# Recent Insights into Treatment of Non-Alcoholic Steatohepatitis

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## Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) represents a major public health issue worldwide. The main characteristic of NAFLD is the accumulation of lipids in hepatocytes to form lipid droplets. The spectrum of NAFLD ranges from non-alcoholic fatty liver (i.e., steatosis) to Non-Alcoholic Steatohepatitis (NASH), a progressive condition increasing in Western Countries and leading to cirrhosis, liver failure and hepatocellular carcinoma. The prevention of NAFLD requires strategies for careful management and monitoring of patients with obesity, diabetes and other components of metabolic syndrome. There is no currently approved treatment that can reverse NASH once it is established. There is no evidence that pioglitazone or vitamin E can improve fibrosis. Life-style changes and bariatric surgery may improve hepatic histology in some patients with NASH. Currently, a few new drugs targeting pathways that have recently been implicated in the development of NAFLD are under development. Therefore, treatment of NASH should be approached as a complex therapy that would take into account etiology of the disease and patient's history. This review summarizes recent insights into the treatment of NASH.

**Keywords:** NAFLD; NASH; Metabolic syndrome; Obeticholic acid; Treatment

## Introduction

For the last two decades, Non-Alcoholic Fatty Liver Disease (NAFLD) and especially Non-Alcoholic Steatohepatitis (NASH) have been the main cause of chronic liver disease in the Western Countries as well as in Middle East, Asia, Africa and South America [1-4]. The increasing prevalence of NAFLD and NASH in developing countries correlates with changes in lifestyle such as consumption of fast-food, soft soda sugar-sweetened beverages and sedentary lifestyle, leading to the increased prevalence of obesity. NAFLD is usually associated with obesity, insulin resistance and type 2 diabetes, dyslipidemia and metabolic syndrome, which affect both adult and pediatric populations [1-11]. NAFLD also increases the risk for development of Cardiovascular Disease (CVD) independently to other risk factors [12,13], making NAFLD not only a liver disease but also a "systemic disease".

NAFLD is a condition ranging from simple Non-Alcoholic Fatty Liver (NAFL) to NASH, steatofibrosis and cryptogenic cirrhosis. The main characteristic of these lesions is fat accumulation in the form of diacylglycerols and triglycerides contained in hepatic lipid droplets. NAFL is usually considered as benign and reversible whereas NASH may progress to liver failure, cirrhosis and hepatocellular carcinoma [2,6,7,9,14].

The diagnosis of NAFLD is made by histology that still remains the gold standard. Diagnosis of NASH is a little more complicated because it encompasses hepatic steatosis, ballooning of hepatocytes, Mallory's body and inflammation, which may or may not be associated fibrosis. For the past years, some groups worked on better defining NASH by developing different scores such as the NASH Activity Score (NAS) or the Steatosis, Activity and Fibrosis (SAF) score [15-18].

In attempts to better understand the onset and progression of NASH and find therapeutic targets for its treatment, a number of different mechanisms have been described [14,19-21]. Mitochondrial dysfunction (e.g. decreased β-oxidation and increased oxidative stress), impaired lipid metabolism (e.g. nuclear receptors and transcription factors implicated in *de novo* lipogenesis) and excretion (e.g. bile acids synthesis and lipoproteins homeostasis) have been raised to play a role [11,14,19-25], but so far, no mechanism specifically linked to NAFLD, and especially NASH, has been pinpointed to explain accumulation of the lipids in the liver and predict the progression of the disease.

Therefore, because prevalence and severity of NASH associated with its hepatic and systemic complications are dramatically increasing worldwide, there is an urgent need to find remedies to decrease or even stop its progression. The first line of intervention is the lifestyle changes. But, academic and pharmacological laboratories are also focused on developing drug therapies. In this review, we will focus on the recent advances in NASH treatments, the failures and the hopes.

## Life style modifications

**Diet:** Lipid overload in the liver is mainly due to high-caloric diets also known as Western Country diets. The high content of lipids such as cholesterol saturated and n-6 unsaturated fatty acids (i.e., decrease in n-3 to n-6 polyunsaturated fatty acids ratio) combined with high sugar content (mainly fructose) has been implicated in the occurrence and progression of NASH [26]. The obvious goal would be to modify the diet by decreasing cholesterol, Saturated Fatty Acid (SFA) and fructose intake, and increase n-3 Polyunsaturated Fatty Acid (PUFA) intake. The whole caloric intake has to be decreased by 25% in calories from the normal diet

based on patient's sex and age according to the World Gastroenterology Organization Global Guidelines [27-30].

Small observational studies showed that a weight loss exceeding 7% of body weight over 1 year improves histology in patients with biopsy-proven NASH [31,32]. More durable weight loss can be achieved in patients with NAFLD by combining diet and exercise for a period longer than one year [33]. It is now recommended that hypocaloric diet should provide 1000-1200 calories per day for women and 1200-1500 calories per day for men with a goal to achieve a weight loss of 0.5-1.0 kg per week based on published guidelines [34].

It has been shown that moderately calorie-restricted diet with changes in macronutrient composition leads to better results compared to a very-low-caloric diet with 5% to 10% weight loss as the goal [35]. Furthermore, low-fat diet associated with weight loss program improves body weight, Body Mass Index (BMI), fatty liver, insulin sensitivity and plasma triglycerides [36]. Importantly, heavy alcohol consumption should be avoided in NAFLD patients [37].

Mediterranean diet due to preparation with olive oil, rich in n-3 PUFA, might be of additional help to modify hepatic lipid content and decrease cardiovascular risks [38]. Recently, a randomized controlled trial named Mediterranean Dietary Intervention for Adults with Non Alcoholic Fatty Liver Disease (MEDINA) enrolling 94 patients with NAFLD associated with insulin resistance and receiving either Mediterranean diet or low-caloric diet during 3 months, showed that Mediterranean diet can result in significant benefits in liver fat and Insulin Resistance (IR), independent of weight loss. These changes are sustained at 12 months. However, liver status was only assessed using magnetic resonance spectroscopy. Hence, this study failed to explore further NAFLD progression [39]. Another study directly assessed effects of Mediterranean diet on liver histology and showed that Mediterranean diet was associated with lower probability of development of high grade of hepatic steatosis and NASH [40,41].

Other studies showed that diets enriched in vegetable oils such as canola, olive pomace and olive oils might improve the grade of hepatic steatosis and insulin sensitivity, especially the combination of olive and canola oils [42]. Indeed, the impact of n-3 PUFA has been investigated in the randomized WELCOME trial and showed that high dose of n-3 PUFA (docosahexaenoic + eicosapentaenoic, Omacor®) did not improve microvascular function but was associated with a decrease in hepatic fat content and improvement in NAFLD severity [43-45].

Other studies have been conducted to test different nutrients enriched in Monounsaturated Fatty Acids (MUFA) and n-3 PUFA such as oily fish/fish oil, nuts and avocado and showed improvement in liver fat and steatosis grading, liver function and insulin sensitivity [46-49]. Some recommendations have been made about the optimal consumption of each nutrient (e.g. avocado, nuts, and olive oil) and the amount per day [48].

Some groups have also studied effects of tea, coffee and caffeine consumption on hepatic steatosis, NASH and fibrosis and showed that there was a negative correlation between caffeine consumption and hepatic fibrosis in overweight or obese patients with NASH [50-52]. In addition, coffee may reduce cardiovascular risk factors by favorably affecting inflammation, insulin sensitivity and decreasing hypertension [53,54]. Thus, caffeine and its derivatives might have a potential to slow down fibrogenesis during NASH progression but further investigation has to be done to assess their effects on NASH development.

In conclusion, the optimal diet for NAFLD patients is still undetermined. However, patients with NAFLD may benefit from a moderate- to low-carbohydrate (40%-45% of total calories) diet, coupled with high MUFA, high n-3 PUFA, low SFA, and low-cholesterol diet.

**Physical activity:** Associated with the unhealthy diet, sedentary lifestyle contributes to the occurrence of obesity and NAFLD. Changes in lifestyle by increasing physical activity have been shown to improve triglyceride turnover and liver fat accumulation leading to increased hepatic insulin sensitivity and whole lipid oxidation in the body and decreased hepatic free fatty acids uptake independently of weight-loss [26,30]. But in both cases, the crucial issue is the motivation. Indeed, the first significant weight-loss improvement might appear after 6 months of training, which may discourage the patients. Therefore, cognitive-behavioral therapy is needed [41,55,56]. A systematic review of data from various randomized controlled trials showed that exercise reduced hepatic fat content [57]. The European guidelines suggest at least 150 min per week of moderate-intensity physical activity and at least 75 min per week of vigorous-intensity physical activity, with additional muscle strengthening exercise twice a week [58].

**Surgery:** Based on the idea that NAFLD/NASH progresses due to the excessive food intake and loss of satiety feeling (e.g. leptin resistance), bariatric surgery may improve NAFLD by reducing food intake [59]. This therapeutic approach is recommended for patients with morbid obesity (>40 kg/ m$^2$) or severe obesity (>35 kg/ m$^2$) associated with complications [60,61]. This surgery is offered to the patients that were not able to lose weight after a period of non-surgical treatment [60,61]. Several techniques have been reported including sleeve gastrectomy, gastric band and Roux-en-Y gastric bypass. Currently, mini-invasive procedures are favored such as laparoscopic approach [62]. Bariatric surgery has been shown to improve insulin sensitivity, dyslipidemia, systemic hypertension, decrease CVD risks and improve the histological and biochemical parameters of NAFL and NASH [63]. Bariatric surgery is also associated with the most rapid, effective and sustained weight loss [64,65]. A recent study showed that bariatric surgery-induced the disappearance of NASH from nearly 85% of 109 morbidly obese patients [66]. However, recently Goossens et al. showed that obese patients with NASH undergoing bariatric surgery had an increased risk of death compared to obese patients without NASH before surgery. This study emphasizes the importance of the systemic perioperative liver biopsy in obese patients undergoing bariatric surgery as a diagnostic and prognostic assessment of the death risk [67], and also suggests that NASH should be managed, in addition to changes in lifestyle, with drug therapy to decrease the death risk for these patients. At the same time another study has found no correlation between NASH and death after bariatric surgery, so the exact relationship between the two, and potential risk factors are still not clear [68].

## Drug therapy

Because in addition to obesity NASH progression is associated with IR/type 2 diabetes and dyslipidemia, the goal for drug therapy is to improve general conditions by increasing the whole body insulin sensitivity, decreasing lipid absorption, hepatic *de novo* lipid synthesis and promoting lipid oxidation in skeletal muscle and liver. In this chapter, we will focus on the recent advances in drug therapies of NASH.

**Insulin sensitizers:** The first idea to treat NASH was to use insulin sensitizers metformin and thiazolidinediones (e.g. pioglitazone, rosiglitazone), which are used extensively to treat IR/type 2 diabetes. Results of the studies of potential effects of metformin on the development of NASH are contradictory. Recently, a meta-analysis showed no benefit of metformin in improving serum aminotransferases or liver histology among NAFLD patients [69]. Thiazolidinediones (TZDs) are selective peroxisome proliferator–activated receptor-gamma agonists. Pioglitazone demonstrated the benefit with histological improvements in NASH patients such as hepatic steatosis, lobular inflammation and ballooning degeneration [70,71]. Furthermore, two recent meta-analyses confirmed the histological improvement of NASH in patients treated with pioglitazone [72,73]. In addition, pioglitazone demonstrated mortality

reduction related to CVD. However, pioglitazone has received black box warning by the Food and Drug Administration due to reports of congestive heart failure. Previously, rosiglitazone has been prohibited in Europe and highly restricted in USA [74-76]. Despite their effects on reducing hepatic gluconeogenesis, intestinal lipid absorption, lipogenesis, lowering serum lipid concentration, improved liver enzymes and increasing global insulin sensitivity, these molecules failed assessments for long-term benefit as they are associated with weight gain, edema, heart failure, risk of CVD and cancer (e.g. bladder cancer) [74-76].

**Antioxidants:** Because NASH development and progression are associated with oxidative stress, another treatment line is the use of antioxidants. Currently, the most commonly used antioxidant is vitamin E supplementation given at 800 IU per day. It improves inflammation and fibrosis induced by suppression of lipid peroxidation and oxidative stress [77-79]. Several studies suggest that vitamin E improves liver enzymes, hepatic steatosis, and liver injury in NASH patients but robust data including prospective evaluation are currently lacking. Furthermore, deleterious effects and an increase in mortality rate have been suggested in a long-term exposure [80]. Further studies are required to clarify the beneficial role of vitamin E monotherapy in NASH patients.

Despite these effects, several studies have evaluated vitamin E. One important study compared effects of vitamin E and insulin sensitizers. 247 biopsy-proven NASH patients without diabetes were randomized to receive pioglitazone 30 mg, vitamin E 800 IU, or placebo for 96 weeks [79]. Vitamin E was superior to placebo with a significantly higher rate of improvement in NASH (43% vs. 19%, *p = 0.001*) whereas pioglitazone was not but showed significant benefits in some of the secondary outcomes (decrease in aminotransferase, hepatic steatosis and lobular inflammation).

Pentoxifylline is another antioxidant that has shown benefit through decreasing oxidative stress. In a randomized trial enrolling 55 biopsy-proven NASH patients, it improved histological features of NASH compared to placebo [81]. This result has been confirmed in a recent meta-analysis [82]. Although these results are encouraging, their validation in a large cohort with a longer follow-up is mandatory.

**Cholesterol-lowering drugs:** Hepatic cholesterol overload due to increasing in hepatic cholesterol synthesis and in intestinal cholesterol absorption participate strongly in the development and progression of NASH [11,12]. A therapeutic strategy is to antagonize both *de novo* hepatic cholesterol synthesis and/or cholesterol absorption by the gut.

Statins inhibit cholesterol synthesis by targeting the Hydroxy-Methyl-Glutaryl-Coenzyme A (HMG-CoA) reductase. Therefore, randomized controlled trials have been conducted in patients with NAFLD. Although statins decreased lipid levels, simvastatin did not induce significant improvement in serum aminotransferase levels, hepatic steatosis, necroinflammatory activity, or stage of fibrosis in NASH patients when liver biopsies were conducted [83].

Recently, a preliminary report from 6 patients showed that rosuvastatin could improve NASH within a year of treatment in patients with dyslipidemia [84]. The next step of this study will be to do randomize control trial in a larger cohort of NAFLD/NASH patients with more than a year of follow-up to assess if these effects on NASH improvement are transient and to assess the side effects of the statins (statin-associated muscle symptoms) that may lead to discontinuation of treatment [85].

Statins combination therapy with ezetimibe, an inhibitor of intestinal cholesterol absorption, has been used to decrease the dose of statin and led to a significant decrease in hepatic cholesterol and plasma LDL level. However, the effect of ezetimibe alone or combined with diet is still controversial in terms of improvement of NASH [86-89]. Thus, the new randomized-control study in a larger cohort of patients using ezetimibe alone or combined with diet and/or statins needs to be conducted to

assess the beneficial effect on NASH features.

**FXR agonists:** Farnesoid X Receptor (FXR) is a nuclear receptor implicated in the regulation of different genes involved mainly in glucose, bile acid and lipid metabolism. Because the main causes of lipid accumulation in NASH are the excess of lipids and carbohydrates from diet, increase in *de novo* lipid synthesis and decrease in lipid oxidation associated with a decrease in hepatic lipid excretion, FXR is becoming one of the most interesting drug targets.

FXR is a bile acid sensor that recognizes specific DNA response elements and binds to them as a heterodimer with retinoid X receptor. Binding of bile acids to FXR leads to the repression of expression of rate-limiting enzymes in the synthesis of bile acids such as cytochrome P450 (CYP) 7A1 and CYP8B1. FXR inhibits *de novo* lipid synthesis by inducing repression of hepatic Sterol Regulatory Element Binding Protein 1c (SREBP1c), a transcription factor important for the synthesis of fatty acids and triglycerides. The FXR also inhibits the transcriptional activity of Carbohydrate Response Element Binding Protein (ChREBP), a transcriptional factor implicated in gluconeogenesis and triglyceride synthesis. In addition, FXR interacts with and inhibits directly ChREBP and hepatocyte nuclear factor 4 alpha (HNF4α) proteins [23]. HNF4α is also implicated in the synthesis of bile acids by increasing CYP7A1 and CYP8B1 [23,90].

Fibroblast Growth Factor 21 (FGF21), one of the FXR target genes mainly expressed in the liver, increases fatty acid oxidation, adiponectin secretion and decreases leptin levels. FGF21 also inhibits lipogenesis by repressing SREBP1c and decreases triglyceride and blood glucose levels [91]. Therefore, FGF21 actions have positive effects on plasma lipid levels and hepatic steatosis [92,93].

At the same time, FXR activates expression of hepatic genes involved in lipoprotein clearance from the plasma such as LDL receptor, SR-B1 (i.e., HDL receptor) and also molecules that regulate lipoprotein lipase [63,94,95]. Therefore, bile acids and derivatives have been investigated as the treatment for NASH.

Ursodeoxycholic Acid (UDCA) is a secondary hydrophilic bile acid usually made by the intestinal microbiota. UDCA is used to treat primary biliary cirrhosis [96]. Then, in several studies UDCA has been tested to treat NASH and controversial conclusions about the efficacy of the treatment to reduce NASH lesions and to improve clinical parameters have been drawn. Some studies have shown that after at least one year of treatment, UDCA improved hepatic steatosis and enzymes and the high dose of UDCA treatment also improved serum fibrosis markers, glycemia and IR [97,98]. On the other hand, a study by Leuschner, et al. [99] did not show any beneficial effect of high-dose of UDCA on overall histology features of NASH. Recently, a long-term study combining UDCA with vitamin E showed improvement in liver function (AST, ALT, γ-GT) among the 101 patients enrolled. Out of those, 10 patients underwent a biopsy and histology were performed before and after treatment. Five patients did not improve their NAS score (0 or-1), one patient had an increased NAS score, and 4 patients showed a decreased of at least -2 points of NAS score. Also, during the study period eighteen (n = 18) patients stopped the treatment because of diarrhea, nausea, pruritus, ineffectiveness, or spontaneously (n = 9) and the other (n = 9) because of pregnancy or normalization of biochemical parameters [78]. These results do not seem very conclusive, which is also supported by a few other papers debating on the subject [100-102]. A larger cohort is needed to understand and to better identify the responder and non-responder patient populations. At this point, UDCA is not recommended for the treatment of NAFLD or NASH by the Food and Drug Administration in USA [37].

Nonetheless, bile acid conjugates have recently been tested such as Ursodeoxycholyl lysophosphatidylethanolamide (UDCA-LPE) that has been used on high-fat diet mouse model and showed a a modification in

fatty acid metabolism, hepatoprotective and anti-inflammatory effects [103-106]. Moreover, it has been shown that UDCA-LPE modulates flawed fatty acid metabolism in mice fed High-Fat Diet (HFD) thus restoring altered lipid profiles and has pronounced anti-inflammatory effects [107]. Further investigations have to be done in patients with NASH.

Obeticholic acid, a synthetic bile acid acting as a ligand of FXR, showed efficacy in animal models [108]. Therefore, a multicenter, double-blind, placebo-controlled (1:1), randomized phase II clinical trial has been conducted by NASH Clinical Research Network in non-cirrhotic and non-alcoholic steatohepatitis (FLINT) [109]. The study showed improvement in histological features of NASH in 50% of 141 patients. These encouraging results have been balanced by side effects as 33 (23%) of 141 patients in the obeticholic acid developed pruritus compared with nine (6%) of 142 in the placebo group. Also, another study showed that obeticholic acid treatment had the same results on NAFLD activity score (-1.7 SD 1.8) as vitamin E (-1.9 SD 2.1) and pioglitazone (-0.7 SD 1.8) [79]. Less than 50% of patients are responders, mainly patients with diabetes, leaving a substantial proportion of patients with NASH without an effective treatment [110].

Singh, et al. [111] performed a Bayesian network meta-analysis combining treatment comparison to assess the efficacy of pharmacological compounds for the treatment of NASH. All studies included in the analysis used biopsy-proven NASH and compared effects of vitamin E, TZDs, pentoxifylline or obeticholic acid to each other or placebo. Interestingly, they showed that depending on the drugs or combination of drugs, improvement of histological features was not the same. Pentoxifylline and Obeticholic acid improved fibrosis, whereas vitamin E, TZDs and Obeticholic acid improved ballooning degeneration. Further investigation using combinations of drugs has to be done to increase efficacy (i.e., every patient with NASH improved) and decrease side effects (i.e., decrease digestive perturbation and pruritus). Another FXR agonist, the newly renamed GS 9674 is currently being evaluated in a phase I study (ClinicalTrials.gov NCT02654002). Hence, bile acid derivatives seem promising for NASH treatments.

## Prebiotics, probiotics and "synbiotics" as treatments of NASH

For more than a decade, microbiota has been in the focus of attention of the scientific community especially when it comes to its role in the development and progression of chronic liver diseases such as NAFLD, cirrhosis and HCC [112-116]. Several studies have suggested that alterations in intestinal microbiota and inflammatory response might play a central role in the development and progression of NASH [117-131]. Following this idea, the goal is to modify gut microbiota by using either specific nutrients, or directly by ingesting specific mix of microbes or by using both approaches also known as prebiotics, probiotics or "synbiotics", respectively.

**Prebiotic treatments:** Prebiotics are defined as a group of nutrients composed mainly of non-digestible carbohydrates (e.g. fibers, fructo-oligosaccharides) that beneficially affect the host by altering the composition and thus the activity of the gut microbiota [132]. Mouse model of NASH treated with Fructo-Oligosaccharides (FOS) showed changes in their gut microbiota and a decrease in n-3 PUFA synthesis modulating hepatic steatosis toward changing in gene expression in the liver [133]. In rats fed HFD, FOS supplementation prevented deleterious effects of HFD such as alterations in lipid profile and hepatic morphologic changes [134]. Also, mice fed HFD supplemented with 6% hydroxypropyl methylcellulose, usually used as emulsifier, thickening and suspending agent, showed improvement on intestinal permeability, insulin resistance, hepatic lipid accumulation, glucocorticoid-related bile acid recycling, oxidative stress, and weight gain [135]. Because thiazolidinedione treatment leads to weight gain, Alligier, et al. [136] used prebiotic treatment to counteract the side effects of TZDs in animal

models. Inulin-type fructan prebiotics decreased adiposity and improved the metabolic response in HFD-fed mice treated with TZDs.

In human clinical trials, FOS has been used to treat NAFLD but did not show efficacy in NASH improvement as observed in animal models. This lack of efficiency in treated patients might be due to power, randomized, controlled clinical trials, involving various centers and the population of different origin, but also to assessing NASH improvement mainly by measuring ALT, a non-specific marker of NASH, and using different doses of prebiotics [137,138]. Recently, a double blinded, placebo controlled, parallel group study, adults (BMI ≥ 25 kg/ m$^2$) with confirmed NAFLD randomized to either a 16 g/day prebiotic or isocaloric diet, was conducted. However, the study is still in progress and no results are available yet [139].

**Probiotic treatments:** A direct approach to modifing intestinal microbiota are probiotics -live microorganisms- which, when administered properly, confer beneficial effects on the host [140]. Therefore using probiotics to modulate gut microbiota and to improve NASH have been considered for a decade [141,142]. Interestingly, studies on mouse models of NASH showed that probiotic VSL#3®, a mixture of eight probiotics, modulated liver fibrosis but did not decrease steatosis and inflammation in liver [143], whereas in ApoE deficient mice, VSL#3® corrected insulin resistance in liver and adipose tissues and protected against development of steatohepatitis [144]. On the other hand, mouse fed high-fat/ high sucrose diet and treated with *Lactobacillus paracasei* showed attenuation of hepatic steatosis and increased M2-dominant Kupffer cells in a NASH model [145]. In rat models of NASH a specific probiotic inducing butyrate production, *Clostridium butyricum* (MIYAIRI 588), showed a beneficial effect in the prevention of NAFLD progression [146]. Supplementation on sodium butyrate in mice fed Western diet were also protected from inflammation in the liver and thus from the development of NASH [147].

Despite the controversial results in animal models, clinical trials using probiotics have been recently conducted with promising results. A first pilot study was conducted on 28 patients with histology-proven NAFLD, who were analyzed in a double-blind randomized clinical trial. Patients were randomized to one of the following treatments during 3 months: group I, treated with one tablet per day with 500 million of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and group II, treated with one placebo tablet (120 mg of starch) showed improvement on liver aminotransferases levels in patients with NAFLD but no data are available on liver histology [148]. Another pilot study including patients with NASH assessed by histology was randomized to receive probiotics (n = 10) or usual care (n = 10) during 6 months. The Lepicol® probiotic formula contains *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium bifidum* combined with fiber (Psyllium husks) and inulin. This proof of concept study showed promising results in reducing liver fat and AST level in NASH patients [149]. The same group conducted a study with a larger group of patients (n= 22 controls and n=16 NASH patients) and looked at gut microbiota. They showed that NASH patients had fecal dysbiosis and changes in microbiota correlate with improvement in hepatic steatosis [150].

**Synbiotic treatments:** Combining both prebiotics and probiotics approaches (i.e., synbiotics) might further improve the effect of each treatment given alone. Based on this idea, a larger study was conducted by another group including random trials that involved patients with NASH assessed by histology before and after the end of the study. Patients were divided into two groups one of which received *Bifidobacterium longum* with FOS and lifestyle modification (i.e., diet and exercise) while the other had lifestyle modification alone. The group of patients receiving *Bifidobacterium longum* with FOS and lifestyle modification had decreased inflammation, serum AST, HOMA-IR, serum endotoxin, hepatic steatosis, and the NASH activity index compared to the lifestyle modification

group. Importantly, a decrease in BMI was observed in both groups of patients suggesting that pre- and/or probiotics have to be associated with a different therapeutic effect [151]. Recently, a randomized, double-blinded, placebo-controlled clinical trial was conducted as a pilot study on 52 patients with NAFLD diagnosed on the basis of the presence of hepatic steatosis on ultrasound examination, fibrosis score determined by transient elastography, and with a persistently elevated ALT concentration (60 U/L) during 6 months before the study. The patients were randomized and the follow-up was conducted at 7, 14, 21 and 28 weeks. Data showed that synbiotic supplementation, in addition to lifestyle modification, has a better outcome compared to lifestyle modification alone for the treatment of NAFLD, at least partially through attenuation of serum inflammatory markers. These effects were seen at the beginning of week 14, and this trend was sustained until the end of the study [152]. The main downsides of this study are that liver histology was not performed, making it difficult to make a conclusion about the improvement of NASH, and uncertainty as to whether these effects will be sustained with longer treatment durations.

Taken together these results underscore the fact that more randomized studies with larger cohorts have to be conducted. Also, more combinations of bacteria need to be tested on a larger number of patients during a longer time period, which will allow to completely changing their gut microbiota. This treatment also has to be associated/combined with other approaches (e.g., control of diet, lifestyle changes) and drug treatments.

## Other drugs under evaluation

The elafibranor, a potent agonist of PPARα/δ, recently completed a phase IIb in 276 patients with NASH in Europe and USA [153]. This drug has shown significant activity on the regression of NASH and on markers of liver fibrosis, as well as significant improvement in cardiovascular risk. Elafibranor should start phase III clinical trials in 2016.

C-C chemokine receptors type 2 and 5 (CCR2 and CCR5, respectively) were shown to be implicated in inflammation and fibrosis. Cenicriviroc, an antagonist of CCR2 and CCR5, is in phase IIb development in a multicenter study. This study included patients with NASH and fibrosis. The goal of this study is to treat patients with cenicriviroc to improve NASH activity score without worsening fibrosis [154].

Finally, simtuzumab and Apoptosis Signal-regulating Kinase 1 (ASK1) inhibitor (GS 4997), are also being evaluated alone or in combination (NCT02466516). ASK1 is implicated in apoptosis process and inflammation. Using ASK1 inhibitor might decrease and limit inflammation during NASH progression [155].

## Next step approaches

**Adiponectin receptor agonists:** Adiponectin is a hormone secreted from adipose tissue and binds to adiponectin receptors AdipoR1 and AdipoR2 to activate LKB1-AMPK and PPARα, respectively. Adiponectin binding to AdipoR1 decreases neoglucogenesis, fasting glucose and SREBP1c activation whereas binding to AdipoR2, it increases fatty acid oxidation and energy expenditure. Thus, adiponectin has antidiabetic effects and limits steatosis development [156].

Recently, a synthetic small molecule named AdipoRon has been tested on obese rodent model db/db mice. Data showed that db/db mice treated orally by AdipoRon have an insulin resistance index and plasma glucose level decreased by acting on white adipose tissue, muscle and liver. In addition, mice fed high fat and treated daily at 30 mg per kg body weight with AdipoRon showed an increase in longevity. Therefore, AdipoRon could achieve the same outcome much like caloric restriction and exercise. Moreover, AdipoRon showed an anti-inflammatory effect [157].

Taken together, small-molecule AdipoR agonists are very promising candidates for NASH treatment. Further experiments have to be done in rodent models that developed NASH to assess their efficacy.

**Honokiol therapy:** Honokiol, a major phenolic constituent isolated from *Magnolia officinalis* extracts, has been reported to have several pharmacological effects, including anti-inflammatory, anti-thrombosis, antioxidant effects and anti-cancerous effects. For few years now honokiol has been shown to have liver protective effects, especially by decreasing fatty liver through the decrease in SREB1c activity and AMPK-LKB1 pathway [158,159]. Therefore, honokiol significantly inhibited SREBP1c maturation and the transcription of lipogenic genes such as Stearoyl-CoA Desaturase-1 (SCD-1) and Fatty Acid Synthases (FAS) in fatty liver [159]. Also, in high-fat diet mouse model of NAFLD, honokiol and magnolol (also a phenolic constituent isolated from *Magnolia officinalis*) showed a significant decrease in fatty acid accumulation in the liver through the activation of AMPK leading to the inhibition of LXRα-SREBP1c pathway [160]. These drugs might be interesting to control fatty liver accumulation but further investigations need to be done, especially regarding efficacy and safety (i.e., assessing side effects) in patients with NASH.

**Nicotinamide riboside drug approach:** One of fatty acid overload mechanisms in patients with hepatic steatosis and NASH is mitochondrial dysfunction leading to a decrease in fatty acid β-oxidation and oxidative phosphorylation, decrease in $NADH/H^+$ oxidation into $NAD^+$ (i.e., $NAD^+$ repletion) by the mitochondrial respiratory chain complex leading to the leak of electrons and overproduction of Reactive Oxygen Species (ROS) and lipid peroxidation damaging the mitochondria and leading to a vicious circle [14,19-22,24,25,161]. Indeed, $NAD^+$ is implicated in numerous physiological processes such as caloric restriction, muscle contraction/heart beating, exercise, circadian rhythms, senescence, kidney, and liver functions.

Recently, Gariani, et al. [162] by using existing liver tissue datasets and high-fat/high-sucrose diet in animal models demonstrated that reduction in hepatic mitochondrial content, function and ATP levels associated with $NAD^+$ depletion leads to an increase in liver weight, lipid content and lipid peroxidation. Therefore, using a precursor of $NAD^+$ biosynthesis (Nicotinamide Riboside) as preventive or therapeutic strategy, the authors demonstrated the prevention or the reversion of the NAFLD toward activating sirtuin-1 and -3 pathway leading to an increase in β-oxidation, mitochondrial content and activity These data led to an increased use of nicotinamide riboside to boost $NAD^+$ biosynthesis to manage the development or progression of NAFLD in clinical trials [163]. In addition, nicotinamide riboside will target and stimulate the mitochondria in the liver to catalyze the excess of free fatty acids, ergo nicotinamide riboside will also increase ROS production, a second hit during the progression of NASH [14,19,21]. These might be a limitation for long-term treatment even if the drug is associated with ROS scavenger such as α-tocopherol (i.e., tocopheryl acetate or vitamin E). Because nicotinamide riboside also is not tissue-specific, long-term treatment might have systemic consequences and then side effects. Therefore, further investigations have to be done before using nicotinamide riboside in double-blind, randomized clinical trial to assess efficacy, side effects and safety (i.e., toxicity on other organs).

## Conclusion

NASH associated with metabolic syndrome can progress to advanced fibrosis and cirrhosis. Weight loss and lifestyle modification have been shown to improve NASH. Other medications used for weight loss and metabolic syndrome have been evaluated such as metformin and thiazolidinediones. Alternative regimens using ursodeoxycholic acid, statins and probiotics as well as bariatric surgery have been evaluated but have not been recommended as first-line treatment for NASH. Vitamin E for NASH patients without diabetes seems to be promising. Many molecules are in the pipeline of the development for the treatment

of NASH, the most advanced being the FXR agonists. The lack of effective treatment for NASH suggests the heterogeneity of patients presenting with the NASH phenotype. Indeed, NASH has many underlying causes of genetic (i.e., differences between individuals) to environment (i.e., geography, diets, socio-cultural differences) with similar histological features. Therefore, no single treatment exists. The best treatment strategy for these patients may be to identify their pathogenic target and develop personalized treatment protocols.

But most importantly, the difficulty in diagnosing NASH without biopsy and therefore, the absence of noninvasive biomarkers makes it difficult to enroll patients in large clinical trials limiting the development of the new molecules.

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