Review

JOURNAL OF HEPATOLOGY

NAFLD: A multisystem disease

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Summary

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries that is predicted to become also the most frequent indication for liver transplantation by 2030. Over the last decade, it has been shown that the clinical burden of NAFLD is not only confined to liver-related morbidity and mortality, but there is now growing evidence that NAFLD is a multisystem disease, affecting extra-hepatic organs and regulatory pathways. For example, NAFLD increases risk of type 2 diabetes mellitus (T2DM), cardiovascular (CVD) and cardiac diseases, and chronic kidney disease (CKD). Although the primary liver pathology in NAFLD affects hepatic structure and function to cause morbidity and mortality from cirrhosis, liver failure and hepatocellular carcinoma, the majority of deaths among NAFLD patients are attributable to CVD. This narrative review focuses on the rapidly expanding body of clinical evidence that supports the concept of NAFLD as a multisystem disease. The review discusses the factors involved in the progression of liver disease in NAFLD and the factors linking NAFLD with other extra-hepatic chronic diseases, such as T2DM, CVD, cardiac diseases and CKD. The review will not discuss NAFLD treatments as these are discussed elsewhere in this issue of the Journal. For this review, PubMed was searched for articles using the keywords "non-alcoholic fatty liver disease" or "fatty liver" combined with "diabetes", "cardiovascular (or cardiac) disease", "cardiovascular mortality" or "chronic kidney disease" between 1990 and 2014. Articles published in languages other than English were excluded.

Keywords: Non-alcoholic fatty liver disease; Hepatocellular carcinoma; Type 2 diabetes; Insulin resistance; Cardiovascular disease; Cardiac disease; Cardiac arrhythmias; Chronic kidney disease.

Received 5 September 2014; received in revised form 6 November 2014; accepted 9 December 2014

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Abbreviations: AF, atrial fibrillation; ATGL, Adipose triglyceride lipase; AST/ALT, aspartate aminotransferase to alanine aminotransferase ratio; APRI, aspartate to platelet ratio index; CRP, C-reactive protein; CVD, cardiovascular disease; CGI-58, Comparative Gene Identification-58; CKD, chronic kidney disease; DAG, di-acyl glycerol; Di-P PA, di-palmitoyl phosphatidic acid; ELF, Enhanced Liver Fibrosis panel; FGF-21, fibroblast growth factor-21; FIB4, Fibrosis-4 Score; GFR, glomerular filtration rate; HCC, hepatocellular carcinoma; HF, heart failure; HR, hazard ratio; IRS-1, insulin receptor substrate-1; IL-6, interleukin 6; LCFAs, long chain fatty acids; LPA, lysophosphatidic acid; LV, left ventricular; MetS, metabolic syndrome; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; NHANES, National Health and Examination Survey; NASH, non-alcoholic steatohepatitis; NF-κB, nuclear factor-kappa B; OR, odds ratio; PTEN, phosphatase and tensin homolog; PA, phosphatidic acid; PAI-1, plasminogen activator inhibitor-1; PKCε, protein kinase Cε; T2DM, type 2 diabetes mellitus; TAG, triacylglycerol; TLR-4, toll-like receptor-4; TNF-α, tumour necrosis factor-α; VLDL, very-low-density lipoprotein.

Introduction

Over the last decade, it has been shown that the clinical burden of non-alcoholic fatty liver disease (NAFLD) is not only confined to liver-related morbidity and mortality, but there is now growing evidence that NAFLD is a multisystem disease, affecting several extra-hepatic organs and regulatory pathways [1]. Since NAFLD has become the predominant cause of chronic liver disease in many parts of the world [2], NAFLD is also potentially contributing to an important burden of extra-hepatic chronic complications. For reasons that are not completely clear, NAFLD is more common in men than women and although precise estimates of incidence rates for NAFLD are uncertain (because of difficulties with establishing a precise diagnosis during sequential follow-up), current incidence rates are approximately 20/10,000 person-years, peaking in the sixth decade of life [3]. Current population-based prevalence of NAFLD is approximately 30–40% in men and 15–20% in women [4] and is even higher in people with type 2 diabetes mellitus (T2DM), occurring in up to 70% of this group of patients [5].

A major focus of the NAFLD-related chronic diseases during the last 10 years has involved chronic liver disease, cardiovascular disease (CVD) and T2DM; e.g., a recent meta-analysis showed that NAFLD increased overall mortality by 57% mainly from liver-related and CVD causes, and increased risk of incident T2DM by approximately twofold [6]. Additionally, and even more recently, increasing attention has also focused on NAFLD-related chronic kidney disease (CKD) and a further recent meta-analysis reported that NAFLD was associated with an approximate twofold increased risk of CKD [7]. Although there is also emerging evidence that NAFLD is linked to other chronic diseases, such as sleep apnea, colorectal cancers, osteoporosis, psoriasis and various endocrinopathies (e.g., polycystic ovary syndrome) [8], because of the limitations on space, this review will focus only

on NAFLD-related extra-hepatic diseases where there is strongest evidence for a possible causal link between NAFLD and pathology in extra-hepatic organs (namely T2DM, CVD and CKD).

This narrative review will discuss NAFLD and: a) liver disease that is relevant to the development of extra-hepatic complications, b) T2DM, c) CVD and cardiac diseases, and d) CKD. The aetiology and pathogenesis of each of these hepatic and extra-hepatic chronic complications will also be briefly discussed.

Key Points

- Liver fat accumulation in NAFLD increases risk of type 2 diabetes mellitus approximately twofold
- Hepatic lipid accumulation (e.g., di-acyl glycerol) in NAFLD impairs insulin signaling (insulin resistance) that contributes to abnormal hepatic metabolism
- Increasing evidence suggests more severe forms of NAFLD further increase risk of type 2 diabetes mellitus
- Both NASH and type 2 diabetes mellitus increase risk of hepatocellular carcinoma
- Mechanisms contributing to the pathogenesis of hepatocellular carcinoma also occur with obesity and insulin resistance (i.e., two common risk factors for type 2 diabetes mellitus)
- Increasing evidence indicates that the presence and severity of NAFLD is associated with an increased prevalence and incidence of cardiovascular disease, independently of established cardiovascular risk factors
- Some evidence also indicates that the presence and severity of NAFLD is associated with an increased prevalence and incidence of chronic kidney disease, independently of multiple cardio-renal risk factors
- It is now becoming increasingly evident that NAFLD is not simply a marker of cardiovascular disease and chronic kidney disease, but also may play a part in the pathogenesis of these extra-hepatic chronic complications
- The clinical implication for these findings is that NAFLD patients may benefit from more intensive surveillance and early treatment interventions to decrease the risk for cardiovascular and kidney complications

NAFLD: diagnosis, development, and progression of liver disease

In clinical practice, an initial diagnosis of NAFLD is usually established with radiological imaging techniques, by the presence of $\geqslant$5% hepatic fat accumulation in the absence of other recognized causes of fatty liver, e.g., alcohol, virus, drugs, autoimmunity.

Because of the limitations of space, this review will not discuss the use of the various techniques for diagnosing NAFLD. However, for a detailed recent review of the radiological imaging modalities available for the assessment of NAFLD see [9]; for more details regarding the sensitivity and specificity of ultrasonography to detect liver fat, the reader is referred to the following [10–12]; and for information, regarding the sensitivity and reproducibility of magnetic resonance techniques to assess liver fat [13,14]. For a discussion of the utility of histological characterization of the liver, using a designated scoring system, see [11,15].

NAFLD is fast becoming one of the most common causes of chronic liver disease worldwide, and is now a major cause of liver-related morbidity and mortality [16]. NAFLD begins with liver lipid accumulation, and marked hepatic fat accumulation is a risk factor for disease progression. Although the major risk factors for hepatic fat and hepatic fibrosis development in NAFLD are well established (e.g., age >50 years, obesity, insulin resistance, T2DM, increased ferritin levels and the patatin-like phospholipase domain-containing 3 (*PNPLA3*) I148M polymorphism) [17–19], the pathological mechanisms by which each of these risk factors (particularly *PNPLA3* genotype) cause NAFLD progression are less well understood. It has been shown that when associated with the I148M gene variant, NAFLD has a lower plasma triacylglycerol profile. This supports the notion that the I148M gene variant inhibits intra-hepatocellular lipolysis rather than stimulates hepatic triacylglycerol synthesis [20]. However, further work is required to establish the precise function of PNPLA3 in the pathogenesis of liver disease progression in NAFLD, as it has also been highlighted in response to this work that a contribution of PNPLA3 lysophosphatidic acid acyltransferase activity could also contribute to altered plasma triacylglycerol composition and concentration [21]. Where there is evidence of advanced hepatic fibrosis, which is easier to establish with some of the newer non-invasive imaging modalities [9,22], complications such as cirrhosis and hepatocellular carcinoma (HCC) are not uncommon. Development of hepatic fibrosis occurs in 40–50% of patients with non-alcoholic steatohepatitis (NASH) and current estimates are that approximately 30–40% of people with NAFLD develop NASH [23]. From a meta-analysis of 40 studies, it has been estimated that NASH increases the risk of liver-related mortality by ~5–10 fold (mainly depending on the degree of hepatic fibrosis present) [6]. With regard to this, Ekstedt *et al.* recently confirmed that hepatic fibrosis stage was the strongest predictor for all-cause and disease-specific mortality in patients with histologically confirmed NAFLD, who were followed-up for a mean period of 26.4 years [24]. In 2009, patients with NASH accounted for ~10% of patients undergoing liver transplantation in the United States; NASH is the third most common indication for liver transplantation in the United States, and considering the spectrum of disease encapsulated within NAFLD, NAFLD is on a trajectory to become the most common indication for liver transplantation [25].

It has been known for many years that obesity and T2DM increase the risk of HCC [26] but the biological explanation for this link remains uncertain. NASH is common in patients with obesity, insulin resistance and T2DM and NASH increases the risk of HCC [27]. That said, it is uncertain whether there is a diabetes/obesity-specific factor that increases risk of HCC or whether there are common pathological mechanisms that occur both in HCC and in T2DM/obesity. For HCC, it has been recently shown that incidence and mortality rates have increased approximately twofold in men and women between 1968 and 2008 [28,29]. Although it is less certain whether simple steatosis increases risk of HCC, it is now becoming clear that NASH is a risk factor for HCC, even in people without cirrhosis [30–32].

The underlying mechanisms by which NASH or T2DM increase risk of developing HCC are not completely understood, but mechanisms involved in liver inflammation, metabolic stress and insulin resistance that are shared between NASH and T2DM may be also involved in HCC development. Activation of certain metabolic or stress-response pathways, involving one-carbon metabolism, nuclear factor-kappa B (NF-κB), phosphatase and tensin homolog (PTEN), and microRNAs occur with HCC [33]. Many of these pathways involved in causing cellular stress also occur within tissues affected by obesity and insulin resistance [34], which are commonly linked and important in the pathogenesis of T2DM. It also has been suggested that chronic activation of toll-like receptor 4 (TLR-4) (i.e., an upstream activator of NF-κB signaling [35]) by gut microbiota-derived ligands, such as lipopolysaccharide may occur. Additionally, gut microbiota-mediated metabolism of bile acids has recently been implicated in HCC development [36]. Gut microbiota also metabolize primary bile acids produced by the liver to secondary bile acids such as deoxycholic acid. In a mouse model of HCC, when deoxycholic acid production was blocked by an inhibitor, HCC development was suppressed [36], implicating the interaction between the gut microbiota and bile production in the pathogenesis of HCC (Fig. 1).

Indeed, the possibility that alterations in the gut microbiota that promote the development of T2DM and NAFLD, and that induce increased risk of HCC, make the gut microbiota an attractive and potentially new therapeutic target in NAFLD (and possibly too in T2DM where there is obesity and insulin resistance). We and others are testing the effects of synbiotic treatment (a combined pro- and pre-biotic therapeutic agent) in NAFLD patients (see INvestigation of SYnbiotic TrEatment (INSYTE) in Non-Alcoholic Fatty Liver Disease (NAFLD), www.clinicaltrials.gov Registration number NCT 01680640). It is likely that further research over the next few years will clarify whether therapeutic modification of the gut microbiota is therapeutically desirable in reducing the development of liver fat and inflammation in the early stages of NAFLD. However, whether therapeutic modification of the gut flora decreases complications of NAFLD such as cirrhosis and HCC will take longer to establish.

NAFLD and diabetes: epidemiology

It has been known for many years that there is a substantial increased risk of mortality from cirrhosis of any aetiology in patients with T2DM [26,37], but the explanation for this increase in risk remains uncertain. Using a large electronic administrative database, we recently analyzed all information available in death certificates in an entire region in Italy to investigate the aetiology of chronic liver disease-associated mortality in people with diabetes (n = 167,621 diabetic individuals aged 30–89 years). Notably, we found that diabetic individuals had an approximately threefold higher risk of dying of chronic liver diseases, mainly associated with a non-virus and non-alcohol-related aetiology, which is largely attributable to NAFLD [38].

Some studies that have examined the relationship between NAFLD and T2DM have used simple biochemical and anthropometric measures as proxies for NAFLD. Serum levels of alanine transaminase (ALT) and gamma-glutamyltransferase (GGT) or the NAFLD fatty liver score and the fatty liver index, which are generated from anthropometric and biochemical measurements have been used as markers for NAFLD, and single biochemical tests are not good markers for assessing NAFLD severity or for guiding clinical management decisions [39]. With that caveat, and, as illustrated by recent work from France, studies that have used biochemical markers as proxies for NAFLD have shown that NAFLD is associated with incident T2DM, independently of multiple potential confounding factors [40]. However, because it is difficult to be certain that abnormal liver function tests are markers for NAFLD, we have excluded studies that have only used biochemical or anthropometric parameters to diagnose NAFLD from this review, since it is often not possible to prove that these subjects had NAFLD. For example, evidence is showing that serum GGT level in particular is a marker of oxidative stress rather than a specific marker of NAFLD-induced liver disease [41].

Among the studies that have used non-invasive imaging techniques (predominantly ultrasonography) to diagnose NAFLD and that have assessed the risk of developing T2DM [42–52], nearly all have shown that NAFLD increases substantially the risk of incident T2DM. As summarized in Table 1, risk of T2DM among these studies varied markedly from a 64% increase [50] to a 5.5-fold increase in risk [44]. This wide inter-study variation in risk might reflect differences in NAFLD severity, since the study by Park *et al.* [49] showed that the incidence rate of T2DM increased progressively according to the ultrasonographic severity of NAFLD at baseline (normal: 7.0%, mild: 9.8%, moderate-to-severe: 17.8%, p <0.001). Even after adjusting for multiple confounders, the hazard ratios (HRs) for T2DM development were significantly higher in the mild-NAFLD group (HR 1.09; 95% CI 0.81–1.48) and in the moderate-to-severe NAFLD group (1.73; 1.00–3.01) than in the no-NAFLD group, respectively. Additionally, the wide inter-study variation in risk of T2DM that was associated with NAFLD might also reflect differences in the number and type of covariates that have been adjusted for (Table 1).

Although there is now convincing evidence that NAFLD increases risk of T2DM, and there is emerging evidence that this risk varies according to NAFLD severity, further evidence is unquestionably needed in non-Asian populations, as all of the studies summarized in Table 1 are in various Asian populations, and most of the evidence obtained to date has been obtained in South Korean people. In addition, since the adjustment for potential confounders has been often incomplete, further prospective studies with a larger panel of established diabetes risk factors will be needed to firmly establish an independent contribution of NAFLD to the development of T2DM.

Although NAFLD is strongly associated with obesity, insulin resistance and T2DM, many people with NAFLD are not obese, and many people with NAFLD do not have T2DM. Consequently, as also shown in Table 1, we investigated the clustering of NAFLD, insulin resistance and obesity in people who developed incident T2DM. Specifically, we compared the impact of obesity, insulin resistance (estimated by homeostasis model assessment) and fatty liver (as detected by ultrasonography) on the risk of incident T2DM at 5-year follow-up in a cohort of over 12,000 South Korean individuals [43]. These data showed that each of these three risk factors was independently associated with increased T2DM risk, and each risk factor (independently) was associated with an approximate doubling of the T2DM risk after adjustment for other recognized risk factors. When all three risk factors occurred together in the same individual (and this occurred in ~50% of people with incident T2DM at follow-up),

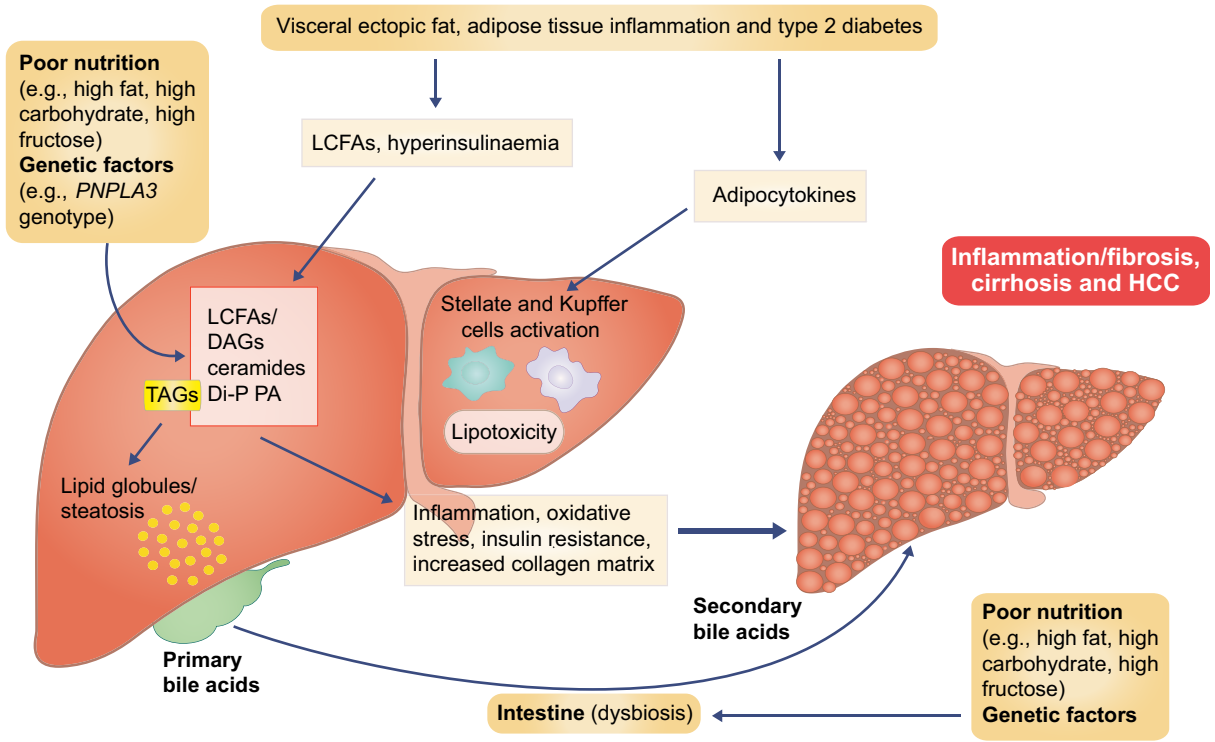

Fig. 1. The influences of visceral ectopic fat accumulation, adipose tissue inflammation, type 2 diabetes, diet and intestinal dysbiosis to promote the development of progressive liver disease in NAFLD. Visceral ectopic fat accumulation, which often occurs with inflammation and type 2 diabetes, causes resistance to insulin action and hepatic necro-inflammation (by Kupffer cell activation) with activation of hepatic stellate cells and increased production of collagen matrix and progression of liver disease. Progression of liver disease over an ill-defined period of time causes advanced liver fibrosis, cirrhosis and, in some cases, hepatocellular carcinoma. Poor diet (particularly high fat and high fructose intakes) along with genetic factors (e.g., *PNPLA3* polymorphisms) may also play a role in NAFLD progression increasing hepatic lipid accumulation and increasing risk of liver fibrosis. Alternations in the diet may cause dysbiosis of the gut microbiota with hepato-toxic effects from secondary bile acids. Dysbiosis may alter the production of short-chain fatty acids (from fermentation of dietary carbohydrate), and increase the production of lipopolysaccharide into the portal circulation (from egress of intestinal bacteria caused by increased intestinal permeability). Such effects create a pro-inflammatory hepatic stimulus that increases risk of progression of NAFLD. (DAG, di-acylglycerol; HCC, hepatocellular carcinoma; LCFAs, long-chain fatty acids; PNPLA3, patatin-like phospholipase domain-containing 3).

there was a fourteen-fold increase in risk of T2DM after adjustment for potential confounders. Notably, in the same observational cohort of Korean people, we further investigated the effect of resolution of fatty liver on risk of incident T2DM at 5-year follow-up to try and establish whether NAFLD improvements were associated with a risk reduction in incident T2DM. Interestingly, these data showed that there was a significant reduction in risk of incident T2DM in those subjects in whom fatty liver on ultrasound resolved over time. In particular, in those subjects, risk of incident T2DM decreased to the background risk of someone who had never had fatty liver [52]. Conversely, the individuals in whom the severity of fatty liver worsened over 5 years (from mild to moderate/severe) showed a marked increase in risk of incident T2DM [adjusted-OR 6.13 (95% CI 2.56–14.68) p <0.001 compared with the risk in people with resolution of fatty liver], supporting the notion that more severe forms of NAFLD are associated with higher risk of incident T2DM.

Rarely when studying the relationship between NAFLD and risk of T2DM, has NAFLD been confirmed, or severity assessed, by liver histology [23]. In a landmark paper from 2006, in a 13.7-year follow-up of 129 patients with biopsy-proven NAFLD, 42 patients with NASH at baseline returned for follow-up biopsy and of these, 30 patients (71%) had T2DM (diagnosed by either fasting glucose/2-h glucose level during an oral glucose tolerance test or by a clinical history of diabetes). Of the patients with simple steatosis at baseline, 21 (46%) had T2DM and this difference was statistically significant (p = 0.01), suggesting that patients with NASH have a higher risk of T2DM than those with simple steatosis.

Whether currently available biomarkers for NAFLD severity are useful for monitoring NAFLD progression (or regression) in people with T2DM is uncertain. Recently, the investigators of the Edinburgh Type 2 Diabetes Study investigated five non-invasive biomarkers to detect liver fibrosis and determined the level of agreement between them in 831 middle-aged patients with T2DM [53]. Patients underwent ultrasound assessment to diagnose the presence or absence of fatty liver. Additionally, measurements of the AST/ALT ratio, aspartate to platelet ratio index (APRI), Enhanced Liver Fibrosis panel (ELF), Fibrosis-4 score (FIB4) and liver stiffness measured by Fibroscan were undertaken. Based on data from the scientific literature, these investigators established thresholds for each of the five biomarkers for identifying liver fibrosis and then determined the level of agreement between the biomarkers to detect liver fibrosis in patients with T2DM. Agreement between the top 5% of the distribution for each biomarker pair was poor. APRI and FIB4 had the best positive agreement at 76.4%, but agreement for all of the other serum biomarker pairs was between 18% and 34%. Agreement with the liver stiffness measurement was also poor (9–16%). Using the top 5% of each biomarker there was good agreement between the biomarkers to exclude the presence of advanced

Table 1. Studies of associations between incident type 2 diabetes mellitus and a) incident fatty liver, b) existing (prevalent) fatty liver, c) increasing severity of fatty liver and d) resolution of fatty liver, diagnosed by non-invasive imaging only.

Study and year	Study design, sample size, population and mean follow up	Diagnosis of NAFLD	Diagnosis of diabetes	Regression modelling adjustments	Risk (HR, RR, OR) [95%CIs] for incident diabetes with incident NAFLD	Risk (HR, RR, OR) [95%CIs] for incident diabetes with prevalent, or existing NAFLD	Risk (HR, RR, OR) [95%CIs] for incident diabetes with worsening of, or more severe NAFLD	Risk (HR, RR, OR) [95%CIs] for incident of diabetes with resolution of NAFLD
Yamada *et al.* 2010 [42]	Retrospective 12,375 Japanese 5 years	Ultrasound	IFG = fasting glucose 6.1-7.0 mmol/L Diabetes = fasting glucose level ≥7.0 mmol/L	Adjusted for age, body mass index (BMI), elevated blood pressure or hypertension, alcohol drinking, smoking status	n.a.	ORs for impaired fasting glucose or diabetes OR 1.91 [1.56-2.34] men OR 2.15 [1.53-3.01] women	n.a.	n.a.
Sung *et al.* 2012 [43]	Retrospective 12,853 Korean 5 years	Ultrasound	Self-report of diabetes, or medical history, or fasting plasma glucose during follow-up	Adjusted for age and sex, alcohol, smoking status, exercise, and educational status, triglyceride, ALT	n.a.	OR 2.73 [1.38-5.41]	n.a.	n.a.
Shibata *et al.* 2007 [44]	Case/control 3189 Japanese 4 years	Ultrasound	Fasting glucose level ≥7.0 mmol/L or 2-h post-load plasma glucose level ≥11.1 mmol/L on a 75-g oral glucose tolerance test	Adjusted for age and BMI	n.a.	HR 5.5 [3.6-8.5]	n.a.	n.a.
Okamoto *et al.* 2003 [45]	Prospective 840 Japanese 10 years	Ultrasound	Fasting glucose ≥6.1 mmol/L or hemoglobin A1c (HbA1c) ≥6.5%	Age, sex, fasting glucose, HbA1c, BMI change during follow up, frequency of examinations, alcohol, family history diabetes	n.a.	OR 1.83 [0.95-3.51]	n.a.	n.a.
Kim *et al.* 2008 [46]	Retrospective 5372 Korean 5 years	Ultrasound	Fasting glucose level ≥7.0 mmol/L or treatments for diabetes.	Age, sex, family history of diabetes, smoking, blood pressure, fasting glucose, BMI, HDL-C, triglyceride, ALT	n.a.	RR 1.51 [1.04-2.20]	RR 2.29 [1.13-4.63] (excluding frequent drinkers = >3x/week)	n.a.
Fan *et al.* 2007 [47]	Case/control design 1146 Chinese 7 years	Ultrasound	Self reported use of diabetes medications and WHO criteria (1999)	Matched for age, sex, occupation, duration of follow up	n.a.	OR 4.63 [3.0-7.0]	n.a.	n.a.
Bae *et al.* 2011 [48]	Retrospective 7949 Korean 4 years	Ultrasound	Fasting glucose ≥7 mmol/L or HbA1C ≥6.5%	Adjusted for age, sex, smoking status, blood pressure, impaired fasting glucose, alcohol consumption, physical activity, BMI, HDL-C, triglycerides	n.a.	HR 1.33 [1.07-1.66]	n.a.	n.a.
Park *et al.* 2012 [49]	Prospective 25,232 Korean Men only 5 years	Ultrasound	Self-report, or medical history, or fasting plasma glucose, or HbA1c ≥6.5% during follow-up	Adjusted for baseline age, sex, waist circumference, insulin and glucose (HOMA-IR), triglyceride, HDL-C, systolic blood pressure, creatinine, hsCRP, family history of diabetes, exercise, MetS	n.a.	Mild fatty liver HR 1.09 [0.81-1.48]	Moderate to severe fatty liver HR 1.73 [1.00-3.01]	n.a.

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Table 1 (*continued*)

Study and year	Study design, sample size, population and mean follow up	Diagnosis of NAFLD	Diagnosis of diabetes	Regression modelling adjustments	Risk (HR, RR, OR) [95%CIs] for incident diabetes with incident NAFLD	Risk (HR, RR, OR) [95%CIs] for incident diabetes with prevalent, or existing NAFLD	Risk (HR, RR, OR) [95%CIs] for incident diabetes with worsening of, or more severe NAFLD	Risk (HR, RR, OR) [95%CIs] for incident of diabetes with resolution of NAFLD
Kasturiratne *et al.* 2013 [50]	Retrospective 2984 Sri Lankan 3 years	Ultrasound	Defined as a past history of diabetes mellitus or fasting glucose level >6.9 mmol/L (125 mg/dl) at baseline.	Adjusted for age, sex, family history of diabetes, waist circumference, hypertension, dyslipidemia, impaired fasting glucose, ALT, BMI	n.a.	HR 1.64 [1.20-1.23]	n.a.	n.a.
Chang *et al.* 2013 [51]	Retrospective 38,291 Korean 5 years	Ultrasound severity of NAFLD assessed with NAFLD fibrosis score.	Self-report, or medical history, or fasting glucose level, or HbA1c ≥6.5% during follow-up	Adjusted for baseline age, sex, waist circumference, insulin and glucose (HOMA-IR), triglyceride, HDL-C, systolic blood pressure, hsCRP, creatinine, family history of diabetes, exercise, MetS	n.a.	Mild fatty liver HR 2.00 [1.79-2.24]	Intermediate and severe fatty liver HR 4.74 [3.67-6.13]	n.a.
Sung *et al.* 2013 [52]	Retrospective 13,218 Korean 5 years	Ultrasound	Self-report, or medical history, or fasting glucose level during follow-up	Adjusted for baseline age, sex, BMI, glucose, insulin, baseline triglyceride, HDL-C, systolic blood pressure, alcohol, smoking, physical activity, and change in BMI between baseline and follow up ALT, AST and GGT	OR 2.49 [1.49-4.14]	Fatty liver at baseline and fatty liver at follow up OR 2.95 [1.91-4.54]	Fatty liver at baseline and worsening in severity at follow up OR 7.38 [3.36-16.22]	OR 0.95 [0.46-1.96]

n.a., not available.

Review

fibrosis, but poor agreement to diagnose advanced fibrosis. Thus, further research is clearly needed to find tools/tests for monitoring disease progression (or resolution) in people with NASH and T2DM, who are at highest risk of developing serious liver disease (namely cirrhosis, liver failure and HCC).

Diabetes development in NAFLD: pathophysiology

When imbalance occurs between energy intake and energy expenditure, or when there is an intrinsic problem with storing excess energy as lipid (triacylglycerol) in adipose tissue depots, lipid occurs in other organs throughout the body. When lipid occurs in tissues or organs not designed to accumulate fat, e.g., liver or omentum, the term 'ectopic fat accumulation' is often used to infer that lipid accumulation has occurred in another site besides adipose tissue [54].

NAFLD is an example of ectopic fat accumulation and this lipid accumulation is usually associated with increased secretion of hepatokines [55], increased gluconeogenesis, decreased glycogen synthesis and inhibition of insulin signaling [56,57]. When excess hepatic lipid accumulates, it often causes insulin resistance and chronic inflammation increasing risk of progressive liver disease with fibrosis, cirrhosis and increased risk of HCC (Fig. 1). Besides liver lipid metabolism, it is now clear that adipose tissue dysfunction/inflammation is crucial in NAFLD pathogenesis and increasing evidence is also now suggesting that dysbiosis of the gut microbiota plays a key role in regulating several intra-hepatic metabolic and inflammatory pathways that contribute to the development and progression of NAFLD. This is possibly through the increased intestinal absorption of multiple bacterial products, such as short-chain fatty acids (e.g., butyrate, propionate and acetate), lipopolysaccharide and endotoxins. For detailed reviews of the role of hepatic lipid accumulation and the gut microbiota in influencing insulin resistance and inflammation in NAFLD, see the following reviews [54,58,59]. Because of the constraints on space, this review will only consider briefly the mechanisms that are potentially involved in the pathogenesis of insulin resistance and inflammation in NAFLD.

Although obesity is strongly associated with hepatic steatosis, excess body fat accumulation is not '*a conditio sine qua non*' for developing NAFLD. In fact, patients with lipodystrophy have marked insulin resistance and commonly develop hepatic steatosis and T2DM, strongly suggesting that it is not body fat mass *per se* that is important, but it is adipose tissue dysfunction that is a key contributor to the pathogenesis of NAFLD [60]. Specifically, increased free fatty acid (FFA) fluxes from the adipose tissue pool increase the availability of long-chain fatty acyl-CoAs for hepatic lipid accumulation, particularly in physically inactive individuals [58], and evidence is accumulating that hepatic lipid accumulation is capable of causing hepatic/peripheral insulin resistance and promoting hepatic inflammation [54,58].

Expansion of peripheral adipose depots provides buffering capacity that may protect the liver from the excessive FFA fluxes that promote hepatic lipid accumulation. Within hepatocytes, long-chain fatty acids (LCFAs) are esterified with glycerol-3-phosphate (derived from glycolysis) to form mono-acylglycerols, di-acylglycerols (DAG) and tri-acylglycerols (TAG) (Fig. 2). Lipid synthesis may increase production of intermediates, such as DAG, di-palmitoyl phosphatic acid (Di-P PA) and other lipid products, such as ceramides; the increased production of these lipid products (particularly DAGs) is very important in causing 'resistance' within the hepatic insulin signaling pathway [61], promoting hepatic inflammation [62–65] and increasing risk of progressive liver disease that occurs with NASH. In the liver, production of ceramides utilizes LCFAs [66], and ceramides can accumulate into the cells via three main routes: 1) the hydrolysis of the membrane phospholipid sphingomyelin, which is coordinated by the enzyme sphingomyelinase; 2) *de novo* synthesis from LCFAs such as palmitate and serine; and 3) a 'salvage' pathway that utilizes sphingosine and forms ceramide [67,68]. Although in the past, it was thought that ceramide was simply a structural molecule, there is some evidence that an increase in membrane ceramide cause insulin resistance (see review [69] and Fig. 2).

The production of DAG has been implemented as a cause of hepatic insulin resistance and the conversion from TAG to DAG is mediated by adipose triglyceride lipase (ATGL). Comparative Gene Identification-58 (CGI-58) is an activator of ATGL and DAG activates protein kinase Cε (PKCε) membrane translocation to inhibit the insulin receptor kinase and decrease insulin signaling [70] (Fig. 2). Hepatic lipids that are not esterified also induce endoplasmic reticulum stress, leading to the activation of c-Jun N-terminal kinases and NF-κB [71], which are two major regulators of inflammatory pathways that also inhibit phosphorylation of insulin receptor substrate-1 (IRS-1) [72], potentially aggravating hepatic insulin resistance and increasing intra-hepatic cytokine production (Fig. 2). Synthesis of lipids such as DAGs is intimately related to inflammatory pathways, and DAGs may also contribute to hepatic production of inflammatory cytokines [e.g., tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6)], and procoagulant factors [e.g., factor VIII, plasminogen activator inhibitor-1 (PAI-1)]. Additionally, intestinal microbiota dysbiosis, perhaps induced by alterations in the diet (Fig. 1), may affect other hepatic lipid pathways, such as those involving bile acid metabolism, consequently increasing hepatic inflammation and fibrosis, and resulting in an increased risk of developing cirrhosis and HCC.

To date, it is unclear whether improvements in NAFLD may ameliorate risk of T2DM or improve glycaemic control in people with NAFLD who have developed T2DM, but it is plausible that resolution of liver fat and improvements in liver lipid metabolism might modify the risk of T2DM via a liver-specific effect. Such a liver-specific effect could be mediated by alteration in the secretion of multiple hepatokines [55] or inflammatory cytokines that influence risk of diabetes. In NAFLD, secretion of diabetogenic hepatokines, such as retinol binding protein (RBP)-4, fetuin-A, fibroblast growth factor (FGF)-21; or inflammatory biomarkers such as C-reactive protein (CRP), TNF-α and IL-6 [73] may directly affect risk of incident T2DM by adversely affecting hepatic gluconeogenesis, glycogen synthesis [56,57] and insulin signaling [74].

NAFLD, CVD and other cardiac diseases: epidemiology

NAFLD and CVD

Patients with NAFLD usually have features of the metabolic syndrome (MetS) and also have a myriad of other emerging CVD risk factors [18,75,76]. This finding has important clinical implications for the development of future CVD events among these patients.

A recent comprehensive meta-analysis of 27 cross-sectional studies reported a strong association between NAFLD detected

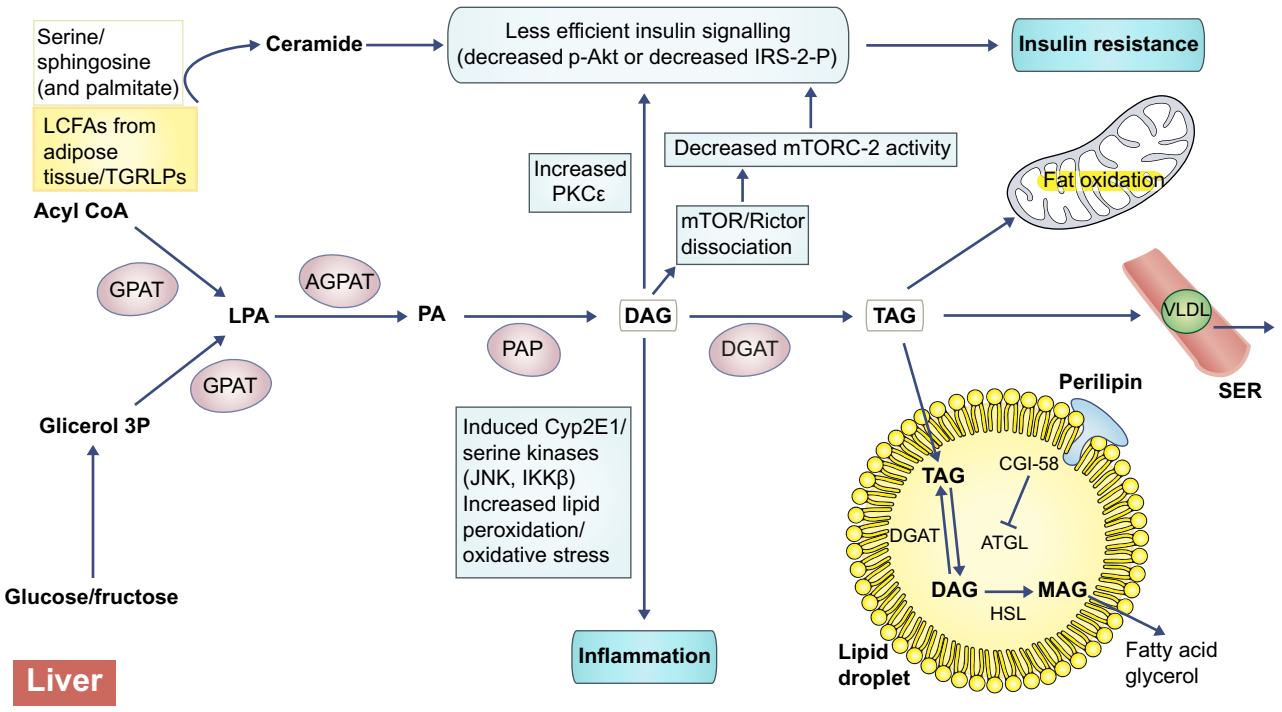

Fig. 2. Lipid induced mechanisms contributing to hepatic insulin resistance and inflammation in NAFLD. Synthesis of lipid intermediates from long-chain fatty acids (LCFAs), e.g., ceramide, lyso-phosphatidic acid, phosphatidic acid (PA), di-acylglycerol (DAG), tri-acylglycerol (TAG) and very-low-density lipoprotein (VLDL) secretion. Synthesis of various species of DAG in particular may promote hepatic insulin resistance and inflammation. Synthesis of ceramide may also increase resistance to insulin action by decreasing efficient insulin signaling. (AGAT, acyl glycerol acyl transferase; AGPAT, acylglycerol phosphate acyltransferase; CGI-58, Comparative Gene Identification-58; Cytochrome P450 2E1; CYP 2E1; DGAT, di-acyl glycerol acyl transferase; IKKβ, IκB kinase β; IRS-2-P, insulin receptor substrate-2 phosphorylation; JNK, c-Jun N-terminal kinase; LPA, lysophosphatidic acid; GPAT, glycerol-3-phosphate acyltransferase; MAG, mono-acyl glycerol; mTOR, mechanistic target of rapamycin; mTORC-2, mechanistic target of rapamycin complex-2; PA, phosphatidic acid; PAP, phosphatidate phosphohydrolase; PKCε, protein kinase Cε; TGRLPs, triglyceride-rich lipoproteins).

by imaging or biopsy and several markers of subclinical atherosclerosis, such as increased carotid intima-media thickness (16 studies), increased coronary artery calcification (7 studies), impaired flow-mediated vasodilation (7 studies) and arterial stiffness (6 studies). All of these associations were independent of classical CVD risk factors and MetS features across a wide range of patient populations [77]. Recently, in a cohort of 755 consecutive otherwise healthy adult men, Moon *et al.* also reported a strong and independent association between NAFLD and carotid artery inflammation (which may reflect plaque vulnerability), as evaluated by ^{18}F-fluorodeoxyglucose positron emission tomography [78].

Several large cross-sectional population and hospital-based studies, involving both patients without diabetes and those with diabetes, have consistently shown that the prevalence of clinical CVD is increased in patients with NAFLD (for more detailed reviews see [75,76,79]). For example, in a national-based cohort of over 11,000 United States adults, NAFLD was associated with an increased prevalence of CVD, independently of multiple CVD risk factors [80]. Similarly, in a large outpatient-cohort study of patients with T2DM, the prevalence of coronary, cerebrovascular and peripheral vascular disease was greater among those with NAFLD than among those without this disease, independently of traditional CVD risk factors, medication use and diabetes-related variables [81]. Finally, in patients referred for clinical coronary angiography, NAFLD was independently associated with increased severity of coronary artery disease [82–84].

To date, there are about 20 retrospective and prospective studies that have assessed the relationship between NAFLD diagnosed on biopsy or imaging and the risk of developing fatal and nonfatal CVD events [23,24,80,82,85–98]. As summarized in Table 2, most of these studies support the notion that CVD is a serious threat to patients with NAFLD. In this table we did not include the large population-based cohort studies that used serum liver enzymes (i.e., surrogate markers of NAFLD) to diagnose NAFLD and that confirmed that mildly elevated serum liver enzyme levels were independent, long-term predictors of incident CVD both in men and in women [75,79].

With regard to biopsy-diagnosed NAFLD (as shown in Table 2) [23,24,88–90,97,98], some retrospective studies with reasonably long follow-up have clearly shown that all-cause, CVD and liver-related mortality were significantly higher in NAFLD patients than in matched control populations. These studies have also shown that the presence and severity of hepatic fibrosis is the main determinant of all-cause and cause-specific mortality, and that CVD is a very common cause of mortality among these patients. Interestingly, some of these studies also reported that patients with NASH, but not those with simple steatosis, had [23,88] an increased risk of all-cause and CVD mortality compared with the reference population. However, it should be noted that all of these studies were retrospective cohort studies with relatively small numbers of patients, who were seen at tertiary care referral centers and full adjustment for potential confounders was not possible. Undoubtedly, all these features limit

Table 2. Principal prospective and retrospective studies of the risk of CVD mortality and morbidity in patients with NAFLD (defined by biopsy or imaging only).

Study and year	Study design, sample size, population and mean follow up	Diagnosis of NAFLD	Main findings
Ekstedt M *et al.* 2006 [23]	Retrospective cohort 129 Swedish NAFLD patients, 13.7 years	Biopsy*	Patients with NASH, but not those with simple steatosis, had higher rates of all-cause (~2-fold), CVD (~2-fold) and liver-related (~10-fold) mortality than the general population matched for age and sex
Ekstedt M *et al.* 2014 [24]	Retrospective cohort 229 Swedish NAFLD patients, 26.4 years	Biopsy*	NAFLD patients had increased risk of death (HR 1.29, 95% CI 1.04-1.59), with a high risk of death from CVD (HR 1.55, 95% CI 1.11-2.15) and liver-related disease (HR 3.2, 95% CI 1.05-9.81). NAFLD activity score (NAS) was not able to predict all-cause death, whereas fibrosis stage predicted all-cause, CVD and liver-related death
Stepanova M *et al.* 2012 [80] and Lazo M *et al.* 2011 [91]	National-based cohort 11,371 US adults from the Third National Health and Nutrition Examination Survey 1988-94, 14.5 years	Ultrasound*	No significant association between NAFLD and all-cause and cause-specific (CVD, cancer and liver) mortality
Wong VW *et al.* 2011 [82]	Prospective cohort 465 Chinese patients with coronary heart disease as diagnosed by coronary angiography, 1.8 years	Ultrasound‖	NAFLD was independently associated with an increased prevalence of CVD at baseline but there was no significant association between NAFLD and risk of incident CVD events
Zhou YJ *et al.* 2012 [85]	Community-based cohort 3543 Chinese adult individuals, 4 years	Ultrasound*	Patients with NAFLD had ~3-fold higher rates of all-cause and CVD mortality than those without NAFLD
Treeprasertsuk S *et al.* 2012 [86]	Retrospective community-based cohort 309 US NAFLD patients, 11.5 years	Ultrasound/ computed tomography¶	Framingham risk score accurately predicted the higher 10-year coronary heart disease risk in NAFLD patients and was the only variable significantly associated with the risk of developing new-onset coronary heart disease events in this patient cohort
Targher G *et al.* 2007 [87]	Prospective cohort 2103 Italian outpatients with type 2 diabetes without viral hepatitis and CVD at baseline, 6.5 years	Ultrasound§	NAFLD was associated with an increased risk of fatal and nonfatal CVD events (HR 1.87, 95% CI 1.2-2.6), independently of age, sex, body mass index, smoking, diabetes duration, hemoglobin A1c, LDL-cholesterol, metabolic syndrome features, medication use
Söderberg C *et al.* 2010 [88]	Retrospective cohort 118 Swedish NAFLD patients, 24 years	Biopsy*	Patients with NASH, but not those with simple steatosis, had higher rates of all-cause (~2-fold), CVD (~2-fold) and liver-related mortality than the general population matched for age and sex
Rafiq N *et al.* 2009 [89]	Retrospective cohort 173 US NAFLD patients, 13 years	Biopsy*	CVD, cancer and liver-related complications were the most common causes of mortality in this cohort of NAFLD patients
Matteoni CA *et al.* 1999 [90]	Retrospective cohort 132 US NAFLD patients, 18 years	Biopsy*	Patients with NASH had higher rates of all-cause and liver-related mortality than those without the disease. CVD mortality did not differ between the groups
Kim D *et al.* 2013 [92]	National-based cohort study 11,154 US adults from the Third National Health and Nutrition Examination Survey, 14.5 years	Ultrasound and advanced fibrosis score systems *	NAFLD was not associated with increased all-cause mortality. However, NAFLD with advanced hepatic fibrosis (defined by NAFLD fibrosis score, APRI index or FIB-4) was independently associated with a 69% increased risk of all-cause mortality. Increase in mortality was almost entirely from CVD causes (for NFS: HR 3.46, 95% CI 1.91-6.25; for APRI: HR 2.53, 95% CI 1.33-4.83; for FIB-4: HR 2.68, 95% CI 1.44-4.99)
Jepsen P *et al.* 2003 [93]	Retrospective cohort 1804 Danish hospitalized patients with NAFLD, 6.2 years	Ultrasound*	Patients with NAFLD had higher rates of all-cause (2.6-fold), CVD (2.1-fold) and liver-related (19.7-fold) mortality than the general population
Haring R *et al.* 2009 [94]	Population-based cohort 4160 German individuals, 7.3 years	Ultrasound*	NAFLD was associated with an increased risk of all-cause and CVD (HR 6.22, 95% CI 1.2-31.6) mortality in men, independently of age, sex, waist circumference, alcohol consumption, physical activity, civil status, equalized income, functional comorbidity index, blood pressure, diabetes status
Hamaguchi M *et al.* 2007 [95]	Community-based cohort 1637 Japanese individuals, 5 years	Ultrasound‡	NAFLD was associated with an increased risk of nonfatal CVD events (HR 4.10, 95% CI 1.6-10.7), independently of age, sex, body mass index, alcohol intake, smoking history, LDL-cholesterol and metabolic syndrome features
Dunn MA *et al.* 2013 [96]	Retrospective cohort 2343 US type 2 diabetics seen in the primary care and specialty clinics of a large integrated delivery network, 5 years	Computed tomography§	No significant association was found between NAFLD and risk of all-cause mortality and cause-specific (CVD, cancer and liver) mortality and morbidity. NAFLD patients (steatosis >30% on imaging) averaged 8 years younger than those without NAFLD
Dam-Larsen S *et al.* 2004 [97]	Retrospective cohort 109 Danish NAFLD patients (without NASH at baseline), 16.7 years	Biopsy*	No significant difference in mortality rates between patients with simple steatosis and the general population
Adams LA *et al.* 2005 [98]	Retrospective cohort 420 US NAFLD patients, 7.6 years	Biopsy/imaging*	Patients with NAFLD (especially those with cirrhosis and NASH) had higher rates of all-cause, CVD and liver-related mortality than the age and sex-matched general population

*Study outcome was all-cause and cause-specific mortality.
‡Study outcome was nonfatal coronary heart disease and stroke.
§Study outcome was a combined end point of CVD mortality and nonfatal myocardial infarction, ischemic stroke and coronary revascularization procedures.
‖Study outcome was a combined end point of CVD mortality and nonfatal myocardial infarction and coronary revascularization procedures.
¶Study outcome was a combined end point of CVD mortality and nonfatal congestive heart failure, angina, myocardial infarction and coronary revascularization procedures.

the generalizability of the findings to a broader patient population.

With regard to imaging-diagnosed NAFLD [80,82,85–87,91–96], several large prospective studies have consistently shown that NAFLD is associated with an increased risk of fatal and non-fatal CVD events, independently of established CVD risk factors both in individuals with, and without T2DM. In contrast, and surprisingly, two recent studies, using data from the Third National Health and Examination Survey (NHANES-III) database of over 11,000 United States adults, have reported that NAFLD was significantly associated with increased CVD prevalence but did not predict the risk of all-cause and cause-specific mortality over 14 years of follow-up [80,91]. The results of these studies may have been influenced by the inclusion of individuals with mild hepatic steatosis within the control arm. Interestingly, however, the latest analyses of the same NHANES-III database found that NAFLD with advanced hepatic fibrosis (defined by non-invasive scoring systems) was independently associated with a ~70% increased risk of all-cause mortality, and that this increase in mortality was almost entirely due to CVD causes [92]. A meta-analysis published in 2011 concluded that patients with NAFLD (diagnosed by imaging or biopsy) had a twofold higher risk of fatal and non-fatal CVD events (OR 2.05, 95% CI 1.81–2.31) than the matched control population, but that the severity of NAFLD histology did not further increase CVD mortality [6]. However, further larger and longer follow-up studies in patients with biopsy-confirmed NAFLD are needed in order to improve understanding and establish whether NAFLD severity affects risk of CVD events. It is important to note that similar to those studies investigating the relationship between NAFLD and diabetes, many of these studies have not been able to adjust for a full range of potential life-style factors and co-morbidities that would have varied among studies but may have impacted on the study results. For example, certain life-style factors may have had a positive effect on NAFLD (e.g., coffee intake, Mediterranean diet). Additionally, the management of certain co-morbidities may also have had a positive effect on NAFLD (e.g., angiotensin converting enzyme inhibitor or angiotensin II receptor blockers as treatments for hypertension, statins for dyslipidaemia, and glucagon-like peptide-1 agonists for T2DM). In addition, lifetime smoking and alcohol intake are not usually considered in many studies that have examined relationships between NAFLD and extra-hepatic disease outcomes.

NAFLD and abnormalities in myocardial metabolism, cardiac function and structure

It is now becoming increasingly evident that NAFLD is associated with abnormalities in myocardial metabolism; for a more detailed review see [79].

Using cardiac magnetic resonance imaging (MRI), Perseghin *et al.* firstly reported that nonobese, nondiabetic, normotensive, young individuals with NAFLD had impaired myocardial energy metabolism (i.e., a lower phosphocreatine/adenosine triphosphate ratio, as measured by ^{31}P-magnetic resonance spectroscopy [MRS]) and excessive fat accumulation in the epicardial area compared with matched control subjects without NAFLD. Interestingly, these myocardial metabolic alterations were detected despite normal left ventricular (LV) morphological features and systolic and diastolic functions [99]. Lautamaki *et al.* [100] and Rijzewijk *et al.* [101] found that T2DM patients with higher intra-hepatic fat content on ^{1}H-MRS had increased myocardial insulin resistance and decreased myocardial perfusion compared with those with lower intra-hepatic fat content; additionally, myocardial insulin resistance was more severe among those with higher intra-hepatic fat content even after adjustment for potential confounders. Again, Rijzewijk *et al.* [102] found that those with higher intra-hepatic fat content had significantly higher myocardial fat content (i.e., cardiac steatosis). Interestingly, in this study cardiac steatosis was a strong predictor of LV diastolic dysfunction [102].

To date, there are plentiful data linking NAFLD with abnormalities in cardiac structure and function both in adolescents and in adults with, or without, co-existing MetS features [79]. For instance, in a small case-control study examining cardiac status by MRI and ^{31}P-MRS in adults with ^{1}H-MRS-diagnosed NAFLD, Hallsworth *et al.* [103] have reported significant changes in cardiac structure and evidence of early LV diastolic dysfunction compared with age-, sex- and body mass index-matched controls, in the absence of cardiac metabolic changes or overt cardiac disease. In a study of T2DM adults without history of CVD and known hepatic diseases, Bonapace *et al.* [104] found that early features of LV diastolic dysfunction could be detected by tissue doppler imaging in those with NAFLD, even if the LV morphology and systolic function were preserved. In addition, there was a positive, graded relationship between the ultrasonographic severity of NAFLD and diastolic dysfunction, independently of hypertension, glycaemic control and other co-existing CVD risk factors [104]. Furthermore, in a community-based cohort of 1886 Korean adults, Kim *et al.* found that ultrasound-diagnosed NAFLD was associated with LV diastolic dysfunction, independently of MetS features and other established CVD risk factors [105].

Interestingly, and most importantly, similar findings have been confirmed also in pediatric NAFLD. Indeed, a number of case-control studies reported that overweight or obese children with NAFLD had echocardiographic features of early LV dysfunction compared with their counterparts without NAFLD [106–110]. These myocardial functional abnormalities were independent of multiple CVD risk factors. Notably, in the study by Pacifico *et al.* [110] when the group of obese children was divided according to the presence of NASH, it was evident that some functional cardiac differences were more pronounced in those with NASH. These observations are intriguing and need to be further investigated. It is important to understand, for example, whether hepatic necro-inflammation or fibrosis *per se* might have a stronger association with the cardiac phenotype than any other hepatic alterations. This is important because it is remarkable that these myocardial changes occur long before the onset of cirrhosis and portal hypertension, suggesting that the cardiac alterations may not be the consequence of the changes in intra-hepatic haemodynamic conditions.

Overall, therefore, from the above-mentioned published studies it is plausible to assume that patients with NAFLD have early changes in myocardial substrate metabolism (e.g., impaired high-energy phosphate metabolism, insulin resistance), producing cardiac functional and structural consequences (e.g., LV dysfunction and hypertrophy) that are potentially linked to an increased risk of congestive heart failure (HF) in this group of patients. With regards to this, two large population-based cohort studies that used elevated serum liver enzymes for diagnosing NAFLD have recently shown that this disease is independently associated with

an increased incidence of HF [111,112]. However, further follow-up studies in well-characterized cohorts of NAFLD patients are needed to better examine the individual contribution of NAFLD to the increased incidence of HF.

NAFLD, cardiac arrhythmias and aortic valve sclerosis

Recently, mildly elevated liver transaminases have been shown to be independently associated with increased incidence of atrial fibrillation (AF) in the Framingham Heart Study cohort [113]. A similar link between elevated serum liver enzymes (mainly serum GGT level) and AF risk was shown in a larger prospective community-based study of 9333 subjects with a mean follow-up of 12 years [114]. More direct evidence of increased risk of AF associated with NAFLD has been recently reported by our group [115,116]. In a case-control study, we found that ultrasound-diagnosed NAFLD was associated with an increased prevalence of AF in hospitalized patients with T2DM and this association was independent of several clinical AF risk factors [115]. In a prospective study, we found that T2DM patients with NAFLD were more likely to develop incident AF over 10 years of follow-up than their counterparts without NAFLD, and that ultrasound-diagnosed NAFLD was associated with a fivefold increased risk of incident AF, independently of MetS features and other common AF risk factors [116].

Interestingly, recent data [117] have shown that NAFLD is also independently linked with prolonged QTc interval, i.e., a powerful predictor of ventricular arrhythmias and sudden cardiac death [118,119], which might explain in part the increased CVD mortality associated with NAFLD. Additionally, in a small study of people without diabetes with histologically proven, non-cirrhotic NAFLD, and an age-, sex- and body mass index-matched control group, there was evidence of cardiac autonomic dysfunction, presenting as orthostatic hypotension, vasovagal syncope or a relative nocturnal hypotension [120].

Finally, the presence of aortic valve sclerosis, i.e., a progressive disease that shares multiple pathogenic risk factors with CVD and is associated with an increased risk of CVD mortality [121,122], has also been linked with NAFLD, independently of established CVD risk factors, in both diabetic and nondiabetic individuals [123,124].

Collectively, as discussed below, although not all data are methodologically solid and most of the studies lack an histological diagnosis of NAFLD, the concept of NAFLD as being an independent contributor to the development of atherosclerosis and other structural and functional cardiac alterations, which subsequently lead to clinical CVD, seems sufficiently substantiated by the current evidence to integrate it in the clinical approach of both the patient with NAFLD and the patient with CVD.

NAFLD and chronic kidney disease: epidemiology

The possible link between NAFLD and CKD has recently attracted considerable scientific interest [1,125,126].

Several large cross-sectional population and hospital-based studies, involving both adults without diabetes and patients with diabetes, have shown that the prevalence of CKD (defined as either decreased estimated glomerular filtration rate [GFR] and/or overt proteinuria) is increased in people with NAFLD [127–136]. These studies have used either ultrasonography or biopsy to diagnose NAFLD and have excluded patients with end-stage renal disease, cirrhosis and those with known causes of chronic liver disease (alcohol abuse, viral hepatitis and use of hepato-toxic drugs in all studies and also hemochromatosis and autoimmune hepatitis in some studies). In these studies, the prevalence of CKD in patients with NAFLD ranged from approximately 20–55% compared to 5–35% in patients without NAFLD. Importantly, most of these studies, including those that used liver biopsy to diagnose NAFLD, reported that the presence and severity of NAFLD was associated with CKD stages, independently of established cardio-renal risk factors [133–136]. These data have been extensively reviewed by our group elsewhere [125,126].

To date, there is a paucity of published data regarding the risk of developing CKD in patients with NAFLD. As summarized in Table 3, four observational studies with reasonably long follow-up have assessed the relationship between NAFLD and the risk of incident CKD [137–140]. In all studies, patients with overt cirrhosis or secondary causes of chronic liver diseases were excluded. In addition, in the two studies published by our group, no participants had ultrasonographic findings suggestive of cirrhosis [137,140].

It is important to note that in all these studies, NAFLD was diagnosed by ultrasonography and the investigators have used a creatinine-based GFR estimating equation instead of a direct GFR measurement to define CKD. The use of direct measurements of GFR should be encouraged as the creatinine-based equations are not accurate in estimating GFR, especially for patients with severe obesity or cirrhosis [141]. As shown in Table 3, it is also important to note that the investigators have used varying degrees of baseline adjustments for potential confounders. None of these published studies have specifically assessed whether a change in NAFLD status (either development of new fatty liver, progression to cirrhosis, or resolution of existing fatty liver) during the follow-up period modified the risk of incident CKD. Finally, no detailed information is available in these studies about specific renal pathology/morphology associated with NAFLD. Notwithstanding these limitations, the published prospective studies [137–140] have consistently reported an independent association between NAFLD and increased risk of incident CKD with HRs for CKD that ranged from approximately 1.3–1.9.

Very recently, in a well-conducted systematic review and meta-analysis (63,902 participants, 20 cross-sectional and 13 longitudinal studies included), Musso *et al.* [7], confirmed that NAFLD as diagnosed by histology, imaging or liver enzyme elevation was significantly associated with an increased risk of prevalent (OR 2.12, 95% CI 1.69–2.66) and incident CKD (HR 1.79, 95% CI 1.65–1.95). Additionally, NASH was associated with a higher prevalence (OR 2.53, 95% CI 1.58–4.05) and incidence (HR 2.12, 95% CI 1.42–3.17) of CKD than simple steatosis [7].

However, further longer prospective studies in larger cohorts of patients with biopsy-proven NAFLD are needed to confirm these findings, and to determine whether improvement in NAFLD (or future treatments for NAFLD) ultimately will prevent or delay the development and progression of CKD. Moreover, because CKD has many potential causes, it also will be of great interest to characterize the renal injury manifestations associated with NAFLD and clarify, in the future, whether NAFLD may selectively contribute to the pathogenesis of different types of kidney disease.

Table 3. Principal prospective and retrospective studies of the risk of developing CKD in patients with NAFLD (defined by imaging only).

Study and year	Study design, sample size, population and follow up	Diagnosis of NAFLD	Main findings
Targher G *et al.* 2008 [137]	Prospective cohort 1760 Italian outpatients with type 2 diabetes with preserved kidney function (mean baseline eGFR 94 ± 10 ml/min/1.73 m^2) and not proteinuria, who did not have baseline CVD, cirrhosis or viral hepatitis 6.5 years	Ultrasound*	547 subjects developed incident CKD. Cumulative incidence of CKD was significantly higher in those with NAFLD than in those without NAFLD (48% *vs.* 29%). NAFLD was associated with increased risk of incident of CKD (HR 1.49, 95% CI 1.1-2.2) independently of sex, age, body mass index, waist circumference, blood pressure, smoking status, diabetes duration, hemoglobin A1c, triglycerides, HDL-cholesterol, LDL-cholesterol, baseline eGFR, and use of medications
Chang Y *et al.* 2008 [138]	Community-based cohort 8329 non-diabetic and non-hypertensive Korean men with normal kidney function (median baseline eGFR 79 ml/min/1.73 m^2) and not proteinuria at baseline 3.2 years	Ultrasound*	324 subjects developed incident CKD during follow-up. NAFLD was associated with increased risk of incident of CKD (HR 1.60, 95% CI 1.3-2.0), independently of age, body mass index, alcohol consumption, blood pressure, smoking status, fasting glucose, baseline eGFR, triglycerides, HDL-cholesterol, LDL-cholesterol, insulin resistance, C-reactive protein, and incident cases of hypertension and diabetes. Consistent results were observed in all subgroups analysed
Arase Y *et al.* 2011 [139]	Retrospective cohort 5561 Japanese patients with NAFLD and no CKD at baseline (mean eGFR 75 ± 12 ml/min/1.73 m^2 and not overt proteinuria) 5.5 years	Ultrasound*	263 subjects developed incident CKD during follow-up. Among patients with NAFLD, elevated serum gamma-glutamyltransferase level was associated with increased risk of incident of CKD (HR 1.35, 95% CI 1.02-1.8), independently of age, sex, hypertension, diabetes, total cholesterol, triglycerides, HDL-cholesterol, aminotransferases, gamma-glutamyltransferase, hemoglobin, white blood cells, platelets, baseline eGFR
Targher G *et al.* 2014 [140]	Prospective cohort 261 Italian adult outpatients with type 1 diabetes with preserved kidney function (mean baseline eGFR 92 ± 23 ml/min/1.73 m^2) and not macroalbuminuria, who did not have baseline CVD, cirrhosis or viral hepatitis 5.2 years	Ultrasound*	61 subjects developed incident CKD during follow-up. Cumulative incidence of CKD was significantly higher in those with NAFLD than in those without NAFLD (35% *vs.* 11%). NAFLD was associated with increased risk of incident of CKD (HR 1.85, 95% CI 1.03-3.3), independently of age, sex, diabetes duration, hemoglobin A1c, hypertension, baseline eGFR, microalbuminuria. Addition of NAFLD to traditional cardio-renal risk factors improved the discriminatory capability of the regression models for predicting incident CKD

*Study outcome was new-onset CKD defined as occurrence of estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 [eGFR was estimated by using the MDRD (Modification of Diet in Renal Disease) Study equation] and/or overt proteinuria.

NAFLD, cardiovascular/cardiac diseases and CKD: pathophysiology

It is beyond the scope of this brief review to discuss in detail the pathophysiological links between NAFLD, cardiovascular/cardiac complications as well as the links between NAFLD and CKD. Detailed discussions of this topic have been published elsewhere [75,76,79,125,126,142]. To date, there is uncertainty as to whether NAFLD is simply a marker or a mediator (pathogenic factor) of cardiovascular/cardiac diseases and CKD. Moreover, uncertainty also exists about the prognostic value of NAFLD in risk stratification for CVD and CKD.

However, although larger, long-term prospective studies are needed, we consider that increasing evidence supports the assertion that NAFLD is not simply a marker but also a pathogenic factor (and probably both) of vascular/cardiac and kidney damage. From the data available in the literature, the association of NAFLD with CKD and especially that with CVD seems to have strength, consistency, specificity, temporality and biological plausibility, satisfying many of the established criteria for a causal relationship.

In respect to mechanisms how NAFLD impacts on cardiovascular/cardiac diseases and CKD, it is important to underline that a clear understanding of the pathophysiological pathways that link NAFLD to the development and progression of these extra-hepatic complications remains lacking because of the intricate biological interactions between NAFLD, visceral obesity and insulin resistance. NAFLD, cardiovascular/cardiac diseases and CKD share many metabolic features and risk factors, leading to the concept that they belong to a complex multisystem disease with several organ manifestations and a complex interplay between the different diseases, with multiple bidirectional cause-effect relationships. The specific contribution of one disease to the others is therefore difficult to discern, and there might be substantial inter-individual variability.

It is likely that there is a pathogenic "cross-talk" between the liver and the expanded and 'dysfunctional' (inflamed) adipose tissue [1,18,58,72,75]. As schematically shown in Fig. 3, the putative underlying mechanisms that link NAFLD, CKD, CVD and other cardiac diseases probably have their origin in expanded and inflamed visceral adipose tissue. This adipose tissue secretes multiple factors that are potentially involved both in the

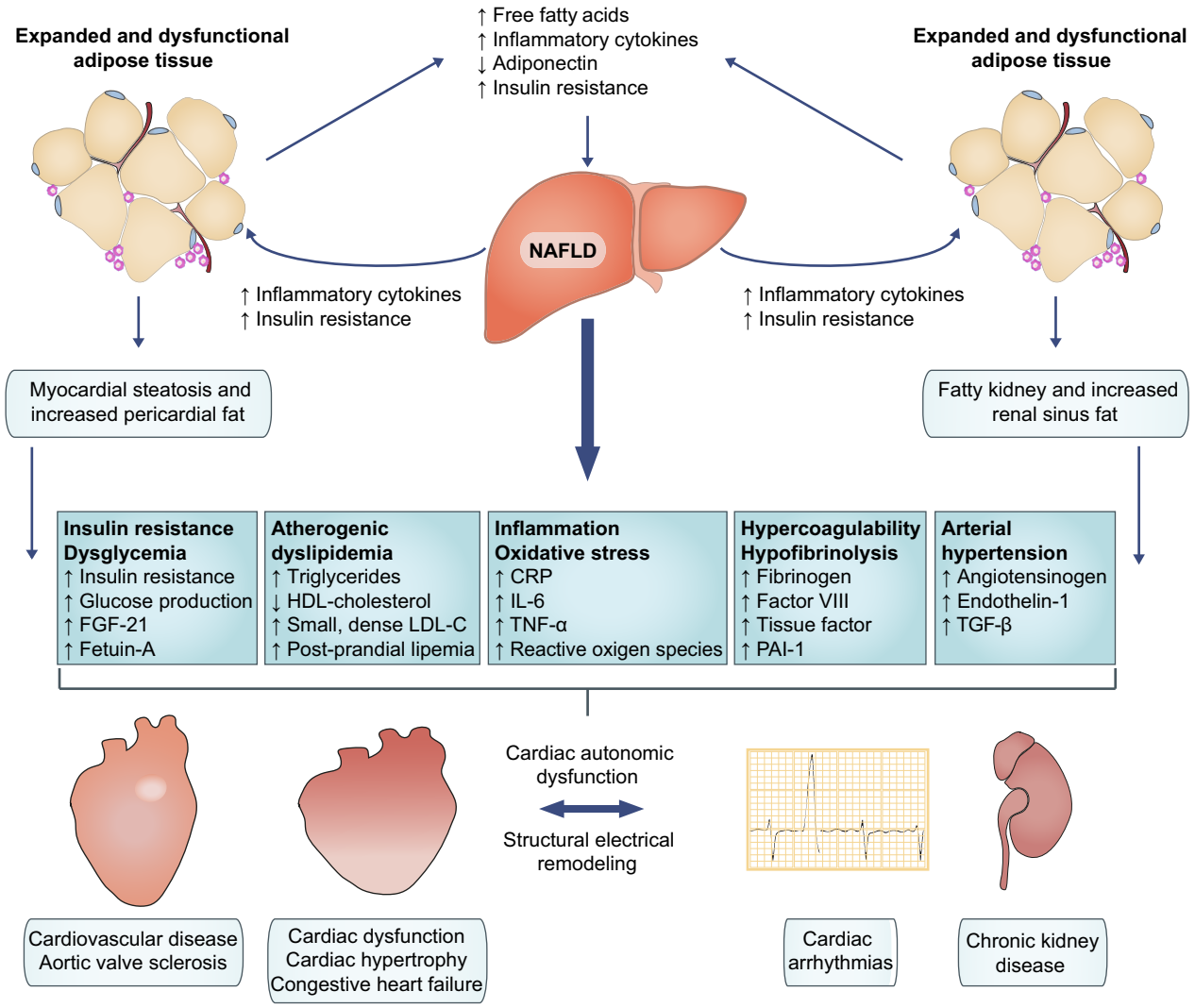

Fig. 3. Schematic representation of the putative mechanisms underlying the contribution of NAFLD to the increased risk of cardiovascular disease (CVD), chronic kidney disease (CKD) and other structural and arrhythmic cardiac complications. The complex and intertwined interactions among NAFLD, abdominal obesity and insulin resistance make it extremely difficult to dissect out the specific role of the liver and the underlying mechanisms responsible for the association between NAFLD and the risk of developing CVD, CKD and other structural cardiac complications (i.e., aortic valve sclerosis, cardiac dysfunction/hypertrophy, congestive heart failure and atrial fibrillation). NAFLD might be associated with such complications either as a consequence of shared cardio-metabolic risk factors and co-morbidities or as a marker of ectopic fat accumulation in other organs. For instance, myocardial steatosis and increased pericardial fat volume as well as fatty kidney and increased renal sinus fat volume may exert local adverse effects that result in structural and functional derangements of the myocardium and kidneys. However, in this dangerous and intricate scenario, growing evidence indicates that NAFLD is not only a simple marker of vascular/cardiac and kidney damage but also may play a part in the pathophysiology of CVD, CKD and other cardiac complications. Indeed, NAFLD may directly contribute to the development and progression of these vascular/cardiac complications through the hepatic production of lipids, atherogenic lipoproteins, the induction of hepatic/peripheral insulin resistance and dysglycaemia (i.e., increased hepatic glucose production), and the systemic release of numerous potentially pathogenic mediators (i.e., pro-inflammatory biomarkers, pro-oxidant molecules, and pro-coagulant and pro-fibrogenic factors). (CRP, C-reactive protein; FGF-21, fibroblast growth factor-21; HDL, high-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; PAI-1, plasminogen activator inhibitor-1; TNF, tumour necrosis factor).

atherogenesis and in the development of insulin resistance and NAFLD [54,58,71,72,75,143–145]. As briefly discussed above when considering the link between NAFLD and T2DM, emerging evidence also suggests that altered gut microbiota can influence the development and progression of NAFLD, possibly through the increased intestinal absorption of multiple bacterial products [59]. In this complex situation, the liver may function both as the target organ of the resulting systemic abnormalities and the source of several pathogenic mediators that may amplify vascular/cardiac and kidney damage. Indeed, NAFLD, especially its necro-inflammatory variant (NASH), may exacerbate hepatic/peripheral insulin resistance, cause atherogenic dyslipidemia, and release a myriad of pro-inflammatory molecules and vasoactive and thrombogenic molecules that play important roles in the pathophysiology of cardiovascular/cardiac diseases and CKD [1,18,75,125,126,143–146]. In this dangerous scenario (as also depicted in Fig. 3), emerging evidence also suggests that the coexistence of obesity-related increases in fat accumulation in

Table 4. Putative targeted screening measures in high-risk individuals for the assessment of cardiovascular disease (CVD) risk among patients with diagnosed NAFLD.

Assessment of the coexisting risk factors		Physical examination	Laboratory tests
A	Prior history of myocardial infarction, angina, heart failure, stroke or other clinical CVD manifestations (i.e., patients in secondary prevention for CVD)	Body weight	Total cholesterol
B	Family history for premature ischemic heart disease (i.e., age of onset <55 years for men and <65 years for women in first-degree relatives) or type 2 diabetes	Height	Triglycerides
C	Cigarette smoking	Body mass index	HDL cholesterol
D	Diabetes mellitus (i.e., fasting glucose level ≥7 mmol/L or HbA1c ≥6.5% or 2-h glucose level ≥11.1 mmol/L during a 75-g OGTT or a "random" glucose level ≥11.1 mmol/L in presence of hyperglycaemic symptoms)	Waist circumference	LDL cholesterol
E	Hyperlipidemia (principally hypercholesterolemia or atherogenic dyslipidemia; if LDL cholesterol >4.9 mmol/L exclude presence of familial hypercholesterolemia)	Blood pressure	Fasting glucose
F	Hypertension (i.e., blood pressure ≥140/90 mmHg or on treatment)	Arterial bruits and pulse examination (including ankle brachial pressure index)	Hemoglobin A1c (HbA1c)
G	Obesity (i.e., for Europeans: BMI ≥30 kg/m^2 and/or waist circumference ≥102 cm in men and ≥88 cm in women)		Estimated glomerular filtration rate (serum creatinine)
H	Metabolic syndrome (i.e., based on the 2009 definition proposed by the International Diabetes Federation and the American Heart Association/National Heart, Lung, and Blood Institute)		Urinalysis
I	Chronic kidney disease (i.e., eGFR <60 ml/min/1.73 m^2 and/or abnormal albuminuria)		Albuminuria
			Fasting insulin (for calculating HOMA-estimated insulin resistance, principally in non diabetics) 75-g oral glucose tolerance test (in patients with impaired fasting glycaemia and/or obesity)
			CVD risk estimation (by using risk calculators, e.g., Framingham risk score or QRISK2)
			Carotid artery ultrasonography (in most cases) or high resolution computed tomography coronary artery calcium score

All patients with NAFLD should be screened for CVD risk, and the assessments should be periodically repeated (every 1 to 2 years), depending on the clustering of CVD risk factors. Most of these clinical and laboratory data along with sex and age can be useful in clinical practice for the estimation of the global (total) CVD risk by using risk assessment systems based on either the Framingham risk score or other available risk score calculators such as QRISK2. However, the use of these CVD risk score calculators needs to be validated by future studies in larger cohorts of NAFLD patients of various ethnic backgrounds in order to substantiate their clinical relevance as a foundation for the primary prevention of CVD in this group of patients. In general, current management of the CVD risk among NAFLD patients widely overlaps with the guidelines for the treatment of CVD risk factors, which are adopted for the general adult population.

the myocardium/pericardium and kidney may additionally exert local adverse effects that result in structural and functional derangements in the myocardium, kidney and vasculature.

Conclusions

The last decade has been an exciting one for investigators and clinicians interested in understanding the broader consequences of NAFLD for chronic liver disease, HCC and for extra-hepatic diseases, such as type 2 diabetes, CVD and cardiac disease and CKD. More frequently do patients with NAFLD die from extra-hepatic complications of NAFLD (mainly from CVD causes) than liver disease *per se* and clearly with the epidemic of obesity and T2DM the prevalence of NAFLD is likely to markedly increase. Table 4 suggests putative targeted screening measures in high-risk individuals for the assessment of global CVD risk among patients with diagnosed NAFLD [147].

There is now firm evidence that NAFLD is an important risk factor for T2DM and there is increasing evidence that NAFLD not only contributes to the development and progression of CVD but also to cardiac diseases (e.g., LV dysfunction and hypertrophy, AF and heart valve calcification) and CKD. Further research is needed to understand the biological mechanisms by which NAFLD influences risk of HCC and these extra-hepatic diseases not least to establish whether there are key 'common threads' (e.g., insulin resistance and activation of inflammatory pathways) that link NAFLD to the development of extra-hepatic diseases.

An improved knowledge of the pathophysiological links between NAFLD and these extra-hepatic complications will not only help develop new pharmacological treatments for this liver disease *per se*, but may also help decrease the global burden of these very common non-communicable diseases that we now know share a 'common soil' with NAFLD.

Financial support

CDB is supported in part by the Southampton National Institute for Health Research Biomedical Research Centre. GT is supported

in part by grants from the University School of Medicine of Verona.

Conflict of interest

Both authors have no relationships with industry that give rise to a conflict of interest. CDB is Principal Investigator of the WELCOME (Wessex Evaluation of fatty Liver and Cardiovascular markers in NAFLD with OMacor thErapy) study funded by the National Institute for Health Research and Diabetes UK. The WELCOME study has tested the effects of high dose (4 grams daily) Omacor (Lovaza) (Abbott) in people with NAFLD (www.clinicaltrials.gov registration number NCT00760513). CDB is Principal Investigator of the 'INvestigation of SYnbiotic TreatmEnt in NAFLD' (INSYTE) study www.clinicaltrials.gov registration number NCT01680640 funded by the National Institute for Health Research. The INSYTE study is testing the effects of a synbiotic (Chr Hansen Denmark) on liver fat, disease biomarkers and intestinal microbiota in NAFLD.

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