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REVIEW

Novel therapeutic concepts

Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor?

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Non-alcoholic fatty liver disease (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. Non-alcoholic fatty liver disease 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, increased oxidative stress, deranged adipokine profile, hypercoaguability, endothelial dysfunction, and accelerated progression of atherosclerosis. This ultimately leads to a dysfunctional cardiometabolic phenotype with cardiovascular mortality representing the main mode of premature death in NAFLD. This review is aimed at introducing NAFLD to the clinical cardiologist by discussing in-depth the evidence to date linking NAFLD with cardiovascular disease, reviewing the likely mechanisms underlying this association, as well as summarizing from a cardiologist's perspective, current and potential future treatment options for this increasingly prevalent disease.

Keywords Non-alcoholic fatty liver disease • Cardiovascular disease • Insulin resistance • Risk factor • Ectopic fat

Introduction

The Greek physician Galen considered the liver to be the most essential organ of the human body, stating it was 'the principal instrument of sanguification' in ~200 AD.[1] To today's cardiologist, the liver is viewed mainly as an uncelebrated obstacle to increasing the dose of the ubiquitous statin! However, 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 multi-faceted condition is essential for practising cardiologists, given that it affects 20–33% of the general population.[2] 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,[3] with most cases unrecognized. With this in mind, the potential future burden of NAFLD on public health-care utilization and costs is likely to be significant.[4] As such, the cardiometabolic risk conferred by NAFLD merits increased collaborative study between diabetologists, hepatologists, and especially cardiologists, given that CV disease appears to largely influence major clinical outcomes in NAFLD.[5–10] This review aims to present to the clinical cardiologist a state-of-the-art 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.

In preparing this review, Medline was searched for English-language articles including the keywords 'non-alcoholic fatty liver disease' combined with 'cardiovascular disease', 'coronary disease', 'pathogenesis', 'diagnosis','treatment' between 1990 and 2010. The bibliographies of identified reports were also explored for additional sources of information.

Definition of non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease is the most common cause of chronic liver disease in the general population and is present

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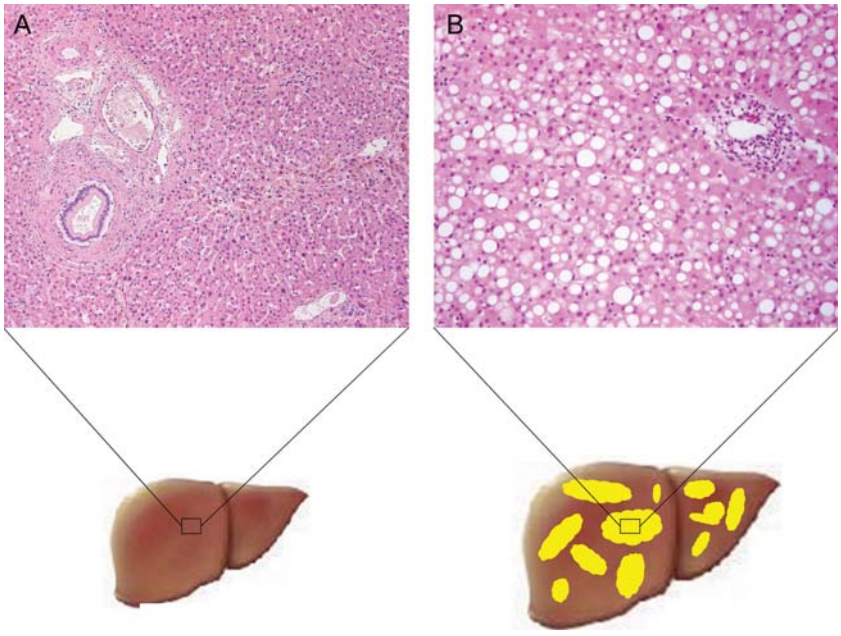

Figure 1 (*A*) Histological section of normal liver tissue compared with (*B*) simple steatosis, showing fat accumulation in hepatocytes.

when fatty infiltration affects >5% of hepatocytes, in the presence of <20 g (2.5 U) of alcohol consumption per day, without evidence of other causes of liver disease (*Figure 1*).[11] Non-alcoholic fatty liver disease 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.[11,12] Non-alcoholic 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 (*Figure 2*).[13] Insulin resistance and obesity, both key features of the MetS, are strongly associated with NAFLD progression.[14] The prevalence of NAFLD in subjects with MetS is increased four-fold compared with those without the disease and 30% of NAFLD subjects have MetS.[2] Despite MetS itself conferring an approximate doubling of CV mortality risk,[15] 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.[16]

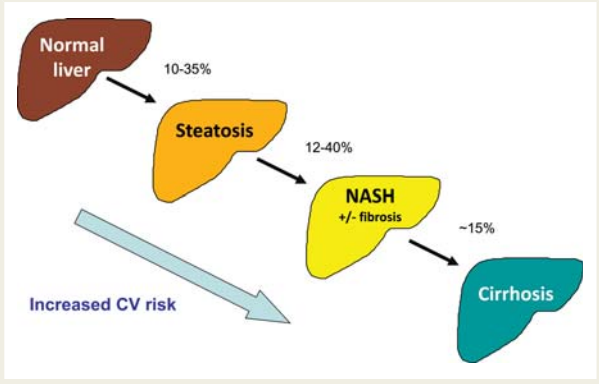

Figure 2 Variable progression of non-alcoholic fatty liver disease (usually over several years), with different grades of severity in each stage of simple steatosis and non-alcoholic steatohepatitis. These stages are generally reversible, apart from more severe forms of non-alcoholic steatohepatitis + fibrosis. Cardiovascular risk is increased as non-alcoholic fatty liver disease becomes more severe.[3,9,10]

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,[17] and although alanine aminotransferase (ALT) levels have shown to be the best single biochemical correlate of hepatic steatosis,[18] they do not distinguish between varying stages of NASH and can be normal in histologically severe disease.[19] 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.[20] Although magnetic resonance spectroscopy (MRS) has excellent sensitivity in detecting and accurately quantifying hepatic steatosis (*Figure 3*), none of the non-invasive modalities can detect inflammation

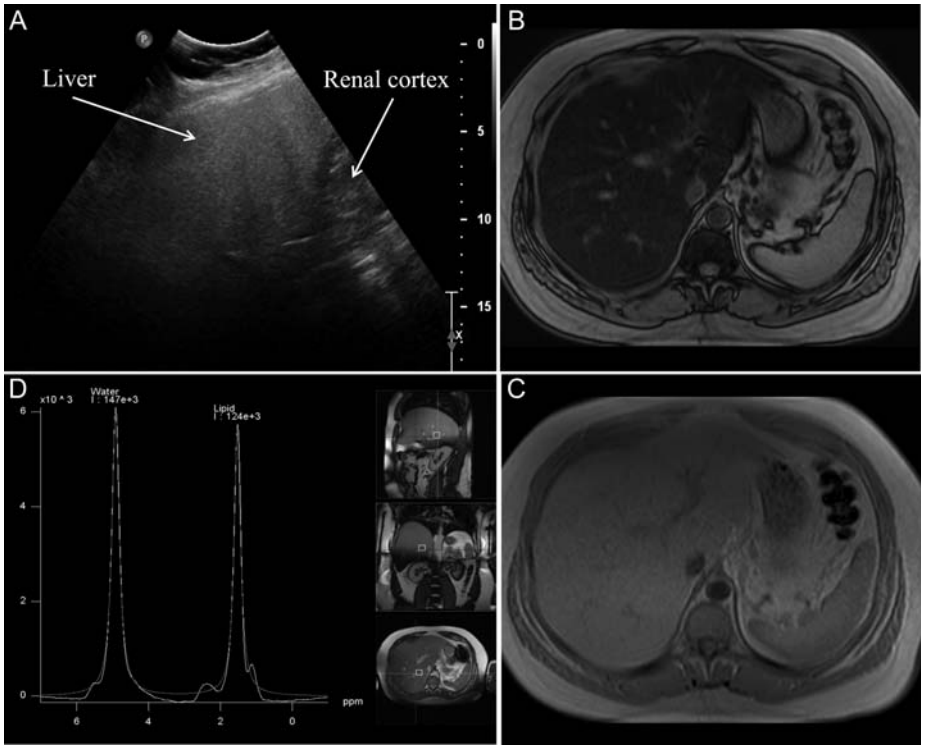

Figure 3 Liver imaging of a 35-year-old man with severe diffuse hepatic fatty infiltration. (*A*) Ultrasound scan showing diffuse increased echogenicity of liver parenchyma compared with renal cortex. (*B*) Chemical shift magnetic resonance imaging showing marked hepatic signal drop-off during out-of-phase image compared with in-phase (*C*) image, suggesting significant diffuse fatty liver. (*D*) Single-voxel magnetic resonance spectroscopy measuring area under lipid spectrum (second peak) relative to water spectrum (first peak), allowing accurate quantification of hepatic fat of 85% in the same patient.

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.[21] 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.[20]

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 population[5–10,22–29] (*Table 1*). As NAFLD is the commonest cause of abnormal liver enzymes in developed countries,[20] 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).[22–24] Additionally, a meta-analysis of 10 pooled studies confirmed the independent association between elevated GGT and adverse CV events.[25] However, GGT is also expressed in atherosclerotic plaques and has a role in oxidative stress,[30] as well as being associated with all components of the MetS.[31] Alanine aminotransferase has been reported to be more closely related to liver fat content than GGT.[14] Similarly, several large population-based cohort studies have reported an independent association between elevated ALT and CV mortality after adjusting for CV risk factors.[26–28] Importantly, the correlation of raised ALT or GGT with CV disease in these studies may simply reflect their significant association with IR,[32] 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[5–7] (*Table 1*). Of note, Hamaguchi *et al.*[6] 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

Table 1 Main epidemiological studies relating non-alcoholic fatty liver disease to increased cardiovascular risk

Authors	Study characteristics [N–O assessment of quality[a]]	Diagnosis of NAFLD	Main findings	Risk estimates (95% CI or *P*-value)	Comments/limitations
Ruttmann *et al.*[22]	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 RFs, alcohol and BMI	HR: men 1.66 (1.40–1.98), women 1.64 (1.36–1.97)	Poor sensitivity of GGT in NAFLD
Wannamethee *et al.*[23]	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 RFs, alcohol, and BMI	RR: 1.42 (1.12–1.80)	Men-only cohort. Poor sensitivity of GGT in NAFLD
Lee *et al.*[24]	Finnish population-based cohort ($n = 28\,838$), median F/U of 11.9 years [3,1,3]	Liver enzymes (GGT)	CHD mortality and non-fatal MI increased in NAFLD independent of CV RFs and alcohol	HR: men 1.20 (1.10–1.31), women 1.14 (1.03–1.27)[25]	Poor sensitivity of GGT in NAFLD
Fraser *et al.*[25]	Meta-analysis of 10 pooled population-based cohort studies[b]	Liver enzymes (GGT)	CV events (fatal and non-fatal) increased in NAFLD after adjustment for CV RFs and alcohol	HR: 1.34 (1.22–1.48)	Heterogeneity of studies ($I^2 = 73\%$), GGT poor marker of NAFLD
Fraser *et al.*[25]	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	ALT: HR 0.94 (0.65–1.37), GGT: HR 1.17 (0.93–1.48)	Women-only cohort, ALT/GGT not sensitive markers of NAFLD, relatively short follow-up
Schindhelm *et al.*[26]	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 RFs	HR: 1.88 (1.21–2.92)	ALT not a sensitive marker of NAFLD
Dunn *et al.*[27]	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 RFs	HR: 8.15 (2.00–33.20)	ALT not a sensitive marker of NAFLD
Yun *et al.*[28]	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 RFs, alcohol, BMI, and socio-economic status	RR: 2.26 (1.22–4.19)	ALT not a sensitive marker of NAFLD
Targher *et al.*[5]	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 RFs, diabetes control, and MetS	HR: 1.87 (1.20–2.60)	Exclusive diabetic cohort, liver ultrasound poor sensitivity with liver fat $< 30\%$
Hamaguchi *et al.*[6]	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 RFs and MetS	OR: 4.12 (1.58–10.75)	Largely volunteer-reported CV events, 25% lost to F/U, use of ultrasound to diagnose NAFLD
Haring *et al.*[7]	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 RFs	HR: men 6.22 (1.22–31.62), women 0.98 (0.11–8.84)	Significantly older age and increased baseline CV disease in men vs. women, inadequate NAFLD sample size in women → type 2 error?
Adams *et al.*[9]	Community-based North American 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	SMR: 1.34 (1.003–1.76)	Liver ultrasound poor sensitivity with liver fat $< 30\%$, wide variability of length of follow-up

Continued

Table 1 *Continued*

Authors	Study characteristics [N–O assessment of quality[a]]	Diagnosis of NAFLD	Main findings	Risk estimates (95% CI or *P*-value)	Comments/limitations
Ekstedt *et al.*[10]	Swedish hospital-based consecutive biopsy cases ($n = 129$), mean F/U of 13.7 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	RR: 1.38 ($P = 0.006$)	No diabetes screening at baseline, small sample size (due to liver biopsy as diagnostic modality)
Soderberg *et al.*[8]	Swedish hospital-based consecutive biopsy cases ($n = 118$), median F/U of 24 years [3,0,3]	Liver biopsy	Increased total mortality in NAFLD was predominantly CV related, compared with matched reference population	SMR: 1.69 (1.24–2.25)	Small sample size (due to liver biopsy as diagnostic modality)
Schwimmer *et al.*[29]	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	OR: 1.80 ($P < 0.001$)	Limitations with autopsy studies

NAFLD, non-alcoholic fatty liver disease; F/U, follow-up; GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase; CV, cardiovascular; RFs, risk factors; BMI, body mass index; CHD, coronary heart disease; MI, myocardial infarction; MetS, metabolic syndrome; NASH, non-alcoholic steatohepatitis; CI, confidence interval; HR, hazard ratio; RR, relative risk; OR, odds ratio; SMR, standardized mortality ratio.
[a]N–O, Newcastle–Ottawa Scale for assessing the quality of non-randomized studies based on study selection (0–4), comparability (0–2), outcome/exposure (0–3).[146]
[b]Meta-analysis adhered to the Meta-analysis of observational studies in Epidemiology (MOOSE) group standards of reporting.[147]

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.[8,10] 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 ($P = 0.06$), primarily from CV-related causes over a median follow-up of 24 years.[8] However, these studies are limited by modest sample sizes and inclusion of select cohorts requiring liver biopsy for clinical reasons, which therefore necessitate cautious interpretation of the reported 'benign' nature of simple steatosis.

Evidence of association of non-alcoholic fatty liver disease 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 (see Supplementary material online, *Table 2*).[33–36] 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.[34] Additionally, we have recently shown a strong association between histological severity of NAFLD and calculated estimates of CV risk [both QRISK2 and Framingham risk score (FRS)] independently of markers of glucose control and obesity.[37]

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.[38] Furthermore, we know that some of the important determinants of NAFLD, such as IR, obesity, and raised triglycerides (TGs), all of which also increase the risk of CV disease, are not generally accounted for in these risk assessment models. Indeed, the FRS is already known to underestimate the risk of CV disease in MetS,[39] which shares many features in common with NAFLD. It might therefore not be appropriate to risk-stratify patients with NAFLD solely based on current CV risk scoring systems. Further research is necessary to determine simple and cost-effective robust biomarkers (or algorithm-based scores) of NAFLD status including its direct cardiometabolic 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 systems.[40] Several studies demonstrate a significantly increased coronary atherosclerotic burden in the presence of NAFLD (see Supplementary material online, *Table 3*),[41–44] 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).[42] 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.[45,46] Furthermore, in the Study of Inherited Risk of Coronary Atherosclerosis (SIRCA) of 860 asymptomatic non-diabetic participants, investigators found that the IR index was a robust and independent predictor of CAC score even after controlling for traditional CV risk factors, MetS, and C-reactive protein.[47]

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 (see Supplementary material online, *Table 4*).[48–51] Although these studies indicate an independent association between NAFLD and increased 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,[52,53] 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 subjects.[54,55] Several studies link NAFLD independently with carotid disease, although a few have described a weaker association after adjusting for MetS (see Supplementary material online, *Table 5*).[35,56–61] Importantly, severity of histological features of NAFLD appears to correlate independently with increasing CIMT,[58] 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.[62]

However, two subsequent studies not included in this meta-analysis did not show an association between NAFLD and increased CIMT (see Supplementary material online, *Table 5*).[63,64] Importantly, both were conducted in primarily diabetic subjects, with one study reporting a majority of their cohort on insulin treatment.[64] Given that insulin therapy is known to decrease liver fat in type 2 diabetics, possibly through reduction in glucose and free fatty acid (FFA) levels,[65] these results must be interpreted with caution. Furthermore, diabetes itself is considered a coronary-risk equivalent and so may have masked the association between NAFLD and carotid disease, especially when analysing relatively small sample sizes. Additionally, neither of these studies evaluated the presence of carotid plaque, which appears to have similar or greater predictive power for CV events than CIMT alone.[66]

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 are in the main secondary to the effects of IR, obesity, and hypertension on cardiac structure and function.[67,68] Only a few studies have focused specifically on NAFLD subjects, and the finding of abnormal LV geometry and diastolic dysfunction has similarly been reported (see Supplementary material online, *Table 6*).[69–71] 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.[70]

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.[72] Just under half of NAFLD patients had an impaired CFR, whereas all controls had normal CFR values, and histological liver fibrosis score was the only independent predictor of impaired CFR. 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.[73]

Studies evaluating endothelial dysfunction and myocardial metabolism in non-alcoholic fatty liver disease

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.[36,74] In addition, lower FMD was observed in NASH compared with simple steatosis, again confirming the graded association of CV risk with severity of NAFLD.[36]

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.[75,76] 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.[75] 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.[76] 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, and 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.[73,77]

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 terms of outcomes measured as well as confounding variables not adequately adjusted for, but most importantly, in the method of NAFLD diagnosis and 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.[78] Similarly, adverse CV outcome is likely to be associated with liver fat/inflammation in a monotonic relationship, progressively increasing with more advanced stages of NAFLD.[58,70] This parallels epidemiologic evidence showing a progressive relationship between glucose levels and CV disease extending from well below the diabetic threshold.[79] Ultimately, the development and progression of IR appears to be the 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 and the consequent cardiometabolic dysfunctional cascade.[80] 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 (*Figure 4*).

Visceral fat

Visceral adipose tissue (VAT) appears to have a strong independent positive correlation with liver fat.[18] 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.[81] Therefore, could VAT itself explain the increased CV risk seen with NAFLD, rather than liver fat content *per se*?

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.[82] Furthermore, the 'portal hypothesis' suggests 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.[83] 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.[84] Additionally, high FFA concentrations in patients with angiographic CAD independently predicted CV mortality.[85] 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 (see Supplementary material online, *Table 7*).[12,77,86–95] 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.[96]

However, the mechanisms linking visceral fat or obesity to CV disease are strongly related to IR, which itself is robustly associated with CV risk and atherosclerosis, already reviewed in detail.[97] It is therefore unclear whether VAT actually confers direct CV risk through secreted factors, or indirectly via IR-related processes, or both. Importantly, studies from patients with lipodystrophy suggest that even with little or no adipose tissue, fatty liver, and IR can still develop quite markedly,[98] which undermines the portal hypothesis. Epidemiological and case–control studies also

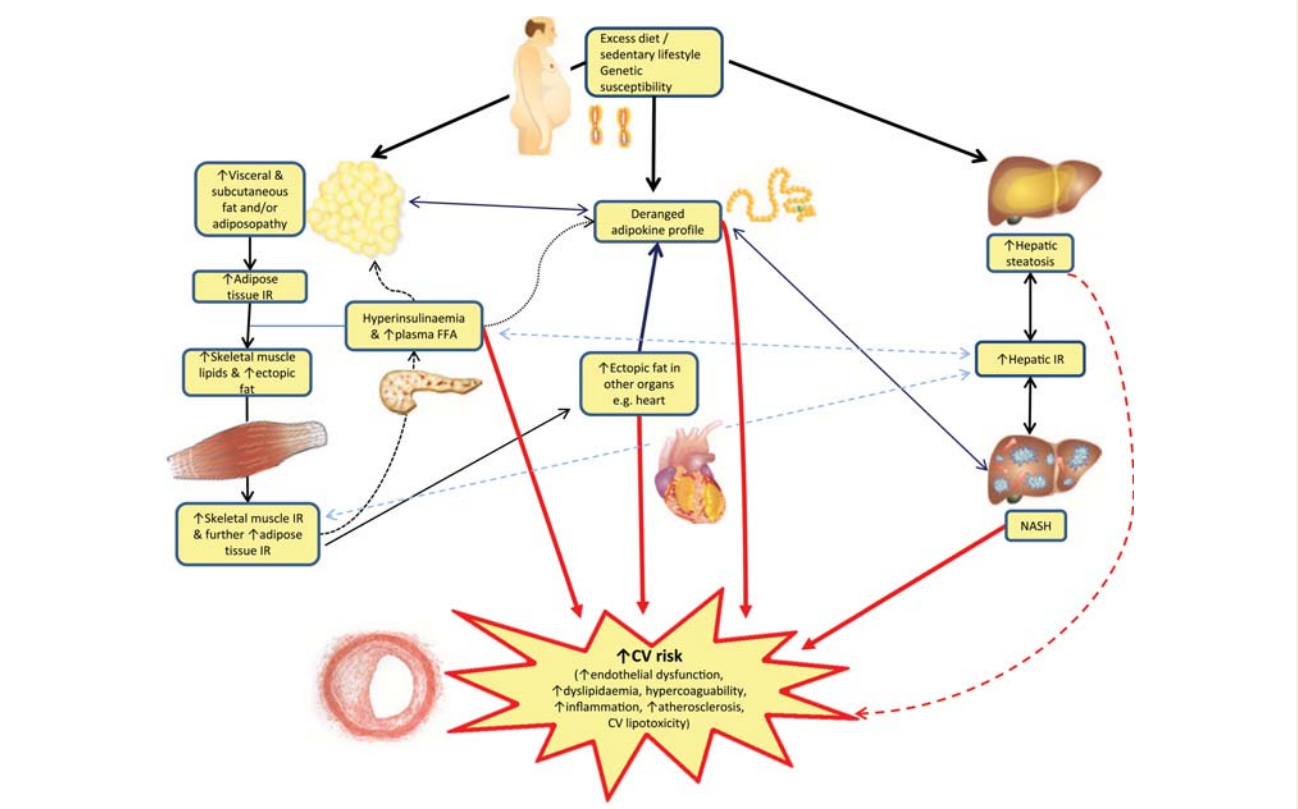

Figure 4 Schematic diagram of the pathophysiological processes involved in non-alcoholic fatty liver disease leading to increased CV risk, highlighting the complex inter-relationships between visceral adipose tissue, adipocytokines, insulin resistance, ectopic fat accumulation and non-alcoholic fatty liver disease. FFA, free fatty acids.

support the key role of liver fat, rather than VAT, as a marker of obesity-related metabolic dysfunction and a strong predictor of multi-organ IR, which is independent of obesity, VAT, or plasma adipokine levels.[80,99,100] Despite these findings, adipose tissue is likely to still contribute to metabolic dysfunction as it is the specific characteristics of adipose tissue rather than the amount that is important. Accordingly, fat cell hypertrophy, macrophage infiltration of adipose tissue causing inflammation, increased adipose tissue lipolytic activity, and adipose tissue hypoxia are all associated with IR.[101] It is therefore plausible that the established link between obesity and CV outcome may in fact be mediated through both ectopic fat accumulation (i.e. liver and cardiac tissue) as well as the effects of adiposopathy or 'sick fat'.[102] This occurs when adipose tissue becomes chronically inflamed and releases proinflammatory adipokines and cytokines that ultimately contribute to atherosclerosis and CV disease. Therefore NAFLD can be considered a sensitive marker of pathological dysfunction of adipose tissue, which appears to be more relevant to CV outcome than simply adipose tissue mass.

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.[103] 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.[104–107] Furthermore, EAT thickness is also positively associated with the presence and severity of angiographic CAD,[106,108,109] and increased epicardial or pericardial fat volume measured by CT are each independently associated with the presence of CAC.[110,111] Importantly, adiponectin expression was found to be significantly lower in epicardial fat isolated from patients with severe CAD compared with those without CAD,[112] and pericardial fat volume also correlates with multiple markers of inflammation and oxidative stress,[113] thus signifying potential similarities in proinflammatory adipokine function between EAT and VAT.

Iacobellis *et al.*[107] have validated a simple echocardiographic method of quantifying EAT involving measurement over the anterior right ventricular wall in the parasternal view, showing an excellent correlation with magnetic resonance imaging-determined values. Furthermore, they have proposed EAT threshold values for cardiometabolic risk stratification,[114] having reported significant correlations of EAT with several anthropometric, CV, and

metabolic risk factors including IR.[115,116] Importantly, pericardial fat volume appears to independently predict major adverse cardiac event risk in asymptomatic subjects, even after adjusting for FRS, CAC score, and BMI.[117]

Weight reduction through exercise training or a low-calorie diet has been shown to decrease EAT thickness, as well as reduce VAT and increase insulin sensitivity.[118,119] Notably, improvement in LV diastolic function correlated better with EAT than waist circumference reduction.[118] Furthermore, increased epicardial fat has a significant negative correlation with cardiac index, and also correlates directly with intramyocardial TG levels.[120] Therefore, it remains unclear whether the LV dysfunction is due to lipotoxicity from excess FFA availability and subsequent oxidative stress, as well as the deleterious effects of increased LV mass, or secondary to adipokine-mediated myocardial inflammation and damage; or both.[121] 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,[120,122] with consequent associated adverse CV outcome.[123]

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 (as summarized in see Supplementary material online, *Table 7*), working in concert and against secreted molecules from adipose tissue, macrophages, and endothelial cells in the context of CV disease initiation and progression.[87,89,91] 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.[124] 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.[86] A similar correlation was found for serum IL-6 levels, as well as serum and hepatic TNF-α in NASH patients.[125] Additionally, hepatic and plasma PAI-1 levels also correlate with the degree of hepatic steatosis.[126] These studies suggest that increased liver-secreted factors in NAFLD play an important role in the pathogenesis of systemic inflammation and atherosclerosis.

Nuclear factor kappa-B (NF-κB) is a hepatocellular transcription factor that plays a key role in intrahepatic inflammation. In rodent models, a high-fat diet results in hepatic steatosis and up-regulation of NF-κB activity, which leads to hepatic production of proinflammatory cytokines IL-6, IL-1β, and TNF-α, as well as activation of Kupffer cells and macrophages, possibly worsening hepatic inflammation.[127] This study also demonstrated that isolated hepatic inflammation in the absence of steatosis through selective activation of NF-κB, resulted in hepatic and skeletal muscle IR. Hepatic steatosis can also induce hepatic inflammation through lipotoxicity and endoplasmic reticulum oxidative stress responses, as well as through mitochondrial dysfunction via increased oxidation of excess fatty acids.[128] Mitochondrial dysfunction and damage are associated with IR and atherosclerosis in several studies,[97] representing a plausible link between NAFLD and increased CV risk.

Dyslipidaemia

Non-alcoholic fatty liver disease is characterized by an atherogenic lipid profile, consisting of high TG levels, low high-density lipoprotein (HDL) cholesterol, an increase in small, dense low-density lipoprotein (LDL) particles, increased very low-density lipoprotein (VLDL) cholesterol levels and elevated apolipoprotein B100 concentration. This type of atherogenic dyslipidaemia is strongly linked to adverse CV outcome.[129] The increased hepatic production of TG-rich VLDL provides a limited compensatory mechanism for reducing liver fat content.[130] 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.[131]

Treatment of non-alcoholic fatty liver disease

Various therapeutic modalities for NAFLD have been postulated and trialled to date and a summary of these treatments, as well as each of its associated CV benefits and risks, are shown in Supplementary material online, *Table 8*.[20,132–140] For a more detailed overview, readers are encouraged to refer to recently published guidelines[20] as well as a meta-analysis of randomized trials for the treatment of NAFLD.[132] To summarize, there is currently no established pharmacological treatment for NAFLD, and lifestyle interventions such as increasing exercise, reducing dietary fat intake, and encouraging weight loss are the only recommended therapeutic strategies with proven benefit. From a cardiologist's perspective, lipid-lowering drugs (e.g. statins), insulin-sensitizers (e.g. thiazolidinediones, metformin) and anti-hypertensive agents have not as yet shown adequate added risk/benefit value in NAFLD over and above already established evidence-based guidelines for the individual treatment of dyslipidaemia, diabetes and hypertension. Given the increased CV risk associated with NAFLD attributed to its pro-atherogenic and pro-inflammatory states, it is perhaps surprising that statins, with their anti-atherosclerotic and pleiotropic (anti-oxidant, anti-inflammatory) effects, have thus far not shown a consistent benefit in NAFLD outcomes. One potential explanation for this could be that statins are also known to indirectly impair insulin sensitivity,[141] which may result in an overall net neutral effect in treating NAFLD. Other possible reasons could include inadequate trial durations to allow inflammatory changes to translate into beneficial clinical outcomes, or the enrolment of low-risk NAFLD cohorts. It is noteworthy that patients with hepatic steatosis have not been shown to be at increased risk for statin hepatotoxicity,[142] and

the Liver Expert Panel stated in a report in 2006 that statins can indeed be safely used in NAFLD and NASH, without the need for routine liver enzyme monitoring.[143] It follows therefore that cardiologists should not be concerned about initiating or continuing statins in patients with NAFLD, unless there is evidence of deranged synthetic liver function or decompensation. However, given the high prevalence of NAFLD in the general population, it would be prudent to routinely check baseline liver enzymes prior to commencing a statin, in order to prevent mistakenly attributing subsequently discovered elevated aminotransferase levels as statin-induced. General practitioners are often too quick to discontinue a statin when confronted with raised aminotransferase levels, when in fact there is no evidence that elevated levels in the absence of raised bilirubin (more than two times the upper limit of normal) reflect drug-induced liver injury.[143] With high-dose statin therapy proving beneficial in certain patient groups, e.g. acute coronary syndromes, one unresolved question is whether NAFLD patients with significantly raised baseline aminotransferase levels (i.e. more than three times the upper limit of normal) should be prescribed high doses at the outset, as these patients are at two to three times the risk of 'transaminitis' compared with moderate or low doses of statins. Until further data emerge proving its safety in this setting, we would recommend a cautious approach and commence statins at a lower dose, up-titrating according to clinical and biochemical response over several weeks. This would also be applicable to other CV drugs which are metabolized by the liver, e.g. amiodarone, nicotinic acid, calcium-channel blockers, and angiotensin-converting enzyme inhibitors, although the vast majority of NAFLD patients has normal synthetic liver function and tolerate these drugs very well.

N-3 long chain polyunsaturated fatty acids (PUFAs) represent a potentially viable pharmacological treatment option in NAFLD. This group of fatty acids has an excellent side-effect profile and in high dose is effective in reducing plasma TGs and FFA levels,[136] both increased in NAFLD and associated with increased CV risk. Animal studies have also shown *n*-3 PUFA to be negative regulators of hepatic lipogenesis and the inflammatory response, as well as improving insulin sensitivity.[137] Although preliminary human trials have already shown a beneficial effect of *n*-3 PUFA in treating NAFLD, they have been limited by small sample sizes, lack of randomization, or placebo arms.[138–140] Furthermore, no studies have yet examined the effect of reducing liver fat on proxy markers of CV risk. We are currently undertaking a randomized double-blind placebo-controlled trial in NAFLD patients to investigate the effect of prolonged treatment with high-dose *n*-3 PUFA (Omacor) on various proxy markers of CV risk and insulin sensitivity in relation to changes in liver fat quantified by MRS (clinicaltrials.gov NCT00760513).

Given the increased CV risk posed by NAFLD and the lack of any established therapeutic option at present, what should a cardiologist do when managing a cardiac patient with concomitant NAFLD? Any patient with documented NASH should be regarded as high risk given that CV mortality is increased approximately two-fold compared with the age-matched general population[10] and each individual CV risk factor should be controlled aggressively to reduce the overall risk. Measurement of fasting TGs, which is often overlooked, should also be undertaken as the TG/HDL ratio appears to not only be a good predictor of IR, but also able to predict CV events independently.[129,144] Currently there are no established guidelines advising assessment or monitoring of simple measures of IR (e.g. waist circumference or TG/HDL ratio) as already exist for LDL-C or blood pressure measurements to optimize outcomes across all secondary care cardiac patients. Certainly in diabetics, a TG/HDL ratio of >2.5 should prompt consideration of adding in a fibrate, nicotinic acid, or *n*-3 PUFA treatment, in addition to statins and lifestyle modification advice.[145] More evidence for optimal thresholds in treating atherogenic dyslipidaemia, common in NAFLD, is needed in younger cohorts and the non-diabetic population.

Conclusion and future directions

Non-alcoholic fatty liver disease is a marker of 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, hypercoaguability, 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.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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