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High-density lipoproteins and non-alcoholic fatty liver disease

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ABSTRACT

Background and aims: Non-alcoholic fatty liver disease (NAFLD), a high incidence liver pathology, is associated with a ~1.5-fold higher cardiovascular disease risk. This phenomenon is generally attributed to the NAFLD-associated increase in circulating levels of pro-atherogenic apolipoprotein B100-containing small dense low-density lipoprotein and plasma hypertriglyceridemia. However, also a significant reduction in cholesterol transported by anti-atherogenic high-density lipoproteins (HDL) is frequently observed in subjects suffering from NAFLD as compared to unaffected people. In this review, we summarize data regarding the relationship between NAFLD and plasma HDL-cholesterol levels, with a special focus on highlighting potential causality between the NAFLD pathology and changes in HDL metabolism.

Methods and results: Publications in PUBMED describing the relationship between HDL levels and NAFLD susceptibility and/or disease severity, either in human clinical settings or genetically-modified mouse models, were critically reviewed for subsequent inclusion in this manuscript. Furthermore, relevant literature describing effects on lipid loading in cultured hepatocytes of models with genetic alterations related to HDL metabolism have been summarized.

Conclusions: Although in vitro observations suggest causality between HDL formation by hepatocytes and protection against NAFLD-like lipid accumulation, current literature remains inconclusive on whether relative HDL deficiency is actually driving the development of fatty liver disease in humans. In light of the current obesity pandemic and the associated marked rise in NAFLD incidence, it is of clear scientific and societal interest to gain further insight into the relationship between HDL-cholesterol levels and fatty liver development to potentially uncover the therapeutic potential of pharmacological HDL level and/or function modulation.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide. It has a staggering overall estimated incidence of ~38%, although its presence within the global population shows broad regional differences and a clear male gender dominance [1]. NAFLD encompasses a variety of disease pathologies that are all characterized by the hepatic accumulation of lipids, i.e. triglycerides and/or cholesteryl esters, in the absence of significant alcohol consumption. The first stages of NAFLD evolve largely asymptomatic and include hepatic steatosis, i.e. initial lipid deposition and associated hepatocyte ballooning, and inflammatory non-alcoholic steatohepatitis (NASH) with and without parallel fibrosis development (Fig. 1). The deposition of lipids within hepatocytes is generally attributed to excessive dietary fat intake and low physical activity. However, it should be acknowledged that fatty acids derived from adipose tissue are, with an estimated contribution of 59%, the primary source of triglycerides stored in livers of NAFLD patients [2]. As such, abdominal obesity and adipose tissue insulin resistance, i.e. a failure of adipocytes to retain fatty acids from fluxing towards the liver, are also major NAFLD risk factors [3,4]. Symptomatic fatty liver disease usually encompasses end-stage liver disease (cirrhosis) or hepatocellular carcinoma, which together form the main cause of liver disease-related mortality. Of note, the risk for fatty liver disease mortality and morbidity is not only dependent on modifiable (environmental/dietary) factors, but also on a person's genetic background [3,5]. More specifically, it has been shown that human individuals carrying single nucleotide variants in the genes for patatin-like phospholipase domain-containing 3 (PNPLA3; rs738409; I148 M), membrane bound O-acyltransferase domain containing 7 (MBOAT7/TMC4/LPIAT1; rs641738), transmembrane 6 superfamily member 2 (TM6SF2; rs58542926; E167K), hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13; rs72613567; altered mRNA splicing),

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mitochondrial amidoxime-reducing component 1 (MARC1; rs2642438) and autophagy-related 7 (ATG7; p.V471A) exhibit a significantly higher risk to develop hepatocellular ballooning, liver fibrosis, cirrhosis or hepatocellular carcinoma [3,6–9]. The mechanisms driving the development of NAFLD appear to be different between subjects suffering from the genetic disease type and those suffering from the metabolically-induced type, with genetics mostly negatively influencing the hepatic redox state whilst substrate surplus primarily underlies the metabolic variant [8].

Subjects with NAFLD display a 1.5- to 2-fold higher cardiovascular disease mortality and morbidity risk [10–14]. This phenomenon is generally attributed to the NAFLD-associated increase in pro-atherogenic apoB100-containing small dense low-density lipoprotein (LDL) levels and plasma hypertriglyceridemia, as reviewed by Deprince et al. [15]. However, human subjects with NAFLD also contain relatively high remnant cholesterol levels [16] and exhibit an increased heart rate and systolic blood pressure [17], which are additional cardiovascular disease risk factors. Importantly, a marked reduction in plasma levels of cholesterol associated with anti-atherogenic high--density lipoproteins (HDL) is frequently detected in human subjects suffering from NAFLD, which also seems to be a significant contributor to the occurrence of cardiovascular complications such as atrial fibrillation in this specific patient population [18]. HDLs constitute an important class of lipid/protein complexes that are essential for reverse cholesterol transport, the process through which excess peripheral cholesterol is removed from the body [19]. As can be seen from Fig. 2, the life cycle of HDL particles in humans starts with the generation of nascent, pre-beta particles through lipidation of hepatocyte-derived apolipoprotein A1 (apoA1) by the ATP-binding cassette transporters ABCA1 and ABCG1 and scavenger receptor BI (SR-BI). Subsequently, after acquisition of phospholipids and triglycerides from apoB-containing lipoproteins, cholesteryl ester-rich fully matured HDL particles with SR-BI present on hepatocytes and adrenocortical cells to selectively transfer their cholesterol load (i.e. without whole particle uptake) for subsequent biliary excretion and conversion into steroid hormones. Please refer to the recent review of Ong et al. for a detailed overview of all processes involved in the formation, maturation, and remodeling of HDL particles [20]. In this review, we summarize current data regarding the relationship between NAFLD and plasma HDL-cholesterol levels, with a special focus on highlighting potential causality between the NAFLD pathology and changes in HDL metabolism.

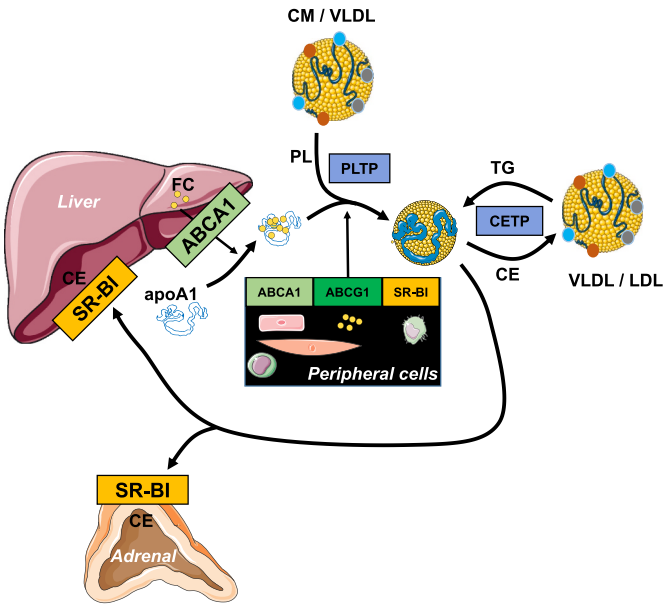

Fig. 2. Schematic overview of the high-density lipoprotein (HDL) life cycle in humans. FC, free cholesterol; PL, phospholipids; TG, triglycerides; CE; cholesteryl esters; CM, chylomicrons; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; apoA1, apolipoprotein A1; ABC, ATP-binding cassette transporter; SR-BI, scavenger receptor BI; PLTP, phospholipid transfer protein; CETP, cholesteryl ester transfer protein.

2. Hepatic steatosis severity in human NAFLD is inversely correlated with plasma HDL-cholesterol levels

Several studies have evaluated the influence of different liver disease stages/pathologies on plasma lipid levels to, for instance, uncover the relative impact of hepatic lipid loading and inflammation on the NAFLD/NASH-associated change in lipidemic state. Zhang et al. have retrospectively analyzed the plasma lipid distribution of human subjects with biopsy- or clinical diagnosis-verified primary biliary cholangitis (PBC), chronic hepatitis, or NAFLD and compared their profiles to those of healthy control subjects [21]. In accordance with the notion that the presence of NAFLD predisposes to the development of a relatively pro-atherogenic lipoprotein profile, subjects with NAFLD within the patient cohort of Zhang et al. exhibited on average the highest plasma LDL-cholesterol levels. Importantly, a parallel reduction in average HDL-cholesterol levels was detected in NAFLD carriers (1.2 mmol/L) as compared to age-matched healthy controls (1.3 mmol/L). In agreement, Ren et al. [22] and DeFilippis et al. [23] have observed that HDL-cholesterol levels are decreased in the context of higher LDL-cholesterol levels and a higher overall plasma atherogenic index (total cholesterol/HDL-cholesterol ratio) in subjects with NAFLD as compared to those without NAFLD. In addition, Yozgat et al. [24] and Karami et al. [25] also found a significant decrease in HDL-cholesterol levels in their non-alcoholic hepatic steatosis patients as compared to patient controls devoid of any fatty liver disease. Furthermore, a clear trend towards a decrease in HDL-cholesterol levels in NAFLD carriers as compared to control subjects was detected by Bril et al. in both

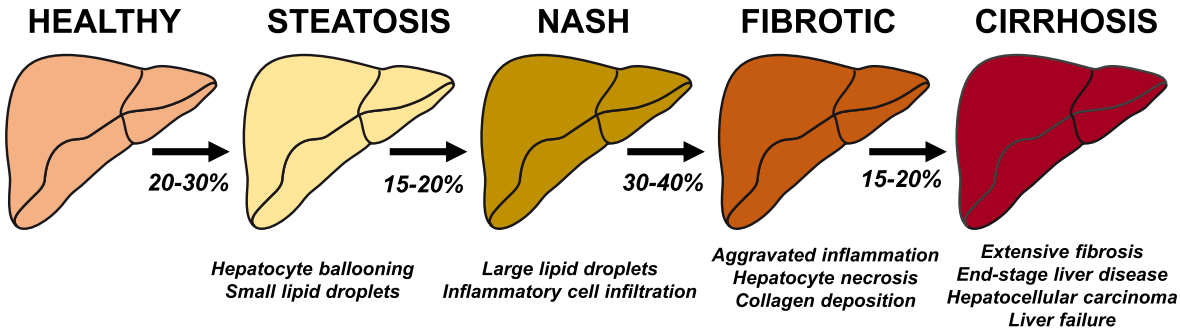

Fig. 1. Schematic overview of the different non-alcoholic fatty liver disease (NAFLD) pathology states. Percentages below arrows indicate the fraction of affected individuals that, on average, is thought to progress from the current pathology to the next disease stage.

non-obese and obese populations [26]. Moreover, Li et al. have observed that a high total cholesterol/HDL-cholesterol ratio and LDL-cholesterol/HDL-cholesterol ratio is associated with a significantly increased NAFLD risk in newly diagnosed type II diabetic patients [27].

An interesting finding from the study by Zhang et al. is that a strong inverse association existed between HDL-cholesterol levels and the hepatic controlled attenuation parameter values, a measure of fat content, in their total study population that also included healthy human controls [21]. Since controlled attenuation parameter values increase with the severity of liver steatosis [28], it can be suggested that HDL-cholesterol levels are inversely correlated to the hepatic steatosis extent. In support, Janac et al. have detected a stepwise reduction in HDL-cholesterol levels with an increasing fatty liver disease index [29]. In addition, an elevated plasma apoB/apoA1 ratio was observed in NAFLD carriers as compared to healthy human subjects [30]. Bril et al. also detected a clear trend towards an increase in the plasma apoB/apoA1 ratio in subjects with NAFLD with an increasing hepatic steatosis grade [26]. Moreover, multiple variable linear regression analysis by DeFilippis et al. uncovered a significant correlation between HDL-cholesterol levels and the CT-derived liver to spleen attenuation ratio (L/S ratio) that decreases in response to hepatic lipid loading [23].

In many clinical studies a negative association has been found between a high systemic inflammatory state and plasma HDL-cholesterol levels in humans. For instance, Kim et al. have shown that subclinical inflammation is associated with and low HDL-cholesterol levels in normal-weight Korean individuals [31]. In addition, Klisic et al. found HDL-cholesterol levels to be a negative predictor of plasma levels of the inflammation marker C-reactive protein in a cohort of healthy postmenopausal women [32]. As such, it has also been investigated whether the inflammatory status of the liver in NAFLD/NASH is related to the observed decrease in plasma HDL levels. In the study by Zhang et al., HDL-cholesterol levels were not different from healthy control values in patients suffering from a hepatitis B or C virus infection [21]. This implies that an increased hepatic inflammatory status is likely not the driving force behind the decrease in HDL-cholesterol levels in NAFLD. Accordingly, Bril et al. did not detect a difference in plasma HDL-cholesterol levels of subjects with NAFLD between those with and without NASH [26]. Furthermore, the apoB to apoA1 ratio (VLDL/LDL to HDL ratio) is not influenced by the liver inflammation grade of subjects with NAFLD [26,33]. The data from Zhang et al. suggest that, in contrast to the relative HDL deficiency in NAFLD, HDL-cholesterol levels are actually relatively high in subjects suffering from PBC, a progressive autoimmune disease that is characterized by destruction of the small bile ducts of the liver leading to intra-hepatic accumulation of bile and other toxins [21]. The observation that PBC is associated with an elevated plasma level of anti-atherogenic HDLs corroborates findings from Loaeza-Del Castillo et al. that plasma triglyceride, LDL-cholesterol, and HDL-cholesterol levels are all higher in PBC patients [34]. Of note, the particularly high HDL levels in human PBC patients may theoretically also explain why the hypercholesterolemia-associated increase in cardiovascular disease risk seen in the general population is frequently not observed in this subgroup of individuals [35].

3. NAFLD is associated with a higher HDL particle turnover and reduction in particle size in humans

The total amount of cholesterol associated with the HDL fraction present in the plasma compartment is dependent on two parameters: the plasma HDL particle number and the cholesterol content of the individual circulating HDL species. To gain insight into the potential mechanism underlying the NAFLD-associated decrease in plasma HDL-cholesterol levels, it is thus important to study the impact of hepatic steatosis on both HDL-related parameters. The core of mature, large spherical HDL particles is filled with cholesteryl esters and surrounded by an unesterified cholesterol/phospholipid-containing shell, whilst small, pre-beta HDL particles are rather disc-like structures or small spheres containing phospholipids and a limited amount of unesterified cholesterol [20]. Particle size is therefore frequently used as surrogate measure of the HDL particle cholesterol content. Interestingly, clinical findings from DeFilippis et al. have suggested that the decrease in HDL-cholesterol levels can possibly be attributed to a decrease in HDL lipidation. HDL particle concentrations – as measured by NMR – were not different between human subjects with mild, moderate, or severe NAFLD and non-diseased controls [23]. In agreement, although it should be noted that the numbers of patients included in this study were relatively low (N = 7–9), McCullough et al. have found the plasma apoA1 pools not to be significantly different between NAFLD patients and healthy controls [30]. However, a significant, step-wise reduction in HDL particle size was detected with increasing NALFD severity, with an average particle diameter of <9 nm in patients versus 9.19 nm in unaffected controls. As a result, the small HDL to large HDL ratio is almost doubled in severely diseased subjects, i.e. those with a L/S ratio of <0.5, as compared to non-diseased subjects with a L/S ratio of ≥1 [23]. In accordance with the notion that HDL size is becoming even smaller during NAFLD disease progression, Castillo-Leon et al. found a shift in the HDL particle distribution profile towards less large HDL species and a higher number of small HDL particles in children with biopsy-proven NAFLD with NASH as compared to NAFLD patients without NASH [33]. As a result, the presence of NASH was associated with a reduction in HDL-cholesterol levels without a difference in the total apoA1 concentration [33]. Morze et al. noted that a positive association existed between apoA1 levels and liver fat in their study cohort of >6000 people from the general population [36]. This unexpected finding contradicts the anticipated inverse correlation between HDL levels and liver steatosis. However, it should be noted that (1) higher apoA1 levels were associated with a reduced odds ratio for NAFLD and that (2) only a limited rise in liver fat content was detected between subjects with the lowest and highest apoA1 levels in the study population from Morze et al. [36]. Furthermore, the observed positive association between apoA1 levels and liver fat highly depended on the presence of a subgroup of HDL particles. Higher levels of apoA1 in HDL that lacks apoC3 were associated with a small, but significant rise in liver fat content, whilst no difference in liver fat values was shown with increasing levels of apoA1 associated with HDL particles also containing apoC3 [36]. In accordance with the notion that both the number as well as the size of HDL particles is reduced in NAFLD, Orozco Morales et al. have found that the decrease in total plasma HDL-cholesterol and apoA1 levels in type II diabetics with NAFLD as compared to those without NAFLD was associated with a re-distribution within the HDL subclasses from relatively large HDL2b particles to small HDL3c particles [37]. A similar shift in the HDL sub-fractions was observed by Nikeghbalian et al. in subjects with a relatively high NAFLD activity score as compared to those with a low NAFLD activity score in healthy liver donors [38]. The NAFLD-associated significant reduction in the HDL2/HDL3 ratio appears to be driven by a decrease in total HDL2 levels in the context of a minor change in total HDL3 levels [38]. The NAFLD-associated decrease in HDL particle size is paralleled by a change in the lipid composition, i.e. a shift towards relatively higher triglyceride amounts at the expense of unesterified and esterified cholesterol levels, at least in type II diabetic patients [37]. Some of the changes in the HDL lipidome detected by Orozco Morales et al. have also been reported for HDL from non-diabetic drug naïve adults with metabolic syndrome, i.e. suffering from obesity, insulin resistance, and mixed dyslipidemia [39] as well as for HDL obtained from cardiovascular disease patients [40]. As such, it can be suggested that HDL dysfunction is the common pathological factor connecting the metabolic syndrome to NAFLD and cardiovascular disease development. HDL particles from NAFLD patients also appear to be characterized by polyunsaturated fatty acid phospholipid depletion and enrichment of saturated fatty acid ceramides [41]. Notably, the fact that HDL particles are somewhat smaller in the steady-state can possibly also be explained by the observation of McCullough et al. that the HDL particle turnover is significantly enhanced in NAFLD patients [30]. An

overview of the NAFLD-associated changes in HDL particle characteristics is presented in Fig. 3.

4. Human NAFLD carriers exhibit an altered HDL protein composition and reduced plasma cholesterol efflux capacity

Although apoA1 is the primary protein constituent of HDL species, it should be acknowledged that the particles can actually contain >50 different proteins. For instance, HDL carries several other cholesterol metabolism-related apolipoprotein subclasses, i.e. apoE and apoA4, as well as the calcium-dependent esterase paraoxonase 1 (PON1) that can hydrolyze lipid peroxides and thereby contributes to HDL's anti-oxidative and anti-inflammatory effects. Given that the presence of a fatty liver appears to be associated with a change in particle size, Rao et al. have investigated in 5 NAFLD and 5 NASH patients and 5 unaffected controls a potential parallel effect of the different liver disease states on the protein composition of HDL [42]. Through applying mass spectrometry on HDL-enriched fractions isolated by fast performance liquid chromatography and size exclusion chromatography, Rao al. identified 120 proteins in samples from subjects with normal livers and simple steatosis as well as those obtained from people with NASH. They did, however, not find a difference in the relative protein amounts of apoA1, apoE, apoA4 or PON1 in the HDL particles between the different disease states. In accordance, van den Berg et al. have observed that the serum activity of PON1 is maintained at normal levels in NAFLD patients, despite the significant reduction in plasma HDL-cholesterol levels [43]. Janac et al. similarly showed that PON1 protein levels and activity are not changed with an increasing fatty liver index in their human patient cohort, whilst they found fatty liver development to be associated with a higher activity of the cholesterol esterification enzyme LCAT [29]. Accordingly, McCullough et al. observed no effect of the presence of a fatty liver on HDL's antioxidant activity [30]. A significant difference was detected by Rao et al. in the levels of 12 proteins in HDL that are primarily associated with the biological pathways negative regulation of endopeptidase activity, platelet degranulation, blood coagulation, complement activation, fibrinolysis, and positive regulation of blood coagulation. More specifically, levels of alpha-2-macroglobulin and apoB were increased in fatty liver disease patients, whilst those of alpha-1B-glycoprotein, alpha-1-antichymotrypsin, corticosteroid-binding globulin, complement factor B, alpha-2-HS-glycoprotein, hemopexin, histidine-rich glycoprotein, plasminogen, and serotransferrin were reduced in HDL fractions of NAFLD/NASH carriers [42]. From these combined findings it can be suggested that the presence of a fatty liver is highly likely associated with an impaired anti-thrombotic action of HDL particles, which may also contribute to the higher cardiovascular disease risk associated with NAFLD. Bril et al. also executed a small-scale proteomic analysis on HDL particles from patients with different stages of fatty liver disease. They found significant reductions in HDL particle protein levels of APOM, APOD, APOC1, and APOC4, LCAT, PON3, and serum amyloid A4 in NAFLD patients with advanced fibrosis as compared to those without a high level of fibrosis [44]. However, the relevance of this latter finding for the anti-atherogenic properties of HDL particles remains currently unknown.

Previous clinical studies by Khera et al. have identified a strong inverse association between the HDL-mediated plasma cholesterol efflux capacity, i.e. a proxy for the initiation of macrophage reverse cholesterol transport, with both carotid intima-media thickness and the likelihood of angiographic coronary artery disease [45]. To further verify a possible effect of fatty liver disease on HDL functionality, van den Berg et al. have therefore measured the ability to generate cholesterol efflux from cultured macrophages of apoB-depleted plasma samples obtained from the human Prevention of REnal and Vascular ENd-stage Disease (PREVEND) cohort. Fatty liver disease carriers in the PREVEND cohort exhibited a reduced cholesterol efflux capacity, which was to be expected based upon the fact that these subjects also exhibited lower apoA1 (cholesterol acceptor) protein levels [46]. A similar reduction in HDL-mediated cholesterol efflux capacity was detected in NAFLD patients in the studies by Di Constanzo et al. [47], Fadaei et al. [48], and McCullough et al. [30]. Multivariate parameter analysis by van den Berg et al. further confirmed the significant correlation between plasma HDL-cholesterol and apoA1 levels and the cholesterol efflux capacity [46]. However, van den Berg et al. also observed a reduced cholesterol efflux capacity in NAFLD patients with plasma HDL-cholesterol levels within the normal, non-diseased range [46]. This latter finding corroborates the common notion that changes in plasma HDL-cholesterol levels do not efficiently predict the overall effect on the (macrophage) reverse cholesterol transport rate/HDL functionality. In this context it is interesting to note that Di Constanzo et al. have observed that the HDL cholesterol efflux capacity is much more decreased in patients that suffer from metabolic disturbances-driven NAFLD than in those with genetic NAFLD without metabolic disturbances (PNPLA3 I148M carriers), which coincides with a significantly elevated pre-beta HDL unesterified/total cholesterol ratio [47]. Furthermore, it is good to acknowledge that having a fatty liver index of >60 also significantly modifies the impact of changes in other variables such as the systemic inflammation status and plasma triglycerides levels on the cholesterol efflux capacity [46].

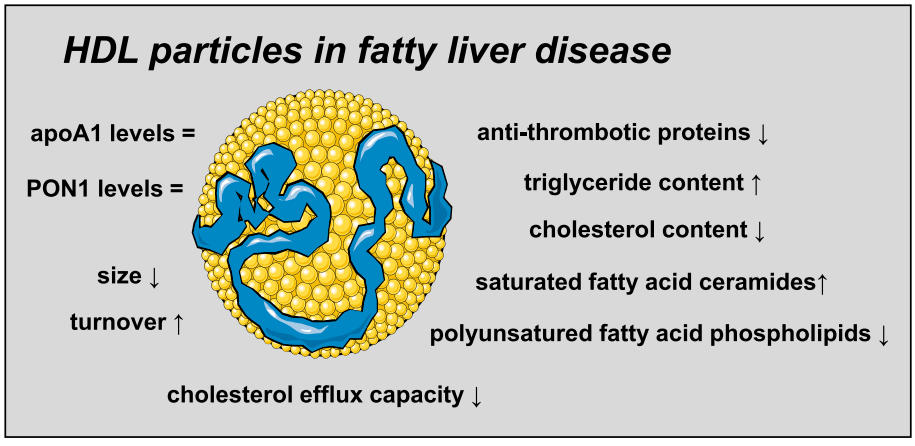

Fig. 3. Summary of the differences with respect to the high-density lipoprotein (HDL) particle shape, content, and functionality detected in human subjects suffering from non-alcoholic fatty liver disease as compared to healthy, unaffected controls. apoA1, apolipoprotein A1; PON1, paraoxonase 1.

5. Changes in HDL formation and metabolism affect NAFLD susceptibility in mice

Although interesting associations have been found in the human, clinical setting, it is difficult to distinguish whether HDL levels are reduced as a result of the presence of NAFLD or if low HDL-cholesterol levels rather predispose to the development of the disease. However, many of this type of cause/consequence related-issues can be efficiently resolved by using animal models. Given the proposed role of HDL-mediated reverse cholesterol transport in the protection against atherosclerosis, a variety of genetically modified mouse models have been developed that exhibit a mutation in proteins involved in the generation and maturation of HDL particles, the ability of different HDL species to accept cholesterol from peripheral cells or to deliver their cholesteryl ester cargo to the liver. Some of these mouse models have also already been applied in experimental settings that involve fatty liver development. As such, preclinical data are available that can shed more light onto the potential contribution of disturbances in HDL metabolism to NAFLD development.

As can be appreciated from our summary of the NAFLD-related findings from mice presented in Table 1, a common approach to induce NAFLD/NASH in wild-type, normolipidemic mice is to feed them either a (Western) diet enriched in fat or a low fat diet deprived of methionine choline. Karavia et al. have utilized a similar type of experimental approach to show that disrupted HDL production in apoA1 knockout mice is associated with an enhanced NAFLD susceptibility [49]. More specifically, they found that livers of apoA1 knockout mice contained significantly higher numbers of fatty hepatocytes as compared to wild-type control mice after feeding a lipid-enriched Western-type diet for 24 weeks. This was paralleled by an increase in hepatic triglyceride stores. In addition, it was observed that adenoviral gene transfer of the functional apoA1milano variant is able to restore plasma HDL-cholesterol levels and reverse the increased NAFLD susceptibility of apoA1 knockout mice [49]. From these combined findings it appears that, in mice, a causal relationship may exist between (genetic) HDL deficiency and NAFLD development. However, it should be noted that the apoA1 genotype-associated effect on hepatic triglyceride levels and fat cell area was not so evident after 18 weeks of Western-type diet feeding in mechanistic follow-up studies in mice performed by Karavia et al. [50]. In accordance with the notion that the inverse relationship between HDL-cholesterol levels and murine fatty liver disease susceptibility is not so clear-cut as suggested from the findings in apoA1 knockout mice, Siggins et al. have actually detected lower triglyceride levels in livers from both male and female HDL deficient PLTP knockout mice as compared to livers from gender-matched C57BL/6 wild-type mice [51]. Female (but not male) PLTP knockout mice exhibit elevated hepatic cholesterol stores under low fat diet feeding conditions [50], whilst cholesterol levels in livers from Western-type diet-fed HDL deficient apoA1 knockout mice are rather decreased as compared Western-type diet-fed controls [49]. Orso et al. have shown that HDL deficiency in mice due to genetic lack of ABCA1 is also associated with a significant decrease in hepatic cholesteryl ester content [52]. Strikingly, Ma et al. found that increasing HDL production through adenoviral overexpression of apoA1 in C57BL/6 wild-type mice fed a methionine choline-deficient is also associated with reduced hepatic lipid stores [53]. The overall effect of changes in HDL-cholesterol levels on NAFLD phenotype may thus be dependent on the specific gene defect underlying the impaired (mature) HDL particle formation, but perhaps also influenced by the type of diet provided. However, the effect of defective cellular HDL-cholesteryl ester selective uptake via SR-BI on hepatic steatosis development does not seem to be dependent on the dietary composition. We have shown that genetic lack of SR-BI protects mice against high fat diet-induced development of fatty liver disease, as judged by the reduced hepatic triglyceride content found in SR-BI knockout mice as compared to wild-type controls after a 12-week high fat diet challenge [54]. A similar significant reduction in hepatic steatosis extent due to SR-BI deficiency was also seen by Karavia et al. in mice fed a Western-type diet for 24 weeks [55]. Impaired clearance of HDL-cholesteryl esters via SR-BI thus consistently seems to protect against the development of NAFLD in mice.

A limitation of using mice to model human lipoprotein metabolism and associated pathologies is that wild-type strains do not express cholesteryl ester transfer protein (CETP) that transfers cholesteryl esters from HDL particles to apoB-containing VLDL and LDL species for subsequent removal of the cholesterol pool from the circulation via LDL receptor-mediated uptake into hepatocytes. To deal with this interspecies genetic variation, mice transgenic for human CETP (including its natural flanking regulatory sequences) have been generated [56]. From studies using these mice it has become clear that (tissue) macrophages such as hepatic Kupffer cells are the primary source of circulating CETP [57,58] and that the presence of CETP also significantly lowers HDL-cholesterol levels and increases VLDL/LDL-cholesterol levels in mice [59,60]. Interestingly, studies by Zhu et al. have shown that CETP activity in mice also significantly impacts the predisposition to high fat diet-induced fatty liver development. Pharmacological inhibition of CETP activity in CETP transgenic mice through treatment with

Table 1
Summary of fatty liver disease findings from studies performed in mice that exhibit a genetic defect in genes critically involved the HDL life cycle.

Genotype	Gender	Age at start	Type of diet	Diet composition	Time on diet	HDL-C	NAFLD extent	Liver lipid phenotype	Reference
ABCA1 KO mice	Unknown	2 weeks-10 months	Western	20.85% raw fat, 0.15% cholesterol, 19.5% casein	10 days	↓	↓	Cholesteryl ester ↓	[52]
apoA1 KO mice	Male	10–12 weeks	Western	17.3% protein, 48.5% carbohydrate, 21.2% fat, 0.2% cholesterol	18 weeks	↓	= /↑	Triglyceride = Cholesterol ↑	[50]
					24 weeks	↓	↑	Triglyceride ↑ Cholesterol ↓	[49]
apoA1 overexpressing C57BL/6 mice	Male	4–6 weeks	Methionine Choline-deficient	Details unknown	1 week	↑	↓	Triglyceride ↓ Cholesterol ↓	[53]
PLTP KO mice	Both	12 weeks	Low fat	18% protein, 5% fat	12 weeks	↓	females: ↓↑ males: ↓	Cholesterol = /↑ Triglyceride ↓	[51]
SR-BI KO mice	Male	8–11 weeks	High fat	45% fat, 20% protein	12 weeks	↑	↓	Triglyceride ↓ Cholesterol=	[54]
	Male	10–12 weeks	Western	17.3% protein, 48.5% carbohydrate, 21.2% fat, 0.2% cholesterol	24 weeks	↑	↓	Triglyceride ↓ Cholesterol ↑	[55]
CETP Tg mice + anacetrapib treatment	Male	Unknown	High fat	60% fat, 20% protein	3 months	↑	↑	Triglyceride ↑ Cholesterol ↓	[61]

Arrows indicate the effect on the specific parameter as compared to normolipidemic wild-type controls or untreated CETP Tg mice, respectively.

anacetrapib is associated with the expected, beneficial shift in the plasma cholesterol profile in the context of atherosclerotic cardiovascular disease, i.e. a reduction in non-HDL-cholesterol levels and an increase in HDL-cholesterol levels [61,62]. However, inhibition of CETP activity actually raises the susceptibility for NAFLD as judged from the finding by Zhu et al. that hepatic triglyceride levels are significantly higher in anacetrapib-treated mice as compared to control-treated mice after a high fat diet challenge [61]. Notably, CETP activity inhibition by anacetrapib increases the pro-inflammatory serum amyloid A content of HDL particles, both in mice fed the regular chow and high fat diets [61]. It can therefore be suggested that the increase in NAFLD/NASH susceptibility associated with anacetrapib exposure may also, at least partially, be secondary to a reduced anti-inflammatory function of HDL. Given that the findings from Zhu et al. highlight that CETP not only modulates HDL-cholesterol levels, but also HDL functionality and fatty liver disease outcome, the predictive value of intervention studies in wild-type mice lacking CETP for the human clinical setting may be limited. In this light it is good to acknowledge that researchers studying human NAFLD in the preclinical setting already make use of APOE*3-Leiden.CETP mice that not only functionally express the human CETP protein but also exhibit a more human-like lipoprotein profile with relatively high non-HDL levels as compared to wild-type mice [62,63].

6. SR-BI deficiency as well as apoA1 and ABCA1 overexpression protect cultured hepatocytes against NAFLD-like cellular lipid accumulation

The development of fatty livers is not only dependent on changes locally within hepatocytes, but also influenced by the activity and functioning of other tissues, i.e. adipose tissue and the pancreas. For instance, the presence of obesity and type II diabetes have been proposed to predispose to the development of severe liver disease [64]. It can thus be difficult to pinpoint whether effects on NAFLD susceptibility of a change in the activity of a HDL metabolism-related gene product in mice are solely due to a direct change in hepatocyte functionality or rather secondary to systemic alterations. Preclinical studies in rodent disease models have therefore been complemented with in vitro cell culture studies that are able to more directly prove causality between changes in HDL metabolism and cellular lipid accumulation. SR-BI may contribute to triglyceride accumulation in NAFLD by directly fluxing fatty acids from the circulation into the hepatocytes, since Wang et al. have observed that genetic SR-BI deficiency is associated with a reduced fatty acid uptake by mouse adipocytes in vitro [65]. However, SR-BI potentially also facilitates NAFLD development through the (selective) uptake of lipids from HDL that subsequently accumulate in cells. More specifically, Sovic et al. have shown that (1) SR-BI overexpression enhances the binding and uptake of HDL by cultured brain capillary endothelial cells and that (2) the fatty acid content of cells increases significantly upon incubation with HDL [66]. Importantly, substantial in vitro evidence has been provided for the hypothesis that the production of apoA1-containing HDL particles reduces hepatic lipid stores and thereby protects against NAFLD development. More specifically, overexpression of apoA1 in human hepatic cell line HepG2 reduces relative mRNA expression levels of key genes involved in the synthesis of fatty acids and diminishes tunicamycin-induced and palmitic acid-induced unesterified cholesterol and triglyceride accumulation [67]. Studies by Ma et al. have found that overexpression of either apoA1 or ABCA1 in the human QSG-7701 hepatocyte cell line also reduces fatty acid synthase and acetyl-CoA carboxylase mRNA expression levels and lowers cellular cholesterol, fatty acid, and triglyceride stores [53]. Induction of ABCA1 expression through exposure of QSG-7701 hepatocytes to berberine, a quaternary benzylisoquinoline plant alkaloid, similarly lowers cellular cholesterol and triglyceride levels [68]. These combined findings underscore that changes in HDL metabolism in hepatocytes can directly impact the susceptibility to NAFLD development.

7. Concluding remarks

Although the presence of NAFLD is frequently associated with low HDL-cholesterol levels, it still remains unclear whether the relative HDL deficiency is secondary to the general dyslipidemia associated with NAFLD or actually the driving force behind the fatty liver development. In vitro observations do clearly suggest causality, with secretion of apoA1 and subsequent HDL particle lipidation by ABCA1 both protecting against cellular lipid accumulation. However, it should be noted that studies in cultured primary hepatocytes or hepatoma-derived cell lines may have limited value in predicting human disease outcomes as (1) relative expression levels of many proteins involved in lipid metabolism in hepatocytes can differ greatly between in vitro and in vivo settings and (2) the potential influence of surrounding liver cells, i.e. (pro-inflammatory) macrophages, hepatic stellate cells, and endothelial cells, on NAFLD outcome is eliminated in vitro. In accordance, Oscarsson et al. have shown that stimulation of hepatic apoA1 and HDL synthesis through treatment with fenofibrate, a synthetic PPARalpha agonist, fails to reduce liver fat content in dyslipidemic, overweight, or obese individuals [69]. It will thus be important to invest in the development of in vitro cell culture systems, i.e. 3D spheroids and organ-on-a-chip approaches, that more reliably model the in vivo (clinical) situation. In this context it is good to acknowledge that zebrafish have recently emerged as a potential novel animal model to study lipid metabolism and the NAFLD pathology. Zebrafish do express CETP that is crucially involved in human lipoprotein metabolism and the function of several scavenger receptors as well as the LDL receptor in hepatic lipid uptake appears to be quite similar between zebrafish, mice, and humans [70–72]. As reviewed by Chang et al., several NAFLD zebrafish models are already in use in which the different disease pathologies are induced through either genetic manipulations, the application of specific diets, or administration of toxins such as thioacetamide [73].

Previous studies have highlighted the potential of Mendelian randomization studies to predict the overall effect of genetically-driven changes in HDL-cholesterol levels on ischemic cardiovascular disease risk in humans [74,75]. Given that the preclinical findings in mice showed ambiguous effects of changes in HDL production on NAFLD outcome, it will be clearly of interest to also investigate a potential causal relationship between genetically low HDL-cholesterol levels and NAFLD risk through applying a Mendelian randomization approach.

Preclinical studies in mice have consistently found that disruption of SR-BI-mediated hepatic lipid uptake is able to reduce NAFLD burden. The small molecule drug ITX 5061 is in clinical development as SR-BI inhibitor as it not only effectively blocks HDL-mediated lipid transfer, but can also diminish hepatitis C virus infection of hepatocytes [76]. However, chronic treatment with ITX 5061 is not anticipated to be a valuable therapeutic approach to overcome NAFLD development as judged from the fact that genetic SR-BI deficiency also predisposes mice and humans to the development of atherosclerotic cardiovascular disease due to disruption of the reverse cholesterol transport pathway [77–79].

In vitro findings from Ma et al. have implied lowering of cellular levels of the bioactive molecule 27-hydroxycholesterol as key element in the protection against lipid accumulation resulting from increased HDL production [53]. In this context it is of interest to acknowledge that several marketed drugs, e.g. bicalutamide (an anti-androgen), anastrozole (an aromatase inhibitor) and dexmedetomidine (an α2-adrenergic agonist), can potently inhibit the activity of cytochrome P450 27A1 that facilitates the conversion of cholesterol into 27-hydroxycholesterol [80]. The impact of these three drugs on NAFLD susceptibility in humans currently remains unknown. Also no preclinical data regarding a potential effect on liver disease outcome of treatment with bicalutamide and anastrozole are present. However, Tao et al. have recently shown that treatment of obese mice with the anxiolytic and sedative drug dexmedetomidine can ameliorate the fatty liver disease extent [81]. Given that clinical studies are ongoing which evaluate the

effect of chronic low dose dexmedetomidine administration in the treatment of insomnia [82], it will be of interest to include liver fat measurements in the test protocols to provide proof for a potential NAFLD-inhibiting action of dexmedetomidine in humans. Furthermore, the application of these compounds in recently developed NAFLD zebrafish models will be helpful in predicting their therapeutic potential.

In conclusion, although in vitro observations suggest causality between HDL formation by hepatocytes and protection against NAFLD-like lipid accumulation, current literature remains inconclusive on whether relative HDL deficiency is actually driving the development of fatty liver disease in humans. In light of the current obesity pandemic and the associated marked rise in NAFLD incidence, it is of clear scientific and societal interest to gain further insight into the relationship between HDL-cholesterol levels and fatty liver development to potentially uncover the therapeutic potential of pharmacological HDL level and/or function modulation. Given the heterogenicity in the cause of the disease, it will be important to take into account that genetic and modifiable risk factors may differentially impact the NAFLD pathogenesis, potential for pharmacological drug treatment, and associated disease outcomes.

CRediT authorship contribution statement

Menno Hoekstra: performed the original literature search and drafted the manuscript. **Miranda Van Eck:** critically reviewed the manuscript before final submission.

Declaration of competing interest

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

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