

# Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases

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## ABSTRACT

Key physiological functions of the liver, including glucose and lipid metabolism, become disturbed in the setting of non-alcoholic fatty liver disease (NAFLD) and may be associated with a systemic inflammatory 'milieu' initiated in part by liver-secreted cytokines and molecules. Consequently, the pathophysiological effects of NAFLD extend beyond the liver with a large body of clinical evidence demonstrating NAFLD to be independently associated with both prevalent and incident cardiovascular disease (CVD), chronic kidney disease (CKD) and type 2 diabetes mellitus (T2DM). The magnitude of risk of developing these extrahepatic diseases parallels the underlying severity of NAFLD, such that patients with non-alcoholic steatohepatitis (NASH) appear to be at greater risk of incident CVD, CKD and T2DM than those with simple steatosis. Other modifiers of risk may include genetic variants (eg, patatin-like phospholipase domain-containing 3 and trans-membrane 6 superfamily member 2 polymorphisms), visceral adipose tissue accumulation, dietary intake and the gut microbiome. Emerging data also suggest that NAFLD may be a risk factor for colonic neoplasia and reduced bone mineral density, especially among men. Importantly, improvement/resolution of NAFLD is associated with a reduced incidence of T2DM and improved kidney function, adding weight to causality and suggesting liver focused treatments may reduce risk of extrahepatic complications. Awareness of these associations is important for the clinicians such that CVD risk factor management, screening for T2DM and CKD are part of the routine management of patients with NAFLD.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a very common pathological condition worldwide that is closely associated with the clinical features of metabolic syndrome and is characterised by substantial interpatient variability in severity and rate of liver disease progression.<sup>1 2</sup>

A purely liver-centric view is that NAFLD represents a spectrum of progressive liver disease occurring in the absence of excessive alcohol consumption that ranges from isolated intrahepatic triglyceride accumulation (simple steatosis), through intrahepatic triglyceride accumulation *plus* inflammation and hepatocyte injury (non-alcoholic steatohepatitis, NASH), and ultimately progresses to fibrosis/cirrhosis and potentially hepatocellular carcinoma.<sup>3</sup> Although a significant proportion of the population has NAFLD, only a minority progresses to advanced liver disease or liver-related death.<sup>3</sup>

However, this liver-centric view does not encompass the wider ramifications of NAFLD. Indeed, NAFLD is just one facet of a multisystem disease (figure 1) that confers substantially increased morbidity and mortality on those patients who are affected and where the most common causes of death are cardiovascular disease (CVD), followed by extrahepatic malignancies and liver-related complications.<sup>4 5</sup>

This review mainly focuses on the principle extrahepatic chronic diseases associated with NAFLD where there is now strongest evidence for a potential causal link: CVD, type 2 diabetes mellitus (T2DM), chronic kidney disease (CKD)<sup>1 2</sup> and, to a lesser extent, certain types of extrahepatic tumours and osteoporosis.

## EPIDEMIOLOGICAL DATA LINKING NAFLD TO RISK OF CVD

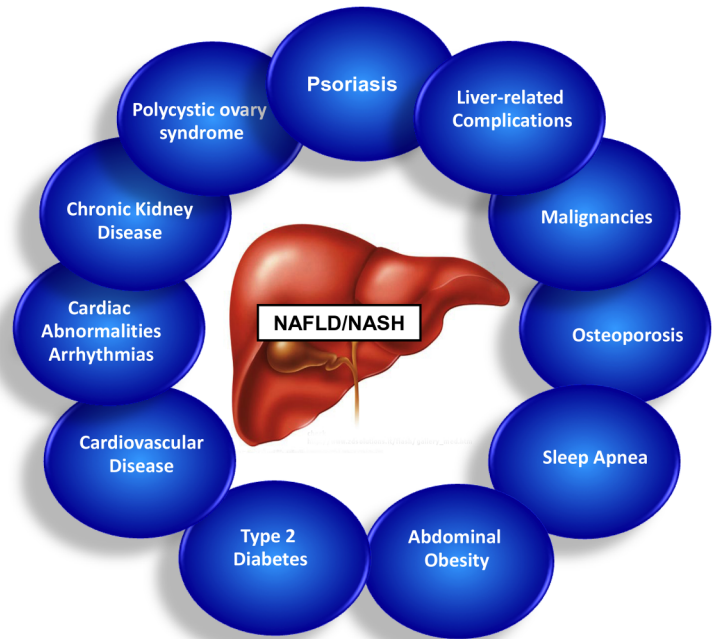
That NAFLD is associated with an increased risk of CVD is unsurprising, given the close associations between NAFLD and the established CVD risk factors encapsulated by the metabolic syndrome, including abdominal obesity, hypertension, atherogenic dyslipidaemia and insulin resistance/dysglycaemia.<sup>6–8</sup> Highlighting the intimacy of these associations is the observation that the increases in hepatic lipid accumulation are directly proportional to the severity of each component of the metabolic syndrome.<sup>9</sup> However, the nature and extent of these associations, whether simply due to a shared underlying aetiology or because the presence of NAFLD confers an additional risk, remains the subject of much scrutiny. This attention is undoubtedly warranted as it has important clinical implications for screening and surveillance strategies in the growing number of patients with NAFLD. Addressing CVD risk in patients with NAFLD is also the aspect of the disease most amenable to medical management and so improving long-term clinical outcomes.

Beyond the associations with traditional CVD risk factors, patients with NAFLD also exhibit a range of non-traditional CVD risk factors, including hyperuricaemia,<sup>10–12</sup> hypoalbuminaemia<sup>13–15</sup> and hypovitaminosis D.<sup>16</sup> The association with CKD, discussed later in this review, also confers an increased risk of CVD.<sup>17</sup> Other potentially contributing risk factors include increased levels of circulating proinflammatory markers (eg, C reactive protein, interleukin (IL) 6, tumour necrosis factor (TNF)- $\alpha$  and other hepatic acute-phase proteins), procoagulant factors (eg, fibrinogen and plasminogen activator inhibitor-1) and adhesion molecules



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**Figure 1** Non-alcoholic fatty liver disease (NAFLD): a multisystem disease. Reported associations between NAFLD and various human diseases.



(eg, vascular adhesion protein-1) that are likely to have been synthesised within the liver, especially in the presence of NASH.<sup>18–23</sup>

Substantial epidemiological evidence links NAFLD with objectively assessed subclinical atherosclerosis and with an increased prevalence of clinically manifest CVD. In case-control studies, NAFLD has been linked with increased carotid artery intima-media thickness,<sup>24–28</sup> increased arterial wall stiffness,<sup>29–30</sup> and impaired endothelium-dependent flow-mediated vasodilatation.<sup>31–32</sup> Similarly, a meta-analysis of seven cross-sectional studies totalling 3497 individuals concluded that ultrasound-diagnosed NAFLD was strongly associated with greater carotid-artery intimal medial thickness and an increased prevalence of carotid atherosclerotic plaques.<sup>33</sup> These findings have since been supported by a larger meta-analysis that incorporated 27 studies and confirmed the association of NAFLD with various markers of subclinical atherosclerosis (including also increased coronary-artery calcium score) independent of traditional CVD risk factors and metabolic syndrome features.<sup>34</sup> Individuals with NAFLD among an occupational cohort of over 10 000 South Korean people had an increased coronary-artery calcium score, independent of multiple CVD risk factors, including insulin resistance.<sup>35</sup> Case-control studies have also reported strong associations of NAFLD with early changes in left ventricular morphology and/or diastolic dysfunction,<sup>36–39</sup> impaired myocardial energy metabolism<sup>40–41</sup> and reduced coronary artery flow.<sup>42</sup> Cohort studies in patients with biopsy-confirmed NAFLD/NASH also clearly demonstrated that CVD is the most common cause of death.<sup>4–5</sup>

Population-based cohort studies, such as the National Health and Nutrition Examination Survey (NHANES-III), also provide evidence of increased CVD prevalence in NAFLD. In approximately 11 500 adult NHANES participants, NAFLD (diagnosed by ultrasonography) remained significantly associated with an increased prevalence of CVD after adjusting for major demographic, clinical and metabolic confounders.<sup>43–44</sup> An Italian study in nearly 3000 outpatients with T2DM also demonstrated that those with ultrasound-diagnosed NAFLD had a higher

prevalence of coronary, cerebrovascular and peripheral vascular disease, independent of traditional CVD risk factors, diabetes-related variables and other potential confounders.<sup>45</sup> Among patients undergoing clinically indicated coronary angiography, the presence of NAFLD also independently correlated with the severity of coronary artery disease.<sup>46–48</sup> Recently, a systematic review and meta-analysis incorporating almost 165 000 participants in 34 studies (21 cross-sectional, 13 cohort studies) has provided further support for the association of NAFLD (diagnosed by biochemistry, imaging or histology) with atherosclerosis, hypertension and both prevalent and incident CVD.<sup>49</sup>

Based on the available data, there seems little doubt that NAFLD is associated with increased CVD prevalence, an association that has been consistently replicated across different patient populations. Whether NAFLD is an independent CVD risk factor or simply a bystander that shares common aetiological factors remains controversial.<sup>3</sup> However, there is a growing body of evidence demonstrating that CVD is a clinical concern in NAFLD, and that patients with NAFLD are more likely to experience a CVD-related death than a liver-related death.<sup>4–5</sup> Although some studies suggested that only patients with NASH rather than those with simple steatosis have an increased CVD mortality compared with the matched control population,<sup>50–51</sup> a subsequent meta-analysis brought this into question. In that meta-analysis patients with NAFLD (as detected by histology or ultrasonography) had a substantially greater risk of CVD mortality than the matched control population but presence of NASH did not further increase risk of CVD mortality.<sup>52</sup> Further prospective studies in patients with biopsy-characterised NAFLD are needed to address this point, although some studies with a sufficiently long follow-up recently showed that fibrosis stage rather than NASH best predicts long-term survival outcomes of patients with NAFLD.<sup>4–5</sup>

Several studies support an association between NAFLD and incident CVD. Recent retrospective cohort studies reported a significant association between ultrasound-diagnosed NAFLD and the progression of subclinical coronary or carotid

atherosclerosis independent of multiple CVD risk factors. The risk of subclinical carotid/coronary atherosclerosis progression was also higher among patients with NAFLD with increased non-invasive markers of advanced fibrosis at baseline (NAFLD fibrosis score (NFS), fibrosis-4 score or elevated  $\gamma$ -glutamyl transferase levels).<sup>53–54</sup> Additionally, the regression of NAFLD on ultrasound over time was associated with a decreased risk of subclinical carotid atherosclerosis development.<sup>53</sup>

The presence of NAFLD at baseline, although defined clinically using the calculated fatty liver index (FLI), conferred an OR of 1.63 for subsequent development of carotid atherosclerotic plaques in a cohort of 1872 European patients.<sup>55</sup> Recent data also support a bidirectional association between incident NAFLD and CVD risk factors. After controlling for potential confounding factors, including alcohol consumption, in 1051 participants of the Framingham Heart Study, individuals with NAFLD at baseline were more likely to subsequently develop incident hypertension and T2DM than those without NAFLD; conversely, those with metabolic risk factors (hypertriglyceridaemia, hypertension or T2DM) at baseline were more likely to develop incident NAFLD.<sup>56</sup> These findings are also consistent with natural history studies of patients with NAFLD with serial liver biopsies where those with occurrence of incident T2DM or hypertension between biopsies were most likely to exhibit progressive liver fibrosis.<sup>1–2</sup> A large number of observational studies also suggest that NAFLD is predictive of incident CVD events and death.<sup>4–50–51–57–67</sup> Table 1 lists the principal retrospective and prospective studies that have addressed the relationship between NAFLD (defined radiologically or histologically) and risk of CVD events. Many of these studies were also included in an updated meta-analysis that incorporated a total of 16 unique, observational studies with 34 043 adults and captured nearly 2600 CVD outcomes over a median of 6.9-year follow-up.<sup>68</sup> This meta-analysis concluded that the presence of NAFLD (diagnosed by imaging or histology) conferred an OR of 1.64 for fatal and non-fatal incident CVD events, a risk that appeared to increase further with greater severity of NAFLD and remained significant in only those studies where analysis was fully adjusted for potentially confounding covariates.<sup>68</sup> However, some of these studies have suggested that the increase in CVD risk may be limited to subgroups of patients with NAFLD, such as those with advanced fibrosis,<sup>57</sup> T2DM<sup>58</sup> or men with an elevated  $\gamma$ -glutamyl transferase level.<sup>58</sup> It is, therefore, conceivable that additional factors may modify the association between NAFLD and risk of CVD events. One such modifying factor may be genetic variation.

Advances in our understanding of how genetic modifiers influence disease progression through genome-wide association studies are also relevant and have provided important insights into the crosstalk between NAFLD and CVD and how they may become dissociated.<sup>69</sup> Although the widely validated single nucleotide polymorphism, rs738409 (c.444 C>G, p.I148M) in patatin-like phospholipase domain-containing 3 (*PNPLA3*) confers an increased risk of NASH, hepatic fibrosis and hepatocellular carcinoma, it has no apparent effect on CVD risk.<sup>70–72</sup> However, further well designed prospective studies are needed to better clarify this controversial issue.<sup>70–73</sup> Recently, another non-synonymous genetic variant within a gene of unknown function called transmembrane 6 superfamily member 2, *TM6SF2* (rs58542926 c.449 C>T, p.E167K), has been linked with NAFLD.<sup>74</sup> As with *PNPLA3*, carriage of the *TM6SF2* minor (T) allele is associated with greater hepatic steatosis, more severe NASH and greater hepatic fibrosis/cirrhosis,<sup>75–76</sup> but intriguingly, carriage of the more common major (C) allele

promotes very low density lipoprotein excretion, conferring an increased risk of dyslipidaemia and CVD.<sup>76–77</sup> Thus, while in general NAFLD is associated with an increased risk of CVD, carriage of specific genetic modifiers might mean that these may become dissociated. In what has been described as the 'catch-22 paradigm', individuals that possess the *TM6SF2* minor (T) allele will be more prone to liver-related morbidity, while those that carry the more common (C) allele may be at greater risk of CVD.<sup>78</sup> Whether the strength of this effect is sufficient to overcome the effects of many other environmental or genetic variants that influence outcome(s) and so be clinically relevant at an individual level does however remain to be definitely determined.<sup>78</sup> The relatively new field of epigenetics research is also providing exciting insights into the role of DNA methylation in the pathogenesis of NAFLD and how gene-environment interactions may influence CVD.<sup>79–82</sup>

Accumulating evidence also suggests that NAFLD is linked with valvular heart diseases (ie, aortic-valve sclerosis or mitral annulus calcification) and increased risk of cardiac arrhythmias (mainly atrial fibrillation and ventricular tachyarrhythmias) that may partly contribute to explain the increased risk of CVD events observed in patients with NAFLD.<sup>83–87</sup>

Overall, therefore, the available evidence demonstrates the strong association between NAFLD and CVD and supports the view that NAFLD increases CVD risk: a relationship that may be modified by a range of factors including genetic variation. This has important clinical implications that may influence the decision to institute primary prevention strategies with lipid-lowering, antihypertensive or antiplatelet agents. At present, algorithms such as the Framingham risk equation or the Systematic COronary Risk Evaluation (SCORE) risk charts for CVD risk assessment are used to guide initiation of preventative interventions. However, they are based on traditional CVD risk factors and do not include presence of NAFLD. Although the Framingham risk equation has been validated for use in patients with NAFLD,<sup>88</sup> it remains unclear whether the addition of NAFLD as a predictive factor in this or other CVD risk score systems can improve their ability to accurately predict future CVD events. Moreover, randomised clinical trials with CVD outcomes that focus on treatments for liver disease in NAFLD are also required in order to definitely establish a causal relationship between NAFLD and risk of CVD events.

#### EPIDEMIOLOGICAL DATA LINKING NAFLD TO RISK OF CKD

Over the past decade, several population-based and hospital-based studies have shown that the prevalence of CKD (defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m<sup>2</sup>, abnormal albuminuria or overt proteinuria) was markedly increased in patients with NAFLD as diagnosed by imaging<sup>89–98</sup> or histology.<sup>99–103</sup> In these studies, the prevalence of CKD ranged from approximately 20% to 55% among patients with NAFLD compared with 5% to 30% among those without NAFLD.<sup>89–103</sup> Notably, in most of these studies the significant association between NAFLD and CKD persisted after adjustment for hypertension, T2DM and other known risk factors for CKD. Pan *et al* recently reported that after adjustment for visceral fat accumulation and other cardiometabolic risk factors, there was a positive, graded relationship between intrahepatic triglyceride content, as measured by magnetic resonance spectroscopy, and the presence of CKD or abnormal albuminuria in obese individuals.<sup>97</sup> In a population-based study of 8270 Chinese adults with normal kidney function, NAFLD was independently associated with low levels of albuminuria also, defined as urine albumin/creatinine ratio below 30 mg/

**Table 1** Selected observational studies exploring the risk of CVD in patients with NAFLD as diagnosed by histology or imaging (studies were ordered by methodology of NAFLD diagnosis and publication year)

Authors, year (ref.)	Study population	Follow-up length	NAFLD diagnosis	Study outcomes	Main findings
Matteoni <i>et al.</i> , 1999 <sup>61</sup>	Retrospective cohort of 132 US patients with NAFLD and raised serum liver enzymes	18 years	Histology	All-cause and cause-specific mortality	Patients with NASH had higher rates of all-cause and liver-related mortality than those without. CVD death rate did not differ between the groups
Dam-Larsen <i>et al.</i> , 2004 <sup>62</sup>	Retrospective cohort of 109 Danish patients with non-alcoholic simple steatosis (without NASH at baseline)	16.7 years	Histology	All-cause and cause-specific mortality	No significant difference in death rates between patients with simple steatosis and the general population
Adams <i>et al.</i> , 2005 <sup>64</sup>	Retrospective community-based cohort of 420 US patients with NAFLD	7.6 years	Histology and imaging	All-cause and cause-specific mortality	Patients with NAFLD (especially those with cirrhosis and NASH) had higher rates of all-cause, CVD and liver-related mortality than the matched general population
Ekstedt <i>et al.</i> , 2006 <sup>50</sup>	Retrospective cohort of 129 Swedish patients with NAFLD and raised serum liver enzymes	13.7 years	Histology	All-cause and cause-specific mortality	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 reference population
Rafiq <i>et al.</i> , 2009 <sup>53</sup>	Retrospective cohort of 173 US patients with NAFLD and raised serum liver enzymes	13 years	Histology	All-cause and cause-specific mortality	CVD, malignancy and liver-related complications were the most common causes of mortality
Söderberg <i>et al.</i> , 2010 <sup>51</sup>	Retrospective cohort of 118 Swedish patients with NAFLD and raised serum liver enzymes	24 years	Histology	All-cause and cause-specific mortality	Patients with NASH, but not those with simple steatosis, had higher rates of all-cause (~twofold), CVD (~twofold) and liver-related mortality than the matched general population
Ekstedt <i>et al.</i> , 2015 <sup>4</sup>	Retrospective cohort of 229 Swedish patients with biopsy-proven NAFLD	26.4±5.6 years	Histology	All-cause and disease-specific mortality	Patients with NAFLD have increased overall mortality (HR 1.29, 95% CI 1.04 to 1.59), with a high risk of death from CVD (HR 1.55, 95% CI 1.11 to 2.15), and liver-related disease. Stage of fibrosis rather than presence of NASH predicted both overall and disease-specific mortality
Fracanzani <i>et al.</i> , 2016 <sup>193</sup>	Prospective case-control study of 125 Italian patients with NAFLD and 250 age-matched and sex-matched control individuals without known liver diseases	10 years	Histology and ultrasound	Non-fatal CVD events (acute coronary syndrome, coronary revascularisation procedures, ischaemic stroke or transitory ischaemic attacks as combined end point)	NAFLD was independently associated with incident non-fatal CHD events (HR 1.99, 95% CI 1.01 to 3.91)
Jepsen <i>et al.</i> , 2003 <sup>65</sup>	Retrospective cohort of 1800 Danish patients discharged with a hospital diagnosis of NAFLD	6.2 years	Ultrasound and liver enzymes	All-cause and cause-specific mortality	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
Hamaguchi <i>et al.</i> , 2007 <sup>39</sup>	Community-based cohort of 1637 Japanese apparently healthy individuals	5 years	Ultrasound	Non-fatal CHD and stroke	NAFLD was independently associated with increased risk of non-fatal CVD events (HR 4.10, 95% CI 1.6 to 10.7)
Targher <i>et al.</i> , 2007 <sup>45</sup>	Prospective cohort of 2103 Italian individuals with type 2 diabetes without baseline viral hepatitis and CVD	6.5 years	Ultrasound	CVD mortality and non-fatal myocardial infarction, ischaemic stroke and revascularisation procedures (combined end point)	NAFLD was independently associated with increased risk of fatal and non-fatal CVD events (HR 1.87, 95% CI 1.2 to 2.6)
Haring <i>et al.</i> , 2009 <sup>58</sup>	Population-based cohort study of 4160 German adult men and women without baseline viral hepatitis or cirrhosis	7.3 years	Ultrasound	All-cause and cause-specific mortality	NAFLD was independently associated with increased risk of all-cause and CVD mortality in men (HR 6.2, 95% CI 1.2 to 31.6)
Zhou <i>et al.</i> , 2012 <sup>67</sup>	Community-based cohort study of 3543 Chinese adult men and women	4 years	Ultrasound	All-cause and cause-specific mortality	Patients with NAFLD had ~threefold higher rates of all-cause and CVD mortality than those without NAFLD

Continued

Table 1 Continued

Authors, year (ref.)	Study population	Follow-up length	NAFLD diagnosis	Study outcomes	Main findings
Stepanova and Yunossi, 2012 <sup>43</sup> ; Lazo <i>et al</i> , 2011 <sup>44</sup>	Nationally based cohort study of 11 371 US adult participants from the Third National Health and Nutrition Examination Survey, 1988–94	14.5 years	Ultrasound	All-cause and cause-specific mortality	No significant association between NAFLD and all-cause and cause-specific mortality
Pisto <i>et al</i> , 2014 <sup>60</sup>	Population-based, randomly recruited cohort of 988 middle-aged Finnish participants	~18 years	Ultrasound	Fatal and non-fatal CVD events	Severe NAFLD did not significantly predict the risk of CVD events after adjustment for potential confounders, including insulin resistance
Wong <i>et al</i> , 2016 <sup>48</sup>	612 consecutive Chinese patients undergoing coronary angiograms without known liver diseases	6 years	Ultrasound	Fatal and non-fatal CVD events, heart failure or secondary coronary interventions (combined end point)	Patients with NAFLD, compared with those without, were more likely to have >50% stenosis in one or more coronary arteries and therefore require percutaneous coronary intervention. NAFLD was not significantly associated with fatal and non-fatal CVD events
Mantovani <i>et al</i> , 2016 <sup>194</sup>	Retrospective cohort of 286 Italian adults with type 1 diabetes without known liver diseases	5.3±2 years	Ultrasound	Non-fatal ischaemic heart disease, non-fatal ischaemic stroke or coronary/peripheral artery revascularisation procedures (combined end point)	NAFLD was independently associated with an increased risk of non-fatal CVD events (HR 6.73, 95% CI 1.2 to 38)
Treeraprasertsuk <i>et al</i> , 2012 <sup>88</sup>	Community-based cohort of 309 US patients with NAFLD	11.5±4.1 years	Ultrasound and CT	New-onset CVD	Patients with NAFLD have a higher 10-year CHD risk than the general population of the same age and sex
Kim <i>et al</i> , 2013 <sup>57</sup>	Nationally based cohort study of 11 154 US adult participants from the Third National Health and Nutrition Examination Survey, 1988–94	14.5 years	Ultrasound and non-invasive fibrosis markers (NFS/APRI/FIB4)	All-cause and cause-specific mortality	NAFLD not associated with increased mortality. However, advanced fibrosis, as determined by non-invasive fibrosis markers, is a significant predictor of mortality, mainly from CVD causes, independent of other known factors
Zeb <i>et al</i> , 2016 <sup>195</sup>	Prospective cohort study of 4119 US adult participants who were free of CVD and known liver diseases at baseline (The Multi-Ethnic Study of Atherosclerosis)	7.6 years (median)	CT	All-cause mortality and CVD events (myocardial infarction, resuscitated cardiac arrest, angina or coronary revascularisation procedures)	NAFLD was independently associated with incident CHD events (HR 1.42, 95% CI 1.00 to 2.03)

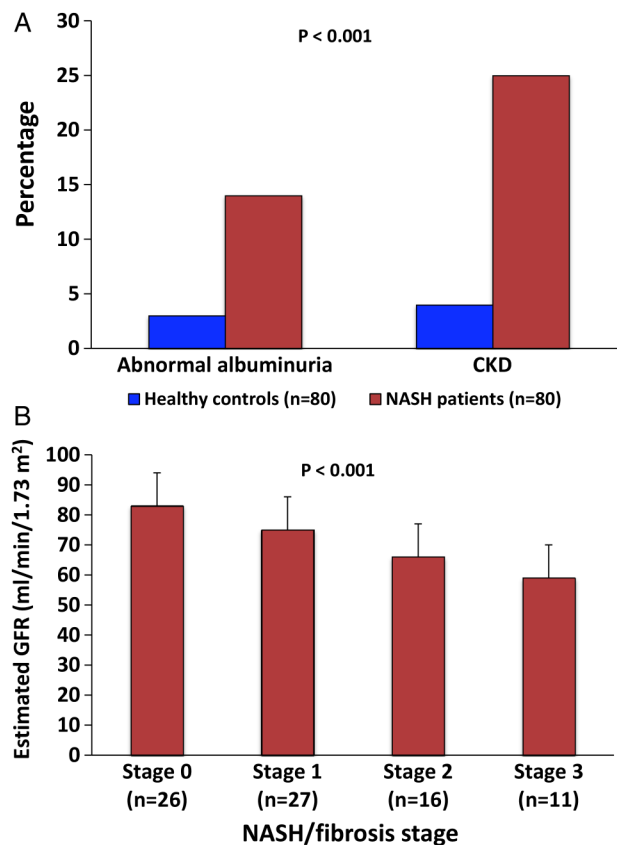
APRI, aspartate aminotransferase to platelet ratio index; CHD, coronary heart disease; CVD, cardiovascular disease; FIB-4, fibrosis-4; NAFLD, non-alcoholic fatty liver disease; NFSH, non-alcoholic steatohepatitis; NFS, NAFLD fibrosis score.

g.<sup>104</sup> The finding of a strong association between NAFLD and early kidney dysfunction (ie, microalbuminuria or eGFR  $<90$  mL/min/1.73 m<sup>2</sup>) has been also documented in a cohort of nearly 600 overweight or obese children.<sup>98</sup> Recognition of this kidney dysfunction in childhood is clinically important because the treatment to reverse it is more likely to be effective if applied early in the disease process. Interestingly, some case-control studies that used liver biopsy to diagnose NAFLD also reported a significant, graded relationship between the severity of NAFLD histology and the presence of either decreased eGFR or abnormal albuminuria.<sup>99–103</sup> For example, in a case-control study, we found that patients with biopsy-confirmed NASH had a greater prevalence of abnormal albuminuria and CKD than matched control subjects without hepatic steatosis, and that the histological severity of NASH was independently associated with decreasing mean eGFR values (figure 2).<sup>99</sup> That said, it is important to underline that none of the abovementioned studies have used renal biopsy to examine the pathology of the CKD; so, it remains currently unknown if NAFLD is associated with a specific type of kidney disease (though it is very likely that NAFLD contributes to kidney damage mainly through accelerated atherogenesis).<sup>89–103</sup>

Although the cross-sectional associations between NAFLD and CKD stages are strong and consistent across a wide range of patient populations, the data on whether NAFLD per se is causally linked to the development and progression of CKD remain debateable.<sup>105 106</sup>

In an occupational cohort of nearly 8500 non-diabetic and non-hypertensive South Korean men with preserved kidney function and no overt proteinuria at baseline who were followed up for a mean period of 3.2 years, NAFLD (diagnosed by ultrasonography) was associated with an increased incidence of CKD (HR 1.60; 95% CI 1.3 to 2.0).<sup>107</sup> This association remained significant after adjusting for body mass index (BMI), blood pressure, insulin resistance, plasma C reactive protein and other potential confounding factors.<sup>107</sup> Similarly, in the Valpolicella Heart Diabetes Study, involving 1760 patients with type 2 diabetes with preserved kidney function who were followed up over a 6.5-year period, the presence of NAFLD on ultrasonography was associated with an increased incidence of CKD (HR 1.69; 95% CI 1.3 to 2.6) independently of traditional cardiorenal risk factors, diabetes-related variables and medication use.<sup>108</sup> Consistent with these findings, in a longitudinal cohort study of 261 type 1 diabetic 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 incidence of CKD. Figure 3 shows a Kaplan-Meier analysis of incidence curves for CKD over the follow-up period in patients with and without NAFLD at baseline.<sup>109</sup> Notably, measurement of NAFLD improved risk prediction for CKD in this patient cohort, independently of traditional cardiorenal risk factors (age, sex, duration of diabetes, haemoglobin A1c, hypertension, baseline eGFR and microalbuminuria (ie, the last two factors being the strongest known risk factors for CKD)).<sup>109</sup>

A 2014 systematic review and meta-analysis of 33 observational studies (including 20 cross-sectional and 13 prospective studies and involving nearly 64 000 individuals) examined the relationship between NAFLD and risk of CKD.<sup>110</sup> NAFLD was diagnosed by biochemistry, imaging or histology, and CKD as either eGFR  $<60$  mL/min/1.73 m<sup>2</sup> or proteinuria. The results of this meta-analysis showed that NAFLD was associated with a nearly twofold increase in the prevalence and incidence of CKD. Similarly, although only a few studies used liver biopsy to



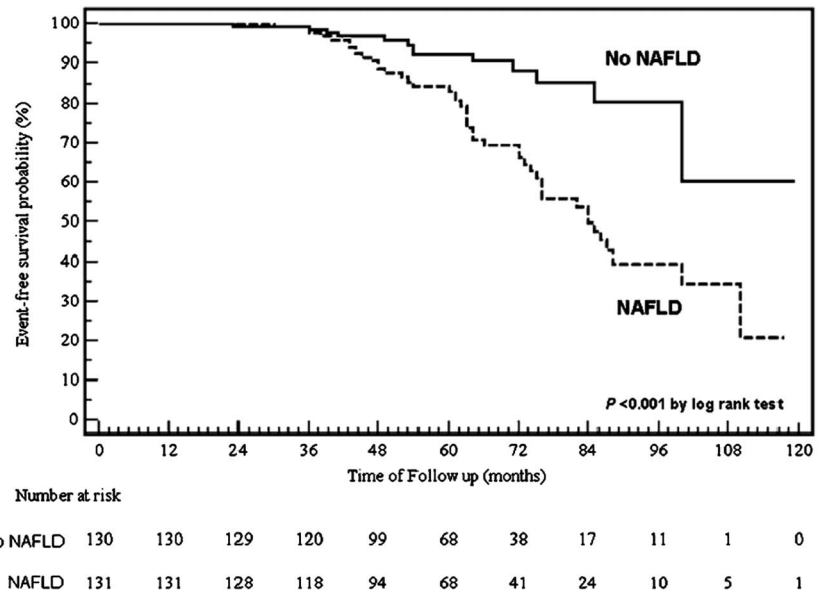
**Figure 2** Kidney function in patients with non-alcoholic steatohepatitis (NASH). (A) Shows the prevalence of both abnormal albuminuria (ie, urinary albumin-to-creatinine ratio  $\geq 30$  mg/g) and chronic kidney disease (CKD, defined as estimated glomerular filtration rate (eGFR), Modification of Diet in Renal Disease (MDRD)  $<60$  mL/min/1.73 m<sup>2</sup> or abnormal albuminuria) in 80 overweight patients with biopsy-proven NASH and 80 non-steatotic control individuals who were matched for age, sex and body mass index. (B) Shows the adjusted means ( $\pm$ SDs) of eGFR in patients with NASH according to the histological stage of hepatic fibrosis (from 0, indicating no fibrosis, to 3, indicating advanced fibrosis; patients with cirrhosis (ie, those with stage 4 fibrosis) were not included in the study). Data have been adjusted for age, sex, body mass index, waist circumference, hypertension status, plasma triglyceride concentrations and insulin resistance (as estimated on the basis of a homeostasis model assessment) (adapted from Targher *et al* [99]).

diagnose NAFLD, the presence of histologically confirmed NASH was associated with an approximately 2.5-fold increased prevalence and incidence of CKD than simple steatosis. Moreover, the presence of advanced hepatic fibrosis was associated with a remarkably greater prevalence and incidence of CKD than non-advanced fibrosis. In all of these analyses, the significant association between NAFLD and CKD persisted after adjustment for pre-existing diabetes, hypertension and other cardiorenal risk factors.<sup>110</sup>

In line with these observations, in a cohort of 261 patients with biopsy-proven NASH, who were treated with lifestyle modifications for 52 weeks, Vilar-Gomez *et al*<sup>111</sup> found that improvement in histological NASH-related end points, achieved by weight loss, was independently associated with improvement in kidney function parameters.

Collectively, these findings provide robust evidence of a strong association between the presence and severity of NAFLD with the stage and risk of developing CKD. Therefore, these findings call for a more active and systematic search for CKD in

**Figure 3** Risk of incident chronic kidney disease (CKD) in patients with type 1 diabetes and non-alcoholic fatty liver disease (NAFLD). Incidence curves for CKD (defined as occurrence of estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m<sup>2</sup> or macroalbuminuria) in a cohort of 261 adults with type 1 diabetes with (dotted line) and without (solid line) NAFLD on ultrasonography over a mean follow-up period of 5.2 years. All patients had preserved kidney function (eGFR<sub>MDRD</sub> ≥ 60 mL/min/1.73 m<sup>2</sup>) and no macroalbuminuria at baseline. Patients with known liver diseases and those with a prior history of cardiovascular disease were excluded from the study (adapted from Targher *et al* [109]).



patients with NAFLD. However, it is important to underline that the quality of some of the published studies is limited, and that causality remains to be definitely proven in larger clinical trials with incident CKD outcomes that focus on treatments for liver disease in NAFLD. Notably, all these studies used creatinine-based equations to estimate GFR (which do not perform well in patients with cirrhosis or severe obesity) instead of direct GFR measurements to define CKD. Furthermore, no detailed information was available in these studies about specific renal pathology/morphology associated with NAFLD. Further research is also needed to better elucidate the underlying mechanisms by which NAFLD contributes to the development and progression of CKD.

#### EPIDEMIOLOGICAL DATA LINKING NAFLD TO RISK OF TYPE 2 DIABETES

Obesity and insulin resistance are key pathogenic factors for NAFLD and T2DM, and thus these two diseases commonly coexist; NAFLD is present in up to 75% of patients with T2DM, whereas the prevalence of T2DM in adults with NAFLD is approximately 10%–18%.<sup>112–114</sup> In the absence of T2DM, NAFLD is a marker of insulin resistance independent of obesity or visceral adiposity, and predicts a greater deterioration in insulin sensitivity with weight gain compared with matched non-NAFLD individuals.<sup>115</sup> Concomitant with these mechanistic observations, there is now robust evidence from observational studies demonstrating an increased risk of incident T2DM following a diagnosis of NAFLD. It is also notable that, in dual biopsy studies, the development of incident T2DM was the strongest predictor of progression to NASH and liver fibrosis.<sup>1</sup>

A significant association between mildly elevated serum liver enzymes and increased risk of incident T2DM was described nearly two decades ago.<sup>116</sup> A meta-analysis of 20 observational studies (involving a total of 117 000 subjects belonging to different ethnic groups) found a 1.6–2.0-fold increased risk of incident T2DM when comparing the highest versus the lowest quartiles of serum aminotransferase levels over a median follow-up of 5 years.<sup>117</sup> Nevertheless, serum aminotransferase levels are relatively insensitive markers of NAFLD. Predictive non-invasive scores, such as the FLI or the NAFLD fatty liver score, incorporate serum liver enzymes with various metabolic

variables, such as BMI and serum triglyceride levels, into their diagnostic algorithms, resulting in greater diagnostic accuracy for NAFLD than serum liver enzymes alone. Both of these non-invasive scores have been demonstrated to predict incident T2DM, however it is difficult to distinguish whether it is NAFLD *itself* or the metabolic variables within these scores, which are responsible for the increased T2DM risk.<sup>118</sup>

More robust evidence of the existence of an association between NAFLD and incident T2DM arises from multiple large cohort studies with a median follow-up period of at least 5 years that used ultrasonography or, less commonly, CT to diagnose NAFLD (table 2). After adjusting for several potential confounding factors, NAFLD has been associated with a 1.5–2-fold increased risk of new-onset T2DM over a 5–10-year follow-up period.<sup>119–129</sup> It is notable that the increased T2DM risk associated with NAFLD was observed in both sexes; however it was typically higher in men, mirroring the male predominance in T2DM.<sup>124 128 129</sup>

Notably, the risk of new-onset T2DM appears to diminish over time following the improvement or resolution of NAFLD, with some Asian cohort studies demonstrating T2DM incidence returning to that of subjects without fatty liver on ultrasonography.<sup>129–131</sup> This is likely to be closely related to temporal changes in body weight, although one study found NAFLD resolution to be associated with a lower incidence of T2DM independently of change in BMI.<sup>130</sup> Another study found NAFLD improvement was only associated with a reduced risk of incident T2DM in those who had a concomitant BMI reduction over time.<sup>129</sup>

NAFLD appears to be additive to established metabolic risk factors of obesity and insulin resistance in increasing the risk of incident T2DM.<sup>120 127 128 132</sup> One cohort study of 12 853 South Korean non-diabetic individuals found that the risk of incident T2DM over 5 years increased 2.7-fold with a baseline diagnosis of fatty liver on ultrasound, and 3.7-fold with insulin resistance determined by the homoeostasis model of assessment, whereas their combination increased the risk to 6.7-fold.<sup>132</sup> Nevertheless, the interaction between insulin resistance, metabolic syndrome and NAFLD is complex with several studies suggesting the association between NAFLD and incident T2DM to be modified by the severity of underlying insulin resistance or baseline body weight; two studies have also found that the risk of incident

**Table 2** Retrospective or prospective cohort studies examining NAFLD (as detected either by ultrasonography or by CT) as a risk factor for incident T2DM

Authors, year (ref.)	Country	Sample size	Follow-up (median, years)	T2DM diagnosis	T2DM% at follow-up	Adjusted OR(s) ( $\pm$ 95% CI)	Adjusted variables
Okamoto <i>et al</i> , 2003 <sup>124</sup>	Japan	840	10.0	FBG $\geq$ 140 mg/dL ( $\geq$ 7.8 mmol/L) or HbA1c $\geq$ 6.5% or T2DM medications	14.1% (men) 4.3% (women)	1.8 (0.9 to 3.5)	Age, sex, BMI, weight gain, FBG, HbA1c, alcohol intake, family history of diabetes
Kim <i>et al</i> , 2008 <sup>122</sup>	South Korea	5372	5.0	FBG $\geq$ 126 mg/dL ( $\geq$ 7.0 mmol/L) or T2DM medications or past history	4.3%	1.5 (1.1 to 2.2)	Age, sex, BMI, smoking, family history of diabetes, ALT, FBG, triglycerides, HDL-cholesterol
Yamada <i>et al</i> , 2010 <sup>128</sup>	Japan	12 375	5.0	FBG $\geq$ 126 mg/dL or T2DM medications	1% (men) 0.5% (women)	1.9 (1.9 to 2.8) men 2.1 (2.5 to 4.7) women	Age, BMI, blood pressure, alcohol intake, smoking
Sung and Kim, 2011 <sup>127</sup>	South Korea	11 091	5.0	Not reported	1.6%	2.05 (1.35 to 3.12)	Age, sex, BMI, alcohol, education, smoking, exercise
Chang <i>et al</i> , 2013 <sup>119</sup>	South Korea	38 291	5.1	FBG $\geq$ 126 mg/dL or HbA1c $\geq$ 6.5% or T2DM medications	1.1% annual incidence	2.0 (1.8 to 2.2) for low NFS 4.7 (3.7 to 6.1) for intermediate/high NFS	Sex, smoking, alcohol intake, exercise, family history of diabetes, total cholesterol, triglycerides, HDL-cholesterol, CRP, HOMA-insulin resistance
Park <i>et al</i> , 2013 <sup>125</sup>	South Korea	25 232 (men)	5.0	FBG $\geq$ 126 mg/dL or HbA1c $\geq$ 6.5% or T2DM medications or past history	8.4%	1.73 (1.00 to 3.01)	Age, waist circumference, triglycerides, HDL-cholesterol, CRP, HOMA-insulin resistance, creatinine, blood pressure, family history of diabetes, exercise, MetS
Shah <i>et al</i> , 2015 <sup>126</sup>	USA	3153	9.1	FBG $\geq$ 126 mg/dL or T2DM medications or past history	6.9%	2.06 (1.52 to 2.79)*	Age, sex, race, BMI, waist circumference, family history of diabetes, blood pressure, FBG, HDL-cholesterol, triglycerides, exercise, CRP, statin use
Yamazaki <i>et al</i> , 2015 <sup>129</sup>	Japan	4604	11.3	FBG $\geq$ 126 mg/dL or HbA1c $\geq$ 6.5% or T2DM medications or past history	6.1%	2.37 (1.60 to 3.52)	Age, sex, BMI, impaired fasting glycaemia, family history, dyslipidaemia, blood pressure, exercise
Ming <i>et al</i> , 2015 <sup>123</sup>	China	508	5.0	FBG $\geq$ 126 mg/dL or 2-hour OGTT $\geq$ 200 mg/dL or T2DM medications	3.9%	4.46 (1.86 to 10.73)	Age, sex, BMI, education, smoking, alcohol intake, exercise, family history, blood pressure, FBG, HDL-cholesterol, triglycerides
Chen <i>et al</i> , 2016 <sup>120</sup>	China	6542	6.0	FBG $\geq$ 126 mg/dL or T2DM medications or past history	5.6%	2.17 (1.53 to 3.01)*	Age, BMI, triglycerides, impaired fasting glycaemia status
Fukada <i>et al</i> , 2016 <sup>121</sup>	Japan	4629	12.8	FBG $\geq$ 126 mg/dL or HbA1c $\geq$ 6.5% or T2DM medications	7.6%	3.6 (2.1 to 5.8) for lean 6.8 (5.2 to 8.9) for obese	Age, sex, smoking, alcohol intake, exercise, HbA1c, family history of diabetes

Only cohort studies with a median follow-up period of at least 5 years are presented. The study by Shah *et al* is the only one in which the diagnosis of NAFLD was based on CT. The study by Ming *et al* is the only one in which the diagnosis of diabetes mellitus was also based on 2-hour OGTT glucose levels.

\* HR presented.

ALT, alanine aminotransferase; BMI, body mass index; CRP, C reactive protein; FBG, fasting blood glucose; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.



T2DM with NAFLD is limited to patients with either baseline-impaired fasting glycaemia or those with high baseline levels of insulin resistance.<sup>127 133</sup> Similarly, a Finnish study of hypertensive patients followed for 21 years found that NAFLD was associated with an increased risk of incident T2DM when coexisting with the metabolic syndrome, but not in its absence.<sup>134</sup> Surprisingly, however, two Japanese studies found the magnitude of risk of incident T2DM to be higher in patients who have NAFLD but are lean or have lower BMI values.<sup>121 128</sup> This finding might be, at least in part, due to the lower sensitivity of ultrasonography for detecting NAFLD with increasing BMI, potentially leading to some misclassification bias.

The severity of underlying NAFLD appears to influence the magnitude of future risk of T2DM, with increasing risk that parallels the severity of hepatic steatosis on ultrasonography or CT.<sup>122 125 126</sup> Importantly, in these studies the T2DM risk associated with NAFLD remained significant even after adjustment for a range of common T2DM risk factors. Similarly, a Korean study found that only patients with NAFLD with elevated alanine-aminotransferase levels had an increased risk of incident T2DM, which may reflect more severe hepatic steatosis or the histologically more aggressive subtype of NASH.<sup>135</sup> Correspondingly, patients with NASH have a higher risk of developing T2DM compared with those with simple steatosis, although it is not clear whether this risk is independent of confounding factors such as obesity.<sup>50</sup> Lastly, after stratification of patients with NAFLD according to likelihood of advanced hepatic fibrosis by the NFS, those with high or intermediate NFS values are more than twice as likely to develop T2DM than those with low NFS values.<sup>119</sup> Although there is now convincing evidence that NAFLD is strongly associated with the risk of incident CKD in patients with type 1 or type 2 diabetes (as discussed previously), it remains unclear whether NAFLD or NASH are also independent risk factors for diabetic retinopathy (another microvascular complication of diabetes), with evidence limited only to cross-sectional studies with conflicting conclusions.<sup>89 136</sup>

It is important to underline that the majority of observational studies that examined the relationship between NAFLD and risk of incident T2DM are retrospective and originate from Asia where large populations undergo regular health check-ups including liver ultrasonography. Only one study from the USA found CT-determined hepatic steatosis to be independently associated with increased risk of incident T2DM in a population that included Caucasians, Blacks and Hispanics; however whether this risk differs according to race is unknown as a stratified analysis was not performed.<sup>126</sup> Additionally, as Asian and non-Asian populations have different adipose tissue distributions and cultural/genetic backgrounds, further evidence is needed in different ethnic and racial groups. Finally, the study by Ming *et al*<sup>123</sup> was the only one in which the diagnosis of diabetes was also based on 2-hour postload plasma glucose levels.

Currently, it is unclear whether NAFLD is causally related to T2DM development or is simply a marker of other shared risk factors, such as visceral adipose tissue. Notably, some genetic conditions leading to intrahepatic triglyceride accumulation, such as familial hypobetalipoproteinaemia, do not lead to increased insulin resistance, and genetic polymorphisms in the *PNPLA3* gene (that also correlate with higher intrahepatic triglyceride content and increased risk of NASH, but are not systematically associated with insulin resistance and metabolic syndrome features) only appear to associate with T2DM in the presence of obesity, suggesting that other factors in addition to hepatic steatosis are important for the development of T2DM.<sup>69 137 138</sup>

Despite the above caveats, a large number of cohort studies clearly demonstrated NAFLD to be associated with an approximate doubling of risk of incident T2DM. This association appears to be dose-dependent and is ameliorated with NAFLD improvement or resolution over time. Consequently, current clinical guidelines recommend routine screening of patients with NAFLD for T2DM with fasting blood glucose or haemoglobin A1c levels or with a 75 g oral glucose tolerance test in high-risk groups.<sup>139</sup>

#### EPIDEMIOLOGICAL DATA LINKING NAFLD TO RISK OF COLORECTAL ADENOMAS/CANCER AND OTHER EXTRAHEPATIC NEOPLASMS

Patients with NAFLD typically have a range of metabolic risk factors associated with the development of neoplasia, including T2DM and obesity; consequently, extrahepatic malignancy is the second most common cause of death among patients with NAFLD.<sup>4 5</sup> In addition, one community-based cohort study of patients with T2DM suggested that NAFLD was associated with an increased risk of death from malignancy, suggesting an independent effect of NAFLD over and above coexisting metabolic risk factors.<sup>140</sup>

The strongest association between NAFLD and extrahepatic neoplasia has been reported with colorectal adenomas (as summarised in table 3). One Taiwanese study of 1522 individuals found a diagnosis of NAFLD in subjects with a normal baseline colonoscopy was associated with a 45% increased risk of future adenoma on subsequent colonoscopy 5 years later.<sup>141</sup> This association remained significant after adjustment for age, sex, smoking and metabolic risk factors. Two subsequent meta-analyses of five observational studies each, demonstrated a 1.5–1.7-fold increased risk of colorectal adenomas.<sup>142 143</sup> NAFLD was also associated with multiple colorectal adenomas ( $\geq 3$ ) and a tendency towards proximal lesions (right-sided colonic adenomas). Whether the risk of colorectal adenoma increases with NASH compared with simple steatosis is unclear with two relatively small cross-sectional studies demonstrating conflicting results.<sup>144 145</sup> Recently, a large cross-sectional study of 26 540 asymptomatic individuals from South Korea however, found the risk for any colorectal neoplasia and advanced colorectal neoplasia to be particularly increased in patients with NAFLD with high NFS or other non-invasive fibrosis scores.<sup>146</sup>

The risk of developing colorectal cancer also appears to be increased, with two large cohort studies of Korean and Chinese individuals undergoing colonoscopy (n=2315 and n=5517, respectively) finding a diagnosis of NAFLD on ultrasound to be significantly associated with an increased cancer risk of 1.87 (95% CI 1.4 to 2.6) and 3.08 (95% CI 1.02 to 9.3), respectively.<sup>147 148</sup> Preliminary reports have also linked NAFLD with the occurrence of gastric and prostate cancers<sup>149</sup>, however further data are required before any definitive conclusions can be made. Further research is also needed to establish whether risk of these extrahepatic cancers differs with severity of NAFLD.

Nevertheless, the studies to date have been predominately cross-sectional, limiting any inference about causality and largely restricted to Asian populations. Currently, although a diagnosis of NAFLD is not sufficient to recommend screening colonoscopy, evaluation of colonic symptoms and ensuring patients are enrolled in colorectal cancer screening programmes as per recommendations for the general population is strongly recommended. Further evidence is needed to clarify the risk in patients aged 40–50 years who are not currently within routine screening guidelines. However, if the potential adverse impact of NAFLD on risk of colorectal adenomas/cancer will be

**Table 3** Cohort studies examining NAFLD as a possible risk factor for colorectal neoplasia

Authors, year (ref.)	Country	Study design	Sample size	Follow-up (median, years)	Adenoma (%)		Carcinoma (%)		Adjusted OR(s) ( $\pm$ 95% CI)		Adjusted variables
					Non-NAFLD	NAFLD	Non-NAFLD	NAFLD	Adenoma	Carcinoma	
Hwang <i>et al</i> , 2010 <sup>136</sup>	South Korea	Cross-sectional	2917	NA	16	24	–	–	1.28 (1.03 to 1.60)	–	Age, sex, smoking, T2DM, blood pressure, MetS
Touzin <i>et al</i> , 2011 <sup>145</sup>	USA	Cross-sectional	233	NA	25	24	–	–	–	–	–
Wong <i>et al</i> , 2011 <sup>144</sup>	Hong Kong	Cross-sectional	380	NA	22	35	0.6	1	1.61 (0.90 to 2.90)	–	Age, sex, BMI, smoking, family history, blood pressure, T2DM, aspirin use
Stadlmayr <i>et al</i> , 2011 <sup>137</sup>	Austria	Cross-sectional	1211	NA	17	28	0.1	0.9	1.47 (1.08 to 2.00)	–	Age, sex, BMI, glucose intolerance status
Lin <i>et al</i> , 2014 <sup>148</sup>	China	Cross-sectional	2315	NA	56	44	18	29	0.86 (0.61 to 1.20)	1.87 (1.36 to 2.57)	Age, sex, smoking, BMI, alcohol intake, T2DM, blood pressure, lipids, serum liver enzymes
Bhatt <i>et al</i> , 2015 <sup>198</sup>	USA	Cross-sectional	591*	NA	21	32	0	0	1.95 (1.09 to 3.48)	–	Age, ethnicity, BMI, blood pressure, alcohol intake, smoking, MELD or Child-Pugh classes
Basyigit <i>et al</i> , 2015 <sup>199</sup>	Turkey	Cross-sectional	127	NA	26	20	24	5	0.63 (0.46 to 1.65)	0.13 (0.12 to 0.31)	Age, sex, obesity, T2DM, MetS
Ahn <i>et al</i> , 2017 <sup>146</sup>	South Korea	Cross-sectional	26 540	NA	29	38	–	–	1.10 (1.03 to 1.17)	–	Age, sex, BMI, smoking, alcohol intake, family history, aspirin use, MetS factors
Lee <i>et al</i> , 2012 <sup>147</sup>	South Korea	Cohort	5517 (women)	7 years	–	–	–	–	1.94 (1.11 to 3.40)	3.08 (1.02 to 9.34)	Age, smoking, MetS
Huang <i>et al</i> , 2013 <sup>141</sup>	Taiwan	Cohort	1522†	5 years	39	56	–	–	1.45 (1.07 to 1.98)	–	Age, sex, BMI, smoking, blood pressure, T2DM, MetS

\*All patients being evaluated for liver transplantation.

†All had negative baseline colonoscopy.

BMI, body mass index; MELD, model for end-stage liver disease; MetS, metabolic syndrome; NA, not applicable; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus.

confirmed in future large-scale prospective studies, there may be significant implications for screening and surveillance strategies given the growing number of patients with NAFLD.

### EPIDEMIOLOGICAL DATA LINKING NAFLD TO RISK OF OSTEOPOROSIS

NAFLD is associated with multiple factors linked to decreased bone mineral density (BMD), including T2DM, obesity, insulin resistance, chronic inflammation, vitamin D<sub>3</sub> deficiency and low levels of physical activity.<sup>150</sup> Consequently, several cross-sectional and case-control studies involving both adults and adolescents have found a significant association between low BMD or osteoporosis and NAFLD.<sup>150</sup> Worryingly, this association has been also reported in the paediatric age group, with one study of 38 obese children with biopsy-proven NAFLD reporting significantly lower BMD compared with 38 non-steatotic children matched for age, sex, ethnicity and BMI.<sup>151</sup> The degree of BMD fell with increasing severity of liver disease with patients with NASH having a lower level than those with simple steatosis.

In adults, the association between NAFLD and osteoporosis is less consistent, with a meta-analysis of a total of 1276 individuals finding no significant differences in BMD between subjects with or without NAFLD, whereas a significant association with BMI was found.<sup>152</sup> Significant heterogeneity between studies however, limits conclusions from this meta-analysis. Subsequently, three large cohort studies totalling over 2500 individuals, all found a significant inverse association between BMD and NAFLD, although interestingly the association was limited to men and not seen in postmenopausal women.<sup>153–155</sup> The association between NAFLD and BMD was modest, however, and remained significant in these studies even after adjusting for several potential confounding factors, including age, weight, alcohol intake, smoking and metabolic risk factors. Increasing ultrasonographic severity of hepatic steatosis as well as serum alanine-aminotransferase levels correlated inversely with BMD.<sup>153</sup> A further cohort study of 7797 Chinese adults aged >40 years confirms the existence of a sex-specific association with a higher rate of recent osteoporotic fractures in men with NAFLD compared with those without (3.6% vs 1.7%,  $p=0.003$ ), with no difference seen in women.<sup>156</sup> Overall, men with NAFLD had a 2.5-fold increased risk of recent osteoporotic fractures even after adjustment for age, smoking, alcohol intake, physical activity, metabolic risk factors, kidney function and use of glucocorticoids or osteoporosis medications.<sup>156</sup> The male-specific association suggests a sex-specific interaction between hepatic steatosis and bone metabolism, or that other factors in women, such as oestrogen status, may dominate the risk of osteoporosis.

Collectively, these findings argue for awareness of BMD among patients with NAFLD. However, it is important to note that the available data are limited to cross-sectional studies largely from Asian populations. The majority of studies focus on BMD rather than bone fracture rate and further evidence is required to clarify whether patients with NAFLD (in particular men) should be screened for osteoporosis.

### PUTATIVE MECHANISMS LINKING NAFLD TO EXTRAHEPATIC CONDITIONS

Extrahepatic pathological conditions in NAFLD are closely linked to the presence of local (ie, in the liver, adipose tissue, vessels and heart) and systemic inflammation. Chronic low-grade inflammation accompanies many metabolic disorders, such as T2DM or NAFLD.<sup>157</sup> It has been demonstrated that inflammatory pathways such as IL-1-type cytokines are driving forces of disease processes in NAFLD and correlate with prognosis of

liver disease.<sup>158–159</sup> Hepatic inflammation might be responsible for the overall degree of hepatic fibrosis and thereby prognosis of this disorder and might also control insulin resistance. Hepatic/peripheral insulin resistance (ie, a hallmark in most patients with NAFLD) and metabolic inflammation are frequently observed in parallel, and research from the past has tried to connect these two phenomena.<sup>160</sup> The origin of chronic inflammatory processes observed in NAFLD is still a matter of discussion as multiple parallel ‘hits’ might control and regulate disease process.<sup>161</sup> Besides lipotoxicity, the GI tract with its significantly altered microbiota could reflect one of the early events in disease development. In addition, abdominal visceral adipose tissue accumulation is another ‘hot’ candidate reflecting a major site of chronic inflammation in NAFLD as adipose tissue inflammation is commonly observed in obesity, T2DM and NAFLD. Indeed, it has been shown that the inflamed (‘dysfunctional’) adipose tissue in obesity might generate almost 50% of circulating IL-6 thereby contributing substantially to systemic chronic inflammation.<sup>162</sup> The importance of visceral adipose tissue as site of chronic inflammation is further supported by some studies demonstrating that the lack of adipocyte 5’ AMP-activated protein kinase worsens insulin resistance and liver disease by affecting brown and beige adipose tissue function.<sup>163</sup> Additionally, adipose tissue-specific insulin receptor knockout mice develop more severe NAFLD with histological evidence of ballooning degeneration further supporting the notion that the adipose tissue is of key importance in the development and progression of NAFLD.<sup>164</sup> In contrast, recent research has found that adipose tissue type I interferon signalling may protect from metabolic dysfunction.<sup>165</sup>

### Nutrition and dietary factors

Nutrition is critically involved in the pathogenesis of NAFLD, besides the fact that NAFLD is mainly a disease of the obese population. Overnutrition and certain dietary factors have been demonstrated to induce chronic low-grade inflammation.<sup>166–167</sup> Epidemiological studies have shown that diet can affect inflammatory processes and the immune system directly, and also through interactions with the gut microbiota. Western diets are able to promote chronic inflammatory processes by many pathways. A high salt content may induce IL-17 producing T helper cells and thereby inflammation.<sup>168</sup> Dietary fatty acids promote inflammation through several mechanisms, including direct effects on immune cells, activation of toll-like receptors and cytokine cascades,<sup>169</sup> and affect intestinal permeability.<sup>170</sup> Mice with a loss of junctional adhesion molecule A (JAM-A) have a defective intestinal epithelial barrier and exhibit more prominent NASH, and colonic tissue from patients with NAFLD have lower levels of JAM-A and higher levels of inflammation compared with healthy controls.<sup>171</sup> In healthy individuals, a high-fat Western diet induces endotoxaemia and, therefore, might contribute to the observed chronic inflammation in NAFLD.<sup>172</sup> A diet rich in saturated milk-derived fatty acids worsens colitis in IL-10 deficient mice via profound changes of the gut microbiota and an increased Th1 cell response.<sup>173</sup> Many studies have also associated dietary phosphatidylcholine and L-carnitine consumption with generation of specific metabolites (ie, trimethylamine (TMA) and TMA N-oxide) and future CVD events.<sup>174</sup> Not surprisingly, dietary interventions might be able to reverse a Western-diet induced liver phenotype as shown, for example, for citrulline.<sup>175</sup> Overall, the composition of Western diets might contribute to the development/progression of NAFLD via generation of overweight and obesity, and induce activation of specific inflammatory pathways.

## Gut microbiota

Evidence is increasing that the gut microbiota might contribute to the pathogenesis of NAFLD. NAFLD reflects a prototypical disease of innate immunity and it is expected that certain bacteria in the intestinal tract activate various pathways of innate immunity in the case of disease.<sup>176</sup> In several mouse models, the microbiota affects hepatic steatosis and fat storage. Human studies from the past years have also supported a role for the microbiota in NAFLD. *Actinobacteria* were increased whereas *Bacteroidetes* were reduced dependent on liver disease activity as demonstrated in a paediatric study population.<sup>177</sup> At species levels, *Oscillobacter* decreased in NAFLD whereas *Ruminococcus*, *Blautia* and *Dorea* increased especially in patients with NASH.<sup>177</sup> Another study assessed the gut microbiota and severity of histology-proven NAFLD in 57 patients.<sup>178</sup> *Bacteroides* abundance increased depending on severity of disease, whereas *Prevotella* abundance decreased. *Ruminococcus* abundance increased in more severe disease, especially if advanced hepatic fibrosis was diagnosed.<sup>178</sup> Overall, many studies from the past years clearly highlighted a role for the gut microbiota in obesity-related disorders supporting the assumption that NASH might be associated with a 'microbiome signature' which could contribute to initiation of inflammation. It has to be acknowledged, however, that these early studies are rather descriptive and neither studies (either preclinical or clinical) have so far identified certain bacterial strains, which act proinflammatory and are indeed involved in disease pathogenesis. The altered microbiome in patients with NAFLD could also be involved in the development of NAFLD-associated malignancy both in the liver<sup>179</sup> and in the GI tract.<sup>180</sup>

## Cytokines/adipocytokines

It is well established that proinflammatory cytokines and transcription factors are highly expressed in various tissues, such as the adipose tissue or liver, in NAFLD and a large number of different immune cells and, especially, tissue macrophages may contribute to the inflammatory phenotype. Various proinflammatory cytokines, such as TNF- $\alpha$  or IL-6, are activated in different tissues but especially in adipose tissue and in the liver of patients with NAFLD/NASH.<sup>181</sup> There is substantial evidence that activation of the transcription of nuclear factor- $\kappa$ B (NF- $\kappa$ B) and downstream inflammatory signalling pathways are involved in metabolic liver inflammation and associated hepatic insulin resistance.<sup>182 183</sup> Receptor activator of NF- $\kappa$ B, a prototypical activator of NF- $\kappa$ B, regulates hepatic insulin sensitivity.<sup>184</sup> Deletion of hepatic MyD88 results in liver inflammation and hepatic insulin resistance also affecting the gut microbiota composition and metabolome.<sup>185</sup> Therefore, several studies using hepatocyte-specific targeting of genes have now strongly supported the notion that hepatocytes are major metabolic 'players' in the development of metabolic inflammation in and beyond the liver.

Chronic inflammation and cytokine activation is a driving force in the evolution of malignancy and, therefore, it is expected that patients with NAFLD exhibit a high rate of liver and extrahepatic malignancies.<sup>144 186</sup> Chronic IL-6 overexpression has been found to result in the generation of liver tumours.<sup>187</sup> In these studies, the development of obesity-promoted hepatocellular carcinoma was dependent on enhanced production of the tumour-promoting cytokines IL-6 and TNF- $\alpha$ , which cause hepatic inflammation and activation of the oncogenic transcription factor STAT3. Besides several cytokines certain adipocytokines, such as adiponectin and leptin, might contribute to systemic inflammatory processes and associated

pathologies in NAFLD.<sup>188 189</sup> For example, NAFLD is associated with hypoadiponectinaemia and lower adiponectin levels correlate with colorectal cancer/adenoma incidence.<sup>190 191</sup> Low-level plasma adiponectin is especially associated with KRAS-mutant colorectal cancer risk.<sup>192</sup> Therefore, it may be concluded that chronic activation of cytokine/adipocytokine cascades as observed in NAFLD might contribute substantially to the extrahepatic chronic diseases observed in NAFLD ranging from CVD to osteoporosis and especially also hepatic and extrahepatic cancers.

## CONCLUSIONS

This review further reinforces the view that NAFLD is a multi-system disease that affects many extrahepatic organ systems and interacts with the regulation of multiple metabolic pathways. NAFLD is associated with liver-related morbidity and mortality, and with an increased risk of developing important extrahepatic chronic diseases, such as CVD, T2DM and CKD. Emerging data also suggest NAFLD may be a risk factor for colonic adenomas/cancer and decreased BMD, particularly among men. Clear evidence indicates that CVD is the leading cause of death in patients with NAFLD. This implies that patients with NAFLD should undergo careful cardiovascular surveillance. In line with this implication, given that CVD complications dictate the outcome(s) in patients with NAFLD more frequently and to a greater extent than does the progression of liver disease, the recent European clinical practice guidelines have recommended CVD risk assessment in all patients with NAFLD.<sup>139</sup>

There is a growing body of epidemiological and experimental evidence suggesting that NAFLD, especially its necroinflammatory form, exacerbates hepatic/peripheral insulin resistance, predisposes to atherogenic dyslipidaemia and releases a variety of proinflammatory, procoagulant, thrombogenic and profibrogenic factors that may promote the development of CVD, CKD, T2DM and other extrahepatic chronic diseases.

However, although all these mechanisms plausibly link NAFLD to the development and progression of CVD, CKD and other extrahepatic diseases, no studies to date have proven a cause-and-effect relationship and further research is certainly needed to gain mechanistic insights into the pathophysiology linking NAFLD to the development and progression of these extrahepatic chronic diseases.

In the meantime, we believe that the clinical implication of these findings is that a diagnosis of NAFLD may identify a subset of the general population, which is exposed to an increased risk of developing some important extrahepatic chronic diseases. Therefore, patients with NAFLD might benefit from more intensive surveillance or early treatment interventions to decrease the risk of developing CVD, CKD, T2DM and other extrahepatic manifestations. Thus, clinicians who manage patients with NAFLD, especially those with NASH with varying amounts of fibrosis, should focus on liver disease, and should recognise the increased risk of CVD, CKD, T2DM and other serious extrahepatic manifestations of these patients, screen them for conventional risk factors and undertake early, aggressive risk factor modification(s).

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