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REVIEWS

Review

NAFLD and Atherosclerosis: Two Sides of the Same Dysmetabolic Coin?

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The prevalence of non-alcoholic fatty liver disease (NAFLD) is strongly increasing and may put patients at increased risk for atherosclerotic cardiovascular disease (asCVD). Both disease phenotypes often co-occur, in the case of obesity, insulin resistance, diabetes mellitus type 2, and the metabolic syndrome. We explore the pathogenesis of NAFLD, the epidemiology of asCVD in NAFLD patients, shared drivers of both phenotypes, and factors caused by NAFLD that contribute to asCVD. Genetic studies support that NAFLD may drive asCVD through mixed hyperlipidemia. Next, we discuss the prospects of lifestyle improvement and pharmacological treatment of NAFLD for asCVD risk reduction. Finally, we point out that earlier identification of patients with NAFLD should be pursued by increasing awareness of the association of these two phenotypes and collaboration between the involved physicians.

Highlights

Non-alcoholic Fatty Liver Disease (NAFLD) and atherosclerotic cardiovascular disease (asCVD) often co-occur.

Large epidemiological studies, genetics studies, and studies of subclinical atherosclerosis, support the relation between NAFLD and asCVD.

NAFLD–non-alcoholic steatohepatitis (NASH) may directly contribute to asCVD and atherothrombotic events in the hepatic secretion of atherogenic lipoproteins and procoagulant factors.

Genetic studies support the relationship between NAFLD and asCVD, notably via increased VLDL secretion. In particular, they support that genetic variants affecting both liver fat and plasma lipid levels have an effect on asCVD risk, while variants with an effect on liver fat only do not affect asCVD.

Factors that may drive both NAFLD and asCVD are insulin resistance, hypertension, and potentially chronic periodic hypoxia, as observed in obstructive sleep apnea, and intestinal dysbiosis and chronic low grade inflammation.

NAFLD: The Hepatic Component of the Metabolic Syndrome and its Relation with asCVD

In a global trend, NAFLD is becoming a predominant cause of liver dysfunction. NAFLD is defined as hepatic fat accumulation, or hepatic steatosis, in the absence of excessive alcohol consumption (Box 1). It represents a spectrum of liver disease that ranges from steatosis and non-alcoholic steatohepatitis (NASH) to advanced fibrosis and cirrhosis [1]. Eventually this process can progress to hepatocellular carcinoma (HCC) and in a minority of patients, NAFLD with fibrosis progresses directly to HCC without the intermediate phase of cirrhosis [2]. The burgeoning problem of NAFLD–NASH could be regarded as the resultant of an ageing population with ever more and longer exposure to obesity and insulin resistance; on average, advanced stages of NAFLD only develop after decades of exposure to obesity and type 2 diabetes mellitus (T2DM) [3]. NAFLD can be viewed as the hepatic component of the metabolic syndrome and T2DM [4]. Indeed, NAFLD strongly coincides with T2DM and obesity: both epidemiological and pathophysiological studies bolster this statement. The endemic and pathogenic nature of NALFD is exemplified by the finding that NAFLD-related cirrhosis has become a primary cause of liver transplantation in the United States. Of note, NAFLD also has a strong correlation with both asCVD and hypertension [5,6].

This review explores the relationship between NAFLD and asCVD: coincidence of both disease phenotypes in obese patients with the metabolic syndrome, joint pathophysiological pathways, mutual risk factors, and potential future treatments that may reduce both NAFLD and asCVD risk. Lastly, we discuss how healthcare providers such as general practitioners, cardiologists, endocrinologists, and hepatologists could generate a platform to improve the care for patients with progressive stages of NAFLD.

Together, these changes induce NASH: necroptosis of hepatocytes and infiltration of macrophages, with subsequent secretion of proinflammatory markers and procoagulant factors, such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNFα), nuclear factor-κB (NF-κB), monocyte chemoattractant protein-1 (MCP-1), resistin, and plasminogen activator inhibitor-1(PAI-1) [3,8–11]. Furthermore, hypoadiponectinemia and hyperleptinemia are also seen in NAFLD patients, suggesting a link between adipose tissue activity and liver fat accumulation. In this regard, adiponectin has a known antisteatotic, anti-inflammatory, and antifibrotic role [12–14].

Some of these factors, such as TNFα, induce inflammatory cascades but also mediate liver repair. Yet in NASH, the chronic inflammatory state leads to differentiation of stellate cells to myofibroblasts,

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Box 1. NAFLD: An Overview of the Pathogenesis

NAFLD tends to arise in a situation of an overload of energy. In this state, hyperplasia and hypertrophy of adipose tissue develops. Insulin resistance of adipose tissue induces local lipolysis, causing an increased flux of free fatty acids (FFA) towards the liver. Stable isotope studies indicate that most hepatic FFAs in NAFLD originate from this hepatopetal FFA flux: 59%, compared with 26% from hepatic *de novo* lipogenesis (itself upregulated in the hyperinsulinemic state via SREBP1c), while only 15% of the FFA is derived from diet [7]. The liver can compensate for this FFA excess by storing FFA as TGs in lipid droplets (hepatic steatogenesis), which in turn results in increased secretion of TG-rich very low density lipoprotein (VLDL) particles. The characteristic mixed hyperlipidemia phenotype observed in NAFLD patients is a consequence of this phenomenon. Moreover, increased FFA flux does result in increased hepatic mitochondrial beta-oxidation of FFAs and increased turnover of lipid droplets via fusion with lysosomes, a process referred to as lipophagy. When these mechanisms become overwhelmed by influx and synthesis of FFAs, lipotoxicity arises, leading to mitochondrial dysfunction, endoplasmic reticulum stress, and ROS production (see Figure 2 in main text).

which deposit type 1 collagen and produce tissue inhibitors of metalloproteinases (TIMPs) [9,15], leading to the characteristic NAFL-related fibrosis (Figure 1) [16].

Epidemiology: A Close Connection between NAFLD and asCVD

Physicians who treat patients with NAFLD may well acknowledge the strong coincidence of NAFLD, (components of the) metabolic syndrome, and asCVD and these associations are supported by observational studies. The correlation between NAFLD and asCVD has been assessed in multiple recent reviews. Patients who have NASH or advanced NAFLD-related fibrosis are at greater risk for asCVD than those with simple steatosis [17,18]. Indeed, asCVD has been mentioned as the number one cause of death in NAFLD patients [19–21]. In a recent meta-analysis, however, it was shown that asCVD mortality was significantly different between NAFLD participants and non-NAFLD participants [22]. Of note, this meta-analysis included 34 studies and aimed to asses to what extent the risk of asCVD is conferred by NAFLD. Despite the absence of an association between NAFLD and the mortality rate from asCVD, the authors did report an association between NAFLD and prevalent [odds ratio (OR) = 1.81, 95% confidence interval (CI): 1.23–2.66] and incident [hazard ratio (HR) = 1.37, 95% CI: 1.10–1.72] asCVD, increased risk of prevalent (OR = 1.87, 95% CI: 1.47–2.37) and incident (HR = 2.31, 95% CI: 1.46–3.65) coronary artery disease (CAD), prevalent (OR = 1.24, 95% CI: 1.14–1.36) and incident (HR = 1.16, 95% CI: 1.06–1.27) hypertension, as well as prevalent (OR = 1.32, 95% CI: 1.07–1.62) atherosclerosis [22].

Lessons from Genetics

NAFLD and asCVD are both multifactorial conditions which often coincide with each other and with features of the metabolic syndrome such as insulin resistance, dyslipidemia, and hypertension. It is therefore difficult to investigate the potential direct contributions of NAFLD to asCVD. It seems plausible that NAFLD may contribute to atherogenesis via mixed hyperlipidemia. Interestingly, lessons from MR studies provide some support for this notion.

A handful of genetic variants are known to accelerate the development of NAFLD, and one of these also influences asCVD risk. The best established NAFLD-associated gene is *PNPLA3*. *PNPLA3* encodes the adiponutrin protein, which acts as hydrolase in triglyceride (TG) metabolism. A SNP rs738409 C>G changes codon 148 in *PNPLA3,* from isoleucine to methionine (I148M). The I148M allele is strongly associated with increased fat liver content and NAFLD. *In vitro,* the I148M variant has been shown to reduce TG hydrolase activity in lipid droplets, being a key regulator of these droplets in hepatocytes and hepatic stellate cells [23]. Yet a direct mechanistic link with hepatic lipid accumulation has thus far not been established, as murine models have provided conflicting findings. In this regard, Mitsche *et al*. have demonstrated that the PNPLA3 protein may have a role in transferring essential fatty acids from TGs to phospholipids in hepatic lipid droplets [24,25].

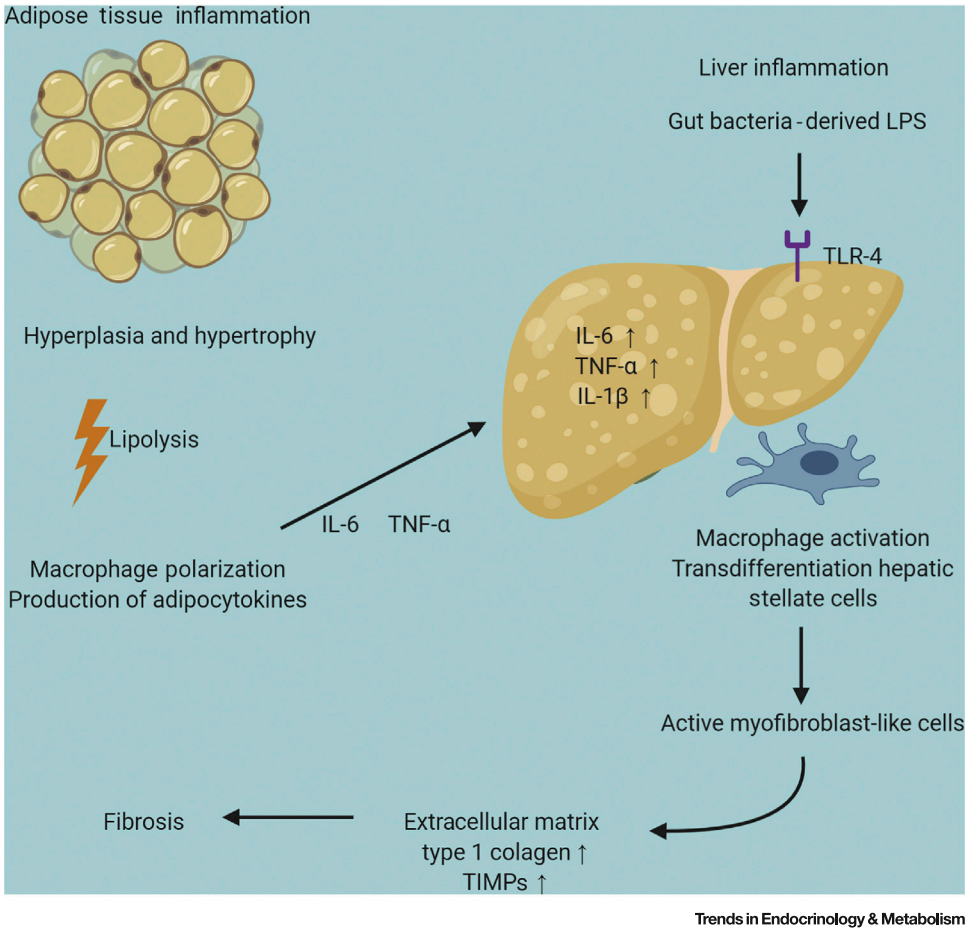

Figure 1. Inflammation Plays an Important Role in NAFLD at Different Levels.
Adipocytokines are produced via macrophage polarization in the adipose tissue and also in the liver. Macrophage activation and transdifferentiation of hepatic stellate cells can induce fibrosis. Gut bacteria-derived LPS has also been related to liver inflammation via TLR-4 receptors. Abbreviations: IL-6, interleukin-6; LPS, lipopolysaccharide; NAFLD, non-alcoholic fatty liver disease; TIMPs, tissue inhibitors of metalloproteinases; TNF-α, tumor necrosis factor alpha.

Using an MR design, the hypothesis that lifelong high liver fat content or diagnosis of NAFLD is a causal risk for ischemic heart disease was tested. The I148M *PNPLA3* allele was used as a proxy for liver fat content. In a meta-analysis of 279 013 individuals from the Copenhagen City Heart Study, the Copenhagen General Population Study, and CARDIoGRAMplusC4D consortium, the main finding was that carriers of the I148M *PNPLA3* allele had higher liver fat content and advanced stages of NAFLD, yet this genetically determined higher liver fat content was not associated with altered plasma lipids or increased risk of IHD (OR = 0.98, 95% CI: 0.94–1.03) [26]. The lack of association between this SNP and CVD has also been previously reviewed [27].

TM6SF2 is another gene associated with NAFLD susceptibility and progression. The variant E167K reduced very low density lipoprotein (VLDL) secretion, causing steatosis, yet reduced ApoB-containing lipoproteins in plasma and also cardiovascular risk [28,29].

In a recently published study from Brouwers *et al.*, a Medline search for 'NAFLD and gene' has identified and clustered 12 NAFLD susceptibility genes. They were subsequently tested for association

with CAD and lipids in the Coronary Artery Disease Genome-Wide Replication and Meta-Analysis plus the Coronary Artery Disease Genetics (CARDIoGRAMplusC4D) Consortium data set. The entire set of 12 NAFLD susceptibility genes was not associated with CAD. However, upon exclusion of genes that relate to NAFLD through impaired VLDL secretion, the remaining gene set did show a positive relation with CAD [30].

Two other NAFLD genes that have emerged are *MBOAT7* and *HSD17B13*. *MBOAT7* is a multispanning transmembrane protein with a six transmembrane domain that has a luminal localization and has a role in remodeling the acyl chain composition of endomembranes. It works in the remodeling cycle of phospholipids and thereby influences the lipotoxic lysophosphatidylcholine [31,32]. *HSD17B13* is a lipid droplet factor in the liver [33]. Although *MBOAT7* seems to have a neutral effect on asCVD, future studies are needed to confirm this and to investigate whether *HSD17B13* influences asCVD risk [34].

Taken together, genetic studies support the relationship between NAFLD and asCVD, notably via altered VLDL secretion and mixed hyperlipidemia. In particular, they support the notion that genetic variants affecting both NAFLD/liver fat and plasma lipid levels have an effect on asCVD risk; some have VLDL overproduction, whilst others have VLDL retention affecting lipids in opposite directions. Future genome-wide association studies (GWAS) providing novel NAFLD loci from large international patient cohorts with liver biopsies are expected in the upcoming years and will further aid in elucidating the relation between NAFLD, asCVD, plasma lipids, and other coincident metabolic factors.

Markers of Subclinical Atherosclerosis in Patients with NAFLD

Coronary Artery Calcium (CAC) Score

CAC scoring by means of computerized tomography (CT) is commonly used to evaluate the extent of coronary atherosclerosis. In a large retrospective longitudinal cohort study with 4731 asymptomatic participants attending regular health screening in South Korea, CAC score progression was compared between subjects with and without NAFLD. NAFLD was diagnosed based on ultrasound standard criteria, including parenchymal brightness, liver-to-kidney contrast, deep beam attenuation, and bright vessel walls. The average duration of follow-up was 3.9 years. The annual rates of CAC progression were 22% in NAFLD participants and 17% in the participants without NAFLD. The multivariable adjusted ratio of progression comparing the two groups was 1.04 (1.02–1.05; $P < 0.001$), indicating that the progression of coronary atherosclerosis was faster in participants with NAFLD at baseline compared with those without NAFLD independent of established risk factors [35]. Similar findings were reported in another Korean study, in which CAC progression was assessed in four different groups based on the presence (+) or absence (–) of NAFLD and metabolic syndrome (MetS): (+)NAFLD (+)MetS, (+)NAFLD (–)MetS, (–)NAFLD (+)MetS, and the control group that was negative for both diseases (–)NAFLD (–)MetS. Both (+)NAFLD groups with and without MetS had a higher risk of CAC progression compared with the control group (–)NAFLD (–)MetS. This supports the association of NAFLD and CAC progression independent of MetS [36].

In a study with 665 patients with NAFLD diagnosed by ultrasound, and who underwent coronary CT, four noninvasive fibrosis markers: fibrosis score (NFS), fibrosis-4 (FIB-4) score, Forn index, and the aspartate aminotransferase to platelet ratio index (APRI), were used to assess their capacity to predict CAC scores. The NFS ($r = 0.157$), FIB-4 score ($r = 0.152$), and APRI ($r = 0.120$) were significantly and independently associated with CAC score >100 [37].

Ultrasonography of Carotid Intima Media Thickness (cIMT)

Pais *et al.* have demonstrated that steatosis is associated with cIMT assessed by ultrasonography. In a longitudinal cohort with 1872 patients with a presence of at least two cardiovascular risk factors, C-IMT increased proportionally with steatosis as measured by fatty liver index, independent of traditional cardiometabolic risk factors. Steatosis was a better predictor of higher cIMT than the presence of T2D or dyslipidemia [38].

The prevalence of NAFLD (diagnosed by ultrasound) and its association with subclinical atherosclerosis (diagnosed by cIMT) was also assessed in a subgroup of Mexican American patients (n = 407) from the Cameron County Hispanic Cohort. NAFLD had a prevalence of 48.8%; nearly one third had also evidence of subclinical atherosclerosis (31.2%). These patients tended to be obese, diabetic, and have the metabolic syndrome. After adjustments for covariates there was an independent association between NAFLD and increased cIMT, only in subjects younger than 45 years [39].

One meta-analysis has included 26 studies, applying the Newcastle-Ottawa Quality Assessment Scale to assure the studies were of high quality. A total of 85 395 participants were analyzed and 24 493 were diagnosed with NAFLD. Subjects with NAFLD exhibited a significant independent association with subclinical atherosclerosis compared with the non-NAFLD group (OR = 1.60, 95% CI: 1.45–1.78). The presence of NAFLD yielded a higher risk of increased carotid artery intima media thickness/plaques, arterial stiffness, coronary artery calcification, and endothelial dysfunction with OR = 1.74, 95% CI: 1.47–2.06; OR = 1.56, 95% CI: 1.24–1.96; OR = 1.40, 95% CI: 1.22–1.60; and OR = 3.73, 95% CI: 0.99–14.09, respectively [40].

Finally, another meta-analysis evaluated the association of NAFLD and subclinical atherosclerosis and CAD, selecting 14 studies. When patients showed at least 50% stenosis at one or more major coronary arteries they were considered to have CAD. NAFLD was assessed by ultrasound and liver biopsy. NAFLD showed a higher prevalence of pathological cIMT, the presence of carotid plaques was higher, NAFLD was associated with a remarkably higher likelihood of CAD than non-NAFLD, using random effects model (OR = 3.31, 95% CI: 2.21–4.95) or fixed effects model (OR = 3.13, 95% CI: 2.36–4.16) [41].

Cardiovascular Risk Scores

As progressive NAFLD increases risk of asCVD, the most commonly used cardiovascular risk factor scoring system for used for cardiovascular risk management, such as the Framingham Risk Score, QRisk2, or SCORE, may not capture and may even underestimate cardiovascular risk in patients with NAFLD. No validated asCVD risk score specific for NAFLD patients has yet been validated or implemented. Yet, a recent interesting study has analyzed the association of mean platelet volume (MPV) and the risk of cardiovascular events in patients with NAFLD and subsequently proposed a specific NAFLD cardiovascular risk score. MPV, age, and diabetes mellitus were independently predictive factors of major adverse cardiovascular events in a modestly sized retrospective cohort of 356 patients and a validation cohort of 111 NAFLD patients. The specific NAFLD cardiovascular risk score outperformed the traditional Framingham Risk Score and Qrisk2, with an area under receiver operating characteristics curve (AUROC) of 0.83 for 1-year risk of major adverse cardiovascular event [42].

Metabolic Drivers of asCVD in Patients with NAFLD

Factors Caused by NAFLD that May in turn Drive asCVD

Mixed Hyperlipidemia

ApoB-containing lipoproteins are widely accepted as a causal factor for asCVD. This low density lipoprotein (LDL) hypothesis is supported by both epidemiological studies, MR studies, as well as multiple randomized controlled trials with LDL-lowering drugs [43]. Excess LDL particles become oxidized in the arterial vessel wall, attracting macrophages, which in turn develop into foam cells, giving rise to plaque formation. Any cause of elevated LDL induces atherogenesis, be is driven by genetics and/or lifestyle. Thus, it is no surprise that, as mentioned above, the relation between NAFLD and asCVD seems to depend, at least for a significant part, on the mixed hyperlipidemia that is frequently co-observed with NAFLD. In the Dallas Heart Study, 60% of patients with mixed hyperlipidemia had hepatic steatosis [44].

Whether the TG component of this mixed hyperlipidemia in itself also contributes to asCVD is less well established, as ApoB itself seems to be related to this risk. MR studies support a causal role for TGs in asCVD, for a review, see Larsen *et al.* [45]. Also, we have just seen the positive results of REDUCE-IT (Reduction of Cardiovascular Events With EPA - Intervention Trial) with eicosapentaenoic

acid to reduce mixed hyperlipidemia (NCT01492361). It is the first TG-lowering drug that turned out positive in a cardiovascular outcome trial (CVOT): a relative risk reduction of 25% for cardiovascular events. However, Ference *et al.* have shown that the associations between TG-lowering *LPL* variants and LDL-C lowering *LDLR* variants, lower TG and LDL-C levels, and lower risk of coronary heart disease, was proportional to absolute change in ApoB levels [46]. In addition another MR analysis using a mixture model did not detect a significant causal effect of genetically determined TG on the risk of CAD [47].

Hypercoagulable State

Another way in which NAFLD–NASH may directly contribute to asCVD and atherothrombotic events is the hepatic secretion of procoagulant factors, notably fibrinogen and plasmin gen activator inhibitor-1, often together with proinflammatory factors such as TNFα and Il-6. The evidence for this contribution has been excellently weighed and balanced by Targher *et al.* [48]. Procoagulant factors are higher in NASH than in simple steatosis and the relation seems to be independent from obesity and other underlying metabolic abnormalities. Yet there is a paucity of large prospective studies, direct measurements of visceral adipose tissue as a potential confounder, and of inclusion of patients with advanced fibrosis or cirrhosis, calling for further studies.

Factors that Have Been Implicated to Drive Both NAFLD and asCVD

- Insulin resistance is considered as one of the root causes of NAFLD [3]. In addition, it leads to hyperglycemia, which affects microvascular and macrovascular homeostasis in multiple ways, favoring atherogenesis. Hyperglycemia has been shown to decrease nitric oxide release, increase vascular cell adhesion molecule expression, partly by induction of AGE-RAGE (receptor for AGE) complexes, and stimulate uptake of oxidized LDL by macrophage receptors such as CD36. In addition, stimulation of smooth muscle cell proliferation and increased platelet activity have been implicated in the relation between insulin resistance/diabetes mellitus and asCVD [49] (Figure 2).
- Hypertension is a well-established risk factor for asCVD events. Increased activity of the renin–angiotensin–aldosterone system has also been implicated in the pathogenesis of NAFLD [50], as it may in itself worsen insulin resistance and it may activate hepatic stellate cells and stimulate fibrosis [51,52].
- Obstructive sleep apnea: obese patients with the metabolic syndrome and NAFLD also frequently suffer from obstructive sleep apnea syndrome (OSAS). Epidemiological studies indicate that obstructive sleep apnea is associated with increases in the incidence and progression of coronary heart disease, heart failure, stroke, and atrial fibrillation [53]. The periodic hypoxia in OSAS has also been implicated as a driver of NAFLD. A large meta-analysis of 18 studies with 2183 participants indeed supported an independent relation of OSAS with NAFLD [54], with ORs for various comparisons ranging between 2 and 3.
- Intestinal dysbiosis and chronic low grade inflammation: patients with obesity and T2DM frequently have small intestinal bacterial overgrowth and increased gut permeability. Bacterial components and metabolites may play a role in driving low grade inflammation of the liver (e.g., lipopolysaccharide) and may contribute to atherogenesis (e.g., trimethylamine *N*-oxide). For a review, see Hardy *et al.* [55].

Intervention Studies

Lifestyle/Weight Reduction Intervention Studies

Weight loss due to caloric restriction and exercise, alone or in combination, is considered to be the corner stone of effective treatment in NAFLD and is thus recommended by the current European Association for the Study of the Liver–European Association for the Study of Diabetes–European Association for the Study of Obesity Clinical Practice Guidelines [56]. Bariatric surgery as a method to lose weight seems also to be beneficial, being associated with significant improvement in histological and biochemical markers of NAFLD [57]. Weight reductions of ≥10% can

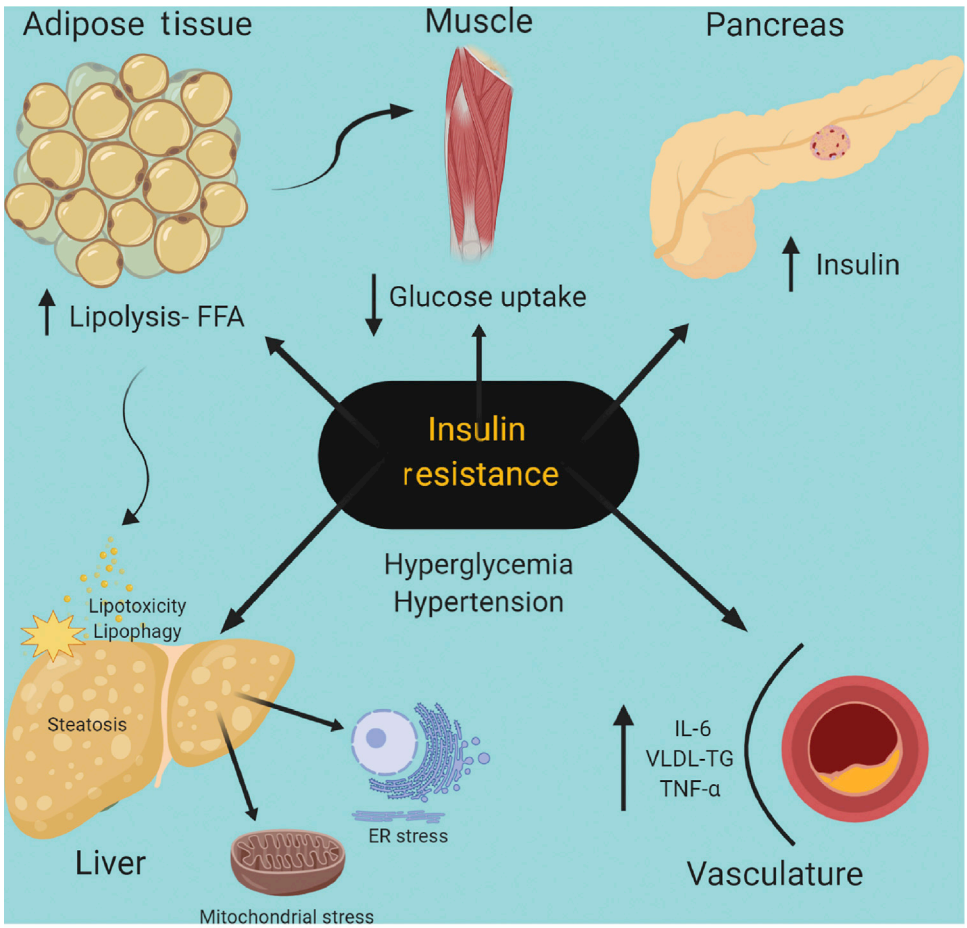

Figure 2. Insulin Resistance Drives Lipolysis of Adipose Tissue and Hyperinsulinemia Drives *De Novo* Lipogenesis.

Both processes induce hepatic steatosis, VLDL secretion leading to hyperlipidemia and to hepatic lipotoxicity. Together with the often co-observed hypertension and hyperglycemia, hyperlipidemia drives atherogenesis. Abbreviations: ER, endoplasmic reticulum; FFA, free fatty acids; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; VLDL, very low density lipoprotein.

resolve NASH and reduce fibrosis; for a review see Romero-Gómez *et al.* [58]. Lifestyle intervention studies are also recommended in patients with high risk for asCVD [59]. Yet, remarkably little effort has been made to assess the effects of lifestyle intervention on asCVD outcomes or surrogates, specifically in NAFLD patients. Here lies a major yet challenging opportunity for clinical researchers (see Outstanding Questions).

Drug Intervention Studies

For the antidiabetic drugs liraglutide, a GLP1-receptor agonist (GLP1-RA) [60], and SGLT2-inhibitor canagliflozin [61], we have seen important positive CVOT in recent years. For drugs that aim to halt and reverse progressive forms of NAFLD, no CVOTs are underway yet, even though the strong relation between NAFLD and asCVD clearly calls for such trials (see Outstanding Questions). The first step here is to obtain positive Phase III trials for progressive stages of NAFLD itself; multiple Phase III trials are underway [62]. In the following paragraph, we discuss the second step: the compounds in development to reduce NAFLD that have potential to reduce asCVD outcomes as well (Figure 3 and Table 1).

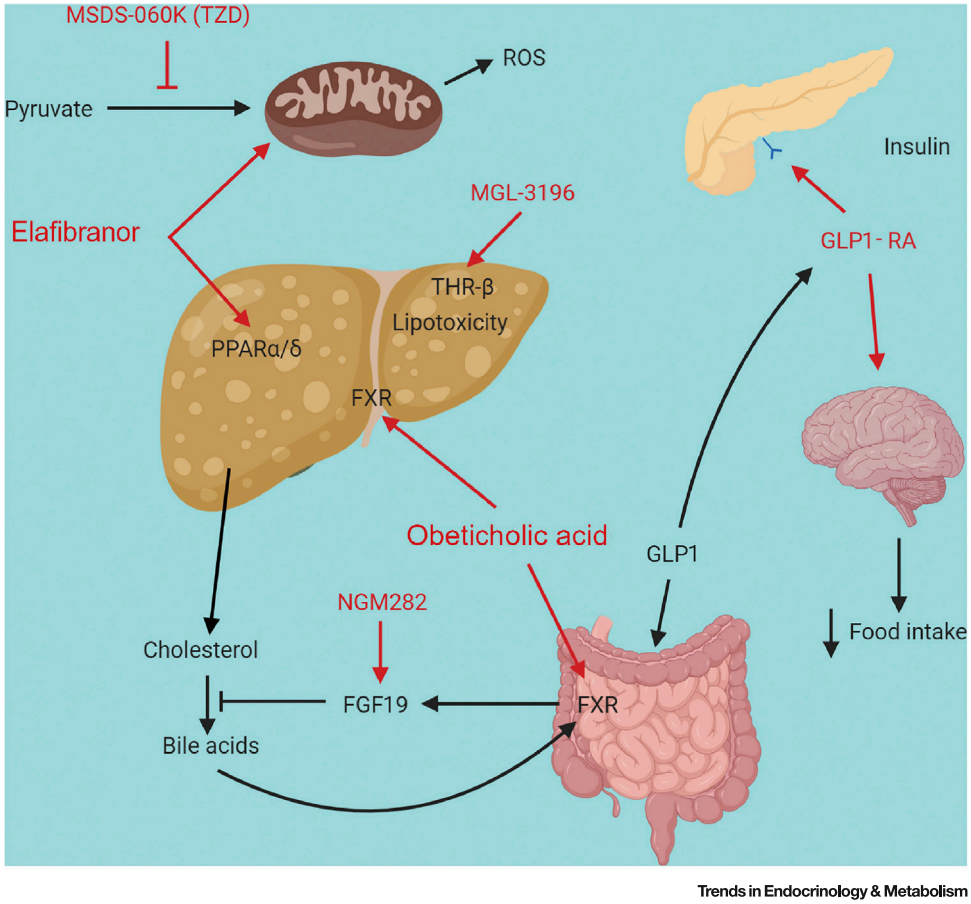

Figure 3. Mechanism of Action of Compounds in Development for NAFLD.
Abbreviations: FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; GLP1-RA, GLP1-receptor agonist; NAFLD, non-alcoholic fatty liver disease; ROS, reactive oxygen species; THR-β, thyroid hormone receptor β; TZD, thiazolidinedione.

GLP1-RAs may have a positive effect in the progression of NAFLD, implicated by the Phase II LEAN trial with liraglutide, which led to a resolution of NASH in 39% of the patients compared with resolution in 9% of the patients in the placebo group [63]. Another Phase II placebo-controlled study with three different dose levels (0.1, 0.2, 0.4 mg) of daily subcutaneous semaglutide in NAFLD patients is underway. The effects on NAFLD may be indirect (i.e., via weight loss), as the liver most probably does not express the GLP1 receptor [64].

Tirzepatide, a novel dual drug [glucose-dependent insulinotropic polypeptide (GIP) + GLP1-RA] is being developed for the treatment of type 2 diabetes. This may be an asset in the future armamentarium to treat patients with NAFLD, as this dual combination has been demonstrated to result in a significant reduction in TGs up to –0.8 mmol/L when compared with GLP1-RA alone. Since the GIP receptor is highly expressed in human adipose tissue it is anticipated that tirzepatide may modify NALFD-NASH via lipolysis and lipogenesis [65].

Thiazolidinediones (TZDs) are a group of antidiabetic drugs that act through two different modes of action: activation of the PPARγ transcription factor (e.g., rosiglitazone, pioglitazone). Treatment with pioglitazone has demonstrated long-term metabolic and histologic improvement in NASH [66] and is thus recommend by some experts for advanced stages of NAFLD. Also, MSDC-0602K, a novel TZD

Table 1. Drugs currently being tested for NAFLD

Drug group	Drug name	Potential cardiovascular effect +/–/?
GLP1-RA	Semaglutide	Probably +
	Liraglutide	
	Dual GIP+GLP1-RA (tirzepatide)	Probably +
TZD	Pioglitazone	?
TZD	MSDC-0602K	?
PPAR-α/δ agonist	Elafibranor	?
THR-β	MGL-3196 (resmetirom)	+
Bile acid pathway	FXR agonist (Obeticholic acid) FGF19 analog (NGM282)	–?
Combination FGF19+ statin	NGM282 + rosuvastatin	+
Chemokine ligand 2/C-C chemokine receptor 2 pathway	Cenicriviroc	?

drug that also targets mitochondrial pyruvate carrier (MPC) has been shown to decrease the transport of pyruvate to the mitochondrial matrix and decreases the Krebs cycle activity with consequently less reactive oxygen species (ROS) formation. Whether MPC-targeted drugs will have a better profile in resolving NASH without the possible side-effects of glitazones has yet to be demonstrated [67], interim results of the Phase II EMMINENCE (a study to evaluate the safety, tolerability, and efficacy of MSDC 0602K in patients with NASH) trial presented at ILC 2019 were favorable, with improved glycemic control and reduced liver enzymes [68]. MMONARCh (a study of MSDC-0602K, a modulator of the mitochondrial pyruvate carrier for outcomes in patients with NASH and diabetes, assessed for resolution of NASH and improved glycemic control), a placebo-controlled Phase III outcomes study of MSDC-0602K or placebo given orally once daily in patients with NAFLD and diabetes is expected to start in September 2019.

Elafibranor is a dual PPAR-α/δ agonist that increased FAS receptors and mitochondrial beta-oxidation. A Phase IIb placebo-controlled study, the GOLDEN-505 trial, did not meet the primary endpoint in the intention-to-treat analysis but a *post hoc* analysis showed a resolving NASH without worsening hepatic fibrosis with 120 mg elafibranor in those with advanced stages of NASH. There was no difference in cardiac events. A Phase III placebo-controlled study, RESOLVE-IT (a multicenter, randomized, double-blind, placebo-controlled Phase III study to evaluate the efficacy and safety of elafibranor in patients with NASH and fibrosis, is ongoing in patients with biopsy-proven NASH [69].

MGL-3196 (resmetirom) is a highly selective thyroid hormone receptor β (THR-β) agonist, a pyridazinone analog novel heterocycle. Thyroid hormone (TH) has beneficial metabolic effects that are mediated by THR-β isoform, which is the predominant TH receptor in the liver. Being highly specific, MGL-3196 does not bind to THR-α, the isoform related to most adverse effects of TH on heart and bone. It has a lipid lowering effect, and a Phase II trial in NASH resulted in significant reductions in hepatic fat, liver enzymes, fibrosis biomarkers, atherogenic lipids, and improvement in NASH on serial liver biopsy. A 52-week placebo-controlled Phase III study is planned to start in 2019, with daily doses of 80 or 100 mg [70].

The FXR-FGF19 axis also is an active target for therapeutic approaches to NASH. Bile acids stimulate the nuclear receptor farnesoid X receptor (FXR) in the ileum, which results in the release of fibroblast growth factor 19 (FGF19) in the portal circulation. Once in the hepatocyte, FGF19 inhibits the enzyme CYP7A1 involved in the synthesis of primary bile acids from cholesterol.

Recently, the Phase III trial REGENERATE, with the FXR ligand obeticholic acid, was presented as the first positive NAFLD outcome trial (NCT02548351). It gave a reduction in NASH and fibrosis, yet at the cost of frequent pruritus and plasma LDL cholesterol elevation. As activation of FXR inhibits the primary bile acids synthesis from cholesterol, dyslipidemia is not a surprising side effect but it will likely reduce the cardiovascular benefit of obeticholic acid despite its positive effects on NAFLD.

As one can expect, adding statin to FXR-FGF19 therapy should diminish the deleterious side effect of dyslipidemia. Indeed, Rinella *et al*. have demonstrated in a multicenter study that adding rosuvastatin to NGM282, an analog of FGF19, after documented increase in cholesterol from baseline, there was a significant reduction in LDL and TGs and increased HDL. Also, there was a significant reduction in liver fat content, as measured by magnetic resonance imaging-estimated proton density fat fraction [71]. A placebo-controlled Phase IIb study of three different doses of subcutaneous NGM282 is currently recruiting patients with biopsy-proven NASH.

Cenicriviroc is an oral inhibitor of the CC-L2/CCR2 pathway, which is involved in the hepatic recruitment of the macrophages and seems to have positive effects on NASH treatment, as seen in the CENTAUR study at a dose of 150 mg orally, once daily, but its effects on cardiovascular disease are not established [72].

Concluding Remarks and Implications for Physicians Who See Patients with NAFLD and asCVD

There is a strong coincidence of NAFLD and asCVD. These two conditions share common drivers, most notably insulin resistance and other elements of the metabolic syndrome. NAFLD itself also likely drives asCVD, by means of the mixed hyperlipidemia and induction of a hypercoagulable state that comes with NAFLD. Cardiologists should be aware that patients with asCVD may have progressive forms of NAFLD, while hepatologists should be aware that patients with progressive NAFLD have a markedly increased risk of asCVD. Endocrinologists, cardiometabolic internists, and general practitioners also provide care for these patients and are likely required to have the same level of awareness for the interrelation of NAFLD and asCVD (see Outstanding Questions). Physicians are to perform vigilant cardiovascular risk management, potentially in a multidisciplinary setting, in the best interest of the patients involved. At present, it is unknown whether lifestyle interventions with proven effectiveness for NAFLD also lower asCVD risk in these patients. In the future, we will likely learn whether new drugs, especially those that may reduce the mixed hyperlipidemia and/or insulin resistance in NAFLD, can also reduce asCVD events.

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Disclaimer Statement

M.N. is in the Scientific Advisory Board of Caelus Health. G.K.H. has served as consultant and speaker for biotech and pharmaceutical companies that develop molecules that influence lipoprotein metabolism, including Regeneron, Pfizer, MSD, Sanofi, and Amgen. Until April 2019 G.K.H. served as principal investigator for clinical trials conducted with a.o. Amgen, Sanofi, Eli Lilly, Novartis, Kowa, Genzyme, Cerenis, Pfizer, Dezima, and Astra Zeneca. The Department of Vascular Medicine receives the honoraria and investigator fees for sponsor driven studies/lectures for companies with approved lipid-lowering therapy in The Netherlands. Since April 2019, G.K.H. is partly employed by Novo

Nordisk (0.7 FTE) and the AMC (0.3 FTE). G.K.H. has no active patents nor shares or ownership of listed companies. D.S.G. and A.G.H. have no personal conflicts of interest pertaining to this manuscript.

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Outstanding Questions

Will future GWAS studies providing novel NAFLD loci from large international patient cohorts with liver biopsies further elucidate the relation between NAFLD, asCVD, plasma lipids, and other coincident metabolic factors?

Can lifestyle intervention in NAFLD patients reduce asCVD risk?

Can future drugs that may reduce steatohepatitis and fibrosis in NAFLD also reduce asCVD risk in NAFLD patients?

Will improved awareness of NAFLD among health care providers who see patients with NAFLD (i.e., general practitioners, endocrinologists, lipidologists, cardiologists, and hepatologists), and improved collaboration between these physicians, result in a reduction of advanced NAFLD severity and will it reduce asCVD risk?

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