Administration with Orange Juice, Water, or Omeprazole in Cystinosis. *Advances in Therapy* 35:199-209.
- [14] Prescott, L.F., Swainson, C.P., Forrest, A.R.W., Newton, R.W., Wright, N., Matthew, H., 1974. Successful treatment of severe paracetamol overdose with cysteamine. *The Lancet* 303:588-592.
- [15] Prescott, L.F., Sutherland, G.R., Park, J., Smith, I.J., Proudfoot, A.T., 1976. Cysteamine, methionine, and penicillamine in the treatment of paracetamol poisoning. *The Lancet* 308:109-113.
- [16] Schwimmer, J.B., Lavine, J.E., Wilson, L.A., Neuschwander-Tetri, B.A., Xanthakos, S.A., Kohli, R., et al., 2016. In Children With Nonalcoholic Fatty Liver Disease, Cysteamine Bitartrate Delayed Release Improves Liver Enzymes but Does Not Reduce Disease Activity Scores. *Gastroenterology* 151:1141-1154.
- [17] Choi, S.S., Diehl, A.M., 2008. Hepatic triglyceride synthesis and nonalcoholic fatty liver disease. *Current Opinion in Lipidology* 19:295-300.
- [18] Scorletti, E., Byrne, C.D., 2018. Omega-3 fatty acids and non-alcoholic fatty liver disease: Evidence of efficacy and mechanism of action. *Molecular Aspects of Medicine* 64:135-146.
- [19] Corte, C.D., Carpino, G., De Vito, R., De Stefanis, C., Alisi, A., Cianfarani, S., et al., 2016. Docosahexanoic acid plus Vitamin D treatment improves features of NAFLD in children with serum Vitamin D deficiency: Results from a single centre trial. *PloS One* 11:e0168216.
- [20] Zöhrer, E., Alisi, A., Jahnel, J., Mosca, A., Della Corte, C., Crudele, A., et al., 2017. Efficacy of docosahexaenoic acid–choline–vitamin E in paediatric NASH: A randomized controlled clinical trial. *Applied Physiology, Nutrition and Metabolism* 42:948-954.
- [21] Fujisawa, K., Takami, T., Matsuzaki, A., Matsumoto, T., Yamamoto, N., Terai, S., et al., 2017. Evaluation of the effects of L-carnitine on medaka (*Oryzias latipes*) fatty liver. *Scientific Reports* 7: 2749.
- [22] Romano, M., Vacante, M., Cristaldi, E., Colonna, V., Gargante, M.P., Cammalleri, L., et al., 2008. L-carnitine treatment reduces steatosis in patients with chronic hepatitis C treated with  $\alpha$ -interferon and ribavirin. *Digestive Diseases and Sciences* 53:1114-1121.
- [23] Malaguarnera, M., Gargante, M.P., Russo, C., Antic, T., Vacante, M., Malaguarnera, M., et al., 2010. L-carnitine supplementation to diet: A new tool in treatment of nonalcoholic steatohepatitis a randomized and controlled clinical trial. *American Journal of Gastroenterology* 105:1338-1345.
- [24] Sakata, N., Phillips, T.E., Dixon, J.L., 2001. Distribution, transport, and degradation of apolipoprotein B-100 in HepG2 cells. *Journal of Lipid Research* 42:1947-1958.
- [25] Daneschvar, H.L., Aronson, M.D., Smetana, G.W., 2016. FDA-Approved Anti-Obesity Drugs in the United States. *American Journal of Medicine* 129:879.e871-879.e876.
- [26] Ohki, T., Isogawa, A., Iwamoto, M., Ohsugi, M., Yoshida, H., Toda, N., et al., 2012. The effectiveness of liraglutide in nonalcoholic fatty liver disease patients with type 2 diabetes mellitus compared to sitagliptin and pioglitazone. *The Scientific World Journal* 2012:496453.
- [27] Angelico, F., Del Ben, M., Conti, R., Francioso, S., Feole, K., Fiorello, S., et al., 2005. Insulin resistance, the metabolic syndrome, and nonalcoholic fatty liver disease. *Journal of Clinical Endocrinology and Metabolism* 90:1578-1582.
- [28] Zhu, W., Feng, P.P., He, K., Li, S.W., Gong, J.P., 2018. Liraglutide protects non-alcoholic fatty liver disease via inhibiting NLRP3 inflammasome activation in a mouse model induced by high-fat diet. *Biochemical and Biophysical Research Communications* 505:523-529.
- [29] Armstrong, M.J., Gaunt, P., Aithal, G.P., Barton, D., Hull, D., Parker, R., et al., 2016. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): A multicentre, double-blind, randomised, placebo-controlled phase 2 study. *The Lancet* 387:679-690.

- [30] Haukeland, J.W., Konopski, Z., Eggesbø, H.B., Von Volkmann, H.L., Raschpichler, G., Bjørø, K., et al., 2009. Metformin in patients with non-alcoholic fatty liver disease: A randomized, controlled trial. *Scandinavian Journal of Gastroenterology* 44:853-860.
- [31] Mazza, A., Fruci, B., Garinis, G.A., Giuliano, S., Malaguarnera, R., Belfiore, A., 2011. The role of metformin in the management of NAFLD. *Experimental Diabetes Research* 2012: 716404.
- [32] Bugianesi, E., Gentilcore, E., Manini, R., Natale, S., Vanni, E., Villanova, N., et al., 2005. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *American Journal of Gastroenterology* 100:1082-1090.
- [33] Shields, W.W., Thompson, K.E., Grice, G.A., Harrison, S.A., Coyle, W.J., 2009. The Effect of Metformin and Standard Therapy versus Standard Therapy alone in Nondiabetic Patients with Insulin Resistance and Nonalcoholic Steatohepatitis (NASH): A Pilot Trial. *Therapeutic Advances in Gastroenterology* 2:157-163.
- [34] Lavine, J.E., Schwimmer, J.B., Van Natta, M.L., Molleston, J.P., Murray, K.F., Rosenthal, P., et al., 2011. Effect of Vitamin e or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents the tonic randomized controlled trial. *Journal of the American Medical Association* 305:1659-1668.
- [35] Makri, E., Cholongitas, E., Tziomalos, K., 2016. Emerging role of obeticholic acid in the management of nonalcoholic fatty liver disease. *World Journal of Gastroenterology* 22:9039-9043.
- [36] Abenavoli, L., Falalyeyeva, T., Boccuto, L., Tsyryuk, O., Kobyljak, N., 2018. Obeticholic acid: A new era in the treatment of nonalcoholic fatty liver disease. *Pharmaceuticals* 11:104.
- [37] Neuschwander-Tetri, B.A., Loomba, R., Sanyal, A.J., Lavine, J.E., Van Natta, M.L., Abdelmalek, M.F., et al., 2015. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): A multicentre, randomised, placebo-controlled trial. *The Lancet* 385:956-965.
- [38] Younossi, Z.M., Ratziu, V., Loomba, R., Rinella, M., Anstee, Q.M., Goodman, Z., et al., 2019. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *The Lancet* 394:2184-2196.
- [39] Harrison, S.A., Bashir, M.R., Guy, C.D., Zhou, R., Moylan, C.A., Frias, J.P., et al., 2019. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *The Lancet* 394:2012-2024.
- [40] Du, J., Ma, Y.Y., Yu, C.H., Li, Y.M., 2014. Effects of pentoxifylline on nonalcoholic fatty liver disease: A meta-analysis. *World Journal of Gastroenterology* 20:569-577.
- [41] Adams, L.A., Zein, C.O., Angulo, P., Lindor, K.D., 2004. A pilot trial of pentoxifylline in nonalcoholic steatohepatitis. *The American Journal of Gastroenterology* 99:2365.
- [42] Ye, J.H., Chao, J., Chang, M.L., Peng, W.H., Cheng, H.Y., Liao, J.W., et al., 2016. Pentoxifylline ameliorates non-alcoholic fatty liver disease in hyperglycaemic and dyslipidaemic mice by upregulating fatty acid  $\beta$ -oxidation. *Scientific Reports* 6:33102.
- [43] van Wagner, L.B., Koppe, S.W.P., Brunt, E.M., Gottstein, J., Gardikiotes, K., Green, R.M., et al., 2011. Pentoxifylline for the treatment of non-alcoholic steatohepatitis: A randomized controlled trial. *Annals of Hepatology* 10:277-286.
- [44] Zein, C.O., Yerian, L.M., Gogate, P., Lopez, R., Kirwan, J.P., Feldstein, A.E., et al., 2011. Pentoxifylline improves nonalcoholic steatohepatitis: A randomized placebo-controlled trial. *Hepatology* 54:1610-1619.
- [45] Ahn, H.Y., Kim, H.H., Hwang, J.-Y., Park, C., Cho, B.Y., Park, Y.J., 2018. Effects of Pioglitazone on Nonalcoholic Fatty Liver Disease in the Absence of Constitutive Androstane Receptor Expression. *PPAR Research* 2018: 9568269.
- [46] Belfort, R., Harrison, S.A., Brown, K., Darland, C., Finch, J., Hardies, J., et al., 2006. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *New England Journal of Medicine* 355:2297-2307.

- [47] Aithal, G.P., Thomas, J.A., Kaye, P.V., Lawson, A., Ryder, S.D., Spendlove, I., et al., 2008. Randomized, Placebo-Controlled Trial of Pioglitazone in Nondiabetic Subjects With Nonalcoholic Steatohepatitis. *Gastroenterology* 135:1176-1184.
- [48] Sanyal, A.J., Chalasani, N., Kowdley, K.V., McCullough, A., Diehl, A.M., Bass, N.M., et al., 2010. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *New England Journal of Medicine* 362:1675-1685.
- [49] Cusi, K., Orsak, B., Bril, F., Lomonaco, R., Hecht, J., Ortiz-Lopez, C., et al., 2016. Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus a randomized trial. *Annals of Internal Medicine* 165:305-315.
- [50] Fiordaliso, M., Kok, N., Desager, J.P., Goethals, F., Deboyser, D., Roberfroid, M., et al., 1995. Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. *Lipids* 30:163-167.
- [51] Bomhof, M.R., Parnell, J.A., Ramay, H.R., Crotty, P., Rioux, K.P., Probert, C.S., et al., 2019. Histological improvement of non-alcoholic steatohepatitis with a prebiotic: a pilot clinical trial. *European Journal of Nutrition* 58:1735-1745.
- [52] Malaguarnera, M., Vacante, M., Antic, T., Giordano, M., Chisari, G., Acquaviva, R., et al., 2012. *Bifidobacterium longum* with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. *Digestive Diseases and Sciences*. 57:545-553.
- [53] Rzonca, S., Suva, L., Gaddy, D., Montague, D., Lecka-Czernik, B., 2004. Bone is a target for the antidiabetic compound rosiglitazone. *Endocrinology* 145:401-406.
- [54] Ratziu, V., Giral, P., Jacqueminet, S., Charlotte, F., Hartemann-Heurtier, A., Serfaty, L., et al., 2008. Rosiglitazone for Nonalcoholic Steatohepatitis: One-Year Results of the Randomized Placebo-Controlled Fatty Liver Improvement With Rosiglitazone Therapy (FLIRT) Trial. *Gastroenterology* 135:100-110.
- [55] Ratziu, V., Charlotte, F., Bernhardt, C., Giral, P., Halbron, M., Lenaour, G., et al., 2010. Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: Results of the Fatty Liver Improvement by Rosiglitazone Therapy (FLIRT 2) extension trial. *Hepatology* 51:445-453.
- [56] Torres, D.M., Jones, F.J., Shaw, J.C., Williams, C.D., Ward, J.A., Harrison, S.A., 2011. Rosiglitazone versus rosiglitazone and metformin versus rosiglitazone and losartan in the treatment of nonalcoholic steatohepatitis in humans: A 12-month randomized, prospective, open-label trial. *Hepatology* 54:1631-1639.
- [57] Loomba, R., Lawitz, E., Mantry, P.S., Jayakumar, S., Caldwell, S.H., Arnold, H., et al., 2018. The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: A randomized, phase 2 trial. *Hepatology* 67:549-559.
- [58] Wah Kheong, C., Nik Mustapha, N.R., Mahadeva, S., 2017. A Randomized Trial of Silymarin for the Treatment of Nonalcoholic Steatohepatitis. *Clinical Gastroenterology and Hepatology* 15:1940-1949.e1948.
- [59] Navarro, V.J., Belle, S.H., D'Amato, M., Adfhal, N., Brunt, E.M., Fried, M.W., et al., 2019. Silymarin in non-cirrhotics with non-alcoholic steatohepatitis: A randomized, double-blind, placebo controlled trial. *PLoS One* 14:e0221683.
- [60] Lindor, K.D., Kowdley, K.V., Heathcote, E.J., Harrison, M.E., Jorgensen, R., Angulo, P., et al., 2004. Ursodeoxycholic Acid for Treatment of Nonalcoholic Steatohepatitis: Results of a Randomized Trial. *Hepatology* 39:770-778.
- [61] Leuschner, U.F.H., Lindenthal, B., Herrmann, G., Arnold, J.C., Rössle, M., Cordes, H.J., et al., 2010. High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: A double-blind, randomized, placebo-controlled trial. *Hepatology* 52:472-479.

- [62] Dufour, J., Oneta, C.M., Gonvers, J., Bihl, F., Cerny, A., Cereda, J., et al., 2006. Randomized Placebo-Controlled Trial of Ursodeoxycholic Acid With Vitamin E in Nonalcoholic Steatohepatitis. *Clinical Gastroenterology and Hepatology* 4:1537-1543.
- [63] Sanyal, A.J., Mofrad, P.S., Contos, M.J., Sargeant, C., Luketic, V.A., Sterling, R.K., et al., 2004. A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. *Clinical Gastroenterology and Hepatology* 2:1107-1115.
- [64] Friedman, S.L., Ratziu, V., Harrison, S.A., Abdelmalek, M.F., Aithal, G.P., Caballeria, J., et al., 2018. A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. *Hepatology* 67:1754-1767.
- [65] Ratziu, V., Harrison, S.A., Francque, S., Bedossa, P., Leheret, P., Serfaty, L., et al., 2016. Elafibranor, an Agonist of the Peroxisome Proliferator-Activated Receptor- $\alpha$  and - $\delta$ , Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening. *Gastroenterology* 150(5):1147-1159.e1145.
- [66] Ferretti, G., Bacchetti, T., Sahebkar, A., 2015. Effect of statin therapy on paraoxonase-1 status: A systematic review and meta-analysis of 25 clinical trials. *Progress in Lipid Research* 60:50-73.
- [67] Sahebkar, A., Kotani, K., Serban, C., Ursoniu, S., Mikhailidis, D.P., Jones, S.R., et al., 2015. Statin therapy reduces plasma endothelin-1 concentrations: A meta-analysis of 15 randomized controlled trials. *Atherosclerosis* 241(2):433-442.
- [68] Serban, C., Sahebkar, A., Ursoniu, S., Mikhailidis, D.P., Rizzo, M., Lip, G.Y.H., et al., 2015. A systematic review and meta-analysis of the effect of statins on plasma asymmetric dimethylarginine concentrations. *Scientific Reports* 5:09902.
- [69] Sahebkar, A., Serban, C., Mikhailidis, D.P., Undas, A., Lip, G.Y.H., Muntner, P., et al., 2015. Association between statin use and plasma d-dimer levels: A systematic review and meta-analysis of randomised controlled trials. *Thrombosis and Haemostasis* 114(3):546-557.
- [70] Parizadeh, S.M.R., Azarpazhooh, M.R., Moohebati, M., Nematy, M., Ghayour-Mobarhan, M., Tavallaie, S., et al., 2011. Simvastatin therapy reduces prooxidant-antioxidant balance: Results of a placebo-controlled cross-over trial. *Lipids* 46(4):333-340.
- [71] Chruściel, P., Sahebkar, A., Rembek-Wieliczko, M., Serban, M.-C., Ursoniu, S., Mikhailidis, D.P., et al., 2016. Impact of statin therapy on plasma adiponectin concentrations: A systematic review and meta-analysis of 43 randomized controlled trial arms. *Atherosclerosis* 253:194-208.
- [72] Sahebkar, A., Serban, C., Ursoniu, S., Mikhailidis, D.P., Undas, A., Lip, G.Y.H., et al., 2016. The impact of statin therapy on plasma levels of von Willebrand factor antigen: Systematic review and meta-analysis of Randomised placebo-controlled trials. *Thrombosis and Haemostasis* 115(3):520-532.
- [73] Newsome, J.S., 2016. Evaluation of statin treatment for nonalcoholic fatty liver disease. *Journal of Pharmacy Technology* 32:169-173.
- [74] Dongiovanni, P., Petta, S., Mannisto, V., Mancina, R.M., Pipitone, R., Karja, V., et al., 2015. Statin use and non-alcoholic steatohepatitis in at risk individuals. *Journal of Hepatology* 63:705-712.
- [75] Kargiotis, K., Athyros, V.G., Giouleme, O., Katsiki, N., Katsiki, E., Anagnostis, P., et al., 2015. Resolution of non-alcoholic steatohepatitis by rosuvastatin monotherapy in patients with metabolic syndrome. *World J Gastroenterol* 21:7860-7868.
- [76] European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the Management of Non-Alcoholic Fatty Liver Disease, 2016. *Obesity Facts* 9:65-90.
- [77] Athyros, V.G., Alexandrides, T.K., Bilianou, H., Cholongitas, E., Doulas, M., Ganotakis, E.S., et al., 2017. The use of statins alone, or in combination with pioglitazone and other drugs, for the treatment of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis and related cardiovascular risk. An Expert Panel Statement. *Metabolism* 71:17-32.

- [78] Nascimbeni, F., Pellegrini, E., Lugari, S., Mondelli, A., Bursi, S., Onfiani, G., et al., 2019. Statins and nonalcoholic fatty liver disease in the era of precision medicine: More friends than foes. *Atherosclerosis* 284:66-74.
- [79] Loomba, R., Sirlin, C.B., Ang, B., Bettencourt, R., Jain, R., Salotti, J., et al., 2015. Ezetimibe for the treatment of nonalcoholic steatohepatitis: assessment by novel magnetic resonance imaging and magnetic resonance elastography in a randomized trial (MOZART trial). *Hepatology* 61:1239-1250.
- [80] Takeshita, Y., Takamura, T., Honda, M., Kita, Y., Zen, Y., Kato, K.-i., et al., 2014. The effects of ezetimibe on non-alcoholic fatty liver disease and glucose metabolism: a randomised controlled trial. *Diabetologia* 57:878-890.
- [81] Ratziu, V., Goodman, Z., Sanyal, A., 2015. Current efforts and trends in the treatment of NASH. *Journal of Hepatology* 62:S65-S75.
- [82] Musso, G., Gambino, R., Cassader, M., Pagano, G., 2010. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology* 52:79-104.
- [83] Li, Y., Liu, L., Wang, B., Wang, J., Chen, D., 2013. Metformin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Biomedical Reports* 1:57-64.
- [84] Vilar Gomez, E., Rodriguez De Miranda, A., Gra Oramas, B., Arus Soler, E., Llanio Navarro, R., Calzadilla Bertot, L., et al., 2009. Clinical trial: A nutritional supplement Viusid, in combination with diet and exercise, in patients with nonalcoholic fatty liver disease. *Alimentary Pharmacology and Therapeutics* 30:999-1009.
- [85] De Vincentis, A., Rahmani, Z., Muley, M., Vespasiani-Gentilucci, U., Ruggiero, S., Zamani, P., et al., 2020. Long noncoding RNAs in nonalcoholic fatty liver disease and liver fibrosis: state-of-the-art and perspectives in diagnosis and treatment. *Drug Discovery Today*. <https://doi.org/10.1016/j.drudis.2020.05.009>
- [86] Sumida, Y., Nakajima, A., Itoh, Y., 2014. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World Journal of Gastroenterology* 20:475.
- [87] Perakakis, N., Polyzos, S.A., Yazdani, A., Sala-Vila, A., Kountouras, J., Anastasilakis, A.D., et al., 2019. Non-invasive diagnosis of non-alcoholic steatohepatitis and fibrosis with the use of omics and supervised learning: A proof of concept study. *Metabolism* 101:154005.
- [88] Perakakis, N., Yazdani, A., Karniadakis, G.E., Mantzoros, C., 2018. Omics, big data and machine learning as tools to propel understanding of biological mechanisms and to discover novel diagnostics and therapeutics. *Metabolism-Clinical and Experimental* 87:A1-A9.

Table 1. Characteristics of liver biopsy-based randomized controlled trials in non-alcoholic liver disease and non-alcoholic steatohepatitis.

Agent	Population (Type & Number)	Comparator group	Age	Dose	Treatment duration (Week)	Effect <sub>1</sub>	Adverse events <sup>4</sup>	Ref.
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Bicyclol	NAFLD with IFG 31	Vitamin E 100 mg 3 times daily	Adults	25 mg three times daily	24	+	Mild abdominal distension and dizziness	[12]
Cysteamine Bitartrate (CB)	NAFLD activity scores of 4 or higher 169	Placebo	Adolescents	twice daily (300-450 mg) age dependent	52	+ <sup>2</sup>	Gastrointestinal adverse events	[16]
Docosahexaenoic Acid Plus Vitamin D	NAFLD and vitamin D deficiency 43	Placebo	Children and adolescents	daily DHA (500 mg) plus vitamin D (800 IU) once daily	24	+ <sup>3</sup>	-	[19]
Docosahexaenoic acid-choline-vitamin E	NASH 40	Placebo	Children and adolescents	combining 250 mg of DHA, 39 UI of vitamin E and 201 mg of choline	24	+ <sup>3</sup>	-	[20]
L-Carnitine	NASH 74	Placebo	Adults	2 g/day	24	+	Nausea, moderate headache, and abdominal pain	[23]
Liraglutide	NASH 52	Placebo	Adults	subcutaneous injections of liraglutide (1.8 mg daily)	48	+	Diarrhea, constipation, and loss of appetite	[29]
Metformin	NAFLD 55	Vitamin E OR prescriptive weight-reducing diet	Adults	2 g/day	48	+ <sup>3</sup>	-	[32]
Metformin	NAFLD 48	Placebo	Adults	500 mg/day increased every week until 2500 mg or 3000 mg	24	-	-	[30]
Metformin	NASH 19	Placebo diet and exercise	Adults	500 mg daily	48	-	-	[33]
Metformin	NAFLD 173	Placebo	Adolescents	Daily dosing 1000 mg of	96	+	Nausea, vomiting,	[34]



				metformin			and diarrhea	
Obeticholic acid	NASH 283	Placebo	Adults	25 mg daily	72	+	Pruritus	[37]
Obeticholic acid	NASH 931	Placebo	Adults	10 or 25 mg daily	72	+	Pruritus	[38]
Pentoxifylline	NASH 30	Placebo	Adults	400 mg 3 times daily	48	-	Headache and abdominal cramps	[43]
Pentoxifylline	NASH 55	Placebo	Adults	400 mg 3 times a day	48	+	Nausea	[44]
Pioglitazone	type 2 diabetes and NASH 55	Placebo	Adults	45 mg daily	24	+	Fatigue and mild lower-extremity edema	[46]
Pioglitazone	NASH 74	Placebo	Adults	30 mg/day	48	+	Fluid retention	[47]
Pioglitazone	NASH without diabetes 247	Placebo	Adults	30 mg daily	96	+	-	[48]
Pioglitazone	NASH 101	Placebo	Adults	45 mg/d	72	+	Weight gain*	[49]
Prebiotic (Oligofructose)	NASH 14	Placebo	Children and adolescents	8 g/day for 12 weeks followed by 16 g/day for 24 weeks	36	+	-	[51]
Prebiotic (Bifidobacterium longum with fructo-oligosaccharides)	NASH 66	Placebo and lifestyle modification	Adult	Bifidobacterium longum with fructo-oligosaccharides 2.5 g	24	+	-	[52]
Rosiglitazone	NASH 33	Placebo	Adults	4 mg/day for the first month and 8 mg/day thereafter	48	+	Weight gain*	[54]
Rosiglitazone	NASH 53	Placebo	Adults	8 mg/day	96	-	Asthenia, muscular cramps, swollen legs and weight gain	[55]
Rosiglitazone	NASH 137	4 mg of rosiglitazone and 500 mg of metformin twice-daily or	Adults	4 mg twice-daily	48	-	-	[56]

		4 mg of rosiglitazone twice-daily and 50 mg of losartan once-daily						
Selonsertib	NASH and stage 2 or 3 liver fibrosis 72	125 mg of simtuzumab with or without selonsertib	Adults	6 or 18 mg of selonsertib once daily	24	+	Headache, nausea, sinusitis, nasopharyngitis, upper abdominal pain, back pain, and fatigue.	[57]
Silymarin	NASH and a NAFLD activity score 4 or more 99	Placebo	Adults	700 mg, 3 times daily	48	+	Ureteric calculi	[58]
Silymarin (Legalon®)	NASH without cirrhosis with NAS $\geq 4$	Placebo	Adults	420 mg, 700 mg, 3 times daily	48	-	Ureteric calculi	[59]
Ursodeoxycholic Acid	NASH 166	Placebo	Adults	between 13 and 15 mg/kg/d	96	-	Gastrointestinal adverse events	[60]
Ursodeoxycholic acid	NASH 185	Placebo	Adults	23-28 mg/kg/day	72	+	Diarrhea*	[61]
Vitamin E with Ursodeoxycholic Acid	NASH 48	UDCA with vitamin E OR UDCA with placebo OR placebo/placebo	Adults	12-15 mg per kg per day with vitamin E 400 IU twice a day	96	+	-	[62]
Vitamin E with pioglitazone	NASH 20	vitamin E (400 IU/day)	Adults	vitamin E (400 IU/day) and pioglitazone (30 mg/day)	24	+	-	[63]
Vitamin E	NASH without diabetes 247	Placebo	Adults	800 IU daily	96	+	Weight gain*	[48]
vitamin E	NAFLD	Placebo	Adolescents	Daily dosing	96	+	-	[34]

	173			of 800 IU of vitamin E				
Cenicriviroc	NASH, with NAS $\geq$ 4, and liver fibrosis stages of 1-3 252	Placebo	Adults	150 mg daily	48	+ <sup>2</sup>	Arrhythmia	[64]
Elafibranor	NASH without cirrhosis	Placebo	Adults	80-120 mg daily	52	+ <sup>2</sup>	mild increase in serum creatinine levels*	[65]
Statin	NASH 107	untreated	Adults	-	24	+	-	[74]
Ezetimibe	NAFLD 32	untreated	Adults	10 mg/day	24	+	-	[80]
Resmetirrom	NASH 125	Placebo	Adults	80 mg/day	36	+	Transient mild diarrhea and nausea*	[39]

<sup>1</sup> Positive effect of drugs defined as an improvement in at least in one histological feature.

<sup>2</sup> Although there was no difference between groups in the primary outcome, patients receiving CBDR had significant improvement in secondary outcomes.

<sup>3</sup> Biopsy at the end of therapy was done only in the treatment group for ethical reasons.

<sup>4</sup> Adverse events did not differ by treatment group except those were marked with star (\*).

**DHA:** Docosahexaenoic Acid, **IFG:** impaired fasting glucose, **NAFLD:** non-alcoholic fatty liver disease, **NAS:** NAFLD activity score, **NASH:** non-alcoholic steatohepatitis, **UDCA:** Ursodeoxycholic acid

Table 2. Changes in histological features of the liver with different therapeutic agents.

Agent	Steatosis	Fibrosis	Hepatocyte ballooning	Lobular inflammation	NAS	Ref
Bicyclol	-	-	-	↓	↓	[12]
Cysteamine Bitartrate (CB) <sup>1</sup>	-	-	-	↓	-	[16]
L-carnitine	↓	↓	N	↓	N	[23]
Liraglutide <sup>1</sup>	↓	-	↓	-	-	[29]

Metformin	-	-	↓	-	-	[34]
Vitamin E	-	-	↓	-	↓	[34]
Obeticholic acid	↓	↓	↓	↓	↓	[37]
Obeticholic acid	-	↓	↓ <sup>1</sup>	↓ <sup>1</sup>	-	[38]
Pentoxifylline	↓	↓	-	↓	↓	[44]
Pioglitazone	↓	↓	↓	↓	↓	[46] [48] [49]
Prebiotic (Oligofructose)	↓	-	-	-	↓	[51]
Prebiotic (Bifidobacterium longum with fructo- oligosaccharides)	↓	-	N	-	↓	[52]
Rosiglitazone <sup>1</sup>	↓	-	-	-	-	[54]
Selonsertib <sup>1</sup>	↓	↓	-	↓	↓	[57]
Silymarin	-	↓	-	-	-	[58]
UDCA	-	-	↓	-	-	[61]
DHA Plus Vitamin D <sup>2</sup>	↓	-	↓	↓	↓	[19]
DHA Plus Vitamin E & choline <sup>2</sup>	↓	-	↓	-	↓	[20]
UDCA Plus Vitamin E	↓	-	-	-	-	[62]
Pioglitazone Plus Vitamin E	-	-	↓	↓	-	[63]
Cenicriviroc	-	↓ <sup>1</sup>	-	-	-	[64]
Statin	↓	↓	-	-	-	[74]
Ezetimibe	-	↓	↓	-	-	[80]
Resmetirom	-	-	-	-	↓	[39]

N: Not determined

↑: Increase

↓: Decrease

-: Not significantly effected

<sup>1</sup> Data derived from percentage of "patients with improvement" in histological parameters.

<sup>2</sup> Results of treatment at the end of the study compared to the baseline because the liver biopsy at the end of the study was performed in the active group alone for ethical reasons.

**DHA:** Docosahexaenoic Acid, **NAS:** NAFLD activity score, **UDCA:** Ursodeoxycholic acid

Table 3. Alterations in liver function indices with different therapeutic agents.

	ALT	AST	ALP	GGT	Albumin	Bilirubin	Ref
Bicyclol	↓	-	N	-	N	N	[12]
Cysteamine Bitartrate (CB)	↓	↓	-	↓	N	N	[16]
L-carnitine	↓	↓	N	↓	-	N	[23]
Liraglutide	-	-	-	↓	-	-	[29]

Metformin	↓	↓					[32]
Obeticholic acid	↓	↓	↑	↓	-	↓	[37] [38]
Pentoxifylline	↓	-	N	N	N	N	[44]
Pioglitazone	↓	↓	↓	↓	↓	-	[46][47] [48] [49]
Prebiotic (Bifidobacterium longum with fructo- oligosaccharides)	-	↓	N	N	-	-	[52]
Selonsertib	↓	↓	N	↓	N	N	[57]
UDCA	↓	-	N	↓	N	N	[62] [61]
DHA Plus Vitamin D	↓	↓	N	-	N	N	[19]
DHA Plus Vitamin E & choline	↓	-	N	-	N	N	[20]
UDCA Plus Vitamin E	↓	↓	N	N	N	N	[62]
Elafibranor	↓	N	↓	↓	N	N	[65]
Resmetirom	↓	↓	-	↓	-	-	[39]

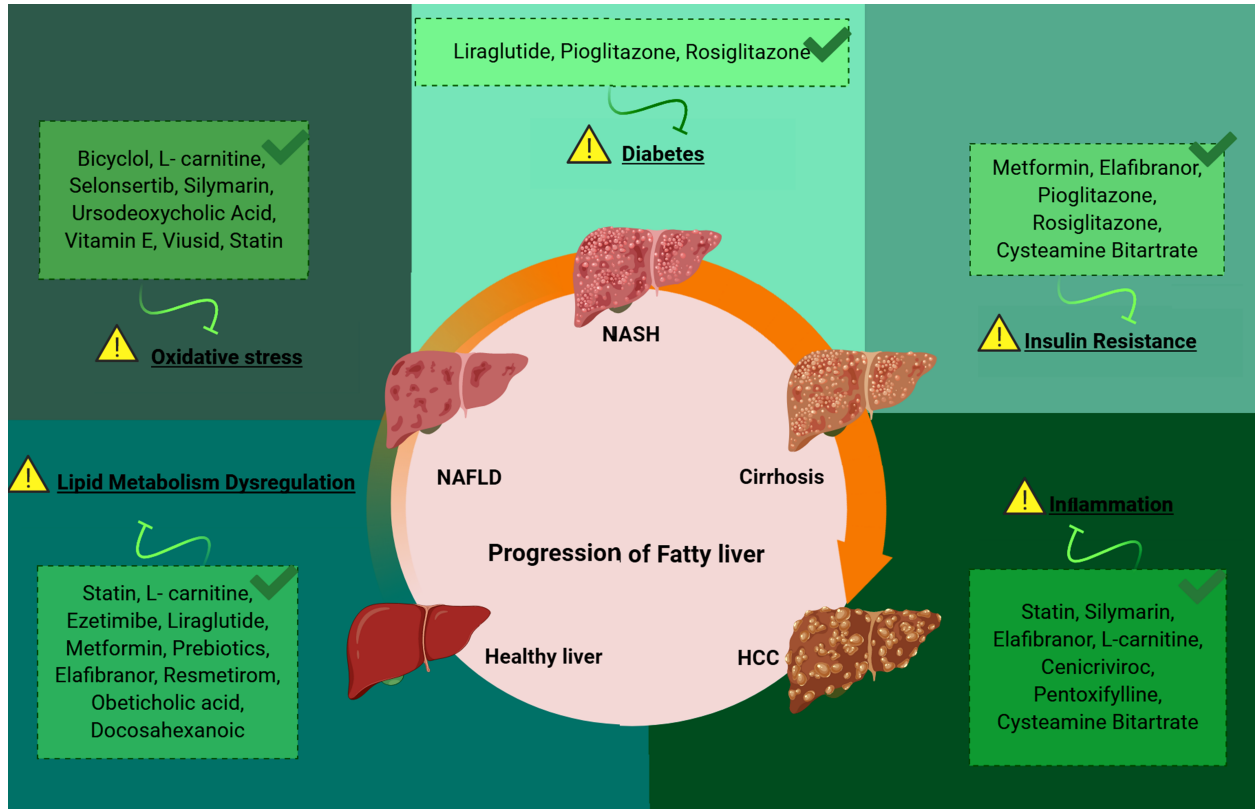
N: Not determined

↑: Increase

↓: Decrease

-: Not significantly affected

**ALP:** Alkaline phosphatase, **ALT:** Alanine transaminase, **AST:** Aspartate transaminase, **DHA:** Docosahexaenoic Acid, **GGT:** Gamma glutamyl transpeptidase, **UDCA:** Ursodeoxycholic acid



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### **Highlights**

- There are no currently approved pharmacological treatments for NASH/NAFLD.
- Confirmation of effective therapies for NAFLD/NASH is challenging due to the limitations of non-biopsy methods.
- We reviewed the efficacy of drugs used in trials of NAFLD/NASH patients that included a liver biopsy as the gold standard.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

DPM has given talks and attended conferences sponsored by Amgen, Libytec and AstraZeneca. Other authors have no competing interests to disclose

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