NONALCOHOLIC FATTY LIVER DISEASE-RELATED RISK OF CARDIOVASCULAR DISEASE AND OTHER CARDIAC COMPLICATIONS

Christopher D. Byrne, MB BCh, PhD[1,2*] Giovanni Targher, MD[3*]

*Both authors contributed equally to the manuscript

[1]Nutrition and Metabolism, Faculty of Medicine, University of Southampton, UK

[2]Southampton National Institute for Health Research Biomedical Research Centre, University Hospital Southampton, Southampton General Hospital, Tremona Road, Southampton, UK

[3]Section of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy

Word count: 291 abstract; 6164 text (*excluding* title page, abstract, references, tables and figure legends). **Figures:** 3, **Tables**: 3

Address for correspondence:
Christopher D. Byrne FRCP FRCPath PhD
Professor Endocrinology & Metabolism
Human Development and Health Academic Unit
Faculty of Medicine
The Institute of Developmental Sciences (IDS)
MP887
University of Southampton
Southampton General Hospital
Southampton SO16 6YD
UK
Email: c.d.byrne@soton.ac.uk

LIST OF ABBREVIATIONS

Apo-B (apolipoprotein B100);

APOE, apolipoprotein E;

AF, atrial fibrillation;

CVD, cardiovascular disease;

GLP-1RA, glucagon-like peptide-1 receptor agonist;

HF, heart failure;

HR, hazard ratio;

HDL-C, high density lipoprotein cholesterol;

LDL, low-density lipoprotein;

MetS, metabolic syndrome;

NAFLD, nonalcoholic fatty liver disease;

NASH, nonalcoholic steatohepatitis;

OR, odds ratio;

95% CI, confidence interval;

PNPLA3, patatin-like phospholipase domain-containing protein 3;

sdLDL, small dense LDL;

SGLT2, sodium-glucose cotransporter-2;

SNP, single nucleotide polymorphism;

TM6SF2, trans-membrane 6 superfamily 2;

T2DM, type 2 diabetes mellitus;

VLDL, very-low density lipoprotein;

ABSTRACT.

Background/Aim: Nonalcoholic fatty liver disease (NAFLD) affects approximately ~25% of the global adult population. The aim of this narrative review is to describe the associations between NAFLD and cardiovascular disease (CVD), arrhythmias, cardiac conduction defects, myocardial remodelling and heart failure. We also discuss the potential mechanisms that mediate or attenuate the strength of these associations, and briefly summarize the effect of treatments that both ameliorate NAFLD and decrease risk of CVD.

Methods. Searches of PubMed were performed by the two authors using the terms listed in the manuscript Appendix. We limited the timeframe to the last decade due to the vast amount of research in the field (up to April 2021) for meta-analyses, reviews and original papers. Only articles published in English were considered.

Results: NAFLD is associated with an increased risk of fatal/nonfatal CVD events and other cardiac and arrhythmic complications (left ventricular hypertrophy, aortic-valve sclerosis and certain arrhythmias), independently of common CVD risk factors. There are probably several underlying mechanisms, including hepatic/systemic insulin resistance, atherogenic dyslipidaemia, hypertension and pro-atherogenic, pro-coagulant and pro-inflammatory mediators released from the steatotic/inflamed liver that may be involved. Some genetic polymorphisms, such as *PNPLA3* (rs738409 C>G) and *TM6SF2* (rs58542926 C>T), may worsen liver disease, but also attenuate the strength of the association between NAFLD and CVD, possibly via their effects on lipoprotein metabolism. Of the currently tested drugs for treating NAFLD that also benefit the vasculature, pioglitazone and GLP-1 receptor agonists are the most promising.

Conclusions: The complex interplay between the liver and cardiometabolic risk factors contributes to CVD, arrhythmias and cardiac disease in NAFLD. There is an urgent need for a multidisciplinary approach to manage both liver disease and cardiometabolic risk, and to test the cardiovascular and cardiac effects of new drugs for NAFLD.

Keywords: Nonalcoholic fatty liver disease; NAFLD; cardiovascular disease; CVD; arrhythmias; conduction defects; heart failure

Introduction

Nonalcoholic fatty liver disease (NAFLD) has become a major health burden, affecting around a quarter of the general population worldwide[1]. Several cohort studies have shown that NAFLD is associated with a higher risk of all-cause mortality, and that cardiovascular disease (CVD) is the leading cause of death in people with NAFLD, followed by extra-hepatic cancers (mainly gastro-intestinal cancers) and liver-related complications[2]. Growing evidence now indicates that NAFLD is not only associated with a higher risk of CVD morbidity and mortality, but also with other cardiac complications, such as arrhythmias (mainly atrial fibrillation and increased QTc interval prolongation), cardiac valvular calcification (mainly aortic-valve sclerosis), and cardiomyopathy (mainly cardiac remodelling and hypertrophy, leading to new-onset heart failure [HF] over time)[3]. In this narrative review, we briefly discuss the current evidence for an association between NAFLD and increased risk of CVD and other cardiac and arrhythmic complications. We also discuss the potential mechanisms that mediate or attenuate the strength of these associations (for CVD), and briefly summarize targeted pharmacological treatments for NAFLD that might also benefit risk of CVD and other cardiac complications. Although there is some very recent evidence to suggest that NAFLD reclassified as metabolic dysfunction-associated fatty liver disease (MAFLD) is also associated with increased risk of CVD[4], it is not within our remint to discuss the associations between MAFLD and CVD and cardiac disease.

Risk of cardiovascular and cardiac diseases

Figure 1 is a schematic figure that illustrates the NAFLD-related risks of subclinical atherosclerosis (as assessed by carotid intima-media thickness or plaques, arterial stiffness and other markers of subclinical atherosclerosis), fatal and nonfatal CVD events, arrhythmias (mostly permanent atrial fibrillation and QTc interval prolongation), certain types of cardiac conduction defects, aortic-valve sclerosis, as well as cardiac remodelling and heart failure in people with NAFLD. These risks have been quantified in recent meta-analyses that are briefly discussed below, and described in **Table 1**.

Cardiovascular disease

A meta-analysis of twenty-six cross-sectional studies (involving a total of ~85,000 individuals) showed a close association between NAFLD and markers of subclinical atherosclerosis[5]. In particular, the investigators reported that NAFLD was associated with greater carotid artery intima–media thickness/plaques, increased arterial stiffness, coronary artery calcification and circulatory endothelial dysfunction, with adjusted pooled odds ratios (OR) ranging from ~1.5 to 3.7. Interestingly, some observational studies, mostly performed in Asian individuals, also reported a strong association between the severity of NAFLD and the long-term risk of progression of subclinical coronary or carotid atherosclerosis[6-8]. Furthermore, the resolution or improvement in NAFLD (on ultrasound examinations) was associated with a lower risk of carotid atherosclerotic development[7,9,10].

Over the last decade, multiple cohort studies have shown that NAFLD (defined radiologically or histologically) is predictive of CVD events. A comprehensive meta-analysis of 16 longitudinal studies (involving around 34,000 individuals)[11] confirmed that imaging-defined or biopsy-proven NAFLD was associated with a ~1.6-fold increased risk of fatal and/or nonfatal CVD events over a median of 6.9 years (random-effects OR 1.64, 95%CI 1.26–2.13). Notably, this risk increased further with greater severity of NAFLD (random-effects OR 2.58; 95%CI 1.78–3.75), and remained significant in those studies with statistical analyses adjusted for established cardiometabolic risk factors.

After the publication of this meta-analysis in 2016[11], other prospective studies have also confirmed that the risk of incident CVD events paralleled the underlying severity of NAFLD, and that the stage of liver fibrosis (rather than other histologic features of NAFLD) was a key prognostic marker for long-term CVD outcomes and overall mortality in people with NAFLD[2]. For example, in a multi-national study of 458 individuals with biopsy-proven NAFLD with advanced fibrosis or compensated cirrhosis, who were followed up for a mean of 5.5 years, Vilar-Gomez et al. found that individuals with NAFLD-cirrhosis developed predominantly liver-related events, whereas those with advanced fibrosis developed predominantly CVD events and extra-hepatic cancers[12]. In a smaller cohort of ~300 adults from the United States with biopsy-confirmed NAFLD, without a prior history of CVD,

Henson et al. reported that the severity of hepatic fibrosis (but not other histological features of NAFLD) was associated with a ~2.9-fold increased risk of incident CVD events over a median of 5.2 years[13]. This association remained even after adjusting for established risk factors and CVD risk scores.

In a nationwide cohort study of 10,568 Swedish individuals with biopsy-proven NAFLD and nearly 50,000 matched control subjects who were followed for a median of 14.2 years, Simon et al. reported that all histological stages of NAFLD were associated with a higher risk of all-cause mortality[14]. This risk increased progressively with worsening NAFLD histology (with adjusted-HRs ranging from ~1.7 for simple steatosis to ~2.7 for non-cirrhotic fibrosis, and ~3.8 for cirrhosis, respectively)[14]. Notably, the excess mortality associated with NAFLD was primarily from extra-hepatic cancers and cirrhosis, followed by CVD and hepatocellular carcinoma[14]. In a nested cohort study of 3,756 subjects from the United States, who underwent coronary contrast-enhanced computed tomography angiography, Meyersohn et al. reported that NAFLD remained associated with a higher risk of incident CVD events after adjustment for established cardiometabolic risk factors and the extent of coronary plaques and stenosis[15]. Recently, in a community-based cohort study involving ~80,000 Chinese individuals followed for a median of 10.3 years, Xu et al. reported that the ultrasonographic severity of NAFLD was associated with a higher risk of future ischaemic stroke events, independently of common CVD risk factors[16].

Risk of arrhythmias and cardiac conduction defects

In the last few years, a number of studies have supported the presence of a strong association between NAFLD and higher risk of certain arrhythmias, such as permanent atrial fibrillation (AF), QTc interval prolongation and ventricular tachyarrhythmias[17]. In a meta-analysis of nine observational studies (five cross-sectional and four longitudinal), Mantovani et al.[18] found that NAFLD was associated with a ~2-fold increased risk of prevalent AF (random-effects OR 2.07, 95%CI 1.38–3.10), independently of common risk factors for AF. Furthermore, meta-analysis of data from longitudinal studies also showed that NAFLD was associated with an increased risk of incident AF, especially in people with established type 2 diabetes mellitus (T2DM) [random-effects

HR 4.96, 95%CI 1.42–17.3][18]. A larger meta-analysis of six longitudinal studies (involving nearly 615,000 participants with and without T2DM) recently confirmed the presence of a significant association between NAFLD and increased incidence of permanent AF over a median of 10 years (random-effects HR 1.65, 95%CI 1.23–2.20)[19]. Compared with non-NAFLD, the absolute risk increase in NAFLD for AF was 1.30 per 1000 person-years[19]. Also, in a retrospective cohort study of patients from the United States undergoing AF ablation, Donnellan et al. reported that imaging-defined NAFLD was significantly associated with higher arrhythmia recurrence rates following AF ablation during a mean follow-up of 2 years[20].

Accumulating evidence shows that the presence and severity of NAFLD (on ultrasound examination) was also associated with a greater risk of QTc interval prolongation (on resting electrocardiogram) and other ventricular tachyarrhythmias (as assessed by 24-hour Holter monitoring), which predisposed to an increased risk of sudden cardiac death[21-24]. Notably, the association between NAFLD and increased risk of QTc interval prolongation remained significant after adjustment for established CVD risk factors and potential confounders.

Some evidence suggests that there is an association between NAFLD and certain types of cardiac conduction defects (mostly persistent first-degree atrio-ventricular block, left anterior hemiblock or right bundle branch block), which are risk factors for cardiac mortality[25,26]. A recent meta-analysis of three cross-sectional studies (involving 3,651 participants) showed that the risk of having the aforementioned cardiac conduction defects was higher in subjects with NAFLD than in those without NAFLD (pooled OR 5.17, 95%CI 1.34–20.0)[27].

Risk of cardiac remodelling, heart failure and aortic-valve sclerosis

Strong evidence supports an association between NAFLD and increased risk of left ventricular (LV) diastolic dysfunction, greater LV hypertrophy, or larger left atrial volume (on echocardiography examinations), in both children and adults, regardless of the presence or absence of metabolic syndrome (MetS) traits[17]. Adverse structural remodelling of the heart has been shown to be a pivotal process in the development and progression of HF[28]. An elegant study of individuals without

diabetes, also showed that myocardial triglyceride, epicardial and pericardial fat depots increased, with increasing levels of liver fat, as assessed by magnetic resonance spectroscopy[29]. Hepatic steatosis was also associated with significant changes in myocardial structure and function (as assessed by cardiac magnetic resonance). In addition, and most importantly, the significant association between hepatic fat content and LV diastolic function persisted after adjustment for cardiometabolic risk factors, myocardial triglyceride content and visceral adipose tissue[29].

A recent meta-analysis of twelve cross-sectional studies (including a total of 280,645 individuals) showed that there was a significant association between NAFLD and higher risk of LV diastolic dysfunction (pooled OR 2.02; 95%CI 1.47–2.79)[30]. Notably, some studies have also shown that this NAFLD-related risk of LV diastolic dysfunction increased progressively with the severity of hepatic fibrosis[31-34]. Recently, in a population-based prospective study of 1,827 black and white middle-aged adults followed for 5 years, VanWagner et al. found that imaging-detected NAFLD was associated with an increased risk of incident LV hypertrophy, abnormal LV geometry, and impaired LV function, independent of established risk factors for HF[35].

Previously, it has been reported that higher serum gamma-glutamyltransferase levels (GGT), within the "normal" range, were associated with an increased risk of new-onset HF, independently of established risk factors for HF and daily alcohol consumption[36-38]. Recently, in a nationwide population-based study in Korea (involving ~308,000 healthy individuals without comorbidities, who underwent annual health check-ups), Roh et al. found that the fatty liver index (FLI), i.e., a validated marker of hepatic steatosis and which includes serum GGT levels, was independently associated with an increased risk of new-onset HF over a median of 5.4 years[39]. Low cardiorespiratory fitness is an established CVD risk factor, and recently it has been shown that increased GGT concentration is associated with low cardiorespiratory fitness in patients with NAFLD[40]. In this study, serum GGT levels accounted for ~25% of the variance in cardiorespiratory fitness levels.

Interestingly, some longitudinal studies have also shown that imaging-defined NAFLD and its severity (assessed by non-invasive fibrosis scores) were independently associated with an

increased risk of both in-hospital and post-discharge, all-cause mortality in patients admitted with acute HF[41-43]. Similarly, it has been reported that there was a high prevalence of NAFLD with increased fibrosis scores in patients with chronic HF, especially amongst those with a preserved LV ejection fraction[44-47]. In addition, patients with HF and coexisting NAFLD had a higher long-term risk of CVD outcomes, compared to their counterparts without coexisting liver disease[44-47].

Some evidence also supports the existence of a significant association between NAFLD and risk of aortic-valve sclerosis, which is associated with CVD morbidity and mortality[48]. In a meta-analysis of three large cross-sectional studies (involving a total of 2639 middle-aged subjects), Di Minno et al. found a significant association between NAFLD and higher prevalence of aortic-valve sclerosis (pooled OR 2.28; 95% CI 1.21–4.28)[49]. However, future prospective studies are needed to corroborate these findings.

Potential mechanisms linking NAFLD, cardiovascular and cardiac diseases

Several pathophysiological mechanisms associated with NAFLD have been proposed that may affect risk of CVD and cardiac complications. These mechanisms involve some NAFLD-related genetic polymorphisms (affecting liver metabolism), atherogenic dyslipidaemia, chronic inflammation, imbalance of pro-coagulant and anti-coagulant factors, insulin resistance, increased oxidative stress, and decreased adiponectin signalling CVD[2,3,50,51]. As discussed above, the risk of CVD events parallels the underlying severity of NAFLD, and that the stage of liver fibrosis is the strongest histological predictor of adverse liver-related outcomes, overall mortality and CVD events in people with NAFLD[2,52]. It is beyond the scope and constraints of this brief review to discuss all of the underlying mechanisms that are potentially involved in the development of CVD and cardiac diseases, and the reader is referred to other recent reviews on the subject[2,3,53-55]. For the purposes of this review, we have elected to focus this section on the potential role of altered liver lipid metabolism, and how altered lipid metabolism influences lipoprotein metabolism as a mediator of increased CVD risk in NAFLD. We have also focussed on recent genetic analyses as results from these studies are providing some insight into how certain NAFLD-related genetic polymorphisms may disconnect the severity of liver disease from the associated increased risk of CVD. These

polymorphisms (discussed below) may therefore modulate the strength of the association between NAFLD and CVD risk.

Obesity, metabolic syndrome, sex and atherogenic dyslipidaemia

Obesity is a well-recognised risk factor for NAFLD and subjects with metabolically healthy obesity have a greater risk of NAFLD development and progression compared to normal weight metabolically healthy individuals[56]. However, this risk is generally lower than that of metabolically unhealthy patients, suggesting a stronger adverse effect of coexisting MetS-associated factors rather than obesity *per se* on the severity of NAFLD[56]. Similarly, the metabolic/cardiovascular phenotype has recently been characterised in lean patients with NAFLD[57]. In this study, a total of 3,043 subjects (cohort I) and 1,048 subjects (cohort II) without chronic liver diseases other than NAFLD, who underwent screening colonoscopy between 2010 and 2020, were assigned to one of the following four patient groups: lean patients without NAFLD, lean NAFLD, overweight NAFLD (BMI 25-30 kg/m^2), and obese NAFLD (BMI >30 kg/m^2). Diagnosis of NAFLD was established using ultrasound (cohort I) or controlled attenuation parameter (cohort II). Data from this study showed that NAFLD in lean patients was associated with the MetS and increased CVD risk[57]. Recent studies have focused on obesity and insulin resistance, but the link between NAFLD and CVD persists regardless of traditional risk factors. Increasing amounts of evidence support the evolving notion that sex is an important modifier of disease outcomes[58] and the incidence and prevalence of CVD events and mortality may differ according to sex in patients with NAFLD. For example, advanced liver fibrosis may be associated with a higher risk of ischemic stroke in women but not men[59]. Similarly, in a recent meta-regression the authors of this study analyzed the impact of age on both sexes, in order to examine differences between the sexes and differential effects sex and ageing on CVD events and all cause mortality[60]. In 108,711 people with NAFLD, of which 44% were women and 56% were men, all-cause mortality was 1.5 times higher in women compared to men. CVD events and mortality were also two times higher in women compared to men. In meta-regression analysis, women had higher mortality with advancing age, starting at age 42 years[60].

There is a growing body of evidence emphasising the links between the pathogenesis of NAFLD/NASH and mechanisms of metabolic dysfunction, through liver lipid accumulation, insulin resistance, low-grade inflammation, apoptosis, and fibrogenic remodelling within the liver[53,54]. The liver plays a key role in contributing to the features of MetS[61], and the MetS includes atherogenic dyslipidaemia, increased blood pressure, dysglycaemia/T2DM, and abdominal obesity[61]. **Figure 2** illustrates schematically the complex links and overlaps between NAFLD, MetS features, insulin resistance, T2DM and increased risk of CVD and cardiac diseases. In patients with newly diagnosed T2DM, the presence of MetS is independently associated with incident CVD events[62] and an increasing number of individual features of MetS present at the time of diagnosis of T2DM are associated with a linear increase in CVD risk, with an almost 5-fold increase when all MetS features are present, compared with one MetS feature alone[62]. Similarly, in persons without T2DM and CVD, not only is MetS common (occurring in up to ~20% of these subjects), but also the presence of MetS is associated with an increased risk of CVD and T2DM[63]. Since the features of MetS are common in people with NAFLD (see below), the presence of MetS features also potentially increases the risk of comorbidities such as T2DM and chronic kidney disease that further increase risk of CVD and cardiac disease[64,65,66]. Recently, in a study of the US population in the Third National Health and Nutrition Examination Survey, the prevalence of NAFLD was 18.2% and individuals with MetS or individuals with component features of MetS were very common [MetS, 43.2%; increased waist circumference, 31.2%; impaired fasting glycaemia/T2DM, 41.2%; high triglyceride (TG) levels, 34.7%; low high-density lipoprotein (HDL) cholesterol, 27.8%; and high blood pressure, 29.2%][67]. There was also a remarkably higher prevalence of NAFLD in subjects with MetS than in controls (adjusted-OR 11.5; 95%CI 8.9–14.7). The severity of NAFLD was also noted to increase with the increasing numbers of component MetS features. Importantly, individuals with impaired fasting glycaemia/T2DM or those with five metabolic features also had higher rates of advanced fibrosis (18.6% and 30.3%, respectively), and in keeping with the accepted notion that NAFLD is rare in people without MetS features, among those individuals without any metabolic abnormality the prevalence of NAFLD was only ~5%[67].

As mentioned above, dyslipidaemia associated with MetS is a major risk factor for NAFLD, and is known to increase CVD. In 1990, the atherogenic dyslipidaemia that occurs with MetS was described and was referred to as the atherogenic lipoprotein phenotype[68]. This type of dyslipidaemia focuses on: a) the cluster of increased plasma levels of TG and apolipoprotein B100 (apo-B) (within very low and intermediate density lipoproteins [VLDL and IDL], respectively), and b) decreased HDL cholesterol, HDL2 mass, and levels of apolipoprotein A-I. In NAFLD, this dyslipidaemia is commonly present, and is an established risk factor for CVD[69]. With the frequently observed NAFLD-associated increase in TG rich-VLDL, there is also increased cholesterol ester transfer protein activity that regulates the reciprocal exchange of TG from TG rich lipoproteins (such as VLDL) with cholesterol esters from cholesterol ester-rich lipoproteins [such as HDL and low-density lipoprotein (LDL)]. The net effect of these reciprocal lipid exchanges is a consequent decrease in HDL cholesterol concentration and an increase in pro-atherogenic small-dense LDL levels (sdLDL).

NAFLD is strongly associated with higher plasma TGs and lower HDL cholesterol levels[70], and in the Multi-Ethnic Study of Atherosclerosis, the presence of computed tomography-defined NAFLD was associated with the atherogenic lipoprotein phenotype in a dose dependent fashion. Notably, this association persisted even after adjustment for cardiometabolic risk factors and HOMA-estimated insulin resistance, suggesting a possible pathophysiological role for the liver in causing this type of dyslipidaemia[70]. However, whether this atherogenic lipoprotein profile arises because of liver fat accumulation, lipoprotein assembly and secretion or some other metabolic dysfunction (such as insulin resistance) in NAFLD, is not clear.

Although individuals with NAFLD have elevated plasma TG levels,[71-74] which are associated with an increase in CVD risk, the association between hepatic TG accumulation and plasma TG levels is not linear[75,76]. In fact, the association between hepatic TG and hepatic VLDL production is lost when hepatic TG content is higher than 10% (as assessed by magnetic resonance spectroscopy)[75,76]. It is plausible that at hepatic TG content >10%, other factors that are necessary for VLDL assembly, such as phospholipids or apo-B, cannot be produced in adequate amounts to

further increase VLDL production and thus export lipid from the liver. In support of insufficient apo-B availability, in obese individuals, the molar ratio of VLDL-TG (particle TG content) to VLDL-apo-B (the number of particles) is increased with high hepatic TG content, suggesting that the secreted VLDL particles are larger and resemble pro-atherogenic $VLDL_1$[76].

Recently, in a study of 265 individuals with NAFLD confirmed by ultrasonographic findings, the NAFLD fibrosis score and the fibrosis-4 (FIB-4) index were used to assess the probability of liver fibrosis as low, intermediate, and high probability of advanced fibrosis[77]. The authors of this study concluded that the severity of hepatic steatosis, NAFLD fibrosis score, and FIB-4 index were significantly associated with the atherogenic lipid profile. Similarly, in a case-control study of individuals with NAFLD *versus* lean and obese controls, atherogenic dyslipidemia was related to increased insulin-induced hepatic lipid synthesis in those with NAFLD[78], and there was a non-significant trend towards a worse atherogenic lipid profile in individuals with NASH[78]. Thus, the atherogenic lipoprotein profile (or atherogenic lipoprotein phenotype as discussed above) may be 'driven' (at least in part) by hepatic TG content and insulin resistance, and this lipid profile may also worsen with the development of more severe liver disease (i.e., NASH).

Although in NAFLD, the liver condition is defined by accumulation of hepatic TG and TG is exported from the liver in VLDL, the VLDL lipoprotein also contains other lipids such as cholesterol. Moreover, the cholesterol content of VLDL is known to be highly correlated with the TG concentration[79]. Treatment to decrease plasma TG levels may also decrease risk of CVD, since a recent systematic review and meta-regression analysis of randomized controlled trials, showed that a 1 mmol/L reduction in plasma TG levels was associated with a 16% decrease in risk for major CVD events (relative risk 0.84; 95%CI 0.75–0.94)[80].Omega-3 long-chain fatty acids at high dose specifically (e.g. 4 g/day) have a powerful effect to lower plasma TG concentrations mainly through an effect on VLDL secretion[81] and are used clinically at this dose to treat hypertriglyceridaemia. However, it still remains not proven whether omega-3 long-chain fatty acids (such as eicosapentanoic acid or docosahexanoic acid) are in any way beneficial to the treatment of liver disease in NAFLD[82-84].

Most (although by no means all) individuals with NAFLD are overweight or obese. Individuals with obesity have higher concentrations of VLDL cholesterol and an increased risk of myocardial infarction. However, whether that increase in VLDL cholesterol explains the excess myocardial infarction risk associated with obesity has been uncertain. In an important recent study utilising the Copenhagen General Population cohort where during follow-up incident myocardial infarction events were recorded; the authors studied 29,010 individuals free of myocardial infarction at baseline, nested within 109,751 individuals from the wider cohort[85]. During 10 years of follow-up, 2,306 individuals developed myocardial infarction. Cholesterol content was measured with magnetic resonance spectroscopy in large and small VLDLs, in intermediate-density lipoprotein (IDL) and in LDL and, interestingly, cholesterol content in large and small VLDLs combined, explained 40% (95%CI 27%-53%) of the excess risk of myocardial infarction associated with higher body mass index. In contrast, IDL and LDL cholesterol did not explain excess risk of myocardial infarction, whereas (and as expected) systolic blood pressure and T2DM explained 17% (11%-23%) and 8.6% (3.2%-14%), respectively[85]. Intriguingly, although it is not known how many of these obese subjects had NAFLD, (it is likely to have been >50%), these novel findings support a focus on lowering cholesterol in VLDL, for prevention of CVD events in individuals with obesity (many of whom will have NAFLD).

Polymorphisms in key genes influencing liver disease severity and affecting lipoproteins and CVD risk

Polymorphisms in certain genes predispose individuals to developing more severe liver disease in NAFLD, e.g., *patatin-like phospholipase domain-containing protein 3* (PNPLA3); *trans-membrane 6 super family 2* (TM6SF2); *glucokinase regulator* (GCKR); *membrane bound O-acyltransferase domain containing 7* (MBOAT7)[86]. In addition, in a recent large multi-cohort exome-wide association study focussed on serum alanine aminotransferase levels, a sequence variant of *APOE* has been also identified that is associated with NAFLD,[87] and this genetic variant is well known to be associated with higher risks of both Alzheimer's disease[88] and dyslipidaemia[89].

It is beyond the scope of this review to discuss the underlying mechanisms by which each of these polymorphisms act to influence the severity of liver disease in NAFLD. Rather, two of these gene polymorphisms (i.e., *PNPLA3 rs738409 c.444 C>G p.I148M* and *TM6SF2 rs58542926 C>T E167K*) are potentially insightful in informing why NAFLD might act to increase risk of CVD. These two gene polymorphisms potentially provide insight, because they are both associated with increased severity of liver disease; yet act to decrease fasting plasma TG concentrations, and are associated with lower risk of CVD.

The first of these polymorphisms that was found to be associated with more severe liver disease in NAFLD, was a non-synonymous single-nucleotide polymorphism in *PNPLA3* (rs738409 c.444 C>G p.I148M)[90]. PNPLA3 encodes a lipid droplet protein that is involved in the lipolysis of TG within the lipid globule. In a Mendelian randomisation study and meta-analysis there was a slightly reduced risk of incident CVD in those with *PNPLA3 148M* (OR 0.98, 95%CI 0.96–1.00)[91]. Similar results were then obtained by screening DNA sequence variants on an exome-focused genotyping array in >300,000 participants with replication in >280,000 participants[92]. In this latter analysis focussed on both *PNPLA3* 148M and *TM6SF2* E167K both polymorphisms were associated with higher hepatic fat levels, and higher risk for T2DM (as expected)[92]. However, both *PNPLA3 148M* and *TM6SF2 E167K* were associated with lower serum TG levels (which is a good proxy for VLDL concentrations in the fasted state) and interestingly, a lower risk of CVD (OR 0.96, 95%CI 0.94–0.97 for *PNPLA3 148M*; and OR 0.95, 95%CI 0.93–0.98 for *TM6SF2 E167K*, respectively). TM6SF2 is implicated in the assembly of TGs and apo-B in VLDL secretion from hepatocytes[93-95]. The *TM6SF2* rs58542926 C>T polymorphism encodes a glutamate to lysine substitution at position 167, thereby leading to loss-of-function[96-98] and is associated with lower levels of fasting TGs that reflects lower levels of VLDL[97-99]. It has been suggested that *TM6SF2 E167K*, induces increased hepatic TG content, by reducing apo-B particle secretion[50] and *TM6SF2 E167K* is also well done to be associated with a lower risk of myocardial infarction[94] and CVD events[96-98].

Interesting new data lends further support to the importance of the relationship between altered liver lipids, modified lipoprotein metabolism and increased CVD risk in NAFLD. Recently, a large

multi-cohort exome-wide association study focussed on altered serum aminotransferase levels, and identified a sequence variant of *APOE* that is associated with NAFLD[87]. rs429358 encodes for a missense p.Cys112Arg in *APOE*, which defines the *APOE4* allele and which is associated with dyslipidaemia[100]. These data showed that this genetic variant is associated with *lower* liver fat content and may protect against NAFLD development. Transcriptomic analyses also found a down-regulation of the TG metabolism in the liver that may occur as a result of decreased hepatic TG content in carriers of this *APOE* genetic variant. Carriers of the minor allele also had higher plasma TG and LDL-C concentrations. *APOE* plays a major role in lipid fluxes between tissues during fasting and refeeding[101] and, recently, a heterozygote autosomal dominant mutation p.(Leu167del) in the *APOE* gene has also been shown to also cause familial hypercholesterolaemia (FH)[102,103]. Interestingly, these patients with FH seem to have a better lipid-lowering response to statin treatment compared with other patients with FH who have mutations in other genes, such as the LDL-receptor that cause FH[103]. *APOE* increases clearance of TG-rich lipoproteins and after initial hydrolysis by lipoprotein lipase, facilitates transfer of lipid into muscle and adipose tissue[88]. Thus, the *APOE* rs429358 may decrease the clearance of circulating lipoproteins and possibly hepatic reuptake of lipid, or also by influencing the efflux of cholesterol in hepatocytes[88]. This genetic variant is also known to be associated with a higher risk of both Alzheimer's disease[88] and dyslipidaemia; and carriers of the genotype, known as the ε4 allele of the *APOE* gene (*APOE* ε4), have higher total and LDL cholesterol levels than non-carriers. The *APOE* ε4 allele is a strong genetic risk factor for CVD events in the general population[89,104] but to date whether there is link between NAFLD and altered risk of neurologic disease is not clear.

Finally, the relative impact of MetS *versus PNPLA3* rs738409 or *TM6SF2* rs58542926 on overall and CVD-specific mortality in subjects with, or without NAFLD has been investigated[105]. 958 middle-aged Finns, 249 with NAFLD (as assessed by ultrasonography), were followed for ~20 years, and mortality data were gathered from the National Death Registry. After adjustment for common CVD risk factors, MetS was shown to be a strong risk factor for overall mortality in subjects with NAFLD (HR 2.05, 95%CI 1.01–4.17), whereas both *PNPLA3* rs738409 (HR 1.05; 95%CI 0.65–1.69) and

TM6SF2 rs58542926 (HR 0.72; 95%CI 0.37–1.41) did not affect overall mortality. In this analysis, in contrast to the genetic polymorphisms, MetS was also a significant risk factor for CVD mortality.

Thus, in subjects with MetS, in which the atherogenic lipoprotein phenotype is present with increased VLDL levels, this metabolic phenotype seems to be a key factor associated with CVD outcomes and all-cause mortality. In contrast, although both *PNPLA3* rs738409 and *TM6SF2* rs58542926 genotypes are associated with increased severity of liver disease and increased risk of incident diabetes, these two genotypes are not associated with increased all-cause and CVD mortality. Therefore, these data and those described above, suggest that both *PNPLA3* rs738409 and *TM6SF2* rs58542926 genotypes may have a modulating influence on CVD risk in individuals with NAFLD whereby they decrease the strength of the association between NAFLD and CVD risk. In contrast, the MetS features in NAFLD seem to have a stronger effect to influence the association between NAFLD and CVD risk. **Figure 3** schematically illustrates the putative mechanisms and risk factors contributing to increase risk of CVD and cardiac diseases in people with NAFLD.

Pharmacological treatments for NAFLD that also decrease risk of CVD

The mainstay of management in NAFLD is both to ameliorate liver disease and to decrease risk of associated conditions, such as T2DM and CVD. Since T2DM is also a strong risk factor for CVD and cardiac disease, it is important to optimize treatment of T2DM as well as associated CVD and cardiac risk factors. Although drug treatment may be necessary, lifestyle interventions are very important to ameliorate liver disease and benefit CVD risk factors. **Table 2** shows the key lifestyle intervention changes that ameliorate the early stages of liver disease in NAFLD, as currently recommended by the EASL-EASO-EASD and American Association for the Study of Liver Diseases (AASLD) practice[106,107] guidelines. Dietary recommendations should consider total energy restriction and exclusion of NAFLD-promoting components (e.g., processed food that is high in saturated fat and beverages that are high in added fructose or sucrose). A Mediterranean diet is the most recommended dietary style for NAFLD[108] and, importantly, a recent systematic review and meta-analysis found that the Mediterranean diet is also an appropriate diet to follow for improving

hyperglycaemia and decreasing CVD risk factors[109]. Additionally, physical exercise even without dietary intervention has been found to reduce liver fat[110-112] and it is well accepted that physical activity and exercise are important in the treatment of T2DM and the amelioration of CVD risk factors. Current recommendations are that pharmacotherapy specifically for liver disease in NAFLD, should be reserved only for individuals with NASH; particularly for those with significant fibrosis. However, for those subjects with less severe disease, who are at high risk of liver disease progression (i.e., individuals with T2DM, MetS, or chronically elevated serum aminotransferase levels), this may represent a high-risk group of people who warrant treatment to prevent disease progression.

Recently, with no licensed treatment for NASH, there has been an increase in interest in testing the efficacy of novel therapeutic agents for the treatment of NASH. In phase-2 and phase-3 randomized controlled trials, the endpoints have focussed on testing the treatment-efficacy on histological resolution of NASH, with either no worsening, or amelioration, of liver fibrosis, assessed by changes in liver histology. Although showing efficacy in the liver is important for licensing authorities for the approval of any new drug for the treatment of NASH, it is important to underline that subjects with NASH die as frequently from CVD, as they do from liver disease. Therefore, we reason it is crucially important to test the efficacy of new drugs for NAFLD/NASH also on the cardiovascular system (and also on diabetes).

It is not our aim in this brief review to describe the effects of all drugs that have been tested in NASH. Rather, it is our intention to illustrate the effects of drugs that benefit the liver and that also have clinically important benefits on the vasculature. Of those drugs that may benefit NAFLD and that also have clinically important effects on the vasculature (shown in **Table 3**), pioglitazone has proven efficacy in NASH[113,114] and is the '*forgotten cardio-protective'* drug that was licensed almost 20 years ago for treatment of T2DM. The cardiovascular benefits of pioglitazone have been recently discussed in an excellent review on the subject by DeFronzo and colleagues[115]. Briefly, although treatment with pioglitazone increases subcutaneous fat, this drug also decreases ectopic fat depots (including liver fat content) and chronic inflammation. Specific benefits on the vasculature may be

mediated by improvements in the atherogenic lipoprotein phenotype, as discussed above (i.e., pioglitazone decreases plasma TGs and sdLDL-C, and increases HDL-C), as well as improvements in blood pressure, haemoglobin A1c and insulin resistance[115].

Another commonly used drug class for treatment of T2DM are glucagon-like peptide-1 receptor agonists (GLP-1RAs), and these drugs have also shown considerable promise in the treatment of both NASH and CVD risk. In contrast to pioglitazone, GLP-1RAs are also proving to be very effective in the treatment of obesity, which is commonly present in subjects with NAFLD. A recent systematic review supports the use of GLP-1RAs to improve hepatic steatosis, as detected either by imaging techniques or histology[116]. Liraglutide treatment was tested in individuals with either biochemistry-based or imaging-defined NAFLD by the LEAD (Liraglutide Effect and Action in Diabetes) program and LEAD-2 study[117] and in subjects with biopsy-proven NASH in the LEAN (Liraglutide Efficacy and Action in NASH) trial[118]. Liraglutide promoted both improvement of hepatic steatosis and the resolution of NASH[118] but did not improve liver fibrosis[118]. Recently, a phase-2 placebo-controlled randomized trial with once-daily subcutaneous semaglutide at a dose of 0.1, 0.2, or 0.4 mg or corresponding placebo showed a similar benefit in NASH. In this trial, the mean percent weight loss was 13% in the 0.4-mg group and 1% in the placebo group[119]. The percentage of subjects in whom NASH resolution was achieved with no worsening of fibrosis was 40% in the 0.1-mg group, 36% in the 0.2-mg group, 59% in the 0.4-mg group, and 17% in the placebo group ($p<0.001$ for semaglutide 0.4 mg vs. placebo)[119]. Importantly, in a recent trial of obesity treatment, semaglutide at a dose of 2.4 mg weekly was very effective in promoting weight loss in this patient group[120]. In this remarkable 68-week randomized controlled trial, the mean change in body weight from baseline to week 68, was a very impressive -14.9% in the semaglutide group, as compared with -2.4% with placebo, with an estimated treatment difference of -12.4% (95%CI -13.4 to -11.5; $p<0.001$). Given that high doses of GLP-1RAs enable considerable weight loss, this therapy would seem a very logical choice for most individuals with NAFLD who need to lose weight (regardless of the presence of T2DM). Furthermore, in the LEADER trial and in a meta-analysis in 2019, GLP-1RAs reduced the risk of CVD events in patients with T2DM[121,122]. Thus, this class of drug is likely to become an important treatment option for patients with NAFLD, as there is increasing evidence

of benefit of these drugs on the liver. That said, it remains uncertain whether any liver-specific benefit of the class in patients with NAFLD, is independent of weight loss.

The efficacy of GLP-1 agonists and sodium-glucose cotransporter-2 (SGLT2) inhibitors in subjects with T2DM at varying CVD and renal risk has recently been assessed in a systematic review and meta-analysis[123]. A total of 764 trials were included in such meta-analysis that included 421,346 T2DM patients. Both classes of drugs lowered all-cause mortality, cardiovascular mortality, non-fatal myocardial infarction, and kidney failure. SGLT-2 inhibitors reduced mortality and admission to hospital for heart failure more than GLP-1 receptor agonists, and GLP-1 receptor agonists reduced non-fatal stroke more than SGLT-2 inhibitors (which appeared to have no effect)[123]. SGLT2 inhibitors have clinically relevant effects and are recommended for the treatment of chronic heart failure with reduced ejection fraction. A recent meta-analysis suggested a very small overall benefit of treatment with SGLT2 inhibitors in NAFLD to decrease liver fat content[124]. However, the effects of treatment have been inconsistent[125,126] and further research is needed in patients with NASH.

Conclusions

NAFLD is associated with an increased risk of CVD events and other cardiac complications (e.g., left ventricular hypertrophy, aortic-valve sclerosis and certain arrhythmias), independently of common cardiovascular risk factors. There are probably multiple pathophysiological mechanisms by which NAFLD/NASH may increase the risk of CVD and other cardiac complications, e.g., NAFLD/NASH exacerbates hepatic and systemic insulin resistance, promotes atherogenic dyslipidaemia and releases several pro-atherogenic, pro-coagulant and pro-inflammatory mediators. Some genetic polymorphisms, such as the *PNAPL3* I148M and *TM6SF2* E167K variants, may modulate the strength of the significant positive association between NAFLD and increased CVD risk. Despite both genotypes being associated with a greater susceptibility to increased disease severity in NAFLD, these two genotypes attenuate the strength of the association between NAFLD and CVD, probably through modifying effects on lipoprotein metabolism. Of the currently available drugs that have shown some efficacy in treating NAFLD or NASH, pioglitazone

and GLP-1RAs (mainly liraglutide and semaglutide) are the most promising to date. Both of these drugs have proven benefits not only in treating T2DM, but also benefitting CVD. The complex interplay between the liver and cardiometabolic risk factors in NAFLD highlights an urgent need for a multidisciplinary and holistic approach to manage both liver disease and cardiometabolic risk[55]. For new drugs where efficacy to treat liver disease is being tested in NAFLD, it is also crucially important to assess any cardiovascular and cardiac benefit, or harm.

Acknowledgments

CDB is supported in part by the Southampton NIHR Biomedical Research Centre (IS-BRC-20004), UK. GT is supported by grants from the University of Verona, Italy.

FIGURE LEGENDS

Figure 1. Magnitude of the NAFLD-related risks of subclinical atherosclerosis, fatal and nonfatal CVD events and other cardiac and arrhythmic complications, as quantified by recent systematic reviews and meta-analyses. Data are derived from meta-analytic studies summarized in Table 1.

Figure 2. Risk factors influencing cardiovascular and cardiac risk in NAFLD. NAFLD is closely associated with insulin resistance and the metabolic syndrome (MetS). The thresholds for defining each of the five easily measured features of the MetS[61] are shown in the figure. With hypertriglyceridaemia and decreased levels of high-density lipoprotein cholesterol (HDL-C), there is often increased levels of small dense low-density lipoprotein (sdLDL). The combination of hypertriglyceridaemia, decreased HDL-C and increased sdLDL is a common atherogenic dyslipidaemia in NAFLD and this type of dyslipidaemia is referred to as the atherogenic lipoprotein phenotype[68]. Although LDL-C concentrations are usually not increased in patients with NAFLD and MetS, sdLDL is often increased and is a proatherogenic lipoprotein. Many patients with insulin resistance and NAFLD also have type 2 diabetes (T2DM) that is diagnosed when a patient's plasma glucose concentration is ≥7.0 mmol/L or HbA1c concentration is ≥48 mmol/mol on two or more occasions. The presence of co-existing T2DM is also an important cardiovascular disease (CVD) and cardiac disease risk factor. Although pancreatic β cell function may be decreased with development of T2DM, decreased β cell function does not adversely affect risk of cardiovascular or cardiac disease. The presence of *PNPLA3 rs738409 c.444 C>G p.I148M* and *TM6SF2 rs58542926 C>T E167K* genotypes contribute to an increase in severity of liver disease (and also increase risk of developing T2DM), but both genotypes may attenuate the risk of developing CVD events.

Figure 3. Putative mechanisms and risk factors contributing to increased risk of type 2 diabetes, cardiovascular (CVD) and cardiac disease in NAFLD. NAFLD affects a variety of vasoactive and thrombogenic molecules and proinflammatory factors that potentially increase risk of CVD. In individuals with NAFLD, the atherogenic lipoprotein phenotype (ALP) occurs, that

increases risk of CVD. The ALP is defined by the combination of increased very low-density lipoprotein (VLDL), i.e., fasting hypertriglyceridaemia, decreased HDL-C and increased sdLDL levels. With central obesity that is very common with the metabolic syndrome and NAFLD, expanded and inflamed (dysfunctional) visceral adipose tissue undergoes increased lipolysis that increases delivery of proinflammatory cytokines, long-chain fatty acids (LCFAs) and glycerol to the liver. With expanded/inflamed adipose tissue, there is also decreased adiponectin that is strongly associated with greater insulin resistance. With increased fluxes of LCFAs, proinflammatory cytokines and decreased fluxes of adiponectin to the liver, there is an increased hepatic synthesis of various lipid species. These lipid species, including ceramide, di-palmitoyl phosphatidic acid (di-P PA), di-acylglycerol (DAG) and tri-acylglycerol (TAG), that may act to promote: a) hepatic insulin resistance (via decreased Akt phosphorylation and decreased insulin signaling); b) hepatic inflammation, (via lipid-induced increased protein kinase C epsilon (PKC-ε)), and c) accumulation of a lipid globule (see review for further detail[54]). With remodeling of the lipid globule, and synthesis of phospholipids, cholesterol esters and apolipoproteins, VLDL particles are assembled for export from the liver. With NAFLD, particularly in the absence of coexisting genetic polymorphisms that are well known to be associated with increased accumulation of the lipid globule and lower levels of VLDL (e.g., *PNPLA3 rs738409 c.444 C>G p.I148M* and *TM6SF2 rs58542926 C>T E167K*), there is an increase in fasting triglyceride concentrations that contributes to the ALP described above (and see text). With increased hepatic insulin resistance and increased flux of glycerol and LCFAs to the liver, there is also increased acetyl Co-A mediated activation of pyruvate carboxylase (catalyzing the irreversible conversion of pyruvate to oxaloacetate). With decreased insulin action relative to glucagon action, there is increased activity of phosphoenol-pyruvate carboxykinase (PEPCK), catalyzing the key rate limiting step in hepatic gluconeogenesis, i.e., the conversion of oxaloacetate to phosphoenolpyruvate leading to increased hepatic glucose output in the fasting state. Thus, increased gluconeogenesis in the fasting state increases hepatic glucose production that increases plasma fasting glucose concentrations. Increased peripheral insulin resistance in adipose tissue (and also skeletal muscle that is not shown on the schematic) increases risk of developing type 2 diabetes, CVD and other cardiac complications.

Table 1. Meta-analytic quantification of the excess of CVD risk (markers of subclinical atherosclerosis and adverse cardiovascular outcomes), cardiac arrhythmias (permanent atrial fibrillation and certain cardiac conduction defects), cardiomyopathy (mainly left ventricular dysfunction) or aortic-valve sclerosis in subjects with NAFLD.

Investigators, Year [Ref.]	Main characteristics of meta-analytic studies	CVD/cardiac markers or clinical outcomes	Random-effects odds ratios or hazard ratios (95% confidence intervals)
Subclinical atherosclerosis markers			
Zhou YY et al., 2018 [5]	26 cross-sectional studies involving a total of 85,395 individuals (29% with NAFLD)	Increased carotid artery intima-media thickness or plaques	1.74 (1.47-2.06)
		Increased arterial stiffness	1.56 (1.24-1.96)
		Coronary artery calcification	1.40 (1.22-1.60)
		Endothelial dysfunction	3.73 (0.99-14.1)
Fatal and non-fatal CVD outcomes [§]			
Targher G et al., 2016 [11]	16 longitudinal studies involving a total of 34,043 individuals (36% with NAFLD); median follow-up of 6.9 years	Any fatal or nonfatal CVD events	1.64 (1.26-2.13)
		Fatal CVD events (only)	1.31 (0.87-1.97)
		Fatal and nonfatal CVD events (combined endpoint)	1.63 (1.06-2.48)
		Nonfatal CVD events (only)	2.52 (1.52-4.18)
	6 longitudinal studies involving patients with "more severe" NAFLD[§]	Fatal CVD events (only)	3.28 (2.26-4.77)
		Fatal and nonfatal CVD events (combined endpoint)	1.94 (1.17-3.21)
Permanent atrial fibrillation			
Mantovani A et al., 2019 [18]	5 cross-sectional and 4 longitudinal studies involving a total of 364,919 individuals (43% with NAFLD)	Prevalent atrial fibrillation	2.07 (1.38-3.10) 5.17 (2.05-13.0) for type 2 diabetics only
		Incident atrial fibrillation	1.34 (0.92-1.95) 4.96 (1.42-17.3) for type 2 diabetics only
Cai X et al., 2020 [19]	6 longitudinal studies involving a total of 614,673 individuals (~40% with NAFLD); median follow-up of 10 years	Incident atrial fibrillation	1.65 (1.23-2.20)
Cardiac conduction defects			
Wijarnpreecha K et al., 2020 [27]	3 cross-sectional studies involving a total of 3,651 individuals (~30% with NAFLD)	Persistent cardiac conduction defects on resting electrocardiogram[#]	5.17 (1.34-20.0)
Left ventricular dysfunction			
Wijarnpreecha K et al., 2018 [30]	12 cross-sectional studies involving a total of 280,645 individuals (~30% with NAFLD)	Left ventricular diastolic dysfunction on echocardiography examination	2.02 (1.47-2.79)
Aortic-valve sclerosis			
Di Minno MN et al., 2016 [49]	3 cross-sectional studies involving a total of 2,639	Aortic-valve sclerosis on echocardiography examination	2.28 (1.21-4.28)

	individuals (44% with NAFLD)		

§ Fatal and/or non-fatal CVD events were defined as cardiovascular death, non-fatal CVD events (i.e., acute myocardial infarction, angina, ischemic stroke or coronary revascularization procedures), or both.

Persistent first-degree atrio-ventricular block, right bundle branch block or left anterior hemi-block.

Table 2. Recommended lifestyle modifications to ameliorate NAFLD and decrease CVD risk (based on clinical guidelines from the US, Europe and the UK[106,107,127])

Nutrition
• 5-10% weight loss target
• Mediterranean diet (i.e., high intake of vegetables, legumes, whole grains, olive oil, fish, seafood, nuts, fruits, and low intake of red meat, processed meats, and sweets)
• Avoid alcohol intake (notably, recent evidence indicates that the "safe" level of alcohol consumption is zero or near to zero)
• Avoid fructose, soft drinks, processed meat, and saturated fatty acids
• Low-carbohydrate ketogenic diets
Physical activity
• Moderate intensity aerobic physical activities (~150 min/week)
• Resistance training (alternatively to aerobic physical activities)
• 3-5 sessions weekly
Other
• Avoid use of cigarette smoking

Table 3. Drugs that may benefit NAFLD and that also benefit risk of cardiovascular or cardiac disease[113-116,118-122,124-127]

Drug/drug class	Mechanism of action	Studies	Primary endpoint(s)	Side-effects	Comments and recommendation
Pioglitazone	PPAR-gamma agonist	Multiple studies & Meta-analysis[113,114]	Improvement in NAS >2 without fibrosis worsening Improvement of fibrosis.	Weight gain (usually 2-4% of body weight). Fluid retention. Bone fractures (mostly in post-menopausal women)	Guidelines recommend pioglitazone in adults with biopsy-proven NASH, regardless of the presence of T2DM[106]. May also benefit liver fibrosis[113]. Not yet approved by most national Medicine agencies outside T2DM. Off-label use for NAFLD/NASH treatment requires the patient's consent. Cardiovascular benefits to decrease risk of myocardial infarction and ischaemic stroke in patients with T2DM or prediabetes[115]
GLP-1RAs (mainly liraglutide and semaglutide)	GLP-1 receptor agonist	Multiple studies[118,119,121,122]	Resolution of NASH Improvement of hepatic steatosis, hepatocyte ballooning. No effects on liver fibrosis.	Loss of appetite. Gastrointestinal side effects, including nausea.	Premature to consider in NASH, unless specifically being used as a treatment for T2DM and obesity. Current controlled trials with histological end-points are available only for liraglutide[118] and semaglutide[119]. Cardiorenal benefits in large controlled trials enrolling patients with T2DM[121,122].
SGLT-2 inhibitors (mainly canagliflozin, empagliflozin and dapagliflozin)	Inhibition of SGLT-2 (kidney proximal convoluted tubule)	Multiple studies[123-126]	Improvement of serum aminotransferase levels. Improvement of liver fat content.	Genitourinary infections. Diabetic ketoacidosis. Hypotension.	Premature to consider in NASH. No current trials with histological end-points are available. Relevant cardiorenal benefits in large controlled trials enrolling subjects with and without T2DM, including benefits to treat chronic heart failure with decreased left ventricular ejection fraction[123,124].

Appendix

Methods. Search strategy and selection criteria.

References for this clinical narrative Review were identified by the two authors through searches of PubMed with the search terms “atherosclerosis”, “cardiovascular disease”, “coronary heart disease”, “CHD”, “coronary artery disease”, “CAD”, “stroke”, “cardiovascular risk”, “heart failure”, “dyslipidemia”, “triglyceride”, “blood pressure”, “biomarkers”, “genetic epidemiological studies”, “genetic”, “insulin resistance”, “liver enzymes”, “NAFLD”, “nonalcoholic fatty liver”, “NAFL”, NAFLD”, “liver/hepatic steatosis”, “nonalcoholic steatohepatitis”, “NASH”, “fatty liver”, “metabolic syndrome”, “obesity”, “treatment”. We have limited the timeframe to the last decade due to the vast amount of research in the field (up to April 2021) for meta-analyses, reviews and original papers. We have considered the relevant literature cited in these papers. Only articles published in English were considered. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review.

References

1. Powell EE, Wong VW, Rinella M. Non-alcoholic fatty liver disease. *Lancet.* 2021. Jun 5;397(10290):2212-2224. doi: 10.1016/S0140-6736(20)32511-3. Epub 2021 Apr 21

2. Targher G, Byrne CD, Tilg H. NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications. *Gut.* 2020;69(9):1691-1705.
3. Targher G, Corey KE, Byrne CD. NAFLD, and cardiovascular and cardiac diseases: Factors influencing risk, prediction and treatment. *Diabetes Metab.* 2021;47(2):101215.
4. Guerreiro GTS, Longo L, Fonseca MA, de Souza VEG, Álvares-da-Silva MR. Does the risk of cardiovascular events differ between biopsy-proven NAFLD and MAFLD? *Hepatology international.* 2021;15(2):380-391.
5. Zhou YY, Zhou XD, Wu SJ, et al. Nonalcoholic fatty liver disease contributes to subclinical atherosclerosis: A systematic review and meta-analysis. *Hepatol Commun.* 2018;2(4):376-392.
6. Lee HH, Cho Y, Choi YJ, et al. Non-alcoholic steatohepatitis and progression of carotid atherosclerosis in patients with type 2 diabetes: a Korean cohort study. *Cardiovascular diabetology.* 2020;19(1):81.
7. Sinn DH, Cho SJ, Gu S, et al. Persistent Nonalcoholic Fatty Liver Disease Increases Risk for Carotid Atherosclerosis. *Gastroenterology.* 2016;151(3):481-488.e481.
8. Sinn DH, Kang D, Chang Y, et al. Non-alcoholic fatty liver disease and progression of coronary artery calcium score: a retrospective cohort study. *Gut.* 2017;66(2):323-329.
9. Lee J, Kim HS, Cho YK, et al. Association between noninvasive assessment of liver fibrosis and coronary artery calcification progression in patients with nonalcoholic fatty liver disease. *Scientific reports.* 2020;10(1):18323.
10. Bhatia L, Scorletti E, Curzen N, Clough GF, Calder PC, Byrne CD. Improvement in non-alcoholic fatty liver disease severity is associated with a reduction in carotid intima-media thickness progression. *Atherosclerosis.* 2016;246:13-20.
11. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. *J Hepatol.* 2016;65(3):589-600.
12. Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, et al. Fibrosis Severity as a Determinant of Cause-Specific Mortality in Patients With Advanced Nonalcoholic Fatty Liver Disease: A Multi-National Cohort Study. *Gastroenterology.* 2018;155(2):443-457.e417.
13. Henson JB, Simon TG, Kaplan A, Osganian S, Masia R, Corey KE. Advanced fibrosis is associated with incident cardiovascular disease in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther.* 2020;51(7):728-736.
14. Simon TG, Roelstraete B, Khalili H, Hagström H, Ludvigsson JF. Mortality in biopsy-confirmed nonalcoholic fatty liver disease: results from a nationwide cohort. *Gut.* 2020:gutjnl-2020-322786.
15. Meyersohn NM, Mayrhofer T, Corey KE, et al. Association of Hepatic Steatosis With Major Adverse Cardiovascular Events, Independent of Coronary Artery Disease. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2020. Jul 21;S1542-3565(20)30992-7. doi: 10.1016/j.cgh.2020.07.030

16. Xu J, Dai L, Zhang Y, et al. Severity of Nonalcoholic Fatty Liver Disease and Risk of Future Ischemic Stroke Events. *Stroke.* 2021;52(1):103-110.
17. Anstee QM, Mantovani A, Tilg H, Targher G. Risk of cardiomyopathy and cardiac arrhythmias in patients with nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol.* 2018;15(7):425-439.
18. Mantovani A, Dauriz M, Sandri D, et al. Association between non-alcoholic fatty liver disease and risk of atrial fibrillation in adult individuals: An updated meta-analysis. *Liver Int.* 2019;39(4):758-769.
19. Cai X, Zheng S, Liu Y, Zhang Y, Lu J, Huang Y. Nonalcoholic fatty liver disease is associated with increased risk of atrial fibrillation. *Liver Int.* 2020;40(7):1594-1600.

20. Donnellan E, Cotter TG, Wazni OM, et al. Impact of Nonalcoholic Fatty Liver Disease on Arrhythmia Recurrence Following Atrial Fibrillation Ablation. *JACC Clinical electrophysiology.* 2020;6(10):1278-1287.
21. Targher G, Valbusa F, Bonapace S, et al. Association of nonalcoholic fatty liver disease with QTc interval in patients with type 2 diabetes. *NutrMetab CardiovascDis.* 2014;24(6):663-669.
22. Hung CS, Tseng PH, Tu CH, et al. Nonalcoholic Fatty Liver Disease Is Associated With QT Prolongation in the General Population. *Journal of the American Heart Association.* 2015;4(7):e001820
23. Chung TH, Shim JY, Lee YJ. Nonalcoholic Fatty Liver Disease as a Risk Factor for Prolonged Corrected QT Interval in Apparently Healthy Korean Women. *Journal of gastrointestinal and liver diseases : JGLD.* 2020;29(1):59-64.
24. Mantovani A, Rigamonti A, Bonapace S, et al. Nonalcoholic Fatty Liver Disease Is Associated With Ventricular Arrhythmias in Patients With Type 2 Diabetes Referred for Clinically Indicated 24-Hour Holter Monitoring. *Diabetes Care.* 2016;39(8):1416-1423.
25. Mantovani A, Rigolon R, Pichiri I, et al. Nonalcoholic fatty liver disease is associated with an increased risk of heart block in hospitalized patients with type 2 diabetes mellitus. *PLoS One.* 2017;12(10):e0185459.
26. Mangi MA, Minhas AM, Rehman H, Pathan F, Liang H, Beidas S. Association of Non-alcoholic Fatty Liver Disease with Conduction Defects on Electrocardiogram. *Cureus.* 2017;9(3):e1107.
27. Wijarnpreecha K, Panjawatanan P, Kroner PT, Cheungpasitporn W, Ungprasert P. Association between cardiac conduction defect and nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Annals of gastroenterology.* 2020;33(6):661-666.
28. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *J Am Coll Cardiol.* 2000;35(3):569-582.
29. Granér M, Nyman K, Siren R, et al. Ectopic fat depots and left ventricular function in nondiabetic men with nonalcoholic fatty liver disease. *Circulation Cardiovascular imaging.* 2015;8(1).
30. Wijarnpreecha K, Lou S, Panjawatanan P, et al. Association between diastolic cardiac dysfunction and nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver.* 2018;50(11):1166-1175.
31. Chung GE, Lee JH, Lee H, et al. Nonalcoholic fatty liver disease and advanced fibrosis are associated with left ventricular diastolic dysfunction. *Atherosclerosis.* 2018;272:137-144.
32. Lee YH, Kim KJ, Yoo ME, et al. Association of non-alcoholic steatohepatitis with subclinical myocardial dysfunction in non-cirrhotic patients. *J Hepatol.* 2018;68(4):764-772.
33. Petta S, Argano C, Colomba D, et al. Epicardial fat, cardiac geometry and cardiac function in patients with non-alcoholic fatty liver disease: association with the severity of liver disease. *J Hepatol.* 2015;62(4):928-933.
34. Simon TG, Bamira DG, Chung RT, Weiner RB, Corey KE. Nonalcoholic Steatohepatitis is Associated with Cardiac Remodeling and Dysfunction. *Obesity (Silver Spring, Md).* 2017;25(8):1313-1316.
35. VanWagner LB, Wilcox JE, Ning H, et al. Longitudinal Association of Non-Alcoholic Fatty Liver Disease With Changes in Myocardial Structure and Function: The CARDIA Study. *Journal of the American Heart Association.* 2020;9(4):e014279.
36. Wannamethee SG, Whincup PH, Shaper AG, Lennon L, Sattar N. Gamma-glutamyltransferase, hepatic enzymes, and risk of incident heart failure in older men. *ArterioscIerThrombVascBiol.* 2012;32(3):830-835.
37. Dhingra R, Gona P, Wang TJ, Fox CS, D'Agostino RB, Sr., Vasan RS. Serum gamma-glutamyl transferase and risk of heart failure in the community. *Arterioscler Thromb Vasc Biol.* 2010;30(9):1855-1860.
38. Wang Y, Tuomilehto J, Jousilahti P, et al. Serum γ-glutamyltransferase and the risk of heart failure in men and women in Finland. *Heart.* 2013;99(3):163-167.

39. Roh JH, Park JH, Lee H, et al. Higher fatty liver index is associated with increased risk of new onset heart failure in healthy adults: a nationwide population-based study in Korea. *BMC cardiovascular disorders.* 2020;20(1):204.
40. Afolabi PR, Scorletti E, Calder PC, Byrne CD. Factors independently associated with cardiorespiratory fitness in patients with non-alcoholic fatty liver disease. *Liver Int.* 2020;40(12):2998-3007.
41. Valbusa F, Bonapace S, Agnoletti D, et al. Nonalcoholic fatty liver disease and increased risk of 1-year all-cause and cardiac hospital readmissions in elderly patients admitted for acute heart failure. *PLoS One.* 2017;12(3):e0173398.
42. Valbusa F, Agnoletti D, Scala L, et al. Non-alcoholic fatty liver disease and increased risk of all-cause mortality in elderly patients admitted for acute heart failure. *Int J Cardiol.* 2018;265:162-168.
43. Takahashi T, Watanabe T, Shishido T, et al. The impact of non-alcoholic fatty liver disease fibrosis score on cardiac prognosis in patients with chronic heart failure. *Heart and vessels.* 2018;33(7):733-739.
44. Stahl EP, Dhindsa DS, Lee SK, Sandesara PB, Chalasani NP, Sperling LS. Nonalcoholic Fatty Liver Disease and the Heart: JACC State-of-the-Art Review. *J Am Coll Cardiol.* 2019;73(8):948-963.
45. Packer M. Atrial Fibrillation and Heart Failure With Preserved Ejection Fraction in Patients With Nonalcoholic Fatty Liver Disease. *Am J Med.* 2020;133(2):170-177.
46. Peters AE, Pandey A, Ayers C, et al. Association of liver fibrosis risk scores with clinical outcomes in patients with heart failure with preserved ejection fraction: findings from TOPCAT. *ESC heart failure.* 2021;8(2):842-848.
47. Miller A, McNamara J, Hummel SL, Konerman MC, Tincopa MA. Prevalence and staging of non-alcoholic fatty liver disease among patients with heart failure with preserved ejection fraction. *Scientific reports.* 2020;10(1):12440.
48. Otto CM, Lind BK, Kitzman DW, Gersh BJ, Siscovick DS. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. *N Engl J Med.* 1999;341(3):142-147.
49. Di Minno MN, Di Minno A, Ambrosino P, Songia P, Tremoli E, Poggio P. Aortic valve sclerosis as a marker of atherosclerosis: Novel insights from hepatic steatosis. *Int J Cardiol.* 2016;217:1-6.
50. Prill S, Caddeo A, Baselli G, et al. The TM6SF2 E167K genetic variant induces lipid biosynthesis and reduces apolipoprotein B secretion in human hepatic 3D spheroids. *Scientific reports.* 2019;9(1):11585.
51. Deprince A, Haas JT, Staels B. Dysregulated lipid metabolism links NAFLD to cardiovascular disease. *Molecular metabolism.* 2020;42:101092.
52. Taylor RS, Taylor RJ, Bayliss S, et al. Association Between Fibrosis Stage and Outcomes of Patients With Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. *Gastroenterology.* 2020;158(6):1611-1625.e1612.
53. Kasper P, Martin A, Lang S, et al. NAFLD and cardiovascular diseases: a clinical review. *Clinical research in cardiology : official journal of the German Cardiac Society.* 2020. Jul 21. doi: 10.1007/s00392-020-01709-7. Online ahead of print
54. Cariou B, Byrne CD, Loomba R, Sanyal AJ. Nonalcoholic fatty liver disease as a metabolic disease in humans: A literature review. *Diabetes Obes Metab.* 2021;23(5):1069-1083.
55. Targher G, Tilg H, Byrne CD. Non-alcoholic fatty liver disease: a multisystem disease requiring a multidisciplinary and holistic approach. *The lancet Gastroenterology & hepatology.* 2021. Jul;6(7):578-588. doi: 10.1016/S2468-1253(21)00020-0. Epub 2021 May 4
56. Lonardo A, Mantovani A, Lugari S, Targher G. Epidemiology and pathophysiology of the association between NAFLD and metabolically healthy or metabolically unhealthy obesity. *Ann Hepatol.* 2020;19(4):359-366.
57. Semmler G, Wernly S, Bachmayer S, et al. Nonalcoholic Fatty Liver Disease in Lean Subjects: Associations With Metabolic Dysregulation and Cardiovascular Risk-A Single-Center Cross-Sectional Study. *Clinical and translational gastroenterology.* 2021;12(4):e00326.

58. Mauvais-Jarvis F, Bairey Merz N, Barnes PJ, et al. Sex and gender: modifiers of health, disease, and medicine. *Lancet.* 2020;396(10250):565-582.
59. Parikh NS, Koh I, VanWagner LB, Elkind MSV, Zakai NA, Cushman M. Liver Fibrosis is Associated with Ischemic Stroke Risk in Women but not Men: The REGARDS Study. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association.* 2021;30(7):105788.
60. Khalid YS, Dasu NR, Suga H, et al. Increased cardiovascular events and mortality in females with NAFLD: a meta-analysis. *American journal of cardiovascular disease.* 2020;10(3):258-271.
61. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and international association for the Study of Obesity. *Circulation.* 2009;120(16):1640-1645.
62. Guzder RN, Gatling W, Mullee MA, Byrne CD. Impact of metabolic syndrome criteria on cardiovascular disease risk in people with newly diagnosed type 2 diabetes. *Diabetologia.* 2006;49(1):49-55.
63. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation.* 2005;112(20):3066-3072.
64. Gastaldelli A, Cusi K. From NASH to diabetes and from diabetes to NASH: Mechanisms and treatment options. *JHEP reports : innovation in hepatology.* 2019;1(4):312-328.
65. Targher G, Byrne CD. A Perspective on Metabolic Syndrome and Nonalcoholic Fatty Liver Disease. *Metab SyndrRelat Disord.* 2015. Aug;13(6):235-8. doi: 10.1089/met.2015.1502. Epub 2015 Feb 25
66. Francque S, De Maeght S, Adler M, et al. High prevalence of advanced fibrosis in association with the metabolic syndrome in a Belgian prospective cohort of NAFLD patients with elevated ALT. Results of the Belgian NAFLD registry. *Acta gastro-enterologica Belgica.* 2011;74(1):9-16.
67. Jinjuvadia R, Antaki F, Lohia P, Liangpunsakul S. The Association Between Nonalcoholic Fatty Liver Disease and Metabolic Abnormalities in The United States Population. *Journal of clinical gastroenterology.* 2017;51(2):160-166.
68. Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation.* 1990;82(2):495-506.
69. Zhang QQ, Lu LG. Nonalcoholic Fatty Liver Disease: Dyslipidemia, Risk for Cardiovascular Complications, and Treatment Strategy. *Journal of clinical and translational hepatology.* 2015;3(1):78-84.
70. DeFilippis AP, Blaha MJ, Martin SS, et al. Nonalcoholic fatty liver disease and serum lipoproteins: the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis.* 2013;227(2):429-436.
71. Peng K, Mo Z, Tian G. Serum Lipid Abnormalities and Nonalcoholic Fatty Liver Disease in Adult Males. *The American journal of the medical sciences.* 2017;353(3):236-241.
72. Bril F, Sninsky JJ, Baca AM, et al. Hepatic Steatosis and Insulin Resistance, But Not Steatohepatitis, Promote Atherogenic Dyslipidemia in NAFLD. *J Clin Endocrinol Metab.* 2016;101(2):644-652.
73. Yang MH, Sung J, Gwak GY. The associations between apolipoprotein B, A1, and the B/A1 ratio and nonalcoholic fatty liver disease in both normal-weight and overweight Korean population. *J Clin Lipidol.* 2016;10(2):289-298.
74. Chen Z, Qin H, Qiu S, Chen G, Chen Y. Correlation of triglyceride to high-density lipoprotein cholesterol ratio with nonalcoholic fatty liver disease among the non-obese Chinese population with normal blood lipid levels: a retrospective cohort research. *Lipids in health and disease.* 2019;18(1):162.
75. Mittendorfer B, Yoshino M, Patterson BW, Klein S. VLDL Triglyceride Kinetics in Lean, Overweight, and Obese Men and Women. *J Clin Endocrinol Metab.* 2016;101(11):4151-4160.
76. Fabbrini E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology.* 2008;134(2):424-431.
77. Tutunchi H, Naeini F, Ebrahimi-Mameghani M, Mobasseri M, Naghshi S, Ostadrahimi A. The association of the steatosis severity, NAFLD fibrosis score, and FIB-4 index with atherogenic

dyslipidemia in adult patients with NAFLD: A cross- sectional study. *International journal of clinical practice.* 2021:e14131.
78. Siddiqui MS, Fuchs M, Idowu MO, et al. Severity of nonalcoholic fatty liver disease and progression to cirrhosis are associated with atherogenic lipoprotein profile. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2015;13(5):1000-1008.e1003.
79. Varbo A, Benn M, Tybjærg-Hansen A, Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol.* 2013;61(4):427-436.
80. Marston NA, Giugliano RP, Im K, et al. Association Between Triglyceride Lowering and Reduction of Cardiovascular Risk Across Multiple Lipid-Lowering Therapeutic Classes: A Systematic Review and Meta-Regression Analysis of Randomized Controlled Trials. *Circulation.* 2019;140(16):1308-1317.
81. Byrne CD, Wang TW, Hales CN. Control of Hep G2-cell triacylglycerol and apolipoprotein B synthesis and secretion by polyunsaturated non-esterified fatty acids and insulin. *The Biochemical journal.* 1992;288 (Pt 1)(Pt 1):101-107.
82. Argo CK, Patrie JT, Lackner C, et al. Effects of n-3 fish oil on metabolic and histological parameters in NASH: A double-blind, randomized, placebo-controlled trial. *J Hepatol.* 2015;62(1):190-197.
83. Sanyal AJ, Abdelmalek MF, Suzuki A, Cummings OW, Chojkier M. No significant effects of ethyl-eicosapentanoic acid on histologic features of nonalcoholic steatohepatitis in a phase 2 trial. *Gastroenterology.* 2014;147(2):377-384.
84. Scorletti E, Bhatia L, McCormick KG, et al. Effects of purified eicosapentaenoic and docosahexaenoic acids in nonalcoholic fatty liver disease: results from the Welcome* study. *Hepatology.* 2014;60(4):1211-1221.
85. Johansen M, Nielsen SF, Afzal S, Vedel-Krogh S, Davey Smith G, Nordestgaard BG. Very Low-Density Lipoprotein Cholesterol May Mediate a Substantial Component of the Effect of Obesity on Myocardial Infarction Risk: The Copenhagen General Population Study. *Clin Chem.* 2021;67(1):276-287.
86. Carlsson B, Lindén D, Brolén G, et al. Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther.* 2020;51(12):1305-1320.
87. Jamialahmadi O, Mancina RM, Ciociola E, et al. Exome-Wide Association Study on Alanine Aminotransferase Identifies Sequence Variants in the GPAM and APOE Associated With Fatty Liver Disease. *Gastroenterology.* 2021. Apr;160(5):1634-1646.e7. doi: 10.1053/j.gastro.2020.12.023. Epub 2021 Feb 6.
88. Yassine HN, Finch CE. APOE Alleles and Diet in Brain Aging and Alzheimer's Disease. *Frontiers in Aging Neuroscience.* 2020 Jun 10;12:150. doi: 10.3389/fnagi.2020.00150. eCollection 2020.
89. Marais AD. Apolipoprotein E in lipoprotein metabolism, health and cardiovascular disease. *Pathology.* 2019;51(2):165-176.
90. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *NatGenet.* 2008;40(12):1461-1465.
91. Lauridsen BK, Stender S, Kristensen TS, et al. Liver fat content, non-alcoholic fatty liver disease, and ischaemic heart disease: Mendelian randomization and meta-analysis of 279 013 individuals. *Eur Heart J.* 2018;39(5):385-393.
92. Liu DJ, Peloso GM, Yu H, et al. Exome-wide association study of plasma lipids in >300,000 individuals. *Nature genetics.* 2017;49(12):1758-1766.
93. Speliotes EK, Yerges-Armstrong LM, Wu J, et al. Genome-Wide Association Analysis Identifies Variants Associated with Nonalcoholic Fatty Liver Disease That Have Distinct Effects on Metabolic Traits. *PLOS Genetics.* 2011;7(3):e1001324.
94. Holmen OL, Zhang H, Fan Y, et al. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nature genetics.* 2014;46(4):345-351.

95. Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nature genetics.* 2014;46(4):352-356.
96. Nobili V, Alisi A, Valenti L, Miele L, Feldstein AE, Alkhouri N. NAFLD in children: new genes, new diagnostic modalities and new drugs. *Nat Rev Gastroenterol Hepatol.* 2019;16(9):517-530.
97. Anstee QM, Day CP. The Genetics of Nonalcoholic Fatty Liver Disease: Spotlight on PNPLA3 and TM6SF2. *Seminars in liver disease.* 2015;35(3):270-290.
98. Eslam M, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: Clinical impact. *J Hepatol.* 2018;68(2):268-279.
99. Santos RD, Valenti L, Romeo S. Does nonalcoholic fatty liver disease cause cardiovascular disease? Current knowledge and gaps. *Atherosclerosis.* 2019;282:110-120.
100. Smolková B, Bonassi S, Buociková V, et al. Genetic determinants of quantitative traits associated with cardiovascular disease risk. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis.* 2015;778:18-25.
101. Blum CB. Dynamics of apolipoprotein E metabolism in humans. *Journal of Lipid Research.* 1982;23(9):1308-1316.
102. Awan Z, Choi HY, Stitziel N, et al. APOE p.Leu167del mutation in familial hypercholesterolemia. *Atherosclerosis.* 2013;231(2):218-222.
103. Bea AM, Lamiquiz-Moneo I, Marco-Benedí V, et al. Lipid-lowering response in subjects with the p.(Leu167del) mutation in the APOE gene. *Atherosclerosis.* 2019;282:143-147.
104. Rasmussen KL. Plasma levels of apolipoprotein E, APOE genotype and risk of dementia and ischemic heart disease: A review. *Atherosclerosis.* 2016;255:145-155.
105. Käräjämäki AJ, Hukkanen J, Kauma H, Kesäniemi YA, Ukkola O. Metabolic syndrome but not genetic polymorphisms known to induce NAFLD predicts increased total mortality in subjects with NAFLD (OPERA study). *Scandinavian journal of clinical and laboratory investigation.* 2020;80(2):106-113.
106. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol.* 2016;64(6):1388-1402.
107. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology.* 2018;67(1):328-357.
108. Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. *J Hepatol.* 2017;67(4):829-846.
109. Asbaghi O, Choghakhori R, Ashtary-Larky D, Abbasnezhad A. Effects of the Mediterranean diet on cardiovascular risk factors in non-alcoholic fatty liver disease patients: A systematic review and meta-analysis. *Clinical nutrition ESPEN.* 2020;37:148-156.
110. Katsagoni CN, Georgoulis M, Papatheodoridis GV, Panagiotakos DB, Kontogianni MD. Effects of lifestyle interventions on clinical characteristics of patients with non-alcoholic fatty liver disease: A meta-analysis. *Metabolism.* 2017;68:119-132.
111. Thoma C, Day CP, Trenell MI. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. *J Hepatol.* 2012;56(1):255-266.
112. Baker CJ, Martinez-Huenchullan SF, D'Souza M, et al. Effect of exercise on hepatic steatosis: Are benefits seen without dietary intervention? A systematic review and meta-analysis. *J Diabetes.* 2021;13(1):63-77.
113. Musso G, Cassader M, Paschetta E, Gambino R. Thiazolidinediones and Advanced Liver Fibrosis in Nonalcoholic Steatohepatitis: A Meta-analysis. *JAMA internal medicine.* 2017;177(5):633-640.
114. Cusi K, Orsak B, Bril F, et al. Long-Term Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus: A Randomized Trial. *Ann Intern Med.* 2016;165(5):305-315.
115. DeFronzo RA, Inzucchi S, Abdul-Ghani M, Nissen SE. Pioglitazone: The forgotten, cost-effective cardioprotective drug for type 2 diabetes. *Diabetes & vascular disease research.* 2019;16(2):133-143.
116. Mantovani A, Byrne CD, Scorletti E, Mantzoros CS, Targher G. Efficacy and safety of anti-hyperglycaemic drugs in patients with non-alcoholic fatty liver disease with or without diabetes: An updated systematic review of randomized controlled trials. *Diabetes Metab.* 2020;46(6):427-441.

117. Armstrong MJ, Houlihan DD, Rowe IA, et al. Safety and efficacy of liraglutide in patients with type 2 diabetes and elevated liver enzymes: individual patient data meta-analysis of the LEAD program. *Aliment Pharmacol Ther.* 2013;37(2):234-242.
118. Armstrong MJ, Gaunt P, Aithal GP, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet.* 2016;387(10019):679-690.
119. Newsome PN, Buchholtz K, Cusi K, et al. A Placebo-Controlled Trial of Subcutaneous Semaglutide in Nonalcoholic Steatohepatitis. *N Engl J Med.* 2021;384(12):1113-1124.
120. Wilding JPH, Batterham RL, Calanna S, et al. Once-Weekly Semaglutide in Adults with Overweight or Obesity. *N Engl J Med.* 2021;384(11):989.
121. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med.* 2016;375(4):311-322.
122. Kristensen SL, Rørth R, Jhund PS, et al. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *The lancet Diabetes & endocrinology.* 2019;7(10):776-785.
123. Palmer SC, Tendal B, Mustafa RA, et al. Sodium-glucose cotransporter protein-2 (SGLT-2) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists for type 2 diabetes: systematic review and network meta-analysis of randomised controlled trials. *Bmj.* 2021;372:m4573.
124. Mantovani A, Petracca G, Csermely A, Beatrice G, Targher G. Sodium-Glucose Cotransporter-2 Inhibitors for Treatment of Nonalcoholic Fatty Liver Disease: A Meta-Analysis of Randomized Controlled Trials. *Metabolites.* 2020;11(1) 22. doi: 10.3390/metabo11010022
125. Marjot T, Green CJ, Charlton CA, et al. Sodium-glucose cotransporter 2 inhibition does not reduce hepatic steatosis in overweight, insulin-resistant patients without type 2 diabetes. *JGH open : an open access journal of gastroenterology and hepatology.* 2020;4(3):433-440.
126. Cusi K, Bril F, Barb D, et al. Effect of canagliflozin treatment on hepatic triglyceride content and glucose metabolism in patients with type 2 diabetes. *Diabetes Obes Metab.* 2019;21(4):812-821.
127. Glen J, Floros L, Day C, Pryke R. Non-alcoholic fatty liver disease (NAFLD): summary of NICE guidance. *Bmj.* 2016;354:i4428.