

## NON-ALCOHOLIC FATTY LIVER DISEASE : AN EMERGING CARDIOVASCULAR RISK FACTOR - EVIDENCE & CONTROVERSIES.



### Cardiology

#### KEYWORDS:

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### ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a marker of pathological ectopic fat accumulation combined with a low-grade chronic inflammatory state. This results in several deleterious pathophysiological processes including abnormal glucose, fatty acid and lipoprotein metabolism, increase oxidative stress, deranged adipokine profile, hypercoagulability, endothelial dysfunction, and accelerated progression of atherosclerosis. NAFLD affects up to a third of the population worldwide and may confer increased cardiometabolic risk with consequent adverse cardiovascular outcomes independent of traditional cardiovascular risk factors and the metabolic syndrome. It is characterized almost universally by insulin resistance and is strongly associated with type 2 diabetes and obesity. This ultimately leads to a dysfunctional cardiometabolic phenotype with cardiovascular mortality representing the main mode of premature death in NAFLD. This article aims at discussing in-depth the evidence to date linking NAFLD with cardiovascular disease & reviewing the likely mechanisms underlying this association.

### Introduction

The role of non-alcoholic fatty liver disease (NAFLD) as a potential independent cardiovascular (CV) risk factor has now gained considerable prominence such that an awareness of this multifaceted condition is essential for practising physicians, given that it affects 20-33% of the general population<sup>1,4</sup>. As the pathogenesis of the condition is closely linked to insulin resistance (IR), its prevalence parallels that of increasing rates of obesity and type 2 diabetes worldwide, with up to 95% of obese persons and 75% of diabetics likely to have NAFLD, with most cases unrecognized. The potential future burden of NAFLD on public health-care utilization and costs is likely to be significant. As such, the cardio-metabolic risk conferred by NAFLD merits increased collaborative study between physicians, diabetologists, hepatologists, and especially cardiologists, given that CV disease appears to largely influence major clinical outcomes in NAFLD.<sup>5-10</sup> This article aims to present to the physicians summary of the evidence linking NAFLD with CV disease, the potential mechanisms underlying this association, as well as its relation to IR, obesity and the metabolic syndrome (MetS) in the context of increased CV risk.

### Definition of non-alcoholic fatty liver disease (NAFLD)

NAFLD is the most common cause of chronic liver disease in the general population and is present when fatty infiltration affects > 5% of hepatocytes, in the presence of <20g (2.5 U) of alcohol consumption per day, without evidence of other causes of liver disease<sup>11</sup>. NAFLD is a slowly progressive condition and represents a spectrum of varying severity of liver disease, ranging from simple steatosis to co-existent inflammation with hepatocyte ballooning and necrosis, variable grades of fibrosis, and ultimately cirrhosis and an increased risk of hepatocellular carcinoma.<sup>11,12</sup> Nonalcoholic steatohepatitis (NASH) represents the more advanced stages of this disease, i.e. the 'inflammatory' component in addition to steatosis, which carries a higher risk of CV disease and mortality than simple steatosis.<sup>13</sup> Insulin resistance and obesity, both key features of the MetS, are strongly associated with NAFLD progression<sup>14</sup>. The prevalence of NAFLD in subjects with MetS is increased four-fold compared with those without the disease and 3% of NAFLD subjects have MetS.<sup>2</sup> Despite MetS itself conferring an approximate doubling of CV mortality risk,<sup>15</sup> there is still abundant evidence linking NAFLD to increased CV disease risk over and above that associated with the MetS criteria, suggesting that NAFLD per se contributes to accelerated atherogenesis.<sup>16</sup>

### Diagnosis of non-alcoholic fatty liver disease

Current laboratory and radiological methods to diagnose NAFLD are either too insensitive or not specific enough to grade disease presence and severity. As the early stages of NAFLD are often asymptomatic, mildly abnormal liver enzymes are usually the only clue pointing to the disease. However up to 70% of NAFLD patients may have normal liver enzymes,<sup>17</sup> and although alanine

aminotransferase (ALT) levels have shown to be the best single biochemical correlate of hepatic steatosis,<sup>18</sup> they do not distinguish between varying stages of NASH and can be normal in histologically severe disease.<sup>19</sup> Furthermore, ultrasound imaging can only detect steatosis when >30% of the liver is affected, but is still recommended as the first-line investigation to 'confirm' the presence of fatty liver due to its widespread availability and low cost.<sup>20</sup> Although magnetic resonance spectroscopy (MRS) has excellent sensitivity in detecting and accurately quantifying hepatic steatosis, none of the non-invasive modalities can detect inflammation and/or fibrosis, i.e. NASH. Consequently, liver biopsy is at present the 'gold-standard' (taking into account potential inaccuracies of sampling variability) for diagnosing NAFLD and staging the degree of NASH and fibrosis by histological assessment, as well as monitoring disease progression.<sup>21</sup> Because of the highly invasive and potentially risky nature of liver biopsy, various algorithms of combined clinical and specialized blood biomarkers, along with advanced imaging methods (e.g. MR/ultrasound elastography) are being developed to allow improved non-invasive detection of disease stage and activity.<sup>20</sup>

### Epidemiology of cardiovascular disease in non-alcoholic fatty liver disease

Numerous epidemiological studies have reported an increased incidence of adverse CV events in NAFLD subjects compared with the general populations<sup>5-10,22-29</sup> (Table 1). As NAFLD is the commonest cause of abnormal liver enzymes in developed countries,<sup>20</sup> many epidemiological studies have employed these as biochemical surrogates of NAFLD. Several studies have shown a significant association between increased gamma-glutamyltransferase (GGT) levels and CV mortality over an average median of 12-year follow-up, even after adjusting for typical CV risk factors and body mass index (BMI).<sup>22-24</sup> Additionally, a meta-analysis of 10 pooled studies confirmed the independent association between elevated GGT and adverse CV events.<sup>25</sup> However, GGT is also expressed in atherosclerotic plaques and has a role in oxidative stress,<sup>30</sup> as well as being associated with all components of the MetS.<sup>31</sup> ALT has been reported to be more closely related to liver fat content than GGT.<sup>14</sup> Similarly, several large population-based cohort studies have reported an independent association between elevated ALT and CV mortality after adjusting for CV risk factors.<sup>26-28</sup> Importantly, the correlation of raised ALT or GGT with CV disease in these studies may simply reflect their significant association with IR,<sup>32</sup> which is itself a strong risk factor for CV disease, rather than as a marker for the presence or severity of NAFLD. Employing ultrasound imaging as a more specific diagnostic determinant of NAFLD than liver enzymes, three large community based prospective cohort studies also documented a significant independent association with CV events<sup>5-7</sup> (Table 1). Of note, Hamaguchi et al.<sup>6</sup> undertook a prospective analysis of 1637 healthy subjects recruited from a health check-up programme, and found 19% with ultrasound evidence of NAFLD. At 5-year follow-up, 5.2% of the NAFLD group suffered an adverse CV

event, compared with 1.0% of the non-NAFLD group ( $P < 0.001$ ). By multivariate analysis, the association between NAFLD and future CV events was shown to be independent of the MetS, as well as conventional cardiac risk factors. Although these studies are strongly indicative of NAFLD as a predictor of CV disease independent of diabetic status, they are limited by the lack of sensitivity of ultrasound determination of NAFLD.

Even so, smaller long-term prospective studies in patients with biopsy-proven NAFLD show significantly higher total mortality rates compared with a matched reference population, with CV disease representing the main mode of death, outnumbering cancer- and liver-related mortality.<sup>8,10</sup> Of note, only subjects with NASH rather than simple steatosis had significantly reduced survival, although in one study even subjects with bland steatosis showed a trend to reduced survival ( $P = .006$ ), primarily from CV-related causes over a median follow-up of 24 years.<sup>8</sup>

#### **Evidence of association of NAFLD with cardiovascular disease**

##### **Cardiovascular risk assessment scores in non-alcoholic fatty liver disease**

Given that traditional CV risk factors are commonly prevalent in NAFLD subjects, investigators have applied validated CV risk prediction scores to evaluate the risk profile of NAFLD patients, with most of these studies showing that NAFLD independently confers an increased CV risk score.<sup>33-36</sup> One study also documented that high-sensitivity C-reactive protein, a well-established marker of adverse CV outcome, was significantly elevated compared with the non-NAFLD group in both sexes. A strong association has been shown between histological severity and calculated estimates of CV risk [both QRISK2 and Framingham risk score (FRS)] independently of markers of glucose control and obesity.<sup>37</sup>

Although these global risk prediction studies may help to describe part of the association between NAFLD and increased CV risk, they are flawed by the inherent limitations of using risk scores based on traditional CV risk factor-derived multivariable statistical models to identify at-risk patients.<sup>38</sup> Furthermore, we know that some of the important determinants of NAFLD, such as IR, obesity, and raised triglycerides (TG), all of which also increase the risk of CV disease in MetS,<sup>39</sup> which shares many features in common with NAFLD status including its direct cardio-metabolic effects, before we can evaluate its added discriminant value when applied to current CV risk prediction models in cohort studies.

##### **Studies evaluating coronary disease in non-alcoholic fatty liver disease**

Coronary artery calcium (CAC) scoring with cardiac computed tomography (CT) is a very sensitive method of demonstrating the presence and extent of coronary atherosclerosis and significantly improving CV risk prediction in asymptomatic individuals beyond traditional risk factor scoring system.<sup>40</sup> Several studies demonstrate a significantly increased coronary atherosclerotic burden in the presence of NAFLD,<sup>41-44</sup> with one study also reporting a significant association between 'vulnerable plaque' and NAFLD in patients undergoing multislice CT for clinical suspicion of coronary artery disease (CAD).<sup>42</sup> This finding is consistent with data showing that NAFLD patients have significantly higher plasma markers of oxidative stress and inflammation, which are in part derived from the diseased liver causing a systemic inflammatory and prothrombotic state.<sup>45-46</sup> A strong association between NAFLD and prevalence of significant CAD determined by coronary angiography has also been consistently reported albeit with variable thresholds of 'significant' CAD between studies.<sup>48-51</sup> Although these studies indicate an independent association between NAFLD and CAD in terms of angiographic appearance even after adjusting for traditional CV risk factors and components of the MetS, none of them evaluated the functional significance of these coronary lesions. Given that the presence of ischaemia rather than coronary anatomy dictate clinical outcome,<sup>52-53</sup> the significance of these findings in association with NAFLD should not be overestimated.

##### **Studies evaluating carotid disease in non-alcoholic fatty liver disease**

Measurement of carotid intima-media thickness (CIMT) and plaque burden by ultrasound is a well-validated and widely accepted screening tool for the prediction of CV disease in asymptomatic subject.<sup>54,55</sup> Several studies link NAFLD independently with carotid disease, although a few have described a weaker association after adjusting for MetS.<sup>35,56-61</sup> Importantly severity of histological features of NAFLD appears to correlate independently with increasing CIMT,<sup>58</sup> concordant with epidemiological data documenting NASH patients having a higher CV risk than simple steatosis. Additionally, a systematic review of seven published studies (total of 3497 subjects) reported a significant association between NAFLD and CIMT, showing an estimated increase of 13% in CIMT for NAFLD cases compared with controls. Prevalence of carotid plaque was also more frequent in NAFLD subjects.<sup>62</sup>

##### **Studies evaluating cardiac function in non-alcoholic fatty liver disease**

Studies in subjects with MetS have consistently shown increased left ventricular (LV) mass index and diastolic function impairment when compared with controls, which could be secondary to the effects of IR, obesity and hypertension on cardiac structure and function.<sup>67,68</sup> Only a few studies have focused specifically on NAFLD subjects, and the finding of abnormal LV geometry and diastolic dysfunction has similarly been reported.<sup>69</sup> One study also demonstrated a strong positive correlation between the degree of diastolic dysfunction and amount of liver fat, with diastolic dysfunction and IR the only independent parameters associated with NAFLD.<sup>70</sup> Another study reported that echocardiographic measures of coronary flow reserve (CFR) were significantly lower in NAFLD compared with healthy controls, after adjusting for obesity, traditional CV risk factors and the presence of MetS.<sup>68</sup> Although they correctly postulated that this result likely reflects impaired coronary endothelial function in the NAFLD group, they were unable to exclude the possibility of these patients having asymptomatic epicardial CAD. The consistent finding of subclinical cardiac dysfunction in an asymptomatic population with NAFLD is perhaps not surprising, given that LV dysfunction and LV mass are strongly correlated with IR, as well as subsequent prognosis.<sup>2</sup>

##### **Studies evaluating endothelial dysfunction and myocardial metabolism in NAFLD.**

Endothelial dysfunction is now recognized as the earliest detectable component in the development of atherosclerosis. In both diabetic and non-diabetic cohorts, studies have shown an independent association between impaired endothelium-dependent flow-mediated dilation (FMD) and NAFLD.<sup>2</sup> In addition, lower FMD was observed in NASH compared with simple steatosis, again confirming the graded association of CV risk with severity of NAFLD.<sup>36</sup> To gain further insight on the causes of subclinical cardiac dysfunction in NAFLD, the effects of hepatic steatosis on myocardial metabolism have also been examined.<sup>2,69</sup> One study found a novel positive association between hepatic fat content and myocardial IR. Patients with high liver fat content not only showed significantly lower whole-body insulin sensitivity as expected, but also reduced myocardial glucose uptake and extraction rate, reduced CFR, and increased plasma levels of inflammatory markers and vascular adhesion molecules. Only liver fat content remained significantly associated with impaired myocardial metabolism even after adjusting for IR, visceral fat mass, and other important variables.<sup>64,69</sup> Another study assessed myocardial energy metabolism in NAFLD, utilizing 31 P-MRS to determine the ratio of phosphocreatine to ATP in a young, healthy cohort.<sup>13</sup> The authors reported significantly impaired LV energy metabolism as well as increased epicardial fat in NAFLD compared with controls. This was despite normal LV morphological features and systolic/diastolic function in both groups, and was independent of usual CV risk factors. This suggests that in patients with hepatic steatosis, abnormalities in myocardial metabolism may precede functional and structural cardiac remodelling, leading to increased LV mass and diastolic dysfunction.

The precipitating factor for this dysfunctional cardiac phenotype appears to be the development of systemic and hepatic IR, leading to hyperinsulinaemia and increased FFA availability with associated myocardial IR. This produces inefficient energy metabolism by cardiomyocytes, switching to fat rather than glucose oxidation in physiologically demanding states & yielding less ATP per oxygen molecule consumed. With progressive workload placing the heart

under increasing strain, this potentiates myocardial dysfunction ultimately leading to myocardial adaptive remodelling and myocardial injury. The excess FFA supply also leads to cardiac lipotoxicity by causing intracellular lipid accumulation and overwhelming normal cardiomyocyte oxidative capacity, resulting in increased oxidative stress and consequent cardiac apoptosis and dysfunction.<sup>2,13</sup>

**Table - Main epidemiological studies relating NAFLD to increased cardiovascular risk**

Authors	Study characteristics [N-O assessment of quality] <sup>a</sup>	Diagnosis of NAFLD	Main findings
Ruttman et al. <sup>22</sup>	Austrian population-based cohort (n=163, 944), median F/U of 12 years [3,1,3]	Liver enzymes (GGT)	CV mortality increased in NAFLD, independent of traditional CV RF <sub>s</sub> , alcohol and BMI
Wannamethee et al. <sup>23</sup>	British population-based cohort (n=7613 middle-aged men), median F/U of 11.5 years [3,1,3]	Liver enzymes (GGT)	Total and CHD mortality increased in NAFLD independent of CV RF <sub>s</sub> , alcohol and BMI
Lee et al. <sup>24</sup>	Finnish population-based cohort (n=28, 838), F/U of 11.9 years [3,1,3]	Liver enzymes (GGT)	CHD mortality and non-fatal MI increased in NAFLD independent of CV RF <sub>s</sub> and alcohol
Fraser et al. <sup>25</sup>	Meta-analysis of 10 pooled population-based cohort studies <sup>b</sup>	Liver enzymes (GGT)	CV events (fatal and non-fatal) increased in NAFLD after adjustment for CV RF <sub>s</sub> and alcohol
Fraser et al. <sup>25</sup>	British Women's Heart and Health Study, population-based (n=2961 older women), median F/U of 4.6 years [3,1,3]	Liver enzymes (ALT and GGT)	No independent association between NAFLD and fatal and non-fatal CV events
Schindhelm et al. <sup>26</sup>	Hoorn Study, population-based (n=1439 middle-aged), F/U of 10 years [3,2,2]	Liver enzymes (ALT)	Fatal and non-fatal CHD increased in NAFLD, independent of CV and MetS RF <sub>s</sub>
Dunn et al. <sup>27</sup>	NHANES-III, population-based cohort (n=7574), mean F/U of 8.7 years [3,1,3]	Liver enzymes (ALT)	Total and CV mortality increased in NAFLD but only in 45-54 year age group. Independent of CV RF <sub>s</sub>
Yun et al. <sup>28</sup>	Korean population-based cohort (n=37, 085), median F/U of 5 years [3,1,3]	Liver enzymes (ALT)	CV or diabetes-related mortality increased in NAFLD, independent of CV RF <sub>s</sub> , alcohol, BMI, and socio-economics status
Targher et al. <sup>5</sup>	Valpolicella Heart Diabetes study, community-based diabetic cohort, free of CV disease (n=2103), mean F/U of 6.5 years [4,2,2]	Liver ultrasound	Increased fatal and non-fatal CV events in NAFLD, independent of CV RF <sub>s</sub> , diabetes control, and MetS
Hamaguchi et al. <sup>6</sup>	Japanese community-based healthy cohort (n=1637), mean F/U of 5.8 years [4,2,1]	Liver ultrasound	Increased adverse CV events in NAFLD, independent of CV RF <sub>s</sub> and MetS
Haring et al. <sup>7</sup>	Study of Health in Pomerania population-based German cohort (n=4160 middle-aged), median F/U of 7.3 years [3,1,3]	GGT and liver ultrasound	Increased CV mortality in men with NAFLD and raised GGT (but not women) after adjustment for cardio-metabolic RF <sub>s</sub>
Adams et al. <sup>9</sup>	Community-based North America cohort (n=420), mean F/U 7.6 years [3,0,3]	Majority had liver ultrasound (liver imaging or biopsy in all subjects)	Increased total mortality (mainly CV-related or cancer) in NAFLD compared with matched reference population
Ekstedt et al. <sup>10</sup>	Swedish hospital-based consecutive biopsy cases (n=129), mean F/U of 24 years [3,0,3]	Liver biopsy	Increased total mortality which was primarily CV-related (only in NASH patients but not in simple steatosis) compared with matched reference population
Soderberg et al. <sup>8</sup>	Swedish hospital-based consecutive biopsy cases (n=118), mean F/U of 24 years [3,0,3]	Liver biopsy	Increased total mortality in NAFLD was predominantly CV related, compared with matched reference population
Schwimmer et al. <sup>29</sup>	Cross-sectional consecutive autopsy biopsy cases of child death (n=817) from accidental or unnatural causes [3,0,3]	Liver biopsy (autopsy)	Increased coronary and aortic atherosclerosis in NAFLD, independent of obesity

**N.B:-N-O :New castle-Ottawa Scale for assessing the quality of non-randomised studies based on study selection (0-4), comparability (0-2), outcome/exposure (0-3).**

#### **Pathogenesis of cardiovascular disease in non-alcoholic fatty liver disease**

##### **Insulin resistance**

The majority of the above studies point to an independent link between NAFLD and increased CV risk or adverse CV outcome. However, there is considerable heterogeneity in these studies in the method of NAFLD diagnosis & quantification of severity of NAFLD. This appears to be of paramount importance due to the disparate pathophysiological and metabolic consequences of the various stages of simple steatosis and NASH, both strongly linked to hepatic and peripheral IR. In fact, liver fat content appears to be the best independent predictor of IR in skeletal muscle, adipose tissue, and the liver.<sup>12</sup> Similarly, adverse CV outcome is likely to be associated

with liver fat/inflammation, progressively increasing with more advanced stages of NAFLD.<sup>58,70</sup> This parallels epidemiologic evidence showing a progressive relationship between glucose levels and CV disease extending from well below the diabetic threshold.<sup>57</sup> Ultimately, the development and progression of IR appears to be key mediator in the initiation and propagation of NAFLD, primarily through adverse changes in glucose, fatty acid and lipoprotein metabolism, with both conditions subsequently driving each other in a synergistic fashion. Alterations in cellular FFA transport, possibly through hyperinsulinaemia, are involved in the pathogenesis of ectopic fat distribution by diverting the accumulation of TG away from adipose tissue and towards other key metabolic organs, such as skeletal muscle and liver. This results in impaired insulin signalling in these tissues, and further exacerbates IR & the consequent cardiometabolic dysfunctional cascade.<sup>57,64</sup> These processes are also exacerbated by associated subclinical inflammation, deranged

adipokines, and increased ectopic fat accumulation in other organs including the heart, all ultimately contributing to increased CV risk.

### Visceral fat

Visceral adipose tissue (VAT) appears to have a strong independent positive correlation with liver fat.<sup>18</sup> This is not surprising given that plasma FFAs appear to be the main source of hepatic TGs in NAFLD, arising in part by greater lipolysis from insulin-resistant adipose tissue. This helps to explain somewhat the close association between the MetS and NAFLD, in that increased waist circumference is a mandatory criteria in the International Diabetes Federation guidelines for diagnosing MetS. Additionally, the independent link between centrally obese individuals and increased CV morbidity and mortality is well established.<sup>67</sup> Studies show that increased VAT mass is independently associated with impaired glucose tolerance, IR, and dyslipidaemia, conferring an increased risk of CV disease, irrespective of diabetic status.<sup>48,49</sup> Furthermore, the 'portal hypothesis' suggest that increased VAT lipolysis secondary to IR leads to an elevated flux of FFAs into the portal vein for direct transport to the liver, resulting in increased hepatic fat, which would suggest that visceral fat is an important mediator of liver fat content.<sup>47</sup> In fact, in the Quebec Cardiovascular Study, elevated FFA levels yielded a two-fold increase in the risk of ischaemic heart disease, regardless of the presence of diabetes.<sup>65</sup> Additionally, high FFA concentrations in patients with angiographic CAD independently predicted CV mortality.<sup>13</sup> Apart from being a fat-storage organ, visceral fat is also metabolically active, secreting several adipokines, cytokines, and hormones that serve to regulate inflammation, liver fat, IR, and modify CV disease outcome.<sup>12</sup> Importantly, obesity in certain situations represents a chronic low-grade systemic inflammatory state that contributes to vasculopathy and CV risk through the release of these proinflammatory and atherogenic bioactive molecules.<sup>29</sup>

### Epicardial fat

Given that NAFLD and excessive visceral abdominal fat represent abnormal ectopic fat deposition in the body, with associated VAT-secreted adipocytokines contributing to subclinical inflammation and atherosclerosis, what about the role of epicardial adipose tissue (EAT), itself a visceral fat layer? Its anatomical location and proximity to the myocardium and adventitial layer of the coronary arteries, as well as sharing the same microcirculation, make it an ideal entity to exert a paracrine and vasocrine effect on the heart and its blood vessels.<sup>36</sup> Imaging studies have already shown that epicardial thickness or pericardial (epicardial and paracardial) fat volume correlate with the amount of VAT in both obese and non-obese subjects.<sup>2</sup> Furthermore, EAT thickness is also positively associated with the presence and severity of angiographic CAD, and increased epicardial or pericardial fat volume measured by CT are each independently associated with the presence of CAC.<sup>5</sup> Importantly, adiponectin expression was found to be significantly lower in epicardial fat isolated from patients with severe CAD compared with those without CAD, and pericardial fat volume also correlates with multiple markers of inflammation and oxidative stress, thus signifying potential similarities in proinflammatory adipokine function between EAT and VAT. However, it is likely that increased epicardial and myocardial fat both represent abnormal ectopic fat storage and may indeed be a marker of the cumulative effects of NAFLD and IR in the setting of pathological adiposity, with consequent associated adverse CV outcome.<sup>2</sup>

### Inflammation

The liver is a key metabolic organ and central to the regulation of systemic inflammation. It is a generator as well as a target of various inflammatory and humoral factors, working in concert and against secreted molecules from adipose tissue, macrophages, and endothelial cells in the context of CV disease initiation and progression.<sup>6</sup> Increasing severity of NAFLD likely represents worsening inflammatory and insulin-resistant states, with poorer cardiometabolic outcomes. High-sensitivity C-reactive protein, which is primarily produced by the liver and a marker of

inflammation, is an independent predictor of CV events in several large studies.<sup>3</sup> Similarly, fibrinogen and plasminogen activator inhibitor-1 (PAI-1) also originate from hepatic tissue and are activators of the coagulation system, enhancing atherothrombosis. Targher et al. showed that biopsy-proven NASH patients had significantly higher levels of high-sensitivity C-reactive protein, fibrinogen, and PAI-1 activity compared with controls. Furthermore, the severity of NASH by liver histology correlated significantly with these CV risk biomarkers after adjustment for potential confounders, including IR and visceral adiposity.<sup>5</sup> A similar correlation was found for serum IL-6 levels, as well as serum and hepatic TNF- $\alpha$  in NASH patients.<sup>2</sup> These studies suggest that increased liver-secreted factors in NAFLD play an important role in the pathogenesis of systemic inflammation and atherosclerosis.

### Dyslipidaemia

Non-alcoholic fatty liver disease is characterized by an atherogenic lipid profile, consisting of high TG levels, low high-density lipoprotein (HDL) cholesterol, and increase in small, dense low-density lipoprotein (LDL) particles, increase very low-density lipoprotein (VLDL) cholesterol levels and elevated apolipoprotein B100 concentration. This type of atherogenic dyslipidemia is strongly linked to adverse CV outcome.<sup>2</sup> The increased hepatic production of TG-rich VLDL provides a limited compensatory mechanism for reducing liver fat content. However, this also results in abnormal HDL metabolism causing HDL reduction as well as compositional alterations. In fact, the amount of liver fat has a significant negative correlation with subfractions of HDL known to be athero-protective, which are reduced in NAFLD independently of peripheral insulin sensitivity.<sup>37</sup>

### Conclusion and future directions

Non-alcoholic fatty liver disease is a marker of a pathological ectopic fat accumulation combined with a low-grade chronic inflammatory state affecting adipose tissue and characterized almost universally by IR. This results in several deleterious pathophysiological processes including abnormal glucose, fatty acid, and lipoprotein metabolism, increased oxidative stress, deranged adipokine profile, worsening subclinical inflammation, hypercoagulability, endothelial dysfunction, and progression of atherosclerosis, ultimately leading to a dysfunctional cardiometabolic phenotype with potentially unfavourable CV outcome. There is convincing evidence that worsening grades of NAFLD contribute to progressive cardiometabolic risk, such that NASH represents a marker as well as a mediator of increased CV risk more than simple steatosis. Although future studies should quantify liver fat using MR spectroscopy as a gold-standard, there remains an issue over how to obtain reproducible non-invasive measures of hepatic necroinflammation and fibrosis to document NAFLD (or specifically, NASH) improvement, especially in randomized studies. Importantly, as steatohepatitis becomes more advanced there is often a reduction in liver fat due to replacement of fat-laden hepatocytes with necrosed and fibrotic tissue, rendering liver fat measurements as a marker of NAFLD severity inaccurate. It is therefore imperative that future therapeutic trials in NAFLD also aim to include measurements of a range of validated cardiac, metabolic, and inflammatory biomarkers linked to clinical outcome, to serve as alternative objective measures of the change in NAFLD status and its associated cardiometabolic phenotype. This may also allow better risk prediction when adjusting for the effect of conventional risk factors in determining the true CV risk of NAFLD. Importantly, current research evaluating easily accessible novel biomarkers or combined clinical and biochemical algorithms to accurately grade the severity of NAFLD tend to focus too narrowly on liver-based outcomes, ignoring the detrimental cardiometabolic effects which are often the main cause of adverse clinical events. Furthermore, the cardiometabolic consequences of NAFLD are remarkably heterogeneous in terms of its interplay with visceral adiposity, IR, and subclinical inflammation, and given that approximately a quarter of the general population are estimated to have this condition, a targeted strategy for pharmacological intervention

would be challenging without outcome-based risk stratification. Therefore further research in this area is urgently needed to establish robust methods of predicting increased CV risk, as well as identifying novel treatments to improve the adverse CV outcome currently associated with NAFLD.

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