Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus

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Abstract | Nonalcoholic fatty liver disease (NAFLD) and diabetes mellitus are common diseases that often coexist and might act synergistically to increase the risk of hepatic and extra-hepatic clinical outcomes. NAFLD affects up to 70–80% of patients with type 2 diabetes mellitus and up to 30–40% of adults with type 1 diabetes mellitus. The coexistence of NAFLD and diabetes mellitus increases the risk of developing not only the more severe forms of NAFLD but also chronic vascular complications of diabetes mellitus. Indeed, substantial evidence links NAFLD with an increased risk of developing cardiovascular disease and other cardiac and arrhythmic complications in patients with type 1 diabetes mellitus or type 2 diabetes mellitus. NAFLD is also associated with an increased risk of developing microvascular diabetic complications, especially chronic kidney disease. This Review focuses on the strong association between NAFLD and the risk of chronic vascular complications in patients with type 1 diabetes mellitus or type 2 diabetes mellitus, thereby promoting an increased awareness of the extra-hepatic implications of this increasingly prevalent and burdensome liver disease. We also discuss the putative underlying mechanisms by which NAFLD contributes to vascular diseases, as well as the emerging role of changes in the gut microbiota (dysbiosis) in the pathogenesis of NAFLD and associated vascular diseases.

Nonalcoholic fatty liver disease (NAFLD), in the absence of competing aetiologies of liver injury, is a variable combination of individual histological findings, namely, accumulation of fat (triglycerides) in >5% of hepatocytes, often with a small amount of low-grade sterile inflammation (simple steatosis), steatosis with ballooning degeneration (nonalcoholic steatohepatitis (NASH)), advanced fibrosis and cryptogenic cirrhosis[1,2].

NAFLD has become one of the most common chronic liver diseases in many parts of the world; it occurs in up to 30% of adults in the general population in Western countries, and the prevalence of the disease is even greater in patients with type 2 diabetes mellitus (T2DM), occurring in up to 70–80% of these patients[1–3]. Patients with T2DM and NAFLD are also more likely than patients with NAFLD alone to develop the more severe histological forms of NAFLD (that is, NASH, advanced fibrosis and cirrhosis), which can ultimately lead to hepatocellular carcinoma (HCC)[1–3].

Strong evidence now indicates that the global health burden of NAFLD is not only confined to severe liver-related complications (cirrhosis, end-stage liver disease and HCC, which might require liver transplantation) but also includes major extra-hepatic conditions[4–6]. Indeed, the leading causes of mortality among patients with NAFLD are cardiovascular disease (CVD), followed by extra-hepatic cancers and liver-related complications (such as gastroesophageal varices or bleeding, ascites, end-stage liver disease and HCC)[1–3]. Moreover, as discussed in greater detail in subsequent sections, it has also become increasingly clear that the presence and severity of NAFLD are strongly associated with an increased risk of developing serious extra-hepatic diseases, such as CVD, cardiomyopathy and cardiac arrhythmias as well as chronic kidney disease (CKD), the latter of which is one of the most important chronic complications of diabetes mellitus.

This Review focuses on the adverse effect of NAFLD on the risk of chronic vascular complications of diabetes mellitus (mainly CVD and CKD but also other microvascular complications of diabetes mellitus). The putative pathophysiological mechanisms by which NAFLD might contribute to the development and progression of chronic vascular complications of diabetes mellitus are also discussed. Finally, the principles of NAFLD treatment are critically evaluated.

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Key points

- Convincing epidemiological evidence substantiates a strong association between the presence and severity of nonalcoholic fatty liver disease (NAFLD) and the risk of chronic macrovascular (mainly cardiovascular disease) and microvascular (mainly chronic kidney disease) complications of diabetes mellitus
- NAFLD exacerbates insulin resistance, predisposes to atherogenic dyslipidaemia and causes the release of pro-inflammatory, procoagulant and proatherogenic factors that have a role in the development of chronic vascular complications of diabetes mellitus
- Despite the evidence linking NAFLD to these chronic vascular complications, it has not been definitively established whether a causal association also exists
- These findings call for a more active and systematic search for NAFLD in adult patients with diabetes mellitus with a view to implementing an earlier and more aggressive treatment whenever indicated
- Whether a more liberal screening policy and more aggressive treatment will cost-effectively prevent the development of chronic vascular complications of diabetes mellitus will be the target of future larger studies
- Although further research is needed, correction of intestinal dysbiosis might be a novel therapeutic target to ameliorate the risk of NAFLD and chronic vascular complications of diabetes mellitus

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Diagnosis and epidemiology of NAFLD

The new epidemic in chronic liver diseases is related to the burden of NAFLD that parallels the worldwide increases in levels of obesity and T2DM[1–3]. The prevalence of NAFLD varies by both the specific characteristics of the population studied (for example, studies in patients with different ethnicities, sex and comorbidities) and the sensitivity of the methodologies used for diagnosing NAFLD (serum levels of liver enzymes, imaging techniques or liver biopsy)[1–3].

Diagnostic methods

NAFLD encompasses a histopathological spectrum of liver conditions ranging from simple steatosis to NASH (with varying levels of liver fibrosis) and cirrhosis. The presence of hepatic steatosis (defined by an accumulation of lipid droplets in >5% of hepatocytes) is a prerequisite for the diagnosis of NAFLD. The grading of hepatic steatosis is conventionally based on the proportion of hepatocytes involved (in mild steatosis, up to 33% of hepatocytes are steatotic; in moderate steatosis, 33–66%; and in severe steatosis, >66%). However, in clinical practice, NAFLD remains a diagnosis of exclusion; the lack of a positive diagnostic criterion to define NAFLD is challenging[7,8]. In operational clinical terms[1,9,10] and irrespective of the background population, clinicians have four main criteria to assess NAFLD. First, they have to identify excess hepatic fat content by using various imaging techniques or, in some cases, by liver biopsy, which remains the reference standard for diagnosing NAFLD and staging the severity of necroinflammation and fibrosis in patients with NAFLD. Second, clinicians have to exclude alcoholic, viral, pharmacological, autoimmune and inherited genetic aetiologies of steatotic liver disease. Third, clinicians have to ascertain the coexistence of the typical features of the metabolic syndrome[11]. Fourth, clinicians need to assess the severity of hepatic fibrosis, which is the strongest predictor of disease-specific mortality in NAFLD[1–3,6]. Clearly, the third point might be superfluous when dealing with people with diabetes mellitus.

Possible screening strategies

Screening for NAFLD among patients with established diabetes mellitus should be a multistep process. The presence of (mainly macrovesicular) fat vesicles in at least 5% of hepatocytes defines steatosis histologically[1,2]. However, as liver biopsy is an invasive procedure that cannot be proposed for all patients with suspected NAFLD, both noninvasive biomarkers of hepatic steatosis and imaging techniques have been developed. However, to date, noninvasive biomarkers of steatosis have a limited clinical utility, as they often do not accurately quantify the percentage of intrahepatic fat content assessed histologically[12]. Therefore, imaging techniques are the preferred noninvasive diagnostic tests for assessing fat accumulation in the liver[1–3]. Proton magnetic resonance spectroscopy is the most precise method for measuring hepatic triglyceride content, but it is of limited availability owing to its high costs[1–3]. Ultrasonography is the most widely used imaging method in clinical practice and has an overall sensitivity and specificity for detecting mild-to-moderate hepatic steatosis of nearly 85% and 94%, respectively[13]. The accuracy of ultrasonography might further improve in relation to the local expertise and the availability of newer ultrasound machines[14]. Semi-quantitative ultrasonographic indices might also provide added diagnostic value[14]. For example, the ultrasonographic fatty liver indicator (US-FLI) is a simple and inexpensive score ranging from two to eight that is calculated on the presence of different degrees of liver-to-kidney contrast and on the presence (or absence) of posterior attenuation of the ultrasound beam, vessel blurring, difficult visualization of the gallbladder wall, difficult ultrasound visualization of the diaphragm and areas of focal sparing[15]. A US-FLI score ≥2 accurately detects a minimum amount of 10% steatosis on liver histology (sensitivity 90% and specificity 90%)[15]. Moreover, a US-FLI score ≤4 has a nearly 95% negative predictive value for excluding severe NASH[16]. The controlled attenuation parameter assessed by transient elastography (FibroScan, Echosens) at a cut-off value of 310 dB/m has 80% sensitivity, 71% specificity, an 86% positive predictive value and a 71% negative predictive value for detecting histological steatosis ≥30%[17].

The exclusion of competing aetiologies of chronic liver disease can be achieved in most patients with suspected NAFLD and coexisting features of the metabolic syndrome with a careful medical history (mainly focused on the history of excessive alcohol consumption and drug exposure), by measuring routine laboratory parameters (for example, serum viral markers and serum levels of ferritin and transferrin)[1,2,9] and by using specific questionnaires to exclude excessive alcohol consumption (a threshold of 20 g per day for women and 30 g per day for men is conventionally adopted)[1–3,18].

Determining whether the patient has the metabolic syndrome can be easily achieved by obtaining appropriate family and personal history, physical examination (with measurement of blood pressure, BMI and waist circumference) and a first-level laboratory assessment (namely, fasting plasma levels of glucose and lipids)[1,9]. Interestingly, using the US-FLI might be more effective at identifying the presence of the metabolic syndrome than measuring insulin resistance alone[15,16,19].

Staging of hepatic fibrosis, which is the strongest predictor of both overall and disease-specific mortality in NAFLD[1–3,20], can be implemented either with the use of liver biopsy (the reference standard) or with the use of multiple noninvasive methods. These methods include biochemical score systems (for example, fibrosis-4 (FIB-4) scores >2.67, NAFLD fibrosis score (NFS) >0.676 and enhanced liver fibrosis (ELF) scores ≥10.51 have a good specificity for diagnosing advanced hepatic fibrosis) and physical techniques (for example, liver stiffness measurement (LSM) >9.9 kPa as assessed with transient elastography or with other noninvasive imaging methods also have good accuracy for diagnosing advanced hepatic fibrosis)[20,21]. The serial combination of LSM with FIB-4 or NFS measurements accurately predicts the presence of advanced hepatic fibrosis in NAFLD[21].

We have proposed a novel pragmatic algorithm for the diagnosis and monitoring of NAFLD in patients with established diabetes mellitus (FIG. 1). However, it is important to emphasize that an intense debate on aspects of our algorithm, as well as of similar algorithms, is ongoing and that a validated, widely accepted algorithm for the diagnosis and monitoring of NAFLD in patients with established diabetes mellitus does not yet exist. In particular, screening for NAFLD (in both the general population and high-risk groups of patients) is not universally recommended by all scientific societies[1,2,22,23]. Furthermore, the cost-effectiveness of screening remains controversial[24,25], and liver biopsies should eventually be carried out in at least a (large) proportion of patients submitted to noninvasive screening, given that the diagnosis of NASH remains universally based on histological findings[1,2,19,21,22]. For example, a cross-sectional study published in 2017 that was conducted in approximately 122,000 patients with T2DM found a high prevalence of advanced hepatic fibrosis by use of NFS and other noninvasive scores[26]; however, the substantial variability among the findings provided by such scores (ranging from nearly 9% with the use of the FIB-4 score to nearly 35% with the NFS) strongly supports the need for their further validation in populations with diabetes mellitus[26].

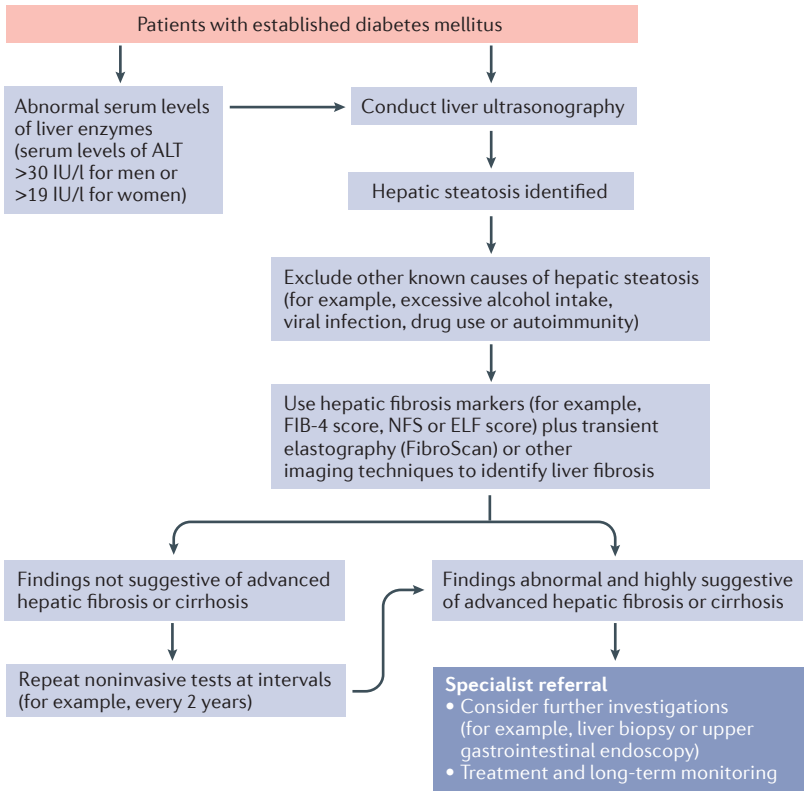

Figure 1 | **Proposed pragmatic algorithm for the management of suspected nonalcoholic fatty liver disease in patients with established diabetes mellitus.** The algorithm has been developed using both available evidence and guidelines[1,2,22,23,45,47] as well as expert opinion where uncertainty existed and evidence was not available. Patients with type 1 or type 2 diabetes mellitus should routinely (approximately every 2 years) undergo diagnostic procedures for nonalcoholic fatty liver disease (NAFLD), which rely on the demonstration of hepatic steatosis. Serum levels of transaminases are not reliable indicators for the screening and diagnosis of NAFLD and should not be used without further investigation in clinical practice. Liver ultrasonography is the preferred first-line imaging method for the diagnosis of NAFLD. The exclusion of competing causes of hepatic steatosis is key for the diagnosis of NAFLD. The algorithm can be used to select patients with NAFLD for liver biopsy, or if biopsy is not undertaken, the noninvasive assessment of advanced liver fibrosis according to panels of specific serum biomarkers (the fibrosis-4 (FIB-4) score, the NAFLD fibrosis score (NFS) or the enhanced liver fibrosis (ELF) score) and transient elastography can be used to select patients for upper gastrointestinal endoscopy (aimed at showing the presence of oesophageal or gastric varices due to portal hypertension). Long-term surveillance for liver-related complications, including hepatocellular carcinoma, should always be undertaken if cirrhosis is present and should be done in selected patients without cirrhosis, such as in patients with advanced liver fibrosis. ALT, alanine transaminase.

Epidemiology

Around one-quarter of adults in the United States and Europe have NAFLD, and the prevalence of NAFLD is even higher in certain areas of South America (~35% in Brazil), the Middle East (~30% in Israel) and Asia (~30–35% in China and South Korea) [27]. This finding further highlights the overwhelming potential clinical and economic burdens imposed by NAFLD, which will probably increase in the future with the projected rise in prevalence of NAFLD[1,2,27]. The scale of the burden of NAFLD also implies that none of the health authorities

around the world can afford to promote screening campaigns aimed at identifying NAFLD in the general population, as it is not feasible to screen all at-risk individuals.

T2DM. Given the unaffordability of population-wide screening, the identification of certain selected cohorts of individuals at high risk of developing NAFLD (such as people with T2DM) seems to be a more fruitful strategy[1,2,11]. Irrespective of the characteristics of the cohorts studied (hospital-based cohorts versus population-based cohorts) and the diagnostic methodologies used for diagnosing NAFLD (imaging versus biopsy), the prevalence of the disease is much greater in patients with T2DM than in the nondiabetic population, ranging from nearly 40% to 100%[28–44] (Supplementary information S1 (table)).

Compared with individuals without diabetes mellitus, patients with established T2DM are also more likely to have more severe histological forms of NAFLD, such as NASH with advanced fibrosis, even in those with fairly normal serum levels of aminotransferases[33,35]. Therefore, serum levels of aminotransferases are not reliable indicators for the screening and diagnosis of NAFLD among patients with T2DM and should not be used to this end in clinical practice[1–4,9,11,45].

Notably, the coexistence of NAFLD and T2DM will worsen the course of both diseases[45–49]. Coexisting T2DM increases the risk of not only NAFLD progression to advanced fibrosis and cirrhosis but also incident HCC, liver-related hospital admissions and liver-related deaths[43,49–55]. In addition, the presence of NAFLD makes achieving good glycaemic control more difficult, increases hepatic and peripheral insulin resistance and exacerbates atherogenic dyslipidaemia[1–3,45,47], thereby further increasing the risk of incident CKD[4–6,56] and major CVD events, particularly in patients with advanced NAFLD[1,57,58].

Collectively, these findings strongly support the assertion that in patients with T2DM, diagnosis of and treatment for NAFLD should be considered a high priority for diabetologists or endocrinologists caring for patients at risk of NAFLD.

T1DM. Compared with our knowledge of the heavy toll imposed on the liver by T2DM, epidemiological data on the presence and effects of NAFLD in patients with T1DM (that is, a disease characterized by an altered portosystemic gradient of insulin and a lower degree of insulin resistance than T2DM) seem to be more variable[59–67] (Supplementary information S2 (table)).

Some studies have reported a (fairly) high prevalence of NAFLD (diagnosed using ultrasonography), with values up to nearly 50% in adult patients with T1DM[60,61]. Others have reported a prevalence of NAFLD (diagnosed using MRI) of 30% in a small group of adults with T1DM[67]. However, some investigators have disputed this notion by reporting a prevalence of NAFLD (diagnosed using MRI) in patients with T1DM that ranged from 0% (in children with T1DM)[63] to nearly 10–15% (in adults with T1DM)[65,66], which is actually a lower prevalence than that observed in the general adult population[1–3,11,27].

We consider that these wide inter-study differences in the prevalence of NAFLD might be, at least in part, due to differences in the imaging techniques used to diagnose NAFLD as well as to differences in age, sex distribution, duration of diabetes mellitus, family history of T2DM, BMI and degree of glycaemic control among the various cohorts of patients with T1DM that were studied. A large, prospective UK study of adult patients with T1DM and T2DM who had undergone liver biopsy has reported that those with T1DM had a risk of developing cirrhosis and portal hypertension that was similar to that observed in patients with T2DM who were matched for sex, age, diabetes mellitus duration, obesity and other potential confounding variables[68]. However, we suggest that further larger studies of well-characterized patients with T1DM are required to better characterize the relationship between NAFLD and T1DM.

Chronic vascular complications

In the past decade, many epidemiological studies have documented that NAFLD, diagnosed either by imaging or by histology, is associated with a substantially increased risk of all-cause and cause-specific (cardiovascular, cancer-related and liver-related) mortality in both patients without diabetes mellitus and those with T2DM[1–3]. Strong evidence indicates that CVD is a clinical concern in NAFLD and that patients with NAFLD are more likely to experience CVD-related death than liver-related death[4–6,69]. Furthermore, several studies have also suggested that NAFLD is closely associated with an increased risk of chronic vascular complications of diabetes mellitus[47].

Macrovascular complications

Substantial epidemiological evidence links NAFLD with various markers of subclinical atherosclerosis (for example, increased arterial stiffness, endothelial dysfunction or increased prevalence of carotid and lower limb atherosclerotic plaques) and with an increased prevalence of clinically manifest CVD across different populations of patients, including people with T2DM[3,4,58,69,70]. In 2017, Guo *et al.*[71] confirmed that ultrasound-diagnosed NAFLD was associated with an increased prevalence of carotid and lower limb atherosclerotic plaques, independent of conventional CVD risk factors, duration of diabetes mellitus, HbA_{1c} levels, insulin resistance, serum levels of liver enzymes and medication use in a large cohort of Chinese individuals with T2DM. Similarly, the Valpolicella Diabetes Heart Study, including 2,392 Italian outpatients with T2DM without secondary causes of chronic liver disease, demonstrated that compared with patients without NAFLD, those with NAFLD diagnosed using ultrasonography had a remarkably greater prevalence of clinically manifest coronary, cerebrovascular and peripheral vascular disease (FIG. 2) independent of age, sex, BMI, waist circumference, smoking status, LDL cholesterol levels, HbA_{1c} levels, duration of diabetes mellitus, presence of the metabolic syndrome and use of hypoglycaemic, antihypertensive, lipid-lowering and antiplatelet medications[28]. Almost similar results were observed in smaller cohorts of adult

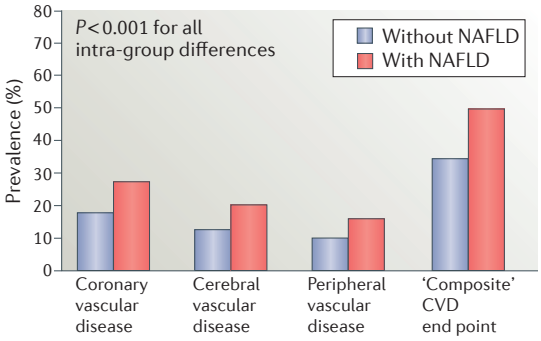

Figure 2 | **Prevalence of clinically manifest cardiovascular disease in patients with type 2 diabetes mellitus.** The prevalence of coronary (defined as myocardial infarction, angina pectoris or coronary revascularization procedures), cerebrovascular (defined as ischaemic stroke, recurrent transient ischaemic attacks, carotid endarterectomy or carotid stenosis >70% as diagnosed by echo-Doppler scanning) and peripheral (defined as intermittent claudication, rest pain as confirmed by echo-Doppler scanning, lower extremity amputations or peripheral revascularization procedures) conditions in 2,392 outpatients with type 2 diabetes mellitus with (red columns) and without (blue columns) nonalcoholic fatty liver disease (NAFLD) diagnosed using ultrasonography (adjusted for age and sex). CVD, cardiovascular disease. © 2007 by the American Diabetes Association® Diabetes Care 2007 May; 30(5): 1212–1218 with permission from the American Diabetes Association®.

patients with T1DM, where NAFLD (diagnosed using ultrasonography) was associated with an increased likelihood of prevalent CVD independent of age, sex, BMI, smoking status, duration of diabetes mellitus, HbA_{1c} levels, systolic blood pressure, plasma levels of lipids and use of antihypertensive, lipid-lowering and antiplatelet medications[60,61]. Moreover, in both patients with and without diabetes mellitus who were referred for clinically indicated coronary angiography, NAFLD was associated with a greater severity of coronary artery disease and with an increased prevalence of high-risk and vulnerable coronary artery plaques independent of the extent and severity of coronary atherosclerosis[70,72–74].

Currently, convincing evidence also indicates that NAFLD is strongly linked with subclinical myocardial remodelling and dysfunction (that is, left ventricular diastolic dysfunction and cardiac hypertrophy), valvular heart diseases (that is, aortic-valve sclerosis and mitral annulus calcification) and cardiac arrhythmias (mainly atrial fibrillation and corrected QT interval prolongation on standard electrocardiograms) in both patients with and without diabetes mellitus[75–84]. Preliminary evidence also suggests that NAFLD (diagnosed using ultrasonography), irrespective of pre-existing diabetes mellitus, is associated with an increased risk of 1-year all-cause and cardiac rehospitalizations in patients admitted with acute heart failure[85].

To date, a number of large hospital-based and population-based cohort studies have reported an increased incidence of fatal and nonfatal CVD events in patients with NAFLD diagnosed with either imaging or biopsy, independent of conventional CVD risk factors, in both patients with and without diabetes mellitus[1,4–6,58,69,70]. For instance, a prospective nested case–control study in 744 Italian outpatients with T2DM who did not have diagnosed CVD at baseline demonstrated that those with ultrasound-diagnosed NAFLD had a nearly twofold increased risk of developing nonfatal coronary heart disease, ischaemic stroke or cardiovascular death over a follow-up period of 5 years. Notably, this association was independent of age, sex, smoking status, diabetes mellitus duration and the presence of the metabolic syndrome as well as serum levels of HbA_{1c}, LDL cholesterol and liver enzymes and the use of hypoglycaemic, antihypertensive, lipid-lowering and antiplatelet medications[86]. Almost identical results were confirmed in a subsequent study with a larger sample size ($n = 2,103$) and a longer follow-up period (6.5 years)[87]. Similarly, in a cohort of 286 adult outpatients with T1DM, the presence of ultrasound-diagnosed NAFLD was associated with a nearly sixfold increased risk of nonfatal CVD events (that is, a combined end point inclusive of nonfatal ischaemic heart disease, ischaemic stroke and coronary or peripheral revascularization procedures) over a mean follow-up period of 5.3 years[88]. Notably, this association was independent of age, sex, BMI, smoking status, diabetes mellitus duration, HbA_{1c} levels, dyslipidaemia, hypertension, CKD, prior ischaemic heart disease and serum levels of γ-glutamyltransferase (GGT1)[88].

Published in 2016, an updated and large meta-analysis that incorporated almost 34,000 individuals with and without T2DM (36.3% with NAFLD) and approximately 2,600 fatal and nonfatal CVD outcomes (>70% of which were CVD deaths) in 16 separate observational prospective and retrospective cohort studies from different countries concluded that the presence of NAFLD (as detected either by imaging or by histology) was associated with a nearly 65% increase in the risk of incident fatal and nonfatal CVD events over a median follow-up period of 6.9 years (random-effect OR 1.64, 95% CI 1.3–2.1) and that this risk increased further with greater severity of NAFLD (random-effect OR 2.58, 95% CI 1.8–3.8)[57]. Sensitivity analyses did not alter these findings. In particular, limiting the analysis to high-quality studies ($n = 10$ studies; random-effect OR 1.54, 95% CI 1.1–2.1) and limiting the analysis to studies with full adjustment for covariates ($n = 5$ studies; random-effect OR 1.69, 95% CI 1.3–2.6) provided overall risk estimates consistent with those generated in the primary analysis[57].

The available data leave little doubt that NAFLD is consistently associated with an increased prevalence of CVD and other cardiac and arrhythmic complications across a wide range of populations of patients, including those with diabetes mellitus. However, whether NAFLD is an independent risk factor for CVD or simply a separate disorder that shares common aetiological factors is still debatable. Although further research is needed to definitively establish a causal association between NAFLD and increased risk of incident fatal and nonfatal CVD events, the current evidence from the published

studies (which have been performed among various ethnic populations with different lifestyle habits) supports the notion that a diagnosis of NAFLD identifies a subset of patients who are at an increased risk of CVD mortality and morbidity over time. In line with this assertion, the recent European clinical practice guidelines for the diagnosis and management of NAFLD have strongly recommended CVD risk assessment in all patients with NAFLD[1].

Microvascular complications

NAFLD is associated with an increased risk of microvascular complications of diabetes mellitus, especially with CKD. In particular, numerous observational studies have consistently shown that NAFLD is associated with an increased prevalence of CKD (defined as an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 with accompanying abnormal albuminuria or overt proteinuria) in both patients without diabetes mellitus and in those with T2DM or T1DM[89]. In the Valpolicella Heart Diabetes Study cohort, which included 2,103 Italian outpatients with T2DM without known chronic liver diseases or CVD at baseline who had available measurements on eGFR, albuminuria and retinopathy[90], it was reported that patients with NAFLD diagnosed using ultrasonography had a nearly twofold increased risk of prevalent CKD or advanced (proliferative or laser-treated) diabetic retinopathy independent of age, sex, BMI, waist circumference, smoking status, hypertension, diabetes mellitus duration, HbA_{1c} levels, plasma levels of lipids and medication use (FIG. 3). Conversely, in a subgroup of 5,963 adult participants (15.8% with established diabetes mellitus) of the National Health and Nutrition Examination Survey-III, the presence of NAFLD diagnosed using ultrasonography was not associated with any degree of retinopathy (detected via fundus photographs) in both individuals with and without known diabetes mellitus after adjusting for multiple covariates[91]. Some other studies in which NAFLD was diagnosed by either ultrasonography or histology

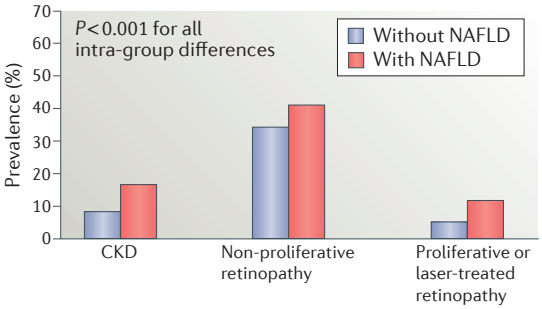

Figure 3 | **Prevalence of diabetic nephropathy and retinopathy in patients with type 2 diabetes mellitus.** The prevalence of chronic kidney disease (CKD; defined as estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 or overt proteinuria) and diabetic retinopathy in 2,103 outpatients with type 2 diabetes mellitus with (red columns) and without (blue columns) nonalcoholic fatty liver disease (NAFLD) diagnosed using ultrasonography (adjusted for age and sex)[90].

have shown that the presence and severity of NAFLD were strongly associated with an increased prevalence of abnormal albuminuria or decreased kidney function in patients with T2DM or prediabetes[89,92,93]. Similarly, some smaller cohorts of Italian adult outpatients with T1DM have shown that ultrasound-diagnosed NAFLD was associated with the presence of diabetic retinopathy or nephropathy, independent of age, sex, BMI, hypertension, diabetes mellitus duration, HbA_{1c} levels and use of antihypertensive and lipid-lowering medications[94,95].

To date, published data are lacking regarding the long-term risk of incident CKD (or other microvascular complications) in patients with coexistent NAFLD and diabetes mellitus. In a subset of 1,760 outpatients with T2DM in the Valpolicella Heart Diabetes Study who had normal kidney function at baseline (that is, after excluding the 343 patients with CKD or macroalbuminuria at baseline), the presence of ultrasound-diagnosed NAFLD was associated with an increased risk of incident CKD (defined as an eGFR <60 ml/min/1.73 m^2 or overt proteinuria) over a follow-up period of 6.5 years independent of age, sex, smoking status, BMI, waist circumference, diabetes mellitus duration, blood pressure, plasma levels of lipids, HbA_{1c} levels, baseline eGFR, microalbuminuria and use of hypoglycaemic, antihypertensive, lipid-lowering and antiplatelet medications (adjusted HR 1.49, 95% CI 1.1–2.2)[96]. Consistent with these findings, in a retrospective cohort study of 261 adult patients with T1DM who had normal kidney function at baseline and who were followed up for a mean period of 5.2 years, NAFLD (diagnosed by ultrasonography) was associated with an approximately threefold increased risk of incident CKD even after adjustment for age, sex, hypertension, diabetes mellitus duration, HbA_{1c} levels and baseline eGFR[97]. Notably, addition of NAFLD to conventional cardiorenal risk factors (that is, age, sex, hypertension, diabetes mellitus duration, HbA_{1c} levels, baseline eGFR and microalbuminuria) improved the discriminatory capability of the regression models to predict the risk of incident CKD[97].

A comprehensive meta-analysis of 33 observational, cross-sectional and prospective studies (including a total of nearly 64,000 individuals) confirmed that the presence and severity of NAFLD, as diagnosed by serum levels of liver enzymes, imaging or histology, were associated with a nearly twofold increase in the prevalence and incidence of CKD. In all these analyses, the statistically significant association between NAFLD and increased risk of CKD persisted after adjustment for pre-existing diabetes mellitus and other established cardiorenal risk factors, such as age, ethnicity, BMI, the metabolic syndrome and smoking status[98].

Finally, some studies also suggest that NAFLD is associated with an increased prevalence of distal symmetric polyneuropathy in patients with T1DM or T2DM[99,100]. Currently, however, not many published studies have evaluated the existence of such associations, and those that have show conflicting results[101,102].

Despite the growing evidence that links NAFLD with the long-term risk of CKD and other microvascular complications in patients with T2DM or T1DM,

a causal association remains to be definitively proved, and additional larger prospective studies in different ethnic populations are needed to establish whether the steatotic (or inflamed) liver actively contributes to the increased risk of microvascular complications observed among patients with diabetes mellitus and NAFLD, a hypothesis that is biologically plausible, as discussed in the next section.

Putative biological mechanisms

Several years ago, it was noted that T2DM and CVD share many risk factors (the 'common soil' hypothesis[103]) and that unlike classic microvascular complications, large-vessel atherosclerosis can precede the development of T2DM. The functionality of pancreatic β-cells, skeletal muscle, liver and adipose tissue is well recognized as important in the development of T2DM. An understanding of the importance of the function of other organs, such as the intestine, brain and kidneys, and of pancreatic α-cells in the development of chronic hyperglycaemia (the 'ominous octet') is also emerging[104]. Thus, rather than vascular disease being a complication of diabetes mellitus, both conditions might have common antecedents (that is, they spring from a 'common soil' (REF. 103)), and those common antecedents might involve the functioning of other key organs beyond the pancreas.

As discussed previously, several authors have shown that NAFLD might be a novel risk factor for CVD, CKD and T2DM[4], and when taken in conjunction with the 'common soil' hypothesis and the 'ominous octet' concept, it is now evident that NAFLD also shares many risk factors with diabetes mellitus and CVD. It is widely accepted that these shared cardiometabolic risk factors revolve around ectopic fat accumulation (abdominal obesity), insulin resistance and other features of the metabolic syndrome[105]. Consequently, when considering the underlying mechanisms by which NAFLD might contribute to the development of chronic vascular complications of diabetes mellitus, it is important to consider not only the influence of the steatotic (or inflamed) liver per se but also the influence of abdominal obesity and other shared cardiometabolic risk factors. In particular, there is crosstalk between the expanded and inflamed visceral adipose tissue and the liver, with the liver acting as both the target organ and the source of the systemic subclinical chronic inflammation and of the abnormalities in coagulation and fibrinolysis (as discussed below) that might promote an increased risk of developing chronic vascular complications of diabetes mellitus[2–4,69,70].

Considerable research interest has focused on the possible pathogenic role of perturbations in the normal intestinal microflora (termed dysbiosis) and abnormalities of normal intestinal function on risk factors for CVD. In discussing the biological mechanisms underpinning the relationship between NAFLD and chronic vascular complications of diabetes mellitus, we discuss the emerging evidence that suggests a link between dysbiosis, intestinal barrier dysfunction, mediators of the gut microbiota and CVD (or CKD)[106–110]. We also discuss potential haemostatic, prothrombotic and pro-inflammatory mediators as well as mechanisms that contribute to oxidative stress that might link NAFLD to chronic vascular complications of diabetes mellitus.

Consequences of dysbiosis

As the liver is the key metabolic organ exposed to high levels of intra-colonic fermentation products (via the portal vein), the changes in specific microbial products, secondary to altered gut microbial composition, and the changes in intestinal permeability and function can affect hepatic structure and function to further increase the risk of NAFLD. Dysbiosis has been described in patients with obesity or other features of the metabolic syndrome[111] and in those with established CVD[107–109], T2DM[112–115], NAFLD[116–118] or CKD[119]. Several potential pathways, factors and processes might link dysbiosis, or mediators of the gut microbiota, and NAFLD to CVD risk factors and vascular and renal diseases (FIG. 4).

Increased gut permeability and release of lipopolysaccharide into the circulation. Dysbiosis is frequently associated with increased production of endotoxins from Gram-negative bacteria, which can damage the intestinal barrier, affect nutrient harvesting and increase gut permeability, with the potential for lipopolysaccharide to enter the portal and systemic circulation and increase the risk of low-grade, chronic inflammation[120,121] (FIG. 5). Lipopolysaccharide causes disruption of the gut intracellular tight junctions, favouring the release of pro-inflammatory cytokines into the circulation and, consequently, into the liver[120–123]. As lipopolysaccharide production provides a direct inflammatory stimulus to the liver via the portal vein, we can speculate that this lipopolysaccharide-mediated inflammatory stimulus might increase the risk of intrahepatic inflammation and oxidative stress.

Altered SCFAs production, trimethyl-amine metabolism and uraemic toxins. Fermentation of dietary fibre in the intestine by anaerobic bacteria, such as *Lactobacilli* and *Bifidobacterium,* forms short-chain fatty acids (SCFAs)[124,125]. SCFAs include acetate, propionate and butyrate, which influence hepatic *de novo* lipogenesis and gluconeogenesis[109,111,118,125]. A meta-analysis has suggested that short-term probiotic treatments have a beneficial effect on insulin resistance in T2DM, which is thought to be mediated by increasing butyrate production[126]. The current list of bacterially derived bioactive molecules that have the potential to adversely influence the vasculature includes trimethylamine (TMA) and/or trimethylamine oxide (TMAO), secondary bile acids, lipopolysaccharide and catecholamines[106–109]. Dysbiosis might also be associated with an increase in uraemic toxins, such as *p*-cresyl sulfate, 4-ethyl phenyl sulfate, hippuric acid, indoxyl sulfate and indole-3 acetic acid[127]. An increase in uraemic toxins is associated with hypertension, and such an effect could mediate another link between dysbiosis and vascular diseases[56].

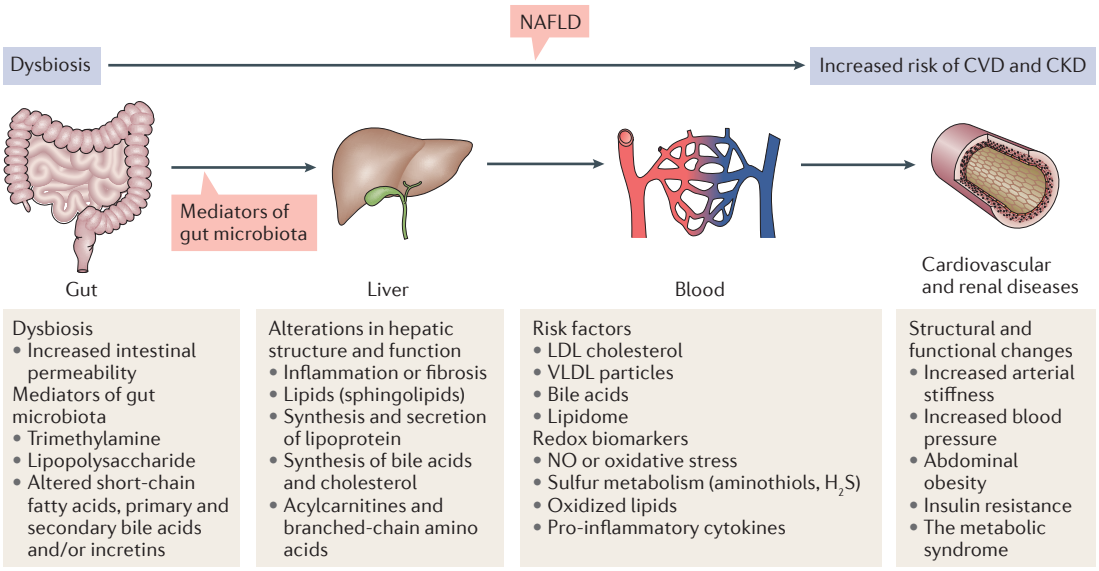

Figure 4 | **Potential pathways, factors and processes that link dysbiosis, mediators of the gut microbiota and alterations in hepatic structure and function to cardiovascular risk factors and vascular and renal diseases.** Dysbiosis (altered gut microbiota) is associated with increased intestinal permeability and intestinal dysfunction. These dysbiosis factors increase the risk of nonalcoholic fatty liver disease (NAFLD) via alterations in several pathways, factors and molecules that modify liver structure and function in NAFLD. These liver-specific changes are associated with an increase in risk factors for vascular disease and the subsequent development over time of vascular and renal diseases via increased insulin resistance, the metabolic syndrome and structural and functional changes affecting both the vasculature and the kidneys. CKD, chronic kidney disease; CVD, cardiovascular disease; NO, nitric oxide.

TMAO is produced in the liver from the oxidation of TMA that is produced as a direct consequence of bacterially dependent metabolism of dietary choline. Increased TMA and/or TMAO levels cause atherogenic lesions in mice and are associated with atherosclerosis in humans[109]. Furthermore, TMAO might exert a marked adverse effect on the vasculature, increasing carotid-artery intimal–medial thickness[128] to promote CVD[107]. Experimentally, it has been shown that TMAO might impair reverse cholesterol transport, induce platelet aggregation, promote foam cell formation and increase expression of the scavenger receptors type A1 and CD36 (REF. 109). Thus, it is plausible to assume that alterations in the levels of SCFAs (via increases in hepatic *de novo* lipogenesis and gluconeogenesis), increases in systemic lipopolysaccharide (via increased intestinal permeability and energy harvesting) and perturbed bile acid metabolism (via decreased hepatic farnesoid X receptor (FXR; also known as bile acid receptor) activity and increased hepatic glucose production; see below) could mediate an increase in the risk of developing T2DM (FIG. 5). Alternatively, in patients with established T2DM, the aforementioned changes could worsen hyperglycaemia and make it more difficult to achieve good glycaemic control.

Altered bile acid metabolism. Primary bile acids, such as cholic acid and chenodeoxycholic acid, are produced by the liver. Bile acids interact with plasma membrane G protein-coupled receptors (such as G protein-coupled bile acid receptor 1 (GPBAR1)), muscarinic receptors and nuclear receptors, such as FXR and pregnane X receptor (PXR; also known as NR1I2)[129]. Bile acid receptors are expressed on cardiovascular tissue cells, such as endothelial cells, vascular smooth cells and cardiomyocytes[129]. Chenodeoxycholic acid is a naturally occurring ligand for FXR[130], and activation of FXR with modified chenodeoxycholic acid compounds, such as obeticholic acid, has marked effects on not only bile acid metabolism but also liver disease; these effects decrease hepatic steatosis and necroinflammation in patients with biopsy-proven, non-cirrhotic NASH. Obeticholic acid treatment also affects cholesterol metabolism, and the levels of LDL cholesterol are markedly increased in treated patients[131]. In addition to regulating bile acid metabolism, FXR activation powerfully influences levels of hepatic glucose production and hepatic glycogen synthesis and storage and also regulates hepatic inflammation[130]. The bile acids are influenced by gut microbiota to produce secondary bile acids, such as deoxycholic acid, ursodeoxycholic acid and lithocholic acid. Different bacteria can have differential effects in producing secondary bile acids, for instance, 7 alpha-dehydroxylating bacteria are capable of generating deoxycholic or ursodeoxycholic acids from precursor bile acids[132,133]. Secondary bile acids are highly hydrophobic and toxic, and increased concentrations of these bile acids in the liver have been linked to inflammation[132] and NAFLD[133].

Evidence also suggests that alteration of bile acid metabolism by the intestinal microbiota influences the risk of CVD by affecting metabolism of LDL cholesterol, vasomotor tone and blood pressure[129,134]. Furthermore, treatment with *Bifidobacterium* might influence cholesterol metabolism by decreasing serum concentrations of

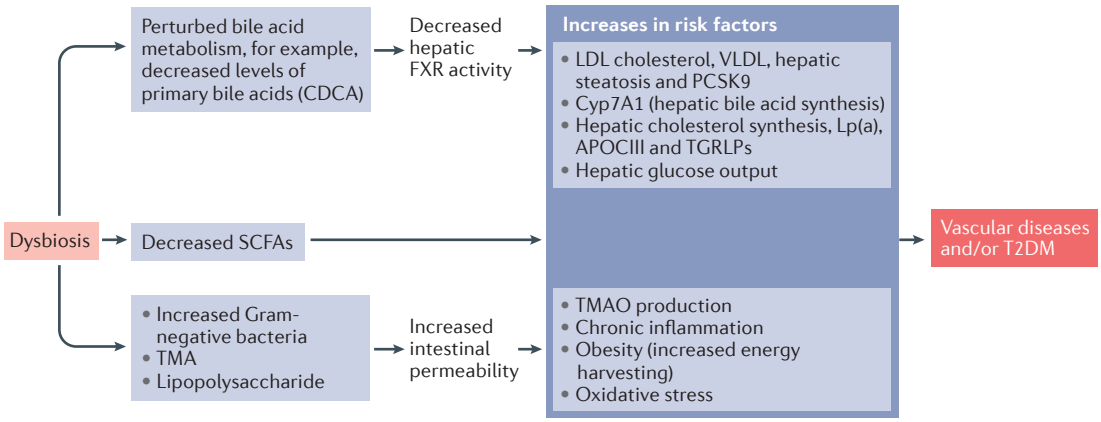

Figure 5 | **Potential pathways linking dysbiosis to cardiovascular disease, type 2 diabetes mellitus and chronic kidney disease.** Intestinal dysbiosis perturbs bile acid metabolism, which affects the levels of bile acids (such as chenodeoxycholic acid (CDCA)), short-chain fatty acids (SCFAs), trimethylamine (TMA) and lipopolysaccharide. Via decreased activity of hepatic nuclear receptor farnesoid X receptor (FXR) in nonalcoholic fatty liver disease (NAFLD) and increased intestinal permeability, a further increase in cardiorenal risk factors occurs with subsequent development of type 2 diabetes mellitus (T2DM) and vascular and renal diseases. APOCIII, apolipoprotein CIII; CYP7A1, cytochrome P450 7A1; Lp(a), lipoprotein (a); PCSK9, proprotein convertase subtilisin/kexin type 9; TGRLPs, triglyceride-rich lipoproteins; TMAO, trimethylamine oxide.

total cholesterol and LDL cholesterol[135]. Treatment with chenodeoxycholic acid also decreases serum levels of the LDL-receptor modulator proprotein convertase subtilisin/kexin type 9 (REF. 136), providing another potentially important mechanism by which bile acids might modify cholesterol metabolism.

Liver-specific pathways

The pathogenesis of NAFLD and its progression to fibrosis are very complex processes that implicate cell interactions between parenchymal (hepatocytes) and nonparenchymal liver cells (Kupffer cells, stellate cells and liver-associated lymphocytes) as well as crosstalk between various immune cell populations in the liver. Among the factors that are potentially implicated in these complex pathophysiological processes, the presence of some environmental factors, such as expanded and inflamed visceral adipose tissue and peripheral insulin resistance, and some genetic factors, such as some specific polymorphisms of patatin-like phospholipase domain-containing 3 (*PNPLA3*) or the transmembrane 6 superfamily member 2 (*TM6SF2*), has an increasingly important pathogenic role[1–4,11]. The development and, more importantly, progression of NAFLD to NASH and advanced fibrosis also result in an increase in a variety of systemic factors (for example, pro-inflammatory and profibrogenic molecules, such as C-reactive protein, tumour necrosis factor, IL-6, endothelin 1, angiotensinogen and transforming growth factor-β) that might increase the risk of developing CVD and CKD[2–5,11,47,56,58]. Other liver-specific pathways that affect atherogenic dyslipidaemia, insulin resistance (for example, α2-HS-glycoprotein (AHSG; also known as fetuin A), fibroblast growth factor 21 (FGF21) and other pro-diabetogenic hepatokines), haemostatic–fibrinolytic factors (for example fibrinogen, coagulation factor VIII, tissue factor and plasminogen activator inhibitor 1 (PAI1)) and increased oxidative stress might also link NAFLD to an increased risk of incident CVD and CKD[2–5,11,47,56,58] (FIG. 6).

Intrahepatic fat accumulation. The development of NAFLD is associated with the intrahepatic accumulation of ceramides and diacylglycerols[4] as well as the secretion of multiple hepatic-derived molecules, such as AHSG, FGF21 and other pro-diabetogenic hepatokines that are able to inhibit the tyrosine kinase activity of the insulin receptor to promote hepatic and peripheral insulin resistance[137] (FIG. 6). Indeed, intrahepatic accumulation of lipid intermediates, such as ceramides and diacylglycerols, inhibits insulin signalling. For example, diacylglycerols activate membrane translocation of protein kinase C ε-type, inhibit the insulin receptor and decrease insulin signalling, and increased ceramide levels in the liver result in activation of inflammatory Toll-like receptor 4 signalling pathways, with consequent impairment of insulin signalling pathways via inhibition of RACα serine/threonine-protein kinase (AKT1) phosphorylation[138,139].

Insulin resistance. The development of hepatic insulin resistance in NAFLD is associated with the features of the metabolic syndrome, such as increased blood pressure, and also with the development of an atherogenic lipoprotein profile[1–4,104,105]. In NASH, key components of the renin–angiotensin–aldosterone system (RAS) are also increased, and RAS activity has a key role in linking NAFLD to vascular disease in CKD[4,5,56,89]. Adipocytes express all components of the RAS, contributing up to 30% of circulating renin, angiotensin-converting enzyme and the vasoconstrictor angiotensin II; however, the liver also expresses RAS constituents, and experimental studies support a role for both systemic and local activation of angiotensin II in NAFLD[140].

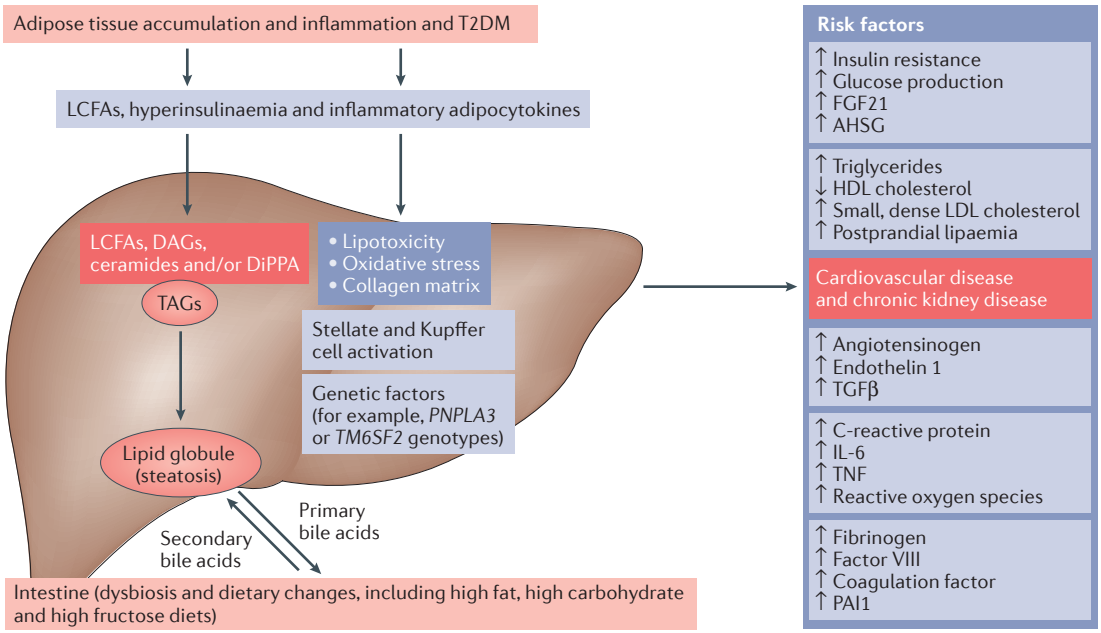

Figure 6 | **Liver-specific pathways linking nonalcoholic fatty liver disease to cardiovascular and chronic kidney disease.** A variety of factors, such as visceral adipose tissue accumulation, low-grade chronic inflammation, type 2 diabetes mellitus (T2DM), dysbiosis and dietary factors, can affect liver-specific pathways to induce (or amplify) lipotoxicity, insulin resistance, oxidative stress and chronic inflammation. Consequent Kupffer cell and stellate cell activation might occur locally as the disease process progresses, with further inflammation, collagen deposition and fibrosis in the liver. All these intrahepatic processes might exacerbate hepatic and systemic insulin resistance, promote the development of atherogenic dyslipidaemia and hypertension and induce the systemic release of multiple pro-inflammatory molecules (such as C-reactive protein, IL-6 and tumour necrosis factor (TNF)), haemostatic–fibrinolytic factors (for example, fibrinogen, coagulation factor VIII and plasminogen activator inhibitor 1 (PAI1)), prooxidant (for instance, reactive oxygen species) and profibrogenic (for example, transforming growth factor-β (TGFβ) and angiotensinogen) mediators that have important roles in the development of both cardiovascular disease and chronic kidney disease. AHSG, α2-HS-glycoprotein; DAGs, diacylglycerols; diPPA, dipalmitoyl phosphatidic acid; FGF21, fibroblast growth factor 21; LCFAs, long chain fatty acids; TAGs, triacyl glycerols.

Atherogenic dyslipidaemia. The specific atherogenic lipoprotein profile that is typically associated with the metabolic syndrome and insulin resistance increases the levels of triglyceride-rich lipoproteins in the circulation[104,140]. The increase in levels of triglyceride-rich lipoproteins in the circulation is also associated with an increase in the activity of cholesteryl ester transfer protein (CETP) [104,140–142]. CETP resides on the surface of the HDL particles and mediates the reciprocal exchange of triglyceride and cholesterol esters between triglyceride-rich lipoproteins and both the HDL and LDL particles. With an increase in triglyceride content of HDL and LDL particles mediated by CETP, both lipoprotein particles are cleared from the circulation, resulting in increased plasma concentrations of small, dense HDL and small, dense LDL particles. Small, dense HDL particles are less efficient than normal HDL lipoproteins in facilitating reverse cholesterol transfer, a process whereby excess cholesterol is cleared from peripheral tissues (including the vasculature) to the liver, and small, dense LDL particles are more atherogenic than normal LDL particles[140–142].

Pro-inflammatory cytokines. In individuals with the metabolic syndrome, insulin resistance and progressive forms of NAFLD (that is, NASH and advanced fibrosis), an upregulation of multiple pro-inflammatory pathways is almost invariably observed. These pro-inflammatory mechanisms influence two main intracellular transcription factor signalling pathways — the nuclear factor-kB (NF-kB) pathway and the mitogen-activated protein kinase (MAPK) pathway[141–143]. Experimental animal data suggest that MAPK8 activation in the adipose tissue causes insulin resistance in the liver[144]. In addition, activation of the NF-kB pathway in NASH might be pivotal in further amplifying the systemic inflammatory response, as the NF-kB pathway mediates an increase in transcription of several different pro-inflammatory genes[145].

Adiponectin, endoplasmic reticulum stress and oxidative stress. Adiponectin is a key adipokine that can affect disease progression in NAFLD, as this adipokine regulates hepatic fat accumulation, necroinflammation and fibrosis[145,146]. In fact, adiponectin exerts anti-inflammatory, anti-fibrotic and anti-atherogenic properties, and low levels of adiponectin are associated with insulin resistance and NASH[146]. Low levels of adiponectin might not only influence the progression of liver disease in NAFLD but also increase the risk of CVD and T2DM (possibly via an effect on NAFLD progression)[146]. Thus, one can speculate that a vicious circle

exists with respect to adiponectin production, NAFLD and T2DM. Low levels of adiponectin might occur with abdominal obesity and insulin resistance, and low levels of adiponectin might promote NAFLD progression to NASH. Development of NASH might further increase the risk of CVD and T2DM.

Hepatic lipids that are not esterified are also able to induce endoplasmic reticulum stress, leading to activation of the MAPK and NF-κB signalling pathways[147]. Hepatic lipids might also induce mitochondrial dysfunction with the generation of free radicals via increased oxidation of excess fatty acids, which causes oxidative stress[148]. Mitochondrial dysfunction is associated with insulin resistance and atherosclerosis in several studies[149], which suggests a plausible mechanistic link between mitochondrial dysfunction, T2DM, NAFLD and CVD (and CKD).

Alterations of haemostatic–fibrinolytic factors. Finally, it is well established that the liver is key to the production of multiple coagulation factors and is also an important site of production of PAI1 and other fibrinolytic proteins[150]. Several case–control studies have shown that the levels of multiple procoagulant factors (for example, fibrinogen, coagulation factor VIII, tissue factor, PAI1 and other haemostatic–fibrinolytic factors) are highest in patients with NASH, intermediate in those with simple steatosis and lowest in control participants without steatosis[151,152], supporting a dose–response relationship between the severity of NAFLD and prothrombotic risk. Studies have also shown that NASH is associated with abnormal intrahepatic expression of most of the aforementioned pro-inflammatory and procoagulant biomarkers[153,154], thus further supporting the notion that the increased circulating levels of these biomarkers result from the upregulation of their synthesis in the steatotic, inflamed or fibrotic liver (FIG. 6).

In summary, evidence clearly suggests that NAFLD increases the risk of chronic vascular complications of diabetes mellitus via a variety of different pathogenic mechanisms. These biological pathways include dysbiosis and perturbed intestinal function, intrahepatic fat accumulation, insulin resistance, atherogenic dyslipidaemia, pro-inflammatory cytokines, increased oxidative stress and alterations of haemostatic–fibrinolytic factors. Despite the biological plausibility of dysbiosis and intestinal dysfunction being a novel mediator that increases the risk of both NAFLD and vascular disease, at present, it remains uncertain whether treatment of dysbiosis favourably modifies the levels of potentially damaging molecules and the pathways leading to NAFLD and chronic vascular and renal damage. Although further research is urgently needed in this area, correction of dysbiosis might be a novel therapeutic target to ameliorate the risk of NAFLD and CVD (and CKD)[108,126,155].

Management of NAFLD in diabetes mellitus

The therapeutic approach to patients with coexistent NAFLD and diabetes mellitus should be multifactorial. Currently, the mainstay of NAFLD management in these patients is to reduce body weight, improve glycaemic control and reduce the modifiable cardiometabolic risk factors (possibly by use of drugs that might have beneficial effects on the liver)[1,2,45,47,156,157].

Lifestyle modification

Lifestyle changes must be suggested to all patients with coexistent NAFLD and diabetes mellitus even though they are difficult to achieve and maintain[1,2,45,47,156,157]. Moreover, a strict control of all coexisting cardiometabolic risk factors (diabetes mellitus, abdominal obesity, atherogenic dyslipidaemia and hypertension) should be pursued, and the higher the risk of progressive liver disease, the more aggressive the treatment strategy should be. However, to date, whether patients with coexistent NAFLD and diabetes mellitus should be treated to specific targets for levels of HbA_{1c}, LDL cholesterol and blood pressure remains uncertain.

Body weight reduction. The importance of losing body weight in patients with T2DM cannot be overemphasized, and in those with NASH, a weight reduction of approximately 5–7% is able to decrease hepatic steatosis; however, an approximate 10% weight reduction is needed to reverse NASH, and a weight loss of ≥10% can also improve or reverse hepatic fibrosis[1,2,45,47,56,156,157]. Given the difficulties in achieving and maintaining this end point through lifestyle modifications, bariatric surgery, which markedly improves all histological lesions of NASH, including hepatic fibrosis, could be suggested to properly selected patients with severe obesity (that is, those with BMI ≥35 kg/m^2 and at least one or more obesity-related comorbidity, such as NASH, T2DM, CVD, sleep apnoea or other respiratory disorders)[1,2,45,56,158,159]. However, while bariatric surgery is undoubtedly effective, there are obvious limitations, including possible complications, patient compliance, service availability and cost.

Diet and smoking. Qualitative and quantitative dietary changes are advisable for all patients with NAFLD. About 1,200–1,600 kcal per day are recommended (depending on the individual patient characteristics, such as age, level of physical activity and presence of comorbidities)[1,2,45,47,156,157]. A low-fat (<30%) diet with less than 10% of calories from saturated fatty acids and fairly low in carbohydrates (<50% of total calories) is suggested[1,2,156,157]. Preference should be given to consuming complex carbohydrates, and patients should avoid simple carbohydrates, which have a higher glycaemic index than complex carbohydrates[160]. Patients with NAFLD should also avoid the lipogenic sugar fructose[160].

The Mediterranean diet seems to be the most useful non-pharmacological option aimed at losing body weight while gaining some beneficial effects on cardiometabolic outcomes[1,2,161]. A high dietary intake of fish and vegetables (but not fruits) has also been associated with protection from developing HCC in NAFLD[47,162]. Conversely, cigarette smoking and even modest amounts of alcohol consumption might increase the risk of HCC in NAFLD[162–164]. Therefore, all patients with NAFLD or NASH should be advised to quit cigarette smoking and avoid any alcohol consumption[1,2,45,47,56,156,157].

Physical exercise. Both aerobic training and resistance training might reduce hepatic steatosis, independent of weight loss[1,2,45,47,56,156,165]. Physical exercise should be individualized on the basis of the patient's attitude and convenience and, ideally, should be maintained indefinitely[165–167]. As patients with NAFLD are often at a high risk of developing CVD[168], a careful cardiac evaluation should be implemented in these patients before submitting them to any vigorous physical efforts.

Pharmacological treatment

A detailed review of drug treatment options for NAFLD and NASH in patients with diabetes mellitus is beyond the scope of this article. Comprehensive review articles of this topic have been published elsewhere[1,2,45,56,156,169].

Currently, there are no licensed pharmacological agents specifically for the treatment of NAFLD. The major issue in this field is the scarcity of high-quality, randomized, blinded, adequately powered controlled trials of sufficient duration and with clinically relevant end points. In line with this consideration, a recent Cochrane review[170] concluded "we are very uncertain about the effectiveness of pharmacological treatments for people with NAFLD, including those with NASH." Some concerns also remain about the long-term safety of the available drugs, necessitating thoughtful balancing of the potential risks and benefits. Therefore, to date, drug treatment is best targeted to patients with NASH, who are at the highest risk of progressive liver disease, or to those patients who have poorly controlled diabetes mellitus[1,2,45,47,56,156,169].

Some drug treatments for NASH might exert (some) beneficial histological effects on hepatic steatosis and necroinflammation and, sometimes, also on hepatic fibrosis in adult patients with T2DM or prediabetes who have biopsy-proven, non-cirrhotic NASH (TABLE 1)[131,171–182]. A central dogma is that any diabetes mellitus treatment might benefit patients with NASH if they have uncontrolled hyperglycaemia[1,2,45,47,56]. In addition to glucose-lowering agents, several classes of drugs might also be considered, such as lipid-lowering agents (for example, statins and ezetimibe), antioxidants (for example, vitamin E) and some more innovative and promising drugs with anti-inflammatory and antifibrotic effects (for example, elafibranor, obeticholic acid and cenicriviroc)[1,2,45,56,156,169]. However, to date, there are no large, well-designed randomized controlled trials examining the effects on liver histology of many of these drugs (and, in many cases, their long-term safety remains to be established). A variety of new drugs are also likely to emerge over the next 5 years, permitting a more stage-based approach to NAFLD management and greater personalization of drug selection. Tailoring pharmacotherapy to the dominant pathogenic pathway in a given patient along with use of combination therapy is likely to represent the future direction in the treatment of patients with NASH (irrespective of the presence of T2DM)[183].

In choosing among the various available drug classes for the treatment of patients with coexistent NAFLD and T2DM, we believe that priority should be given to drugs with actions that are not limited to an individual therapeutic target but that also reduce the risk of CVD events and severe liver-related complications, such as cirrhosis and HCC[168]. Statins are an example of such a pleiotropic class of drugs[184]. For instance, a cross-sectional survey conducted in nearly 350 patients with T2DM and histologically proven NAFLD has shown that the use of statins is inversely associated with the presence and severity of NASH and fibrosis on liver histology[185]. In the same study, the use of insulin or sulfonylureas seemed to be positively associated with the presence and severity of NASH and fibrosis[185].

As shown in TABLE 1, most of the available evidence of efficacy in patients with NASH and T2DM (or prediabetes) relates to the use of pioglitazone, which might improve the natural history of liver disease by reducing its progression to cirrhosis in some patients with biopsy-proven NASH[1,2,45,47,56,156,169,183]. However, long-term safety concerns have limited its use. Metformin is the first-line oral hypoglycaemic agent for T2DM, but it is not currently recommended as a specific treatment for liver disease (mostly owing to its lack of efficacy on hepatic histological end points) in patients with T2DM and NAFLD or NASH[1,2,45,47,56,156,169,183]. Similarly, no robust data exist with histological end points as a primary outcome to formally comment on the effectiveness of the use of the newer glucose-lowering agents (for instance, dipeptidyl peptidase 4 inhibitors, glucagon-like peptide 1 receptor agonists or sodium/glucose cotransporter 2 inhibitors) as treatments for NAFLD or NASH with coexistent T2DM[2,45,47,56,156,169,183]. As also shown in TABLE 1, a phase II multicentre, double-blinded, randomized controlled trial (involving 52 patients with obesity and biopsy-proven NASH with or without T2DM) using liraglutide at 1.8 mg per day subcutaneously has shown promising results in the improvement of hepatic steatosis, necroinflammation and fibrosis after 48 weeks of treatment[177]. However, these findings warrant further evaluation in larger, randomized controlled phase III trials.

On these grounds, an expert panel suggested that, pending forthcoming randomized controlled trials, physicians should consider using pioglitazone or statins alone or preferably in combination with each other or with ezetimibe in those patients with NAFLD or NASH who are at high risk of CVD or HCC (unless contraindicated) for the primary or secondary prevention of CVD and the avoidance of cirrhosis, liver transplantation or HCC, bearing in mind that CVD is the main cause of mortality in patients with NAFLD[186].

In 2016, the UK National Institute for Care and Clinical Excellence (NICE) recommended that in secondary or tertiary care settings only, clinicians should consider treatment with pioglitazone or vitamin E for adults with advanced liver fibrosis (whether they have diabetes mellitus or not). Before prescribing pioglitazone or vitamin E, it was recommended that clinicians take into account any comorbidities and the risk of adverse effects associated with these conditions[187]. However, it is important to note that for all treatments that have been advocated for NAFLD, not all patients respond to treatment. Given that all available treatments have potentially harmful adverse effects, until there are accepted strategies for monitoring responses to therapy, it is difficult to advocate that a treatment be started if there is no regular monitoring of treatment effectiveness.

Table 1 | **Effects of different drug treatments for NASH on liver histology**

Study	Active treatment (sample size); % of patients with established T2DM or prediabetes	Duration of treatment	Main effects on liver histology
Bugianesi *et al.*, 2005 (REF. 171)	MET 2 g per day ($n = 55$) versus vitamin E 800 IU per day ($n = 28$) versus diet modification ($n = 27$); 9% with T2DM	12 months	Vitamin E and diet modification did not produce any beneficial histological effects. MET improved hepatic steatosis, necroinflammation and fibrosis
Belfort *et al.*, 2006 (REF. 172)	PIO 45 mg per day ($n = 29$) versus counselling ($n = 25$); 100% with T2DM or prediabetes	6 months	PIO significantly improved hepatic steatosis, necroinflammation, ballooning and fibrosis versus counselling
Ratziu *et al.*, 2008 (FLIRT trial)[173]	RSG 8 mg per day ($n = 32$) versus PL ($n = 31$); 25% with T2DM	12 months	RSG significantly improved hepatic steatosis without any changes in necroinflammation and fibrosis
Haukeland *et al.*, 2009 (REF. 174)	MET 2.5–3.0 g per day ($n = 24$) versus PL ($n = 24$); 100% with T2DM or prediabetes	6 months	No significant differences in hepatic steatosis, necroinflammation or fibrosis were observed between the MET and PL groups
Ratziu *et al.*, 2010 (FLIRT-2 trial)[175]	RSG 8 mg per day (RSG–RSG, $n = 25$; PL–RSG, $n = 28$). Open-label extension of the FLIRT trial; 25% with T2DM	24 months	RSG administered beyond 1 year did not yield any additional improvement in liver histology
Neuschwander-Tetri *et al.*, 2014 (FLINT trial)[131]	OCA 25 mg per day ($n = 141$) versus PL ($n = 142$); 52% with T2DM	72 weeks	The study was interrupted for superiority, 45% of the OCA group versus 21% of the PL group had significantly improved hepatic steatosis, lobular inflammation, ballooning and fibrosis. A marginally greater resolution of NASH was observed after OCA treatment (22% versus 13%)
Argo *et al.*, 2015 (REF. 176)	N-3 PUFA 3 g per day ($n = 17$) or PL ($n = 17$); 32% with T2DM	12 months	N-3 PUFA did not lead to improvement in the primary outcome of histological activity in patients with NASH (≥2 points NAS reduction). N-3 PUFA led to reduced liver fat by multiple measures
Armstrong *et al.*, 2016 (LEAN trial)[177]	LIRA 1.8 mg per day ($n = 26$) versus PL ($n = 26$); 33% with T2DM	48 weeks (extended to 72 weeks)	LIRA improved hepatic steatosis, ballooning and fibrosis. NASH resolution was significantly greater after LIRA (39% in the LIRA group versus 9% in the PL group)
Ratziu *et al.*, 2016 (GOLDEN-505)[178]	ELA 80 mg per day ($n = 93$) versus ELA 120 mg per day ($n = 91$) versus PL ($n = 92$); 40% with T2DM	52 weeks	NASH resolved without worsening fibrosis in more patients in the ELA 120 mg group versus the PL group (19% versus 12%). In post hoc analyses of patients with an NAS ≥4 ($n = 234$), ELA 120 mg significantly resolved NASH compared with PL (20% versus 11%). Patients with NASH resolution after receiving ELA 120 mg had reduced hepatic fibrosis compared with those without NASH resolution
Cusi *et al.*, 2016 (REF. 179)	A total of 101 patients with T2DM or prediabetes with biopsy-proven NASH were randomized to receive either PIO (45 mg per day) or PL in combination with a low-calorie diet	18 months, followed by an 18-month open-label extension with PIO	Among patients randomly assigned to PIO, 58% achieved the primary histological outcome and 51% had NASH resolution. PIO treatment was also associated with reduced intrahepatic fat content and improved adipose tissue, hepatic and muscle insulin sensitivity. All 18-month metabolic and histological improvements persisted over 36 months of therapy
Joy *et al.*, 2017 (REF. 180)	SITA 100 mg per day ($n = 6$) or PL ($n = 6$); 100% with T2DM	24 weeks	SITA was not significantly better than PL at reducing hepatic fibrosis score or NAS and its individual histological components
Bril *et al.*, 2017 (REF. 181)	Post hoc analysis of statin use in a randomized trial assessing PIO versus PL in 101 patients (86 on statins) with T2DM or prediabetes and biopsy-proven NASH	Up to 36 months	No significant changes in liver histology or hepatic insulin resistance were observed in patients who newly started receiving statins or PL during the trial
Friedman *et al.*, 2017 (CENTAUR trial)[182]	CVC 150 mg per day ($n = 145$) versus PL ($n = 144$); 50% with T2DM	12 months	The primary end point of NAS improvement and resolution of NASH was achieved in a similar proportion of patients on CVC and PL. However, the fibrosis end point (a secondary end point of the trial) was met in significantly more patients on CVC than PL (20% versus 10%)

All results were observed in randomized controlled trials that included adult patients with nonalcoholic steatohepatitis (NASH) and type 2 diabetes mellitus (T2DM) or prediabetes. CVC, cenicriviroc; ELA, elafibranor; IU, international units; LIRA, liraglutide; MET, metformin; NAS, nonalcoholic fatty liver disease activity score; N-3 PUFA, N-3 polyunsaturated fatty acids; OCA, obeticholic acid; PIO, pioglitazone; PL, placebo; RSG, rosiglitazone; SITA, sitagliptin.

Conclusions

Existing guidelines do not advocate screening for liver-related complications in patients with T2DM or T1DM, making the liver a potentially neglected organ and meaning that chronic disease progression to cirrhosis might be largely undetected. However, given the increasingly growing prevalence and incidence of NAFLD in patients with diabetes mellitus and its related hepatic and extra-hepatic complications, NAFLD should always be ruled out in adult patients with T2DM or T1DM.

This Review outlines the strong association between the presence and severity of NAFLD and the risk of chronic vascular complications of diabetes mellitus, mainly, CVD, cardiomyopathy (left ventricular dysfunction and hypertrophy) and CKD. Despite the growing evidence that links NAFLD with CVD, CKD and other microvascular complications of diabetes mellitus, it remains to be definitively established whether a causal association also exists.

In the meantime, however, these findings call for a more active and systematic search for NAFLD in adult patients with T2DM or T1DM with a view to potential earlier treatment. We strongly believe that the possibility of NAFLD should be considered as a part of the routine evaluation of adult patients with T2DM or T1DM, in the same way we search for CVD, CKD and other chronic complications of diabetes mellitus.

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G.T. and C.D.B. declare no competing interests. A.L. is a researcher of a phase III, double-blind, randomized, placebo-controlled, multicentre study evaluating the safety and efficacy of obeticholic acid in patients with nonalcoholic steatohepatitis (EudraCT 2015-002560-16).

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