Nonalcoholic Fatty Liver Disease and Chronic Vascular Complications of Diabetes

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List of abbreviations used

BAs = bile acids
BMI = body mass index
CAP = controlled attenuation parameter
CETP = cholesterol ester transfer protein
CKD = chronic kidney disease
CVD = cardiovascular disease
DAG = di-acyl glycerols
DCA = deoxycholic acid
eGFR = estimated glomerular filtration rate
FIB-4 = fibrosis-4
FXR = farnesoid X receptor
HCC = hepatocellular carcinoma
JNK = C-Jun-N-terminal kinase
LCA = lithocholic acid
LPS = lipopolysaccharide
MetS = metabolic syndrome
NAFLD = nonalcoholic fatty liver disease
NASH = nonalcoholic steatohepatitis
NFS = NAFLD fibrosis score
NF-kB = nuclear factor-kB
PAI-1 = plasminogen activator inhibitor-1
PKC = protein kinase C
RAS = renin-angiotensin-aldosterone system
T1DM = type 1 diabetes mellitus
T2DM = type 2 diabetes mellitus
TGRLP = triglyceride-rich lipoproteins
UDCA = ursodeoxycholic acid
US-FLI = ultrasonographic fatty liver index

ABSTRACT

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

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) describes, in the absence of competing etiologies of liver injury, a variable combination of individual histological elementary findings, namely accumulation of fat in more than 5% of hepatocytes, often with minor degrees of low-grade sterile inflammation (simple steatosis); steatosis *plus* ballooning degeneration (nonalcoholic steatohepatitis [NASH]), advanced fibrosis and "cryptogenic" cirrhosis[1,2].

NAFLD has become among 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 much greater in patients with type 2 diabetes mellitus (T2DM), who are also more likely to develop the more severe histological forms of NAFLD [*i.e.,* NASH, advanced fibrosis, cirrhosis and 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, liver failure, HCC and liver transplantation) but also embraces major extra-hepatic conditions[4-6]. Indeed, the leading causes of mortality among patients with NAFLD are cardiovascular disease (CVD), followed by non-liver malignancy and liver disease[1-3]. Moreover, as it will be discussed in greater detail below, it has also become increasingly clear that the presence and severity of NAFLD is strongly associated with an increased risk of developing serious extra-hepatic diseases, such as, further to CVD, cardiomyopathy and cardiac arrhythmias as well as chronic kidney disease (CKD), which ranks among the most important chronic complications of diabetes.

The present review focuses on the adverse impact of NAFLD on the risk of chronic vascular complications of diabetes (principally CVD, CKD but also other microvascular diabetic complications). In addition, the putative pathophysiological mechanisms by which NAFLD may contribute to the development and progression of chronic vascular complications of diabetes are also discussed. Finally, the principles of NAFLD treatment are also critically evaluated.

2. DIAGNOSIS AND EPIDEMIOLOGY OF NAFLD

2.1 Diagnostic methods

NAFLD remains a diagnosis *"of exclusion"*, which suffers from the contradiction of missing a positive diagnostic criterion useful to define it[7,8]. This implies that in operational terms[1,9,10] and irrespective of the background population, the clinicians will have: a) to identify excess hepatic fat content by using various imaging technologies or, in some cases, by liver biopsy, which remains the "reference standard" for diagnosing NASH and staging the severity of necroinflammation and fibrosis in patients with NAFLD; b) to exclude alcoholic, viral, pharmacological, autoimmune and inherited-genetic etiologies of steatotic liver disease; c) to ascertain the coexistence of the typical features of metabolic syndrome (MetS)[11], and d) to assess the severity of hepatic fibrosis, which is the strongest predictor for disease-specific mortality in NAFLD[1-3,6]. Clearly, point c) may be superfluous when dealing with people with diabetes.

2.2 Possible screening strategies

Screening for NAFLD among patients with established diabetes should be a multistep process.

The presence of fat vesicles in at least 5% of hepatocytes defines steatosis histologically[1,2]. However, since liver biopsy is an invasive procedure that cannot be proposed for all patients with suspected NAFLD, both non-invasive biomarkers of hepatic steatosis and imaging techniques have been developed. To date, non-invasive biomarkers of steatosis have a limited clinical utility, as they often do not accurately quantify hepatic steatosis as assessed histologically[12]. Therefore, imaging techniques are the preferred diagnostic tests for assessing liver fat accumulation. 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[1,2]. Ultrasonography is the most widely used imaging method in clinical practice, and has a diagnostic accuracy of 0.91-0.93 for detecting mild-to-moderate hepatic steatosis and a specificity of 0.88-0.99[13]. The accuracy of ultrasonography may further improve in relation to the local expertise and the availability of newer ultrasound machines[14]. Semi-quantitative ultrasonographic indices may also provide added diagnostic value[14]. For example, the ultrasonographic fatty liver index (US-FLI) with a cut-off value ≥2 detects a minimum amount of 10% steatosis on liver histology (sensitivity 90%, specificity 90%)[15]. Moreover, a US-FLI <4 has also a 94% negative predictive value for excluding severe

NASH[16]. Controlled attenuation parameter (CAP) assessed by transient elastography (FibroScan) at a cut-off value of 310 dB/m has 80% sensitivity, 71% specificity, 86% positive and 71% negative predictive values, respectively, for histological steatosis >30%[17].

The exclusion of competing etiologies of chronic liver disease can be accomplished through medical history, simple biochemical parameters[1,2,9] and specific questionnaires to exclude excessive alcohol consumption[18].

The ascertainment of a full-blown MetS will be accomplished, at baseline and repeated over time, through appropriate family and personal history; physical examination and a first-level laboratory assessment[1,9]. Interestingly, the US-FLI may better differentiate the presence of MetS than insulin resistance alone[15,16,19].

Staging of hepatic fibrosis can be implemented with the use of multiple non-invasive methods. These include both "biochemical" score systems [e.g., fibrosis-4 score (FIB-4) >2.67, NAFLD fibrosis score (NFS) >0.676 and enhanced liver fibrosis score (ELF) >10.51] and "physical" techniques (e.g., liver stiffness measurement [LSM] assessed with transient elastography >9.6 KPa or with other non-invasive imaging methods)[20,21]. The serial combination of LSM with FIB-4/NFS measurements accurately predicts the presence of advanced hepatic fibrosis in NAFLD[21].

In the schematic **Figure 1** we propose a pragmatic algorithm for the diagnosis and monitoring of NAFLD in patients with established diabetes. However, it is important to emphasize that an intense debate on aspects of our 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 does not yet exist. In particular, screening for NAFLD (both in the general population and in high-risk patient groups) is not universally recommended by all scientific societies[1,2,22,23]; that its cost-effectiveness remains controversial[24,25] and that liver biopsy should be eventually carried out in at least a (large) proportion of those submitted to non-invasive screening, given that the diagnosis of NASH remains universally based on histological grounds[1,2,19,21,22]. For example, a recent cross-sectional study conducted in approximately 122,000 patients with T2DM found a high prevalence of advanced

hepatic fibrosis using NFS and other non-invasive scores[26]; however, the significant variability among the findings provided by such scores (ranging from nearly 9% with the use of the FIB-4 score to nearly 35% with NFS) strongly supports the need for their further validation in diabetic populations[26].

2.3 Epidemiology

Around one-quarter of adults in the United States and Europe have NAFLD, and in certain areas of South America and Asia the prevalence of the disease is even higher[27]. This finding further highlights the overwhelming potential clinical and economic burdens imposed by NAFLD, which is now projected to increase further in the foreseeable future[1,27]. The scale of the burden of NAFLD also implies that no Health Authority worldwide can afford to promote any screening campaigns aimed at identifying NAFLD in the general population.

2.3.1 T2DM

On these grounds, the identification of certain selected cohorts of individuals at high risk of developing NAFLD (such as people with T2DM) appears to be a more fruitful strategy[1,2,11] Irrespective of the characteristics of the cohorts studied (hospital-based *vs.* population-based cohorts) and the diagnostic methodologies used for diagnosing NAFLD (imaging *vs.* biopsy), the prevalence of the disease has been found to be much greater in patients with T2DM than in the nondiabetic population, ranging from nearly 40% to 100% (as summarized in the **Supplementary Table 1**)[28-44].

Patients with T2DM are also more likely to have more severe histological forms of NAFLD, such as NASH with advanced fibrosis, even with fairly normal serum aminotransferase levels[33,35]. Therefore, serum aminotransferase levels 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[1-4,9,45].

Notably, the coexistence of NAFLD and T2DM will worsen the course of both diseases[45-49]. Coexisting T2DM not only increases the risk of NAFLD progression to advanced fibrosis and cirrhosis, but also increases the risk of incident HCC, liver-related hospital admissions and liver-related deaths[43,49-55]. Conversely, 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]; thus further increasing the risk of incident CKD[4-6,56] and major CVD events, particularly in those 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.

2.3.2 T1DM

Compared to the heavy toll imposed on the liver by T2DM, epidemiological data on the burden of NAFLD imposed by T1DM (*i.e.*, a disease that is characterized by an altered porto-systemic gradient of insulin and a lesser degree of insulin resistance compared to T2DM) appear to be more variable (summarized in **Supplementary Table 2**)[59-67].

Some authors have reported a (relatively) high prevalence of NAFLD (on ultrasonography), up to nearly 50%, in adult patients with T1DM[60,61]. Others have reported a prevalence of NAFLD (diagnosed by magnetic resonance imaging) of 30% in a small group of adults with T1DM[67]. However, some investigators have disputed this notion by reporting a prevalence of NAFLD (on magnetic resonance imaging) in patients with T1DM that ranged from 0% (in children with T1DM)[63] to nearly 10-15%[65,66], which is a prevalence of the disease definitely lower than that observed in the general adult population.

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, family history of T2DM, body mass index (BMI) and degree of glycaemic control among the various cohorts of T1DM patients 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, which was comparable to that observed in T2DM individuals who were matched for sex, age, diabetes duration, obesity and other potential confounding variables[68]. However, we suggest that further larger studies of well-characterized T1DM patients are certainly required to better characterize the relationship between NAFLD and T1DM.

3. STUDIES LINKING NAFLD TO RISK OF CHRONIC VASCULAR COMPLICATIONS OF DIABETES

In recent years, 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 both in nondiabetic patients and in 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 associated with an increased risk of chronic vascular complications of diabetes[47].

3.1 Macrovascular complications

Substantial epidemiological evidence links NAFLD with various markers of subclinical atherosclerosis (*e.g.*, increased arterial stiffness, endothelial dysfunction or increased carotid and lower limb atherosclerotic plaques) and with an increased prevalence of clinically manifest CVD across different patient populations, including people with T2DM[3.4,58,69,70]. Recently, Guo *et al.* 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, haemoglobin A1c, insulin resistance, serum liver enzyme levels and medication use in a large cohort of Chinese individuals with T2DM[71]. Again, the Valpolicella Diabetes Heart Study, including 2392 Italian outpatients with T2DM without secondary causes of chronic liver disease, demonstrated that those with ultrasound-diagnosed NAFLD had a remarkably greater prevalence of clinically manifest coronary, cerebrovascular and peripheral vascular disease (**Figure 2**), independent of age, sex, BMI, waist circumference, smoking, low-density lipoprotein (LDL)-cholesterol, haemoglobin A1c, duration of diabetes, presence of MetS and use of hypoglycaemic, antihypertensive, lipid-lowering and antiplatelet medications[28]. Almost similar results were observed in smaller cohorts of adult patients with T1DM, where NAFLD (diagnosed by ultrasonography) was associated with higher odds of prevalent CVD, independently of age, sex, BMI, smoking, duration of diabetes, haemoglobin A1c, systolic blood pressure, plasma lipids and use of medications[60,61]. Moreover, in both nondiabetic and diabetic patients 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, independently of the extent and severity of coronary atherosclerosis[70,72-74].

To date, convincing evidence also substantiates the existence of a strong link of NAFLD with subclinical myocardial remodeling and dysfunction (*i.e.,* left ventricular diastolic dysfunction and cardiac hypertrophy), valvular heart diseases (*i.e.,* aortic-valve sclerosis and mitral annulus calcification) and cardiac arrhythmias (mainly atrial fibrillation and QTc interval prolongation on standard electrocardiograms) both in patients with and without diabetes[75-84]. Preliminary evidence also suggests that ultrasound-diagnosed NAFLD, irrespective of pre-existing diabetes, is associated with an increased risk of 1-year all-cause and cardiac re-hospitalizations in patients admitted with acute heart failure[85].

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 patient populations, including those with diabetes. However, whether NAFLD is an independent CVD risk factor or simply a bystander that shares common aetiological factors remains still debatable.

To date, a number of large hospital-based and population-based cohort studies reported an increased incidence of fatal and nonfatal CVD events in patients with either imaging- or biopsy-diagnosed NAFLD, independent of conventional CVD risk factors, both in patients with and without diabetes[1,4-6,58,69,70]. For instance, a prospective nested case-control study in 744 Italian outpatients with T2DM, who were free of diagnosed CVD at baseline, demonstrated that those with ultrasound-diagnosed NAFLD had a nearly twofold increased risk of developing nonfatal coronary heart disease, ischemic stroke or cardiovascular death over a follow-up period of 5 years. Notably, this association was independent of age, sex, smoking, diabetes duration, haemoglobin A1c, LDL-cholesterol, serum liver enzymes, presence of MetS, and 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*=2103) 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 six-fold increased risk of nonfatal CVD events (*i.e.*, combined endpoint inclusive of nonfatal ischaemic heart disease, ischaemic stroke or coronary/peripheral revascularization procedures) over a mean follow-up period of 5.3 years[88]. Notably, this association was independent of age, sex, BMI, smoking, diabetes duration, haemoglobin A1c, dyslipidaemia, hypertension, CKD, prior ischaemic heart disease and serum gamma-glutamyltransferase levels[88].

Recently, an updated and large meta-analysis that incorporated almost 34000 individuals with and without T2DM (36.3% with NAFLD) and approximately 2600 fatal and nonfatal CVD outcomes (>70% CVD deaths) in 16 unique, observational prospective and retrospective cohort studies from different countries concluded that the presence of NAFLD (as detected by either imaging or histology) was associated with a higher risk of incident fatal and nonfatal CVD events over a median follow-up period of 6.9 years (random-effect odds ratio 1.64, 95%CI 1.3-2.1), and that this risk increased further with greater severity of NAFLD (random-effect odds ratio 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 odds ratio 1.54, 95%CI 1.1-2.1) and limiting to studies with full adjustment for covariates (*n*=5 studies; random-effect odds ratio 1.69, 95%CI 1.3-2.6) provided overall risk estimates consistent with the primary analysis[57].

Although further research is needed to definitely establish a causal association between NAFLD and increased risk of incident 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 greater 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].

3.2 Microvascular complications

NAFLD is significantly associated with the risk of microvascular diabetic complications, especially with CKD. In particular, numerous observational studies showed that NAFLD is associated with an increased prevalence of CKD (defined as estimated glomerular

filtration rate [eGFR] <60 ml/min/1.73 m^2 or proteinuria) both in patients without diabetes and in those with T2DM or T1DM[89]. As shown in **Figure 3**, in the Valpolicella Heart Diabetes Study cohort involving 2103 Italian outpatients with T2DM without known chronic liver diseases and CVD at baseline, who had available measurements on eGFR, albuminuria and retinopathy[90], it has reported that patients with ultrasound-diagnosed NAFLD had a nearly twofold increased risk of prevalent CKD or advanced (proliferative or laser-treated) diabetic retinopathy, independently of age, sex, BMI, waist circumference, smoking, hypertension, diabetes duration, haemoglobin A1c, plasma lipids and medication use. Conversely, in a subgroup of 5963 adult participants (15.8% with established diabetes) of the National Health and Nutrition Examination Survey-III, the presence of ultrasound-diagnosed NAFLD was not significantly associated with any degree of retinopathy (detected via fundus photographs) both in individuals with and without known diabetes after adjusting for multiple covariates[91]. Some other studies in which NAFLD was diagnosed by either ultrasonography or histology have shown that the presence and severity of NAFLD was 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, independently of age, sex, BMI, hypertension, diabetes duration, haemoglobin A1c and use of medications[94,95].

To date, there is a paucity of published data regarding the risk of developing CKD (or other microvascular complications) in patients with coexistent NAFLD and diabetes. In a subset of 1760 outpatients with T2DM of the Valpolicella Heart Diabetes Study, who had normal kidney function at baseline (*i.e.*, after excluding those with CKD or macroalbuminuria [n=343]), the presence of ultrasound-diagnosed NAFLD was associated with an increased risk of incident CKD (defined as occurrence of eGFR <60 ml/min/1.73 m^2 or overt proteinuria) over a follow-up period of 6.5 years, independently of age, sex, smoking, BMI, waist circumference, diabetes duration, blood pressure, plasma lipids, haemoglobin A1c, baseline eGFR, albuminuria and use of hypoglycaemic, antihypertensive, lipid-lowering and antiplatelet drugs (adjusted-hazard ratio 1.49; 95%CI 1.1-2.2)[96]. Consistent with these findings, in a retrospective cohort study of 261 T1DM adults with preserved kidney function at baseline, 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 duration, haemoglobin A1c and baseline eGFR[97]. Notably, addition of NAFLD to conventional cardio-renal risk factors significantly improved the discriminatory capability of the regression models for predicting CKD[97].

A comprehensive meta-analysis of 33 observational, cross-sectional and prospective studies (including a total of 64000 individuals) confirmed that the presence and severity of NAFLD, as diagnosed by biochemistry, imaging or histology, was associated with a nearly twofold increase in the prevalence and incidence of CKD. In all of these analyses, the significant association between NAFLD and increased risk of CKD persisted after adjustment for pre-existing diabetes and other established cardio-renal risk factors[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, published studies that have evaluated the existence of such association are very few and have produced conflicting results[101,102].

Despite the growing evidence that links NAFLD with CKD and other microvascular complications in patients with T2DM or T1DM, a causal association remains to be definitely proven, and additional larger studies in different ethnic populations are needed to establish whether the steatotic/inflamed liver may actively contribute to the increased risk of microvascular complications observed among diabetic patients with NAFLD, a hypothesis which is biologically plausible, as discussed below.

4. PUTATIVE BIOLOGICAL MECHANISMS BY WHICH NAFLD CONTRIBUTES TO THE DEVELOPMENT OF CHRONIC VASCULAR COMPLICATIONS OF DIABETES

Several years ago, it was noted that T2DM and CVD share many risk factors (the "common soil" hypothesis[103]) and that unlike classical microvascular complications, large-vessel atherosclerosis can precede the development of T2DM. It has been recognized for many years that functioning of pancreatic β cells, skeletal muscle, liver and adipose tissue are important in the development of T2DM and more recently

functioning of other organs such as the intestine, brain and kidneys, as well as pancreatic α cells, has been highlighted in the development of chronic hyperglycaemia (the so-called "ominous octet")[104]. Thus, rather than vascular disease being a complication of diabetes, both conditions may have common antecedents, *i.e.*, they spring from a "common soil"[103] and those common antecedents involve the functioning of other key organs beyond the pancreas.

As discussed previously, several authors have shown that NAFLD may 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", it is now evident that NAFLD also shares many risk factors with diabetes and CVD. It is widely accepted that these shared risk factors revolve around ectopic fat accumulation (abdominal obesity), insulin resistance and other features of MetS[105]. Consequently, when considering the underlying mechanisms by which NAFLD may contribute to the development of chronic vascular complications of diabetes, it is important not only to consider the influence of the steatotic/inflamed liver *per se*, but also to consider the influence of abdominal obesity and other shared cardiometabolic risk factors. In particular, there is a cross-talk between the expanded/inflamed visceral adipose tissue and the liver, with this latter acting as both the target organ and the source of the systemic subclinical chronic inflammation and abnormalities in coagulation and fibrinolysis (as discussed below) that may promote an increased risk of developing chronic vascular complications of diabetes[2-4,69,70].

Recently, there has also been considerable research interest in the possible pathogenic role of perturbations in the normal intestinal microflora (the so-called "dysbiosis") and abnormalities of normal intestinal function on CVD risk factors. In discussing the biological mechanisms underpinning the relationship between NAFLD and chronic vascular complications of diabetes, we will discuss the emerging evidence that suggests a link between dysbiosis, intestinal barrier dysfunction, gut microbial-mediators and CVD[106-110]. We will also discuss potential haemostatic, prothrombotic and proinflammatory mediators, as well as mechanisms contributing to oxidative stress that may link NAFLD to chronic vascular complications of diabetes.

4.1 Consequences of dysbiosis

Since 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 risk of NAFLD. Dysbiosis[111] has recently been described in patients with MetS, and in those with established CVD[107-109], T2DM[112-115], NAFLD[116-118] or CKD[119]. In **Figure 4** are displayed the potential pathways, factors and processes that link dysbiosis/gut microbial mediators and NAFLD to CVD risk factors and vascular and renal diseases.

4.1.1 *Increased gut permeability and release of lipopolysaccharide into the circulation*

Dysbiosis is frequently associated with increased production of endotoxins from Gram-negative bacteria that can damage the intestinal barrier, affect nutrient harvesting and increase gut permeability with the potential for lipopolysaccharide (LPS) to enter the portal and systemic circulation to increase the inflammatory burden[120,121]. LPS causes disruption of the gut intracellular tight junctions, favouring the release of proinflammatory cytokines into the circulation and, consequently, into the liver[120-123]. Since LPS production provides a direct inflammatory stimulus to the liver *via* the portal vein, LPS might increase risk of hepatic inflammation and oxidative stress.

4.1.2 *Altered short-chain fatty acid production, trimethyl-amine metabolism, uraemic toxins and bile acid metabolism*

Fermentation of dietary fibre in the intestine by anaerobic bacteria, such as *Lactobacilli* and *Bifidobacteria*, forms short-chain fatty acids (SCFAs)[124,125]. SCFAs include acetate, propionate and butyrate that influence hepatic *de novo* lipogenesis, and gluconeogenesis. A meta-analysis of predominantly short-term probiotic treatments in T2DM has suggested a beneficial effect on insulin resistance, 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)/trimethylamine oxide (TMAO), secondary bile acids, LPS, and catecholamines[106-109]. With dysbiosis there may also be an increase in uraemic toxins, such as TMAO, p-cresyl sulfate, 4-ethyl phenyl sulphate, hippuric acid, indoxyl sulfate and indole-3 acetic acid[127].

Increased TMA/TMAO levels have been shown to cause 'atherogenic' lesions in mice and are associated with atherosclerosis in man[109], and it has been suggested that TMAO may 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 may impair reverse cholesterol transport, induce platelet aggregation, promote foam cell formation and increase expression of the scavenger receptors A1 and CD36[109]. 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.

4.1.3 *Altered bile acid metabolism*

Primary bile acids (BAs), such as cholic acid and chenodeoxycholic acid (CDCA), are produced by the liver. BAs interact with plasma membrane G-protein-coupled receptors (*e.g.*, TGR5), muscarinic receptors and nuclear receptors, *e.g.* the farnesoid (FXR) and pregnane (PXR) X receptors[129]. BA receptors are expressed in cardiovascular tissue, *e.g.* endothelial cells vascular smooth cells and cardiomyocytes[129]. CDCA is a naturally occurring ligand for the FXR, and activation of the FXR with modified CDCA compounds, such as obeticholic acid, is now known to have marked effects not only on bile acid metabolism but also on liver disease, cholesterol metabolism and LDL-cholesterol levels in NAFLD[130,131]. Besides regulating bile acid metabolism, FXR activation also powerfully influences levels of lipids such as hepatic glucose output, hepatic glycogen, cholesterol, triglyceride and fatty acids, and also regulates inflammation. The BAs are influenced by gut microbiota to produce secondary bile acids, such as urodeoxycholic acid (UDCA), deoxycholic acid (DCA) and lithocholic acid (LCA). Secondary BAs are highly hydrophobic and toxic, and increased concentrations in the liver have been linked to inflammation[132] and NAFLD[133]. There is also evidence suggesting that alteration of bile acid metabolism by the intestinal microbiota may influence CVD risk by affecting LDL-cholesterol metabolism, vasomotor tone and blood pressure[129, 134]. Furthermore, treatment with *Bifidobacterium* may influence cholesterol metabolism by decreasing serum total cholesterol and LDL-cholesterol concentrations[135]. CDCA treatment has also recently been shown to decrease the LDL-receptor modulator proprotein convertase subtilisin/kexin type 9 (PCSK9)[136], providing another potentially important mechanism by which BAs may modify cholesterol metabolism. **Figure 5** summarizes potential pathways linking dysbiosis to vascular diseases.

4.2 Insulin resistance, atherogenic dyslipidaemia, proinflammatory cytokines, increased haemostatic factors and oxidative stress

The development and, more importantly, progression of NAFLD result in amplifying the risk of vascular damage with an increase in other traditional and nontraditional CVD risk factors, such as atherogenic dyslipidaemia, insulin resistance, proinflammatory cytokines, haemostatic-fibrinolytic factors and increased oxidative stress.

The development of NAFLD is associated with the accumulation of intra-hepatic ceramides and di-acyl glycerols (DAG)[4] as well as the secretion of multiple hepatic-derived molecules, such as fetuin-A and other hepatokines that are able to inhibit the insulin receptor tyrosine kinase to promote hepatic and peripheral insulin resistance[137]. Indeed, intrahepatic accumulation of lipid intermediates, such as ceramides and DAG, inhibits insulin signaling. For example, DAG activates protein kinase C-epsilon (PKC-epsilon) membrane translocation, inhibits the insulin receptor kinase and decreases insulin signaling; and increased hepatic ceramide results in activation of inflammatory toll-like receptor-4 signaling pathways, with consequent impairment of insulin signaling pathways via inhibition of Akt phosphorylation[138,139].

The development of insulin resistance in NAFLD is associated with the features of MetS, such as increased blood pressure and also the development of an atherogenic lipoprotein profile. In NASH, key components of the renin-angiotensin-aldosterone system (RAS) are also increased, and RAS activity plays a key role linking NAFLD to vascular disease in CKD. It is well known that adipocytes express all components of RAS contributing up to 30% of circulating renin, angiotensin-converting enzyme, and the vasoconstrictor angiotensin II, but the liver also expresses RAS constituents, and experimental studies support a role for both systemic and local activation of the angiotensin II in NAFLD[140].

The specific atherogenic lipoprotein profile that is typically associated with the MetS and insulin resistance increases secretion of triglyceride-rich lipoproteins (TGRLP) into the circulation. With an increase in TGRLPs in the circulation there is also an associated increase in cholesterol ester transfer protein (CETP) activity[104,140-142]. CETP resides on high-density lipoproteins (HDL) and mediates the reciprocal exchange of triglyceride

and cholesterol esters between TGRLPs and both the HDL and LDL particles. With a CETP-mediated increase in triglyceride content of HDL and LDL lipoproteins, 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 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].

In individuals with MetS, insulin resistance and progressive forms of NAFLD, an up-regulation of multiple proinflammatory pathways is almost invariably observed. These proinflammatory mechanisms influence two main intracellular transcription factor-signaling pathways, the nuclear factor-kB (NF-kB) pathway and the C-Jun-N-terminal kinase (JNK) pathway[141-143]. Experimental animal data suggest that JNK-1 activation in the adipose tissue causes insulin resistance in the liver[144], and activation of the NF-kB pathway in NASH is potentially pivotal in further amplifying the systemic inflammatory response with an NF-kB pathway-mediated increase in transcription of several different proinflammatory genes[145].

Additionally, adiponectin and leptin are two key adipokines, which may have an impact on disease progression in NAFLD by regulation of hepatic fat accumulation, inflammation and fibrosis[145,146]. Adiponectin exerts anti-inflammatory, anti-fibrotic and anti-atherogenic properties, and therefore low levels of adiponectin are associated with insulin resistance, and NASH may influence progression not only of liver disease and HCC in NAFLD, but also may increase risk of CVD and CKD[146].

Hepatic lipids that are not esterified are also able to induce endoplasmic reticulum stress, leading to activation of JNK kinase and NF-κB[147]. Hepatic lipid may also induce an endoplasmic reticulum oxidative stress response, as well as induce mitochondrial dysfunction with the potential for generation of free radicals, *via* increased oxidation of excess fatty acids[148]. Mitochondrial dysfunction is associated with insulin resistance and atherosclerosis in several studies[149], suggesting a mechanism linking NAFLD and increased risk of CVD and CKD.

Finally, it is known that the liver is key to the production of multiple coagulation factors and is also an important site of production of plasminogen activator inhibitor-1 (PAI-1) and other fibrinolytic proteins[150]. Several case-control studies have shown that the levels of multiple procoagulant factors (*e.g.*, fibrinogen, factor VII, von Willebrand factor, PAI-1 and other haemostatic-fibrinolytic factors) are highest in patients with NASH, intermediate in those with simple steatosis, and lowest in control subjects without steatosis (as reviewed in[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 above-mentioned proinflammatory and procoagulant biomarkers[153,154], thus further supporting the notion that the increased circulating levels of these biomarkers result from the up-regulation of their synthesis, taking place in the steatotic /inflamed/fibrotic liver.

Figure 6 shows the more relevant liver-specific pathways potentially linking NAFLD to increased risk of CVD and CKD.

In summary, recent evidence clearly suggests that NAFLD may increase the risk of chronic vascular complications of diabetes *via* a variety of different pathogenic mechanisms. These biological pathways potentially include dysbiosis and perturbed intestinal function, abdominal obesity, insulin resistance, atherogenic dyslipidaemia, proinflammatory cytokines, increased oxidative stress and alterations of haemostatic-fibrinolytic factors. Despite the biological plausibility of dysbiosis and intestinal dysfunction might also represent a novel mediator increasing risk of both NAFLD and vascular disease, presently, it remains uncertain whether treatment for dysbiosis favourably modifies levels of potentially damaging molecules and pathways leading to NAFLD and to chronic vascular and renal damage. Although further research is urgently needed in this area, correction of dysbiosis might represent a novel therapeutic target to ameliorate both risk of NAFLD and also CVD (and CKD) [108,126,155].

5. MANAGEMENT AND TREATMENT OPTIONS FOR NAFLD IN DIABETES

5.1 Lifestyle modification

The therapeutic approach to patients with coexistent NAFLD and diabetes 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 using drugs with potentially beneficial effects on the liver)[1,2,45,47,156].

Lifestyle changes must be suggested to all patients with coexistent NAFLD and diabetes even though they are difficult to achieve and maintain. Moreover, a strict control of all coexisting cardiometabolic risk factors should be pursued and the higher the risk of progressive liver disease, the more aggressive the treatment should be. However, to date, whether patients with coexistent NAFLD and diabetes should be treated to a specific haemoglobin A1c, LDL-cholesterol, and blood pressure target remains uncertain.

5.1.1 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 nearly 5–7% is able to decrease hepatic steatosis; however, a nearly 8–10% weight reduction is requested to reverse NASH and a weight loss of ≥10% can also improve or reverse hepatic fibrosis[1,2,156,157]. Given the difficulties in achieving and maintaining this endpoint through lifestyle modifications, bariatric surgery, which is able to improve all histological lesions of NASH, including hepatic fibrosis, may be suggested to properly selected severely obese patients[1,2,45,158,159]. However, whilst bariatric surgery is undoubtedly effective, there are obvious limitations, including possible complications, patient compliance, service availability and cost.

5.1.2 Diet and smoking

Qualitative and quantitative dietary changes are advisable for all patients with NAFLD[1,2]. About 1200–1600 kcal/day are recommended. A low-fat (<30%) diet with less than 10% of saturated fatty acid intake, and relatively low in carbohydrate intakes (<50% of total Kcals) is suggested. Preference should be given to complex carbohydrates, avoiding simple ones, which have a higher glycemic index; as well as the lipogenic sugar fructose[160].

Mediterranean diet appears 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 in fish and vegetables (but not in fruits) has been

also associated with a protection from developing HCC. Conversely, cigarette smoking and even modest amount of alcohol consumption may increase HCC risk[162-164].

5.1.3 Physical exercise

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

5.2 Pharmacological treatment

A detailed review of drug treatment options for NAFLD/NASH in patients with diabetes 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 endpoints. In line with this consideration, a recent Cochrane review[170] concluded that *"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 reserved to those patients with NASH, who are at the highest risk of progressive liver disease and/or those patients who have more severely decompensated diabetes[1,2,45,56,156,169].

A central dogma is that any diabetes treatment may benefit patients with NASH if they have uncontrolled hyperglycaemia[45,56]. Further to glucose-lowering agents, several classes of drugs can be taken into consideration, such as lipid-lowering agents; antioxidants; iron depletion; innovative drugs and, in some cases, bariatric (metabolic) surgery[1,2,45,56,156,169]. A variety of drugs will also probably emerge over the next 5 years,

permitting a more stage-based approach of NAFLD and greater personalization of drug selection.

Table 1 summarizes the effects on liver histology of drug treatments for NASH observed in the principal randomized clinical trials that included adults with T2DM or prediabetes with biopsy-proven NASH[131,171-181].

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 those drug principles whose action is not limited to an individual therapeutic target but is also extended to improve the risk of developing CVD events and severe liver-related complications, such as cirrhosis and HCC[168]. Statins are an example of such a pleiotropic class of drugs[182]. 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, while the use of insulin or sulphonylureas appear to be positively associated with the presence and severity of NASH and fibrosis on liver histology[183]. Similarly, the use of pioglitazone, which has to date the most available evidence of efficacy in T2DM patients with NASH, may potentially improve the natural history of the liver disease by reducing its progression to cirrhosis in (some) patients with biopsy-proven NASH[1,2,45,56,156,169]. Conversely, metformin is not currently recommended as a specific treatment for liver disease in T2DM patients with NAFLD or NASH. Furthermore, there are no robust data with clinically relevant endpoints as a primary outcome to formally comment on the effectiveness of the use of the newer glucose-lowering agents (*e.g.*, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 receptor agonists or sodium glucose co-transporter 2 inhibitors) as a treatment for NAFLD/NASH with coexistent diabetes. On these grounds, an Expert Panel recently suggested that, pending forthcoming randomized clinical trials, physicians should consider using pioglitazone, or, statin use in those patients with NAFLD/NASH at high CVD or HCC risk (unless contraindicated), alone or preferably in combination with each other or with ezetimibe, 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 death in patients with NAFLD[184]. 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 or not). Before prescribing pioglitazone or vitamin E, it was recommended that clinicians take into account any comorbidities and the risk of adverse events associated with these conditions[185]. However, that said, 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 side 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 efficacy.

6. CONCLUSIONS

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

This review article supports the existence of a strong association between the presence and severity of NAFLD and the risk of chronic vascular complications of diabetes, 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, 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 entertained 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.

7. KEY POINTS

• Convincing epidemiological evidence substantiates a strong association between the presence and severity of NAFLD and risk of chronic macrovascular (mainly cardiovascular disease) and microvascular (mainly chronic kidney disease) complications of diabetes.

• Evidence suggests that NAFLD exacerbates insulin resistance, predisposes to atherogenic dyslipidaemia and causes the release of a variety of proinflammatory, procoagulant and proatherogenic mediators that play a role in the development of chronic vascular complications of diabetes.

• 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 (with the combined use of serum liver enzymes, liver ultrasonography, FibroScan and non-invasive tests of advanced hepatic fibrosis) with a view to implementing an earlier and more aggressive treatment whenever indicated.

• Whether such a more liberal screening policy and more aggressive treatment will cost-effectively prevent the development of chronic vascular complications of diabetes will be the target of future larger studies.

Competing interests statement

G.T. & C.D.B. declare no competing financial interests. A.L. is a researcher of a phase III, double-blind, randomized, long-term, placebo controlled, multicenter study evaluating the safety and efficacy of obeticholic acid in subjects with NASH (EudraCT 2015-002560-16).

Author Contributions

All authors researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and undertook review and/or editing of the manuscript before submission.

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FIGURES LEGENDS

Figure 1. Proposed pragmatic algorithm for the management of suspected NAFLD in patients with established diabetes mellitus.

The algorithm has been developed using both available evidence and guidelines, as well as expert opinion where uncertainty exists and evidence was not available. Patients with type 1 or type 2 diabetes should routinely undergo diagnostic procedures for the diagnosis of NAFLD, which relies on the demonstration of hepatic steatosis. Serum transaminase levels 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, *e.g.*, excessive alcohol intake, viral hepatitis, autoimmune hepatitis, hemochromatosis or chronic use of steatogenic medications is key for the diagnosis of NAFLD. The algorithm can be used to select those NAFLD patients to submit to liver biopsy, or if biopsy is not undertaken, the non-invasive assessment of advanced liver fibrosis according to panels of specific serum biomarkers (*e.g.*, the fibrosis-4 [FIB-4] score, the NAFLD fibrosis score or the enhanced liver fibrosis [ELF] blood test) and transient elastography (*e.g.*, the FibroScan or other non-invasive imaging techniques that assess liver stiffness) may be used for selecting patients for upper gastrointestinal endoscopy (aimed at showing the presence of esophageal/gastric varices due to portal hypertension) and long-term surveillance for liver-related complications, including hepatocellular carcinoma, always if cirrhosis is present; and in selected non-cirrhotic cases, particularly if advanced fibrosis is present).

Figure 2. Prevalence of clinically manifest cardiovascular disease (CVD) in patients with type 2 diabetes mellitus.

Sex- and age-adjusted prevalence of coronary (defined as myocardial infarction, angina pectoris or coronary revascularization procedures), cerebrovascular (ischemic stroke, recurrent transient ischemic attacks, carotid endarterectomy or carotid stenosis >70% as diagnosed by echo-Doppler scanning) and peripheral (intermittent claudication, rest pain, as confirmed by echo-Doppler scanning, lower extremity amputations or peripheral revascularization procedures) in type 2 diabetic outpatients with (red columns) and without (green columns) ultrasound-diagnosed NAFLD. Data derived from Targher *et al.*[28] (Reproduced with permission).

Figure 3. Prevalence of diabetic nephropathy and retinopathy in patients with type 2 diabetes mellitus.

Age- and sex-adjusted 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 type 2 diabetic outpatients with (red columns) and without (green columns) ultrasound-diagnosed NAFLD. Data derived from Targher *et al.*[90] (Reproduced with permission).

Figure 4. Potential pathways, factors and processes that link dysbiosis, gut microbial mediators and alterations in hepatic structure and function (NAFLD) to cardiovascular risk factors and vascular and renal diseases.

Dysbiosis is associated with increased intestinal permeability and intestinal dysfunction (A). Factors in (A) increase risk of NAFLD via alterations in several pathways, factors and molecules that modify liver structure and function in NAFLD (B). With these liver-specific changes (B), there is an increase in risk factors for vascular disease (C) and the subsequent development over time of vascular and renal diseases via increased insulin resistance, metabolic syndrome and structural and functional changes affecting both the vasculature and the kidneys (D).

Figure 5. Potential pathways linking dysbiosis to cardiovascular and chronic kidney disease.

Intestinal dysbiosis perturbs bile acid metabolism affecting the levels of *e.g.* bile acids (such as chenodeoxycholic acid [CDCA]), short-chain fatty acids (SCFA), trimethylamine and lipopolysaccharide. Via decreased activity of hepatic nuclear receptor farnesoid X receptor (FXR) activity in NAFLD and increased intestinal permeability, there is a further increase in cardio-renal risk factors with subsequent development of vascular and renal diseases.

Figure abbreviations: LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9; Cyp7a1, cytochrome p450 7a1; Lp(a), lipoprotein (a); apoCIII, apolipoprotein CIII; TGRLPs, triglyceride rich lipoproteins; TMAO, trimethylamine oxide.

Figure 6. Liver-specific pathways in NAFLD linking NAFLD to cardiovascular and chronic kidney disease.

A variety of factors, such as visceral adipose tissue accumulation, low-grade chronic inflammation, 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 may occur and the disease process progresses, with further inflammation, collagen deposition and fibrosis into the liver. All of these intrahepatic processes may also increase the production of risk factors for vascular disease with subsequent development of both cardiovascular disease and chronic kidney disease.

Figure abbreviations: DAGs, di-acylglycerols; di-P PA, di-palmitoyl phosphatidic acid; LCFAs, long chain fatty; LDL-C, low-density lipoprotein cholesterol; TAGs, tri-acyl glycerols; PNPLA3, patatin-like phospholipase domain containing 3; TM6SF2, transmembrane 6 superfamily member 2; TGF-beta, transforming growth factor-beta; TNF-alpha, tumor necrosis factor.