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# ABOUT COVER

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Is there a role of lipid-lowering therapies in the management of fatty liver disease?

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Abstract

Atherogenic dyslipidemia is characterized by increased triglyceride-rich lipoproteins and low high-density lipoprotein cholesterol concentrations. It is highly prevalent in non-alcoholic fatty liver disease (NAFLD) and contributes to the increased cardiovascular risk associated with this condition. Alongside insulin resistance it plays an important pathogenetic role in NAFLD/non-alcoholic steatohepatitis (NASH) development and progression. It has been shown that cholesterol-lowering reduces cardiovascular risk more in NAFLD vs non-NAFLD high-risk individuals. This evidence highlights the importance of effective lipid modulation in NAFLD. In this narrative review the effects of the most commonly used lipid-lowering therapies on liver outcomes alongside their therapeutic implications in NAFLD/NASH are critically discussed. Preclinical and clinical evidence suggests that statins reduce hepatic steatosis, inflammation and fibrosis in patients with NAFLD/NASH. Atorvastatin and rosuvastatin showed greater benefits, whereas data for other statins are scarce and sometimes conflicting. Similar studies to those of statins showed efficacy of ezetimibe against hepatic steatosis. However, no significant anti-inflammatory and anti-fibrotic actions of ezetimibe have been shown. Preclinical studies showed that fibrates through peroxisome proliferator-activated receptor (PPAR)α activation may have a role in NAFLD prevention and management. Nevertheless, no relevant benefits have been noted in human studies. Species-related differences in PPARα expression and its activation responsiveness may help explain this discrepancy. Omega-3 fatty acids reduced hepatic steatosis in numerous hetero-
Non-alcoholic fatty liver disease (NAFLD) has become a pandemic with an estimated prevalence of 24%, 30% and 32% in Europe, America and Middle East respectively[1, 2]. It has epidemiologic and pathophysiologic links with obesity, type 2 diabetes mellitus, dyslipidemia, unhealthy lifestyle patterns and the metabolic syndrome[3]. Its increasing prevalence is strongly associated with the corresponding rise in those conditions globally.

NAFLD histologic spectrum varies from steatosis alone to non-alcoholic steatohepatitis (NASH) that also encompasses various degrees of necroinflammation and fibrosis. Cirrhosis with or without portal hypertension leading to death or liver transplantation are the liver-specific endpoints of NAFLD/NASH[4-6]. Concerningly, NASH-associated liver transplantation cases increased by 170% from 2004 to 2013 in the United States[5].

NAFLD is typically asymptomatic or presents with non-specific symptoms particularly in more advanced forms. It is biochemically characterized by variable elevations in liver enzymes, mainly of elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (γ-GT) activities. Of note, liver enzymes may not be sensitive for NAFLD diagnosis, hence many NAFLD cases may be undiagnosed via routine biochemical screening. It was estimated that up to 60% of patients with advanced NAFLD have normal liver enzymes[7, 8]. Coagulopathy and abnormalities of albumin and bilirubin levels are typically encountered in more advance stages of the disease.

Liver imaging aims to reveal hepatic steatosis and determine whether NAFLD is accompanied by a degree of fibrosis. Liver ultrasound and magnetic resonance imaging (MRI) or computed tomography (CT) are the most frequently imaging modalities in this respect[9]. Vibration controlled transient elastography is used to grade the different levels of liver fibrosis[9]. Liver biopsy remains the ‘gold standard’ for the diagnosis and staging of NASH as well as for identifying cirrhosis[10].

Key Words: Non-alcoholic fatty liver; Non-alcoholic steatohepatitis; Statin; Ezetimibe; Fibrates; α-3 fatty acids; Bile acid resins

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Cirrhosis and hepatocellular carcinoma account for 4%-8% and 1%-5% of NAFLD-associated mortality respectively[1]. However, cardiovascular (CV) disease is the major mortality cause accounting for approximately 40% of all deaths[11]. NAFLD has been recognized as a risk factor for CV disease by various analyses[12]. However, it is difficult to dissociate this correlation from the high prevalence of concomitant abnormalities representing traditional CV risk factors in NAFLD. These include, but are not limited to obesity, type 2 diabetes, metabolic syndrome and atherogenic dyslipidemia. In fact, it was suggested that it is not the hepatic steatosis itself but the constellation of those metabolic abnormalities contributing to the increased CV risk in NAFLD[13].

To prevent from the progress and complications of NAFLD its early identification and management is crucial. Mainstay treatment involves lifestyle modifications, such as weight management, alcohol restriction, regular exercise and dietary intervention [14-17]. To date, there are no evidence-based drug therapies recommended for the management of NAFLD/NASH leaving a significant unmet clinical need[18]. Nevertheless, pharmacotherapy to address the increased CV risk through anti-obesity, anti-diabetic and lipid-lowering drugs is commonly used in clinical practice[19].

**Dyslipidemia and NAFLD**

Insulin resistance and the associated dyslipidemia play an important pathogenetic role in NAFLD. Insulin is a key player in lipid metabolism by promoting triglyceride (TG) storage into adipose tissue and by inhibiting hepatic production of the TG-rich very low-density lipoproteins (VLDL). Also, the catabolism of VLDL and their remnants via lipoprotein lipase is partly dependent on insulin action. In contrast, insulin resistance is associated with impaired fat storage in adipose tissue resulting in increased influx of non-esterified fatty acids (NEFAs) to the liver and subsequent hepatic fat accumulation. Besides, high cholesterol diet and increased cholesterol influx to the hepatocytes seems to promote de novo lipogenesis (through mechanisms that are explained further below) and hepatic steatosis. Free unesterified cholesterol further promotes pro-inflammatory and pro-fibrotic pathways which facilitate progression of NAFLD to NASH and/or cirrhosis[13].

Liver NEFAs further serve as substrate for increased VLDL hepatic production. This abnormality alongside an impaired catabolism of TG-rich lipoproteins results in increased concentrations of the atherogenic apolipoprotein(apo) B-rich lipoproteins in NAFLD. Subsequent lipolysis of these lipoproteins results in increased production of the atherogenic small dense LDL particles[13]. Furthermore, cholesteryl ester transfer protein (CETP) facilitates cholesteryl ester shift from high density lipoproteins (HDL) to TG-rich lipoproteins in exchange for TG. In insulin resistant states such as NAFLD, both TG-rich lipoprotein concentration and CETP activity are enhanced. These abnormalities result in TG-enriched HDL particles which are easily eliminated via the hepatic lipase. Also, in NAFLD there is reduced hepatic and intestinal production of the anti-atherogenic apoA1 (the main apolipoprotein of HDL) through decreased adiponectin levels. Through these mechanisms HDL cholesterol (HDL-C) levels are typically reduced in NAFLD. This profile is associated with impaired endothelial function and reverse cholesterol transport by HDL. Other antiatherogenic, including anti-inflammatory, antioxidant and anti-thrombotic effects of HDL are diminished too [20,21].

In summary, the serum lipid profile in NAFLD is similar to the atherogenic dyslipidemia encountered in other insulin resistant states, including the metabolic syndrome and type 2 diabetes. It is characterized by increased TG, non-HDL cholesterol and apoB levels together with low HDL-C and apoA1 Levels. Furthermore, a predominance of the small dense LDL particles further adds to atherogenicity. The same abnormalities seem to contribute to the maintenance and progression of NAFLD. All these considered, aggressive management of dyslipidemia is important to prevent CV disease, while it might be helpful in reducing liver-specific complications in NAFLD.

In this narrative review, a possible therapeutic role of the most commonly used lipid-lowering therapeutics against NAFLD/NASH is discussed.

**Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors)**

Statins are potent cholesterol-lowering agents and evidenced-based drugs to reduce CV outcomes. Their cholesterol-lowering efficacy is expected to be beneficial for NAFLD, which is characterized by aggregation of free cholesterol into hepatocytes[22, 23]. Besides, statins have been suggested to exert variable lipid-independent pleiotropic benefits, including antioxidant, anti-thrombotic, anti-fibrotic and anti-inflammatory actions as well as endothelial function improvement. Except for their protective role against atherothrombosis these effects may play a role in the prevention
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and management of NAFLD/NASH[22,24-26].

Mechanistic implications

Free unesterified cholesterol appears to be toxic to the hepatocytes by promoting liver inflammation and subsequent fibrosis. Namely, cholesterol crystals accumulation into hepatocytes results in inflammatory response via activated Kupffer cells surrounding the steatotic liver cells in crown-like structures. Besides, cholesterol crystals activate NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome within the Kupffer cells. Atorvastatin both alone or in combination with ezetimibe attenuated these effects in high-fat and high-cholesterol diet fed mice after 16 wk. These findings indicated a potential role of cholesterol lowering in NASH prevention[27].

Statins also inhibit the synthesis of isoprenoids, which are mevalonate pathway products. Prenylation/activation of the small guanosine triphosphate (GTP)ases through isoprenoids regulates the intracellular signaling of numerous receptors mediating liver inflammation and fibrosis. Statin-related inhibition of this isoprenoid-dependent process led to significant anti-inflammatory and anti-fibrotic effects in an experimental model of NAFLD[28].

Furthermore, statins may reduce the expression of pro-inflammatory and pro-fibrinogenic mediators. Namely, rosuvastatin significantly decreased the expression of tumor necrosis factor (TNF)α, interleukin (IL)-6, IL-1β, interferon (IFN)-γ and transforming growth factor (TGF)-β1 in a rodent model of hepatocellular carcinoma (HCC) fed with high-fat and high-cholesterol diet. These effects alongside a reduced expression of the vascular epidermal growth factor receptor (VEGFR), the epidermal growth factor receptor (EGFR) and the platelet-derived growth factor (PDGF) suggested a protective role of rosuvastatin against HCC associated with NAFLD/NASH[29].

Furthermore, antioxidant actions of statins may be beneficial against NAFLD/NASH. Peroxisomes play a key role in the maintenance of intracellular redox balance. Statins were suggested to increase the gene expression of peroxisome proliferator-activated receptor α (PPARα), a regulator of peroxisomal and mitochondrial fatty acid oxidation. Through this mechanism statin treatment limited hepatic steatosis and improved peroxisomal and mitochondrial function in an experimental rodent model [24]. Paraoxonase (PON)1 is another liver-derived enzyme, which is linked to HDL. It has a limiting role in oxidative stress and inflammation by hydrolysing peroxides and lactones associated with lipoproteins. Genetic studies have shown that reduced PON1 activity plays a significant pathogenetic role in NASH[30]. Atorvastatin 40 mg/d was associated with increased PON1 activity in 25 NAFLD patients after 8 mo. This effect was accompanied by significantly reduced serum malondialdehyde levels as a marker of lipid peroxidation, suggesting a promising role of this statin in NASH prevention[25].

Also, in vitro and in vivo experimental evidence suggested that the antioxidant and anti-inflammatory actions of statins can prevent hepatic stellate cells (HSCs) activation and subsequent fibrosis in NASH. This benefit may be mediated via reduced expression of pro-inflammatory genes as well as of reactive oxygen species (ROS), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase gp91 phox subunit, α-smooth muscle actin (α-SMA) and nuclear factor-κ (NF-κ) p65 nuclear translocation[31]. An inhibitory effect of statins on HSCs activation may also be mediated via increased endothelial NO synthase (eNOS) together with reduced inducible NO synthase (iNOS) expression[32].

Furthermore, statins may exert anti-fibrotic effects and reduce portal pressure via improving the nitric-oxide (NO)-dependent liver sinusoidal endothelial cells (LSECs) function. LSECs dysfunction in NASH is considered to precede portal hypertension and its subsequent fibrosis via activation of the HSCs[33].

Clinical evidence

Due to their evidence-based anti-atherothrombotic benefits statins have a key role in addressing the increased CV risk in NAFLD. Interestingly, it was suggested that the reduction of CV events by statin use is greater in high-risk patients with vs without NAFLD[34]. Also, long-term statin use was associated with reduced risk of cancer in NAFLD. This protection was greater with increasing the time of statin exposure, becoming significant after 1 year of treatment[35].

Unfortunately, real-life statin use in NAFLD is often limited by the abnormal pre-treatment liver enzymes and the possibility of their further treatment-induced elevations. This is relevant despite several clinical studies showing that statins may improve liver enzyme activities and limit hepatic steatosis in NAFLD/NASH. However, these benefits have not been reflected by relevant recommendations in the
treatment guidelines, possibly awaiting further confirmation by larger scale studies. Current evidence suggests that statin use is overall safe with appropriate liver function test monitoring except for Child-Pugh B and C cirrhosis, particularly when total bilirubin is > 3 mg/dl. In this context, the vast majority of patients with increased aminotransferase activities due to an established chronic liver disease, including NAFLD/NASH, should not be exempt from statin use[36-38].

To date, statin benefits on several markers and surrogates of NAFLD/NASH have been demonstrated in several large population studies as well as in smaller prospective clinical ones. A large-population study tested whether statin therapy can lower NAFLD incidence in healthy individuals or reduce progression to hepatic fibrosis among patients with established NAFLD. Overall, 11,539,409 individuals were recruited and were followed up for 6 years. Fatty liver index (FLI) and BARD score were used for the diagnosis of NAFLD and the assessment of hepatic fibrosis, respectively. Of all study participants, 5,339,901 were NAFLD-free (FLI<30); of those, 164,856 subjects were diagnosed with NAFLD at the end of the follow-up. Statin treatment was associated with reduced incidence of NAFLD (assessed by FLI > 60; adjusted odds ratio (OR:) 0.66; 95% confidence interval [CI] 0.65-0.67). Statin treatment was also associated with reduced progression to hepatic fibrosis (BARD score > 2) among NAFLD patients (adjusted OR: 0.43; 95%CI: 0.42-0.44)[39].

In a multicenter cohort study liver biopsy was performed in 1,201 individuals considered to have NASH. In the biopsy-proven NASH cases statin use was associated with a significant dose-dependent protection against hepatic steatosis, NASH and hepatic fibrosis F2-F4 stage. In statin-treated patients with genetic mutations (PNPLA3 I148M risk alleles, TM6SF2 E167K variant), impaired fasting glucose, type 2 diabetes, increased age and elevated body mass index (BMI), statin use was associated with reduced risk of steatosis (OR: 0.09, 95%CI: 0.01-0.32; P = 0.004), steatohepatitis (OR: 0.25, 95%CI: 0.13-0.47; P < 0.001), and fibrosis F2-F4 stage (OR: 0.42, 95%CI: 0.20-0.80; P = 0.017)[40].

A randomized clinical trial included 5,400 military personnel who were screened through clinical and laboratory checkup. Of those individuals, 604 were diagnosed with NAFLD/NASH and were randomized by 1:1:1:1 to diet/exercise, rosuvastatin, atorvastatin or pitavastatin treatment. After 1 year, changes of 2 non-invasive scores [NAFLD Activity Score (NAS); Fibrosis-4 score (FIB-4)] were assessed. No significant changes in any of these scores were observed in the diet/exercise group. However, statin treatment was associated with significantly reduced NAS and FIB-4 scores at the end of follow up. This benefit was relevant for all statins used in this study[41].

A possible protective role of statin treatment against NAFLD progression to HCC was also demonstrated in observational studies. A case-control study included 102 NAFLD patients with vs without HCC (cases, n = 34 vs controls, n = 68 respectively). In multivariate analysis statin treatment was associated with lower risk of HCC (OR: 0.20, 95%CI: 0.07-0.60, P = 0.004)[42]. Another retrospective cohort study investigated the likelihood of progression to HCC in 18,080 non-cirrhotic NAFLD patients identified in Taiwan’s National Health Insurance Research Database between 1998 and 2012. The median follow-up period was 6.32 years. The 10-year cumulative incidence of HCC was estimated to be 2.73% (95%CI: 1.69%-3.76%). In multivariate analysis statin use was associated with reduced risk of HCC progression (hazard ratio, HR 0.29, 95%CI: 0.12-0.68)[43]. However, all these studies should be interpreted with caution due to their retrospective character. More prospective longitudinal data may be required to establish a protective role of statin treatment against HCC development in NAFLD.

**Atorvastatin**
The Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) study included 1,600 patients with established coronary artery disease, low density lipoprotein cholesterol (LDL-C) > 2.6 mmol/l (100 mg/dl) and TG < 4.5 mmol/l (400 mg/dl). These patients were randomized to atorvastatin 10-80 mg/d or usual care (including statins) to achieve LDL-C < 2.6 mmol/l (100 mg/dl). A post hoc analysis of this study included 437 patients with suspected NAFLD due to moderately abnormal liver function tests: 227 received atorvastatin at a mean dose of 24 mg/d and 210 received usual care. The primary outcome of this analysis was risk reduction by statin vs non-statin treatment for first recurrent CV event in patients with moderately abnormal liver tests (AST and ALT levels < 3 x the upper limit of normal). A relative risk reduction by 68% was noted in the atorvastatin-treated group compared with usual care. Interestingly, this benefit was significantly greater by 39% in the subgroup of patients with suspected NAFLD compared with patients exhibiting normal liver function tests at baseline. This finding suggested that CV benefits of atorvastatin is
more prominent among high-risk patients who have NAFLD compared with the corresponding patients who do not have NAFLD. Also, the use of atorvastatin was safe in NAFLD patients leading to rare treatment discontinuations due to liver enzyme abnormalities (7/880 patients)[34].

Small pilot prospective studies also suggested that atorvastatin monotherapy improves liver-specific outcomes in NAFLD/NASH. Namely, atorvastatin across dose range 10-80 mg/d was given in patients with dyslipidemia and NAFLD for 3-12 mo [44,45]. Treatment improved the lipid profile and was associated with significant reductions of the baseline elevated liver function tests. A large proportion of patients (i.e., 36.3%) showed liver enzyme normalization after 6 mo of treatment with additional patients exhibiting similar benefits with continuation of treatment for up to 1 year. Radiographic regression of liver steatosis assessed by CT was noted too[44].

Atorvastatin was also beneficial when used in combination with other agents. 1005 individuals were randomized to atorvastatin 20 mg/d + vitamin C (1 g/d) + vitamin E (1000 IU/d) vs placebo for 4 years. The combination treatment was associated with a 71% reduced odds of hepatic steatosis development assessed by CT (liver-to-spleen ratio) compared with placebo[46]. Another prospective study included 186 non-diabetic patients with metabolic syndrome and ultrasonographic findings of NAFLD. These patients were randomized to atorvastatin 20 mg/d, (n = 63), fenofibrate 200 mg/d (n = 62) or their combination (n = 61). After 54 wk, liver enzymes and ultrasound findings of NAFLD normalized in 67% of the participants in the atorvastatin, 42% in the fenofibrate and 70% in the combination group[47].

In another phase 2 randomized placebo-controlled trial atorvastatin treatment blunted the increases in LDL-C levels and LDL particle concentration (LDL-pc) associated with obeticholic acid (OCA) in NASH patients. OCA is an agent with promising effects on liver histology and fibrosis, which adversely impacts on lipoprotein metabolism. The latter may be safely alleviated when it is used in combination with atorvastatin[48].

Rosuvastatin

Like atorvastatin, rosuvastatin effectively improved liver-specific endpoints of NAFLD in small pilot studies. Rosuvastatin 10 mg/d treatment was given in 6 non-diabetic non-hypertensive dyslipidemia patients with metabolic syndrome and biopsy-proven NASH. A second biopsy as well as liver ultrasound after 12 mo showed complete resolution of NASH characteristics (steatosis, necroinflammation and fibrosis) in 5/6 patients. Additionally, rosuvastatin was associated with significantly reduced ALT and AST activities by 76 and 61% respectively[49]. Similar were the findings of another small prospective study including 23 NAFLD patients with dyslipidemia who received rosuvastatin 10mg/d. After 8 mo liver enzymes normalized in all patients[50].

In another study rosuvastatin 10 mg/d was given in 20 patients with dyslipidemia, biopsy-proven NASH and metabolic syndrome for 12 mo. Significant improvement of the course of NASH and metabolic syndrome was confirmed through laboratory tests, repeated biopsies and ultrasound assessment at the end of follow-up. Furthermore, significant reductions in serum uric acid and fasting plasma glucose levels implied additional cardiometabolic benefits of rosuvastatin in these patients[51]. Another pilot study included 19 dyslipidemic patients with biopsy-proven NASH treated with rosuvastatin 2.5 mg/d. After 24 mo biopsies in 9 patients showed improved NAS and fibrotic stage in 33.3% patients[52].

Despite this promising evidence long-term (96 wk) rosuvastatin 10 mg/d failed to reduce hepatic steatosis assessed by the liver fat score vs placebo in 147 human immunodeficiency virus (HIV)-positive patients on antiretrovirial treatment. Instead, hepatic steatosis progression was noted both in the rosuvastatin and placebo groups at the end of follow-up. Reassuringly, multivariate regression analysis suggested that this finding was associated with increased inflammatory biomarkers, but not with rosuvastatin treatment[53].

Simvastatin

Data on simvastatin are limited. A retrospective study evaluated the safety and efficacy of simvastatin alone or in combination with ezetimibe in 45 patients with NAFLD, metabolic syndrome, and increased CV risk[54]. Twenty-six patients received simvastatin monotherapy 20 mg/d and 19 simvastatin/ezetimibe 10/10 mg/d. After 6 mo AST and ALT activities were significantly reduced compared with pre-treatment values. Interestingly, simvastatin monotherapy was associated with significantly greater AST/ALT reductions compared with the combination therapy. This finding suggested a potential dose-dependent benefit of simvastatin in this respect[54].
In contrast, a pilot randomized study evaluating the effects of simvastatin 40 mg/d vs placebo in 16 patients with NASH and dyslipidemia did not show similar benefits [55]. Despite significant improvement of the serum lipid profile, liver biopsy performed after 1 year in 10 patients did not show any differences in hepatic steatosis or fibrosis stage between the simvastatin and the placebo group. No significant changes in serum aminotransferases in any of the groups were noted either [55].

**Pravastatin**
Limited clinical data suggest that pravastatin may be a safe and tolerable option in improving the serum lipid profile in NAFLD patients whilst improving NASH-related liver histology. A multicenter randomized clinical trial included 326 hypercholesterolemic patients with known chronic liver disease (64% with NAFLD) treated with high-dose pravastatin (80 mg/d) vs placebo. After 36 wk, pravastatin significantly improved the serum lipid profile. No statistically significant difference between the pravastatin and the placebo group was noted in ALT elevation events (defined as doubling from pre-treatment values): 8% vs 13%, respectively. These results suggest that pravastatin is a safe option to beneficially modify the lipid profile among NAFLD patients [56]. In another small study, pravastatin 20 mg/d was given in 5 participants with biopsy-proven NASH and abnormal liver enzyme activities. After 6 mo, hepatic enzymes normalized in all participants. Also, repeat biopsy in 4/5 participants showed that hepatic steatosis and inflammation were persistent only in 1 and 3 participants, respectively [57].

**Pitavastatin**
Pitavastatin effectively reduces LDL-C, while increasing HDL-C levels, especially in patients with pre-diabetes or diabetes [58]. Its favorable metabolic profile associated with improved insulin resistance and carbohydrate metabolism may be promising for the management and prevention of NAFLD/NASH [59]. It was suggested that these benefits are mediated through increased adiponectin levels and reduced oxidative stress [58].

However, limited clinical evidence is conflicting and cannot firmly establish a beneficial role of this statin against NAFLD. Namely, a 12 wk pitavastatin 2-4 mg/d treatment significantly reduced liver enzyme activities in 97 patients with mildly-to-moderately elevated liver enzyme tests at baseline [44]. This benefit was accompanied by reduced severity of the CT-assessed hepatic steatosis among patients with this abnormality at baseline. Events of moderate to severe ALT elevations at > 100 and > 120 IU/L at 12 wk were rare in pitavastatin-treated patients: 5/97 and 1/97, respectively [60].

Similar were the results of a 12 mo pitavastatin 2 mg/d treatment in 20 NASH patients with dyslipidemia. However, NAS and fibrosis stage in biopsy were not significantly altered [61]. In accordance with this finding a 6-month pitavastatin 4 mg/d treatment did not significantly reduce Hydrogen-1 MRI-assessed hepatic fat compared with placebo in 50 adults with Body Mass Index (BMI) ≥ 27 kg/m² and waist circumference ≥ 102 cm who had not used statins for ≥1 year. In the same study pitavastatin did not favorably improve indices of carbohydrate metabolism, including endogenous glucose production, whole body insulin sensitivity or insulin-induced glucose uptake [62]. Therefore, more data are needed to clarify the effects of this statin on hepatic steatosis, inflammation and fibrosis as well as on the associated metabolic abnormalities.

**Lovastatin**
In one multicenter study 87 patients with NAFLD/NASH and dyslipidemia received lovastatin 10 mg/d for 4 mo. Significant reductions in transaminase and cholesterol levels were noted even within the first 2 mo of treatment. Also, a decreased the AST-to-platelet ratio index (APRI) as a marker of liver fibrosis was noted [63].

**EZETIMIBE**

**Mechanistic implications**
Ezetimibe exerts its mild-to-moderate LDL-C-lowering action via inhibiting intestinal cholesterol absorption through the Niemann-Pick C1-like 1 (NPC1L1) protein [64]. Its additive LDL-C lowering effects to statins were associated with further significant reductions in the risk of CV events in high-risk patients [65,66].
Experimental studies suggested that dietary cholesterol uptake is associated with hepatic steatosis and, hence high cholesterol-fed animals have been extensively used as experimental models of NAFLD/NASH. Increased cholesterol absorption and hepatocyte cholesterol content results in a cholesterol-dependent activation of the liver X receptor α (LXRα). The latter enhances the expression of several transcriptional factors that promote hepatic lipogenesis, including the sterol regulatory element binding protein (SREBP)-1c and the carbohydrate response element-binding protein (ChREBP). Reducing cholesterol absorption via ezetimibe can help reverse this deleterious process[67].

NPC1L1 is also expressed in the human liver and facilitates cholesterol reuptake from bile to hepatocytes[67]. Further experiments suggested that NPC1L1 may be associated with impaired VLDL-TG secretion and subsequent TG hepatic accumulation[68]. NPC1L1 inhibition by ezetimibe ameliorated hepatic steatosis in genetically engineered L1-Tg mice characterized by enhanced hepatic NPC1L1 expression[69,70].

Ezetimibe-related liver de-lipidation can be facilitated through other mechanisms too. Lipoprotein secretion and lipid removal from the liver is mediated by the microsomal TG transfer protein (MTP) whose degradation is inhibited by ezetimibe [71]. Ezetimibe may also enhance the expression of cholesterol efflux transporters like Abcg5/g8[72]. Furthermore, ezetimibe was associated with improved hepatic insulin sensitivity through an upregulation of the small heterodimer partner (SHP). This effect was accompanied by an upregulation of SREBP2 and downregulation of SREBP-1c expression[73]. Also, ezetimibe was associated with a decreased hepatic expression of Cd36 gene. Cd36 is a multifunctional scavenger receptor facilitating fatty acid uptake and oxidation, and it has been associated with dysfunctional fatty metabolism and fatty liver[74].

Besides, according to experimental studies ezetimibe can confer significant protection against inflammation and oxidative stress associated with hepatic steatosis. A proposed mechanism is through activation of the nuclear factor erythroid 2-related factor 2 (Nrf2), a transcriptional factor whose target genes are antioxidant proteins and detoxification enzymes[67].

Further in vitro experimental data suggested that ezetimibe exerts anti-inflammatory benefits against NASH in human liver cells. In this context, ezetimibe may promote autophagy, which plays a key role in hepatic lipid catabolism (lipophagy) and prevents from hepatic steatosis[75]. This effect may be mediated by AMP-activated protein kinase (AMPK) activation and transcription factor EB (TFEB) nuclear translocation associated with the MAPK/ERK pathway. Ezetimibe may also reduce NLPR3 inflammasome activation in macrophages by modulating autophagy and a hepatocyte-driven exosome pathway[76]. Besides, it was shown to reduce hepatic fat content and prevent from the associated NFκB pathway-mediated liver inflammation by high-fat diet[77].

Last, ezetimibe was suggested to exert protective effects against angiogenesis associated with HCC development in NASH[78]. In this context, ezetimibe was also associated with downregulated expression of SKP2 which serves as an oncogene in HCC in a high-fat diet rodent model[74].

Clinical evidence

The effects of ezetimibe on liver-specific outcomes of NAFLD/NASH were mainly evaluated in small interventional studies with similar design to the statin ones. Namely, ezetimibe 10 mg/d was associated with significantly decreased ALT, AST and γ-GT activities in 70 individuals with dyslipidemia and NAFLD. Also, the ultrasonographic progress of steatosis was limited in 38.6% of these patients[79]. Another small study included 8 non-obese individuals with NAFLD who were treated with ezetimibe. Four patients had been receiving ursodeoxycholic acid which was subsequently switched to ezetimibe, while the remaining 4 patients had been previously treatment naive. After 12 mo, ALT activity was significantly reduced by 49.3% and normalized in 4 of 8 patients. Nevertheless, ezetimibe treatment was not associated with any significant ultrasonographic changes of hepatic steatosis[80].

Long-term effects of ezetimibe monotherapy on hepatic steatosis were evaluated in 45 biopsy-proven NAFLD patients. After 2 years of treatment histologically-assessed hepatic steatosis and NAFLD activity scores were significantly reduced. However, no improvement in the fibrosis state was observed[81].

A randomized double-blind placebo-controlled study assessed whether ezetimibe reduces hepatic steatosis in NASH. It included 50 biopsy-proven patients randomized to ezetimibe 10 mg/d or placebo. After 24 wk ezetimibe was associated with reduced liver steatosis assessed by magnetic resonance imaging-derived proton density-fat fraction (MRI-PDFF) compared with baseline. However, no significant difference
between the ezetimibe and the placebo group was noted[82]. Another study assessed
the effects of a 6-month ezetimibe treatment in 10 patients with dyslipidemia and
NASH. Significantly improved activities of AST, ALT, γ-GT as well as reduced LDL-C,
CRP and type IV collagen 7 levels were observed. Histologically-assessed steatosis also
improved in every patients, whilst significant benefits on fibrosis status was noted in
6/10 patients[83].

A further randomized controlled trial included 32 NAFLD patients of whom 17
received ezetimibe, while the rest were controls. After 6 mo ezetimibe significantly
reduced total cholesterol levels by 9.5% compared with control group and ameliorated hepatic fibrosis. Namely, histologically-assessed staging and ballooning scores were
significantly improved in the ezetimibe group compared with baseline. In contrast, the
steatosis score, lobular inflammation and NAS did not significantly change in either
group[84].

The efficacy and safety of ezetimibe 10 mg/d + simvastatin 20 mg/d combination
was evaluated in 19 patients with type 2 diabetes and NAFLD[85]. After 6 mo,
treatment was associated with significantly reduced ALT activity by 48.9%[85]. The
IMPROVE-IT (Improved Reduction of Outcomes: Vytorin Efficacy International Trial)
included 18,144 patients who had been hospitalized for an acute coronary syndrome
within the preceding 10 ds and had LDL-C levels of 1.3-2.6 mmol/1 (50-100 mg/dl) if
they were receiving lipid-lowering therapy or 1.3-3.2 mmol/1 (50-125 mg/dl) if they
were not receiving lipid-lowering therapy. These patients were randomized to
simvastatin 40 mg/d + placebo vs simvastatin 40/d + ezetimibe 10 mg/d. The primary
endpoint was the composite of CV death, myocardial infarction, rehospitalization for
unstable angina, coronary revascularization or stroke. After 7 years the simvastatin +
ezetimibe combination significantly reduced the primary endpoint compared with
simvastatin monotherapy (34.7 vs 32.7%, p = 0.016)[65]. In a sub-analysis, NAFLD
fibrosis score (NFS) was prospectively applied to 14,819 IMPROVE-IT participants.
NFS is a serum-based index, originally developed for the diagnosis of advanced
hepatic fibrosis in patients with NAFLD and it was found to be associated with
increased CV morbidity and mortality. Using validated NFS cutoffs the effect of
treatment on the primary endpoint was assessed. In the IMPROVE-IT patients with
high NFS score (> 0.67) had a 30% increased risk of major CV events compared with
the low-risk group. Simvastatin+ezetimibe combination was associated with a 3.7%
absolute reduction in the risk of recurrent CV events, compared with placebo +
simvastatin (HR 0.85 [0.74–0.98]) translating to a number-needed-to-treat of 27. This
additional CV benefit was not noted in the low-risk group based on NFS. This finding
suggests that ezetimibe confers additional CV protection amongst NAFLD patients
with higher fibrosis score[86].

A meta-analysis of 2 randomized controlled trials (RCTs) + 4 single-arm trials
included 273 participants with NAFLD and NASH. Ezetimibe treatment was not
associated with improved hepatic inflammation and fibrosis, although an
improvement in transaminase levels was observed[87]. Similar were the results of a
more recent meta-analysis of 5 lipid-lowering treatment studies (2 ezetimibe, 1
simvastatin, 1 atorvastatin and 1 any-statin) including 199 patients with NAFLD[88].
Ezetimibe was associated with decreased NAS, but not with reduced hepatic steatosis
[88].

**FIBRATES**

**Mechanistic implications**

TG reduction is the main lipid-lowering action of fibrates accompanied by moderate
increases in HDL-C and mild reductions in LDL-C levels. Despite these favorable
effects their role in CV disease prevention remains controversial[89]. Nevertheless,
several animal studies suggested a promising role of fibrates for the management of
NAFLD. Specifically, fibrates ameliorated hepatic steatosis, necroinflammation and
fibrosis induced by high-fat diet in several experimental studies[90-96].

PPARα activation is their main mechanism of action associated with multiple
benefits on the lipid and glucose metabolism[97]. Through this effect fibrates
upregulate lipoprotein lipase, a key enzyme for the TG-rich lipoprotein catabolism.
This enzyme also plays a key pathogenetic role in hepatic steatosis when downreg-
ulated[96]. Also, PPARα activation through fibrates is expected to reduce hepatic
steatosis mainly via enhanced expression of target genes. These include fatty acid
transport and binding proteins, carnitine palmitoyltransferase II, as well as medium-
and long-chain acyl-CoA dehydrogenase and acyl-CoA oxidase mediating
mitochondrial and peroxisomal FA β-oxidation[97,99,100]. In the same context, fibrate use has been associated with reduced hepatic insulin resistance mostly by enhanced fatty PPARα-related acid β-oxidation. Also, PPARα activation is associated with increased expression of FGF21, a hepatokine enhancing extra-hepatic tissue insulin sensitivity through glucose transporter 1 activation[99].

Additionally, fibrates downregulate the expression of pro-inflammatory cytokines genes, such as TNFα, monocyte chemoattractant protein (MCP)-1, intercellular adhesion molecule (ICAM)-1 and vascular adhesion molecule (VCAM)-1. This suggests a promising role against NASH[97,99]. Fibrates were also suggested to exert antioxidant actions, mainly through reducing fatty acid peroxidation and ROS production. The latter effects appear to be PPARα-dependent. Furthermore, fibrate treatment was associated with amelioration of high-fat diet-induced disturbances in hepatic microvasculature. This effect seems to improve the liver microvascular environment and oxygenation and may be protective against NAFLD in a PPARα-dependent manner[97].

Many of the abovementioned effects may be also mediated by a fibrate-associated increased production of adiponectin, an adipokine that exerts multiple benefits on lipid and glucose metabolism, mainly via reducing hepatic insulin resistance. This adipokine also exerts variable anti-inflammatory, antioxidant and anti-fibrotic actions in the liver being potentially protective against NASH. Besides, fibrate treatment seems to preserve the liver-specific adiponectin receptor (AdipoR2), which is protective against liver steatosis and inflammation[97].

Clinical evidence

The very promising experimental evidence suggesting a therapeutic role of fibrates in NAFLD has not been confirmed by clinical studies. This inconsistency might be explained by differences between animals and humans in PPARα tissue expression, being higher in the former[101]. Another important factor is the difference in PPARα responsiveness to treatment between rodents and humans. Namely, fibrate-related human PPARα activation is less prominent in humans than in rodents at equivalent doses[99,102]. Also, in experimental rodent studies the fibrate doses used were significantly higher than the human equivalent ones in clinical studies[102]. These factors may have obscured any benefits of fibrates in human studies[99,102].

Like statins and ezetimibe, clinical data for fibrates are mainly derived from small clinical studies, in which these drugs were used as monotherapy or in combination with other agents.

In a small study, fenofibrate 200 mg/d was given in 16 individuals with biopsy-proven NAFLD for 48 wk. Fenofibrate significantly reduced triglycerides and increased apolipoprotein A1 levels, while it improved the glycemic status. Significant reductions in liver enzyme activities, including ALT, AST, ALP and γ-GT were noted at the end of follow up. Minor improvements of liver histologic abnormalities were also noted in the fenofibrate-treated patients. Namely, a significantly reduced grade of hepatocellular ballooning degeneration was observed, while the grade of steatosis, lobular inflammation and fibrosis or NAS remained unchanged[103].

Another study included 90 patients with NAFLD who were randomized to fenofibrate 300 mg/d monotherapy or fenofibrate 300 mg/d + pentoxifylline 1200 mg 3 times daily for 24 wk. Pentoxifylline + fenofibrate combination were significantly more beneficial on liver enzyme activities than fenofibrate monotherapy. Namely, ALT/AST/γGT activity was reduced by 50.0/45.6/43.2% in the monotherapy vs 60.6/62.0/54.2% in the combination group. Of note, liver fibrosis assessed by indirect biochemical markers, a direct marker linked to matrix deposition (hyaluronic acid), a cytokine/growth factor linked to liver fibrosis (i.e., TGF-β1), the inflammatory pathway, insulin resistance and liver stiffness (assessed by Fibroscan) were improved more in the combination treatment compared with the fenofibrate monotherapy group. This finding indicated that therapy with pentoxifylline + fenofibrate may have a therapeutic role against NAFLD and progression of NASH to cirrhosis[104].

Another randomized placebo-controlled trial assessed the effects of fenofibrate vs nicotinic acid on intrahepatic TG levels and CV risk in 27 obese patients with NAFLD. Study participants were randomized to fenofibrate 200 mg/d for 8 wk or nicotinic acid extended release 2000 mg/d for 16 wk, or placebo for 8 wk. Both fenofibrate and nicotinic acid significantly lowered circulating very low-density lipoprotein-TG (VLDL-TG) concentrations to a similar extent: by 48.9% and 40.2% respectively. Nevertheless, the hepatic triglyceride content remained unchanged in both treatment groups[105].
Fenofibrate effects on hepatic fat was also compared with free omega-3 carboxylic acid (OM-3CA) treatment in a trial including 78 obese patients with NAFLD. Those patients were randomized to OM-3CA 4 g/d (n = 25), fenofibrate 200 mg/d (n = 27) or placebo (n = 26). After 12 wk both fenofibrate and OM-3CA significantly reduced serum TG levels by 26% (p = 0.02) and 38% (p < 0.001) respectively compared with placebo. However, none of the treatments significantly altered hepatic fat. Interestingly, hepatic fat and volume increased in the fenofibrate compared with the OM-3CA group[106].

Another study included 90 overweight patients with NAFLD who were randomized by 1:1:1 to lifestyle intervention alone or in combination with fenofibrate 200 mg/d or pioglitazone 30/d. Improved ALT and AST activities as well as significant reductions in blood pressure and BMI were noted in all study groups. However, no significant differences between groups were noted in the median changes of ALT/AST activities or BMI[107].

Also, in an abovementioned study the effects of atorvastatin and fenofibrate monotherapies or in combination were assessed in patients with metabolic syndrome and NAFLD. Normalization in the liver biochemistry and ultrasound findings of NAFLD was noted 67, 42 and 70% of participants in the atorvastatin, fenofibrate and combination group respectively[47].

### OTHER LIPID-LOWERING DRUGS

**ω-3 fatty acids**

α-Linolenic acid (ALA), Stearidonic acid (SDA), Eicosapentanoic acid (EPA), Docosapentanoic acid (DPA) and Docosahexaenoic acid (DHA) are ω-3 Polyunsaturated Fatty Acids (PUFAs). It was suggested that ω-3 PUFAs exert multiple benefits on the CV and nervous system, maternal and child health, cancer progression and diabetes[108]. However, their efficacy to reduce CV mortality and morbidity as well as total mortality was not supported by meta-analyses of RCTs[109]. There is some variation between different ω-3 PUFAs in this regard, with the highly purified EPA, docosapent ethyl being more beneficial[110].

An association between ω-3 PUFAs and NAFLD has also been suggested. Specifically, NAFLD patients were shown to have reduced hepatic levels of ω-3 PUFAs. This observation may be relevant for the disease development and progression. Namely, adequate levels of PUFAs were suggested to reduce liver lipogenesis and hepatic inflammation in animal studies[111]. Interestingly, a cross-sectional analysis evaluated a potential prognostic role of ω-3 PUFAs in childhood NAFLD. The study included 223 children (aged 6-18 years) who were recruited from the “Treatment of Nonalcoholic Fatty Liver Disease in Children” trial. Their intake on fish and ω-3 PUFA was estimated based on the Block Brief 2000 Food Frequency Questionnaire. Inadequate consumption of fish and ω-3 PUFA was observed in NAFLD children, and it was linked to increased inflammation of the liver. This finding suggests a significant prognostic role of limited ω-3 fatty acids intake in the development and progression of the disease[112].

The hypothesis of whether ω-3 PUFA supplementation can be beneficial on liver endpoints in NAFLD was assessed in various clinical studies. In a recent meta-analysis including 1,366 NAFLD participants of 22 RCTs, ω-3 PUFA supplements were associated with reduced hepatic fat content compared with placebo (pooled risk ratio 1.52; 95%CI: 1.09-2.13). This benefit was attributed at least in part to favorable changes in BMI, but also to effective lipid modulation by reductions in TG and total cholesterol levels in ω-3 fatty acid-treated patients. However, the heterogeneity of the RCTs (diverse ethnicity and age groups) was considered a significant limitation of this analysis. Other limitations included the small sample size as well as paucity of data from follow up, and significant differences in the treatment plans (related to therapeutic dosages, duration and regimens) of the included trials[113].

A clinical trial included 48 patients with diabetes and NAFLD who were randomized to a combination of probiotics + ω-3 PUFAs vs placebo for 8 wk. Fatty Liver Index and Liver stiffness were determined through Shear Wave Elastography (SWE). Combination therapy ameliorated hepatic fat, improved the serum lipid profile and decreased systemic inflammation[114].

Another study investigated potential benefits of ω-3 PUFAs on NAFLD progression. Thirty NAFLD patients were separated into 2 groups depending on the severity of their disease (moderate vs severe). This distinction was based on biochemical and ultrasonographic characteristics, NAS and FFL. Participants in the severe NAFLD
group received ω-3 PUFA (2 g/d) for 6 mo. At the end of the study, patients displayed increased circulating levels of EPA/DHA and reduced hepatic steatosis[115]. Similar were the results of another study that assessed whether long-term (1-year) ω-3 PUFAs use is beneficial in 56 NAFLD patients: 42 received ω-3 PUFAs (1 g/d) and 14 were controls. ω-3 PUFAs were associated with significantly reduced ALT/AST activities as well as with decreased TGs levels. Ultrasonographically assessed hepatic steatosis also improved in the treatment group[116].

Promising clinical evidence suggested a potential therapeutic role of highly purified EPA against NAFLD/NASH. A pilot study included 23 biopsy-proven NASH patients who were treated with highly purified EPA 2,700 mg/d. After 12 mo, treatment was associated with significantly improved biomarkers or NAFLD and its associated oxidative stress. Those included improved ALT activity as well as reduced fatty acid, TNF receptors 1 and 2, serum ferritin and thioredoxin levels. Also, follow up biopsy after 12 mo showed reduced hepatic steatosis and hepatocyte ballooning as well as improved lobular inflammation and liver fibrosis in 6/7 patients[117]. These benefits were evident even in the absence of any significant changes in body weight, insulin resistance and adiponectin levels[117]. Several mechanisms were proposed to mediate potential benefits of highly purified EPA on NAFLD. These included reduced fatty acid hepatic uptake, increased intrahepatic TG hydrolysis as well as inhibition of the SREBP-1 maturation. All those actions were proven independent of PPARα activation[118].

**Bile acid sequestrant resins**

Bile acid sequestrants (BAS) are lipid-lowering drugs recommended for individuals with increased cholesterol levels, but normal TGs. Both as monotherapy or in combination with other lipid-lowering drugs they may decrease LDL-C by 20% and CV risk[119].

A study including 50 NASH patients assessed whether colesevelam can reduce hepatic fat. These patients were randomized to colesevelam 3.75 g/d or placebo. After 24 wk, colesevelam was associated with slightly worsened hepatic fat and inflammation than placebo. The hepatic outcomes were evaluated by MRI-PDFF and conventional MR spectroscopy (MRS). The clinical impact and the responsible mechanism explaining these findings have not been established and more clinical trials are needed to reach safer conclusions[120].

An ongoing Phase 2 randomized trial (NCT04235205) is testing whether combination therapy of elobixibat (EXB), an ileal bile acid transporter inhibitor and cholestyramine is efficient in the management of NAFLD. This study will include 100 NAFLD patients who will be randomized to 4 treatment groups: EXB 10 mg + colesevelam 4g, EXB 10 mg monotherapy, cholestyramine 4g monotherapy or placebo. Changes in the course of NAFLD will be evaluated by means of biochemical parameters and liver imaging after 16 wk of treatment[121].

**PCSK9 (Proprotein convertase subtilisin/kexin type 9) inhibitors**

To date, there are 2 available fully human monoclonal antibodies (mAbs) inhibiting proprotein convertase subtilisin/kexin type 9 (PCSK9). These drugs are highly efficacious in reducing LDL-C levels by up 60% even when given on top of existing lipid lowering therapy[122]. RCTs showed that this additional LDL-C lowering was associated with additional CV outcome benefits in high-risk populations[123,124].

PCSK9 seems to play a role in the pathogenesis of NAFLD. Increased intrahepatic and circulating PCSK9 levels were associated with enhanced hepatic fatty acid and TG content along with lipid deposition in the muscle and adipose tissue[125]. Whether PCSK9 inhibition with the currently available mAbs, but also with the upcoming small interference RNA PCSK9 inhibitor (inclisiran) can beneficially alter the development and progression of NAFLD remains unknown. There are only 2 studies suggesting a promising role[126,127].

An observational study included 26 patients with Familial Hypercholesterolemia (FH) and NAFLD maintaining increased LDL-C levels despite statins + ezetimibe treatment. These patients were divided into 2 groups based on the TG/HDL median value as a surrogate of atherogenic dyslipidemia. All patients received PCSK9 inhibitors for 6 mo. PCSK9 inhibitors significantly improved hepatic steatosis biomarkers, including triglyceride-glucose index (TyG) and HIS (by -7.5% and -8.4% respectively), particularly in individuals with low TG/HDL. This finding suggests a possible role of PCSK9 inhibitors in the management of NAFLD especially in patients who lack features of atherogenic dyslipidemia[126]. The role of PCSK9 inhibitors in NAFLD was also assessed in a retrospective chart review-based study including 29 NAFLD patients. PCSK9 inhibitors were associated with full resolution of NAFLD
radiologic features in 8 of 11 individuals with liver steatosis. Moreover, transaminase levels improved in all participants[127].

CONCLUSION

Atherogenic dyslipidemia contributes significantly to an increased CV risk in NAFLD. It also plays a key role in NAFLD development and progression. Therefore, its aggressive management through effective lipid lowering drugs is crucial. This was highlighted by the results of a post hoc analysis of the GREACE study suggesting that statin-related CV benefits are more prominent in high-risk NAFLD vs non-NAFLD patients. However, the most commonly used lipid-lowering drug classes have been associated with adverse elevations of the liver enzymes (e.g., statins, fibrates). Hence, clinicians cautiously use them in NAFLD. For this reason, it is important to clarify the safety and efficacy of lipid-lowering drugs in this clinical context.

The amount of evidence in this respect varies amongst different drug classes with more data being available for statins, ezetimibe and fibrates. There is considerable evidence from pre-clinical mechanistic studies suggesting that the lipid-lowering and pleiotropic effects of statins are beneficial for NAFLD/NASH. Besides, these effects may be protective against cirrhosis/HCC development in NAFLD. Also, promising were the results from observational studies about statin effects on liver outcomes. However, due to their retrospective character these results should be viewed with caution.

Smaller prospective studies showed beneficial effects of statins on NAFLD histology, clinical (steatosis and fibrosis) surrogate scores and biochemical biomarkers, especially with atorvastatin and rosuvastatin. Data are more limited and somehow conflicting with other statins. Besides, statin use amongst NAFLD/NASH patients appears to be overall safe. In fact, liver enzyme elevations with statins are limited to a small proportion of NAFLD patients and are not accompanied by any evidence of hepatic dysfunction.

Ezetimibe is associated with more infrequent liver enzyme elevations than statins, hence clinicians comfortably prescribe it in the NAFLD/NASH clinical setting. There is considerable pre-clinical evidence suggesting that ezetimibe is beneficial on hepatic steatosis mainly through its cholesterol lowering effects and its associated reduction of de novo hepatic lipogenesis. Besides, ezetimibe may facilitate liver delipidation by inhibiting MTP degradation and by enhancing the expression of cholesterol efflux transporters. Experimental data also suggest significant anti-inflammatory and antioxidant effects of ezetimibe on the liver tissue through various molecular pathways. Ezetimibe may be protective against HCC development in NAFLD too.

Clinical evidence suggesting benefits of ezetimibe on hepatic outcomes in NAFLD/NASH is limited to small prospective studies, few of which were randomized placebo-controlled. Most studies showed that ezetimibe monotherapy for few mo was associated with improvements of the liver enzyme activities as well as of histologically-assessed hepatic steatosis and relevant surrogate imaging endpoints. However, evidence suggesting benefits on hepatic inflammation and fibrosis is less robust. Given the milder lipid-lowering effects of ezetimibe and its less potent pleiotropy than this of statins it is uncertain whether more longitudinal data would better establish benefits on hepatic inflammation and fibrosis too.

Fibrates exert significant lipid modifying effects against atherogenic dyslipidemia which is very prevalent in NAFLD. Their effects are exhibited mainly through activation of PPARα, which play a key role in various metabolic pathways, especially in favorably modulating key lipid and glucose metabolic pathways involved in NAFLD pathogenesis. In this context, pre-clinical studies suggested that fibrates reduce hepatic steatosis via increasing hepatic insulin sensitivity and by accelerating the catabolism of the TG-rich lipoproteins and fatty acid oxidation. There is also promising preclinical evidence suggesting significant liver anti-inflammatory and antioxidant effects of fibrates.

Despite this promising mechanistic potential fibrate effects on liver steatosis, necroinflammation and fibrosis were poor in clinical studies. Liver enzyme improvement was the only established improved NAFLD surrogate associated with fibrate use, though this evidence should be cautiously interpreted in view of the variability of this biomarker. Fenofibrate combination with pentoxifylline is promising but its benefits require further confirmation by larger scale longitudinal studies with histological endpoints. It should be acknowledged though that the lack of fibrate efficacy in clinical studies as compared with the experimental ones may be explained
by inter-species variation in PPARα expression and responsiveness to drug-induced activation. Also, relatively reduced human equivalent fibrate doses were used in clinical studies compared with the experimental rodent ones.

Omega-3 fatty acids can beneficially modulate lipid abnormalities encountered in NAFLD. Also, low hepatic ω-3 fatty acid levels have been associated with the development of hepatic steatosis. There have been multiple studies to suggest that ω-3 fatty acid supplementation is associated with reduced hepatic steatosis, though these are characterised by significant heterogeneity in design, study populations, type of intervention and study end points. No robust evidence exists to suggest that ω-3 fatty acid reduce hepatic inflammation or fibrosis in NAFLD.

Bile acid sequestrants have not shown any benefits against NAFLD. Instead, deleterious effects of colesevelam on hepatic steatosis and inflammation were shown in one study. Cholesteryramine is currently being evaluated alone or in combination with an ideal bile acid transporter inhibitor under development in NAFLD participants of a Phase 2 RCT. Both circulating and hepatic PCSK9 seems to play a pathogenetic role in hepatic steatosis and may represent an attractive therapeutic target in NAFLD. The currently available PCKS9 inhibitor mAbs have shown promising effects only in small observational studies, particularly among patients without atherogenic dyslipidemia. However, this benefit should be established by large scale longitudinal prospective clinical trials.

Limitations of the studies discussed in this review include their relatively small size and short follow up period. For example, benefits of certain drug classes could arise with their long-term continuation. Likewise, marginal benefits could be missed due to small sample sizes of the studies. NAFLD/NASH development has long natural history and any potential reversal/regression may require long-term treatment too. Designing large-scale liver outcome studies with liver biopsy, which remains the gold-standard method for assessment of hepatic steatosis, necroinflammation and fibrosis is also challenging. Furthermore, the heterogeneity of other liver endpoints assessed in most studies hampers the pooling of data that could help reach safer conclusions. Most studies have assessed the effects of lipid lowering agents on liver enzyme activities, a biomarker which has poor correlation with disease activity and broad variability. Importantly, the predictive value of liver enzyme activity lowering on histological endpoints of NAFLD/NASH via certain interventions has not been validated. Identifying well-validated and easy-to-use disease surrogates remains an ongoing challenge for the assessment of various therapeutic interventions, including lipid-lowering drugs, in NAFLD/NASH.

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