Non-alcoholic fatty liver disease and cardiovascular risk : pathophysiological mechanisms and implications

TITLE

NON-ALCOHOLIC FATTY LIVER DISEASE AND CARDIOVASCULAR RISK: PATHOPHYSIOLOGICAL MECHANISMS AND IMPLICATIONS

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ABBREVIATIONS (order of appearance)

NAFLD	Non-alcoholic fatty liver disease
CV	Cardiovascular
CVD	Cardiovascular disease
MetS	Metabolic Syndrome
AT	Adipose tissue
TG	Triglycerides
DM	Diabetes mellitus
NASH	Non-alcoholic steatohepatitis
CVRF	Cardiovascular risk factor
cIMT	Carotid intima media thickness
LV	Left ventricle
ADMA	Asymmetric dimethyl arginine
LDL	Low-density lipoproteins
HDL	High-density lipoproteins
VLDL	Very low-density lipoproteins
NAFL	non-alcoholic fatty liver (also known as simple steatosis)
VEGF	Vascular endothelial growth factor
CAD	Coronary artery disease
PAI-1	Plasminogen inhibitor activator 1
hsCRP	high sensitive C reactive protein
FetA	Fetuine A
FGF21	Fibroblast growth factor 21
SeP	Selenoprotein P
ANGPTL	Angiopoeitin like protein
TMA	Trimethylamine
TMAO	Trimethylamine-n-oxide
PNPLA3	Patatin-like phospholipase domain containing protein 3

74	TM6SF2	Transmembrane 6 superfamily member 2
75	SNP	Single nucleotide polymorphism

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) has become one of the most frequent chronic liver diseases in the Western society and its prevalence is likely to rise even further. An increasing body of evidence shows that NAFLD is not only a potentially progressive liver disease, but also has systemic consequences. More specifically, evidence points out that NAFLD has to be considered as a significant independent risk factor for subclinical and clinical cardiovascular disease (CVD). Longterm follow-up studies demonstrate cardiovascular mortality to be the most important cause of death in NAFLD patients. Moreover, ample evidence associates NAFLD with endothelial dysfunction, increased pulse wave velocity, increased coronary arterial calcifications and increased carotid intima-media thickness, all established markers for CVD.

Despite of all this evidence, the mechanisms by which NAFLD causally contributes to CVD are not fully elucidated. Furthermore, an extensive overview of all potential pathophysiological mechanisms and the corresponding current data are lacking. In this review we summarize current knowledge, originating from fundamental and clinical research, that mechanistically links NAFLD to CVD. Subsequently, the impact of CVD on current clinical practice and future research in the area of NALFD are discussed.

KEY POINT BOX

- The liver and the cardiovascular system are inextricably linked to each other: the hepato-cardiovascular axis.
- Increased cardiovascular mortality and morbidity are observed in NAFLD.
- NAFLD is not only associated with, but also contributes to the pathogenesis of cardiovascular diseases.
- The contribution of NAFLD to cardiovascular diseases is independent of shared risk factors, e.g. obesity or diabetes mellitus.
- Several potential pathophysiological mechanisms are involved, including endothelial dysfunction, systemic inflammation, systemic oxidative imbalance, dyslipidaemia, organokines and genetic factors.
- Subclinical cardiovascular alterations can be detected easily in NAFLD and screening is potentially cost-effective in selected patient-groups.
- Treatment of NAFLD should include assessment and treatment of extra-hepatic manifestations of NAFLD.
- Treatment of NASH might also result in improvement of cardiovascular disease, though this remains currently to be proven.
- Markers of subclinical cardiovascular disease should become co-primary or secondary endpoints in clinical trials as surrogate for long-term CV benefit.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become a major cause of chronic liver disease in Western societies and will become the main underlying casue for liver transplantation within 10 years [1]. Although awareness amongst physicians increases and the importance is recognised, screening and referral to hepatologists in suspected NAFLD is low in primary care and non-hepatology specialties [2,3]. As a result, NAFLD is relatively underdiagnosed and long-term outcomes of hepatic and extrahepatic manifestations of NAFLD are compromised. Indeed, NAFLD is not only associated with increased liver-related morbidity and mortality, but also with increased mortality of cardiovascular disease (CVD) and cancer [4,5].

The role of NAFLD as an independent cardiovascular risk factor is still debated. Several studies demonstrated unequivocally an increased cardiovascular (CV) mortality in NAFLD [4,6]. Nevertheless, some studies failed to confirm this association, including two large cohort-studies with long-term follow up [7,8]. However, the data should be interpreted with caution because several methodological issues, including retrospective diagnosis based on recorded ultrasound imaging or on biochemistry [7], which is known to poorly correlate with histological NAFLD features [9]. Even in the absence of a significant relation with CV mortality, CVD was still undoubtedly increased in NALFD patients compared to controls [8], supporting the many convincing data that NAFLD independently contributes to (sub)clinical CVD.

Distillation of NAFLD as a separate risk factor is impeded by overlap with other well-established risk factors for CVD, as they are also risk factors for NAFLD itself [5]. Assuming that NAFLD is a contributor to CVD implies the need for knowledge on the underlying pathophysiological mechanisms that explain how NAFLD independently impacts on CVD. An extensive overview of potential mechanisms is currently lacking.

In this review we summarise knowledge, originating from animal research as well as translational and clinical research, about the underlying pathophysiological mechanisms that might link NAFLD to CVD. We subsequently discuss the potential implications of these findings for clinical management of patients with NAFLD and future research goals.

THE CARDIOVASCULAR RISK ASSOCIATED WITH NAFLD

General considerations

The specific contribution of NAFLD to increased CVD risk is, especially in clinical studies, difficult to dissect from the combination of risk factors that are shared by both NAFLD and CVD. The population of NAFLD patients is furthermore probably heterogeneous, in some of whom NAFLD is just part of and victim of the global metabolic derangement whilst in others, the liver is particularly involved in the pathophysiology of the Metabolic Syndrome (MetS) itself, and in the emergence of CVD and other complications [10,11]. Many patients will be somewhere in between, with the liver being diseased because of some metabolic abnormalities, and, once diseased, also contributing significantly to disease progression in terms of MetS, CVD and malignancies. This concept is fundamental in our understanding of NAFLD as part of a systemic disease.

The mechanisms by which the liver might contribute are also complex and heterogeneous. The liver plays a crucial role in lipid and glucose homeostasis and is hence in the centre of cardiometabolic disease. There is a very complex interplay between the gut, visceral and subcutaneous adipose tissues (AT), muscle tissues, the cardiovascular system and the liver [12].

One of the starting points is most probably an imbalance in calorie-intake and expenditure, exceeding the storing-capacity of AT leading to deposition of ectopic fat, including the liver [13].

Once these mechanisms are initiated, a vicious circle starts, after which interplay between the different players is so complex that simple cause-effect relations become extremely difficult to assess (Fig. 1). As the liver is centrally positioned between different players, it is

surprising that the mechanisms contribution to diabetes mellitus (DM) and CVD have gained so little attention. If it can be demonstrated that non-alcoholic steatohepatitis (NASH) livers play a pivotal role in that vicious circle, targeting the liver becomes attractive to break through the circle and halt metabolic and CVD progression.

Determinants of outcomes in NAFLD: subclinical and clinical CVD data

NAFLD encompasses a spectrum of liver diseases, ranging from non-alcoholic fatty liver (NAFL, also known as simple steatosis) over NASH and might lead to advanced fibrosis or cirrhosis and hepatocellular carcinoma (HCC). NAFLD is characterized by excessive fat accumulation in the hepatocytes (steatosis). When steatosis is accompanied by both hepatocellular ballooning degeneration and lobular inflammation, a diagnosis of NASH is made [14]. The natural history of NAFLD and its different subtypes is not so well described, in part because the gold standard for the accurate diagnosis of NASH is liver biopsy, and large long term follow data with repeated biopsies are scarce and should be interpreted with caution because, amongst others, of potential selection bias. Nevertheless, NAFLD is generally considered to run a benign course, with a low (but not completely absent) risk of fibrosis progression, whereas NASH has a significantly higher risk of progressive liver disease [15].

This dichotomous concept has recently been challenged [16]. Singh et al. [17] systematically reviewed and performed a meta-analysis on 11 paired biopsy cohort studies. Although the majority of the patients had stable disease, it was shown that fibrosis progression occurred in both patients with NASH as well as NAFL (annually increase 0.14 and 0.07 fibrosis stage respectively). Moreover, a subset op patients was identified with considerable rapid fibrosis progression. Of note, progressors with baseline NAFL frequently had mild lobular inflammation or ballooning compared to non-progressors and although insufficient for the diagnosis of NASH, these subtle differences might explain their progression and are still in line with the concept of necro-inflammation being the driving force of disease progression

[17]. Another recent paired biopsy study also showed that 44% of the patients with NAFL progressed to NASH, and 37% to fibrosis (including some with progression to stage 3 fibrosis). Of note, at baseline NAFL patients were significantly younger compared to NASH patients, and most of the progressors out of the NAFL group had NASH at follow-up, and frequently had mild lobular inflammation at baseline. Development of type 2 diabetes mellitus was also an important determinant of progression [18].

NAFLD is unambiguously related to increased liver-related and all-cause mortality [4,6]. Importantly, CVD is the main cause of death in NAFLD patients (38% of all causes [19]), with baseline fibrosis being the strongest predictor [4,19]. Earlier studies suggested that the risk was higher in patients with NASH compared to NAFLD [20] In a meta-analysis of 2011 mortality did not differ between NAFL and NASH, though the same analysis was inconclusive on potential differences in incident CVD [21]. While NASH category as such was significantly associated with long-term outcomes [19], fibrosis remained the only prognostic histological feature in subsequent analyses [4,19].

Although valuable information comes from these recent data, several methodological issues make it still difficult to accurately answer the question of the natural history of NAFLD and what drives its progression both as a liver disease and concerning the extrahepatic consequences. NAFLD is a systemic trait with a complex and multidirectional interplay with CVD and the MetS, all dynamic conditions that may substantially fluctuate between 2 time points of evaluation. Fluctuations in life style, body weight and glycaemic control might substantially impact on disease progression, which is probably far from linear. The placebo effect observed in the clinical trials conducted so far (as well as the % of regressors in the paired biopsy studies) also illustrate the dynamic nature of the disease, especially in milder cases [22,23]. Another aspect that deserves to be considered, is the way necro-inflammatory changes are assessed: histological analysis with routine stains is still the reference method used in scoring systems and diagnostic decision trees, but presumably not so very accurate

to assess subtle activation of inflammatory cascades, especially in milder cases. The role of fibrosis as a prognostic marker also needs to be further clarified, as it is not clear whether this is to be considered a reflection of a median disease activity in the period preceding the baseline biopsy and hence an indirect prognostic factor (but presumably stronger than the actual disease activity because the latter only reflects the current status at the time of biopsy) or whether it is a direct prognostic factor by itself. Furthermore, fibrosis is assessed as a static parameter that does not accurately reflect the dynamics of pro- and antifibrogenic processes. The concept that inflammation is the main driver of fibrogenesis and disease progression [24] is hence still important in defining target populations for screening and treatment.

Besides mortality, evidence linking clinical CVD to NAFLD is scarce. Elevated liver enzymes are related to stroke [25] and the prevalence of NAFLD in ST-elevated myocardial infarction is high [26]. In contrast to hard clinical endpoints, ample evidence links NAFLD to subclinical CVD, often independently of other well-established CV risk factors (CVRFs). Patients with NAFLD exhibit endothelial dysfunction of conducting vessels [27], as well of the microvasculature [28]. Arterial stiffness is a well-accepted marker of CVD preceding arterial hypertension [29] and NAFLD is independently associated with increased vascular stiffness [28]. The carotid intimal media thickness (cIMT), a marker for generalised atherosclerotic burden, or the effective carotid plaque burden is also assoctiated with NAFLD [30]. Finally, significant associations are observed of NAFLD with left ventricular (LV) diastolic dysfunction and LV mass [31,32]. For further details about the (sub)clinical data we refer to already published extensive reviews [33–37], since this review focusses on the potential mechanisms underlying this association.

POTENTIAL PATHOPHYSIOLOGICAL MECHANISMS

The mechanisms explaining development of CVD in NAFLD are incompletely understood. NAFLD is part of a complex multisystem disease with multiple bidirectional relationships. Moreover, each individual patient might exhibit a unique combination of causal mechanisms. The next section summarises the different potential mechanisms by which NAFLD may contribute to CVD (Fig. 2; Table 1). These mechanisms are rather complex, incompletely known and often interrelated.

Structural alterations

Hepatic microvasculature shows important alterations in case of NAFLD, with distortion of the sinusoidal pattern, occurrence of sinusoidal blebs, compression of sinusoids by fat-loaden hepatocytes and loss of fenestrae [38–40]. These effects occurred prior to development of inflammation and fibrosis, indicating an early event [38]. These structural alterations are in part held responsible for the observed increase in portal pressure in non-cirrhotic NAFLD both in animals and humans [38,41]. The exact underlying pathophysiological mechanisms behind these alterations aren't known, but may be explained by alterations in angiogenic factors.

The arterial wall exists out of three layers, the intima, media and adventitia. In case of increased arterial stiffness, associated with NAFLD and CVD, the media of the large arteries is structurally altered: its collagen content and crosslinking increases, whereas elastin fibres decrease and become fractioned [42]. Levels of metaloproteinases, as well serum elastase are correlated to arterial stiffness [42]. Interestingly, the latter was increased in NAFLD [43]. Others speculate a potential role of TGF-β [44].

Endothelial dysfunction

Impaired endothelial function is an early step in the process of atherosclerosis, ahead of development of fatty streaks or plaque inflammation [45] and hence crucial in CVD development.

In cirrhosis, intrahepatic and mesenterial endothelial dysfunction are well known [46]. Intrahepatic dysfunction is also described in NAFLD [47] but, intriguingly, in the absence of inflammation or fibrosis [38,48,49], indicating it's an early event that might drive disease progression.

Endothelial dysfunction of the systemic circulation was also observed in NAFLD, more pronounced in NASH [27]. Asymmetric dimethyl arginine (ADMA) is an endogenous antagonist of NO-synthase (NOS), positively linked to CVD. Reduced breakdown, in which the liver plays a dominant role [50], is supposed to cause increased ADMA levels [51]. NAFLD patients exhibit increased levels of circulating ADMA, an association disappearing after correction for metabolic risk factors [50,52]. Other markers for endothelial dysfunction (e.g. endocan) were also increased in NAFLD [53].

An intact endothelial monolayer is important for normal vessel wall functioning. Disruption of this layer plays a role in atherogenesis and is characterised by increased levels of endothelial microparticles (EMPs), indicating endothelial disruption, and endothelial progenitor cells (EPCs), indicating endothelial repair [54]. The levels of circulating EPCs were reduced in NAFLD and their adhesive function was attenuated [55].

Homocysteine and oxidative stress

The liver is the main organ handling amino acids, including homocysteine. Elevated serum homocysteine is frequently reported in NAFLD [56–60], though not consequently [61–63].

Levels of vitamin B_{12} or folic acid, elevating serum homocysteine when impaired, weren't different. Interestingly, plasma homocysteine was lower in those with NASH compared to NAFL [60,63]. Alterations of the homocysteine metabolism were reported, with reduced transsulfuration to cysteine and impaired remethylation to methionine [57,62,64,65] (although polymorphisms altering MTHFR enzyme-function, part of the folate cycle that regulates remethylation of homocysteine, weren't different [58,59]). As a result, oxidative stress within the liver increases (whilst faced with higher levels of beta-oxidation and reduced repletion of glutathione stores), contributing to NASH progression [57,62]. Intrahepatic vasculature is directly influenced by homocysteine, with impaired NO formation and increased intrahepatic vascular resistance [66].

Inborn hyperhomocysteinaemia is related with increased CV mortality, irrespective of the underlying genetic defect. Moreover, observational studies support plasma homocysteine levels as an independent CVRF [67,68]. Homocysteine causes oxidative stress, endothelial dysfunction, impairs redox status and enhances platelet activation, all contributing to CV effects [68]. One study looking directly at associations of NAFLD with serum homocysteine levels and preclinical CVD showed an increased level of homocysteine, associated with elevated oxidative stress in NAFLD [69]. The cIMT and plaque frequency increased in NAFLD. The cIMT was significantly correlated with reduced glutathione, though not directly with homocysteine. Intriguingly, the PIVENS trial evidenced lower levels of homocysteine after treatment [65].

Postprandial increase in serum oxidised LDL (oxLDL) and large VLDL, with parallel decrease of total antioxidative status, is seen in NASH. This oxidative imbalance is related to liver fat content, liver injury and the degree of fibrosis [70]. Postprandial lipaemia is an established CVRF (see below) and an important source of oxidative stress [70]. Not only postprandial, but oxidative stress in general is increased in NASH [71,72]. Oxidative stress is essential in

CV pathophysiology [73], thus all of the above may contribute to CVD development in NAFLD.

Lipid profile

The liver is a central regulator of whole body lipid metabolism by the combined action of *de novo* lipogenesis and breakdown of lipids, as well uptake and secretion of serum lipoproteins [74].

Serum lipid profile correlates significantly with NAFLD severity, with more pronounced disturbances in NASH. Increased levels of TG and LDL and decrease of HDL results in more atherogenic lipid ratios [75]. These differences were less clear in more recent studies, but advanced analyses still reveal pro-atherogenic profiles. Moreover particle composition, subclasses, surface apolipoproteins and phospholipids are of increasing importance with respect to CVD risk [76]. Small dense LDL particles (LDL3 and LDL4) were increased, whilst the large LDL particle LDL1 was decreased in NASH compared with NAFL [77]. Siddiqui *et al*. confirmed the relationship with small dense LDL accompanied with increased VLDL particles and impaired maturation of HDL. Larger VLDL particles impair lipoprotein lipase-mediated clearance, causing higher triglyceride-rich circulating remnants. Concurrently, the activity of sterol regulatory element-binding protein 1 was increased, fuelling cholesterol synthesis [78].

Postprandial lipid profile is compatible with a more atherogenic profile through increased chylomicron remnants, more LDL and less HDL particles [79]. In patients with NAFLD this postprandial mechanism is accentuated with higher levels of triglyceride-rich and enlarged VLDL particles [80,81].

Angiogenic factors

Centrozonal arteries and microvessels are a common finding in NAFLD, even without advanced fibrosis. These alterations are indicative of active angiogenesis, as part of vascular remodelling, in early stages of NAFLD [82]. In line, anti-VEGFR2 treatment was able to improve steatosis and inflammation in mouse models [40].

Coulon *et al.* demonstrated increased serum levels of VEGF in NAFL and NASH compared to controls [83]. Other studies showed no differences [84] or only an increase in NASH [85] compared to controls. Serum sVEGFR1 showed the same trend with comparable increase in NAFL and NASH [83]. Others reported lowered serum levels of sVEGFR1 [84]. Finally, the levels of sVEGFR2 weren't different amongst the groups [83]. At a transcriptional level only NAFL and NASH could be compared, showing increased expression of VEGF and VEGFR2 in livers with NAFL compared to NASH [83], but fold-changes in expression were rather small. Other studies using a threshold of ≥2 times fold-changes failed to demonstrate significant differences [86].

VEGF-A and other VEGF-family members are also implied in CV pathophysiology. Significant associations indicate an active role in atherogenesis and plaque instability, thus contributing to plaque formation and vulnerability [87,88]. The increased levels of VEGF in NAFLD can therefore link the 2 conditions. However, the role of VEGF in atherosclerogenesis is challenged lately, with some negative studies in animal and clinical settings. Local concentrations and not systemic concentrations might explain this discrepancy [88].

High mobility group box 1 (HMGB-1) is a molecule with diverse functions, amongst which induction of angiogenesis [89]. It showed to be capable of inducing liver damage and systemic inflammation [90]. Moreover, it's associated with plaque burden in coronary artery disease (CAD) [91]. Interestingly, in a small study HMGB1 levels in patients with NAFLD and CAD were reduced compared to those without CAD [53].

Haemostasis

The liver is an important and sometimes exclusive source of both pro- and anticoagulant factors [92]. Alterations of these factors have previously been extensively studied in relation to cirrhosis, obesity and MetS, but less in NAFLD.

Most of the studies have low patient numbers or lack histologic diagnosis. Nevertheless they all point towards an increase in prothrombotic factors even after correction for other cardiometabolic risk factors. Hypercoagulability can be linked to atherosclerosis and CVD[93]. The factors VIII, IX, XI and XII were positively correlated to hepatic fat content [94].

The strongest evidence exists regarding plasminogen inhibitor activator 1 (PAI-1). Circulating levels were positively related to hepatic fat content or NASH [95–98]. Verrijken *et al.* analysed a large cohort and proved that fibrinogen, factor VII, von Willebrandfactor, PAI-1 were increased, while antithrombin III was decreased. However, only PAI-1 levels were independently related to NAFLD. Furthermore, PAI-1 serum levels as well as hepatic expression were significantly correlated with histological severity of NAFLD [98]. The increase in PAI-1 expression in NASH strongly supports the concept of increased secretion of PAI-1 in NASH [97,98].

At present, aside from many correlations of prothrombotic factors with NAFLD, there is scarce epidemiological evidence to connect NAFLD directly to thrombosis in absence of cirrhosis [99]. Nonetheless, Tripodi *et al.* elegantly demonstrated a procoagulant imbalance in NAFLD, correlating with disease-severity, using advanced coagulation assays. Decreased protein C activity and increased levels of factor VIII are supposed to be responsible [100].

Whole blood viscosity and related haemorheological factors are related with increased CVD[101], probably due to compromised delivery of substrates and oxygen to tissues. Increased blood viscosity, in part because of dyslipidaemia, was also linked to NAFLD [102,103] and provide an additional link to CVD.

Inflammation and cytokines

The liver contains the largest number of residential macrophages and contains high numbers of immune cells [104]. It's conceivable that cytokines secreted by the diseased liver drain into the systemic circulation and cause secondary CV effects. Systemic inflammation and circulating cyto- and chemokines are associated with CVD [105]. Inflammation fuels CVD via endothelial dysfunction, altered vascular tone, enhanced plaque formation and coagulation [106]. Interestingly, 18 genes were significantly differently expressed in NASH compared to NAFL, all of which were linkable to inflammation and/or plaque formation [107].

The observed increased hepaticovenous pressure gradient in non-cirrhotic NAFLD was positively associated with IL-1β [108]. In NAFLD, hepatic venous blood (representing the outflow tract of the liver) showed a higher M1/2 macophage ratio (hence a more inflammatory pattern) than systemic blood, correlated with a higher hepaticovenous pressure gradient and hence affected vascular function, at least within the liver. Hepaticovenous levels of several cytokines (IL-6, IL1β, TNFα, IL10/IL17 ratio) also correlated with more disturbed parameters of glucose metabolism [108].

Increased circulating markers for systemic inflammation are associated with NAFLD. This link is most pronounced in NASH. Levels of IL-6 were increased in line with histological severity [40,109,110], though not consequently[111]. Hepatic expression of IL-6 was also related to NAFLD, though lost significance when adding metabolic risk factors [109]. Similarly, serum levels and expression of hsCRP were elevated in relation with disease severity

[110,112]. Finally, serum levels of TNFα, CCL3 and sICAM-1 were positively related with NAFLD [97,111]. Hepatic expression of TNF, CXCL10 and IL1RN were congruently elevated [113].

Taking together, these data provide evidence that the liver is indeed directly contributing to the observed systemic inflammation that affects the CV system at distance.

Hepatokines

Hepatokines are a type of organokines (proteins exclusively/predominantly produced by and secreted from a specific tissue) that are less well known than adipokines. Organokines are not only simple markers of their tissue of origin, but can exert active para- and endocrine effects. Several hepatokines have been described and the majority influences insulin sensitivity, although some of them showed to have CV effects [114].

Fetuin-A (FetA) is a hepatokine that inhibits the insulin receptor tyrosine kinase in liver and skeletal muscle causing insulin resistance [114] and it is capable of inducing the expression of inflammatory cytokines and inhibits adiponectin [115]. The expression of FetA is linked with that of key enzymes of lipid and glucose metabolism. NAFLD is related with higher expression, transcription and serum levels of FetA [116,117], and levels in NASH are the highest [117]. Since myocardial infarction and stroke are also related with FetA [118], FetA can be a causative link between NASH and CVD.

Sato *et al.* showed cIMT and endothelial dysfunction to be positively related to FetA levels. In contrast with others, FetA levels were, however, not different between controls and NAFLD. Contrariwise, cIMT was even negatively correlated with FetA levels in NAFLD, as was the degree of liver fibrosis [119], which could be explained by the inhibitory effects of FetA on TGF-β1 signalling. The authors suggest a dual role of FetA, with a kind of self-defensive

mechanism with respect to fibrosis, whilst a positive trend between FetA and steatohepatitis was seen. This inverse relation with fibrosis wasn't reconfirmed in a biopsy-proven series, a discrepancy attributed to the outweighing of inflammation in NASH in case of lower degrees of fibrosis [120]. Finally, and in line with a dual role of FetA, NAFLD could be independently associated with FetA, while the risk of coronarographically diagnosed CAD decreased [121]. More recent analyses confirm the conflicting CV protective and harmful role of FetA, potentially explained by the balance between promoting a CVD risk profile and the capability to decrease vascular calcifications [122].

Fibroblast growth factor 21 (FGF21) has beneficial effects on insulin sensitivity and cholesterol. Despite its favourable effects, increased levels are strongly associated with increased CVD, potentially reflecting an adaptive hypersecretion to FGF21 resistance [123]. In NAFLD both increased serum levels and hepatic expression of FGF21 were seen [124,125], correlating with the degree of steatosis [124]. In a coronarographic study both NAFLD and CAD were positively associated with FGF21, albeit this association was independent of NAFLD [126].

Selenoprotein P (SeP) is increased in NAFLD, and the risk of NAFL increases 7.5 times in the highest plasma level tertile in non-diabetics. Moreover, SeP is positively correlated with insulin resistance, hsCRP and arterial stiffness [127] or cIMT [128]. The close relationship with glucose handling and associated inflammation might explain the effects on CVD. A longitudinal follow-up after bariatric surgery in 10 patients unexpectedly showed decrease of ANGPTL6 (see below) and increase of SeP after 9 months, making the role of SeP more complex. The simultaneous anti-oxidative properties of SeP and potential U-shaped relationship between SeP and DM may explain this paradox [129].

Angiopoetin like proteins (ANGPTL) are considered orphan ligands, because they don't bind to receptors targeted by angiopoeietins. They have pleiotropic effects, including angiogenic

properties and influence on glucose and lipid metabolism. Some of these ANGPTLs are mainly produced by the liver [130,131]. Besides metabolic linkage, ANGTPL's are associated with subclinical CVD [132–134]. Interestingly, patients with NASH, not NAFL, have higher levels of ANGTPL3 [135].

Adipokines

The adipokines are produced by white AT, which is nowadays recognised as an active metabolic player and no longer as a simple storage body compartment. The presence of alterations in circulating adipokines (decreased adiponectin, increased leptin) in MetS or NAFLD is hence not surprising. Adipokines are implicated in both NAFLD and CVD pathogenesis. Nonetheless, since the liver isn't directly involved in their production and since systemic levels are rather similar or lower than portal levels [136–138], the role of adipokines in the link between NAFLD and CVD seems minor. Publications on other adipokines, e.g. visfatin or resistin, are in light of CVD in NAFLD limited. Similarly, there is limited knowledge of the effects of brown AT on CVD.

Gut-liver axis

Intestinal dysbiosis is related to the aetiology of NAFLD and progression to NASH [139–141]. Likewise intestinal dysbiosis can be linked to atherogenesis, myocardial infarction and heart failure [142,143]. Within plaques the presence of intestinal DNA was even demonstrated and associated with CV events [142].

The gut microbiome can secrete different molecules that, via the intestinal wall, end up into the blood stream. The most well known are the secondary bile acids, but also trimethylamine (TMA) and short chain fatty acids (SCFA) are amongst them. These molecules can modulate energy balance, insulin sensitivity and may indirectly influence NAFLD and CVD [143]. More

direct evidence of a link with CVD is found with trimetylamine-N-Oxide (TMAO), considered as a pro-atherogenic compound. Impact on cholesterol metabolism and the promotion of foam cells are assumed to be responsible [143].

TMAO is formed out of TMA by flavin monoxygenase (FMO) in the liver. Genetic analysis of inbred mouse strains couldn't show a positive relation of FMO3, the most active isoform, with hepatic TG content, but FMO5 instead was upregulated [144]. The main substrates for TMA formation are dietary choline, phosphatidylcholine and L-carnitine. Clinically, and in line with many animal models of NASH [145], choline-deficiency and corresponding microbiome alterations are related to NAFLD [146], which seems to imply that this should rather be beneficial towards CVD. The intestinal microbiome activity may contribute to both steatosis, due to relative choline-deficiency, and increased levels of produced TMA out of this choline [147].

Incretins are gastrointestinal peptide hormones regulating postprandial glucose metabolism. Glucose-induced release of glucagon-like peptide 1 (GLP-1), nor gastric inhibitory protein (GIP), was impaired in non-diabetic patients with NAFLD [148]. Treatment with GLP-1 receptor agonists or DPP-4 inhibitors improve liver histology [149] and seems promising. GLP-1 is known to have multiple cardioprotective effects [150]. Furthermore, the exendine-4 (GLP-1 receptor agonist) improved NASH, vessel inflammation and plaque size via the inhibition of macrophage recruitment and activation [151].

Of note, the role of the gut is also particular interest in light of the upcoming treatments, including obeticholic acid, which exerts its action through agonism on the farnesoid X receptor (a receptor of secondary bile acids), located in intestinal epithelial cells and hepatocytes and a key regulator of bile acid homeostasis but possesses important crosstalk with glucose and lipid metabolism [22].

Genetics

There are increasing arguments for a role of genetic modifiers of NAFLD. When restricting to those genes with robust validation or the use of transmission disequilibrium testing, only a handful of genes currently seems to be of interest [152], of which patatin-like phospholipase domain containing protein 3 (PNPLA3) and transmembrane 6 superfamily member 2 (TM6SF2) are most documented.

Familial clustering, ethnic differences and twin studies give arguments for the presence of heritable components. These studies estimate that 18-50% can be attributed to genetic factors [152–154]. In a recent twin study CV risk and NAFLD was specifically studied [153]. Although CV parameters were more frequently abnormal in NAFLD, they failed to establish a role of genetic components in acquiring NAFLD (in contrast to [154]) or the associated CVRFs [153].

Robust data now associate the PNPLA3 rs738409 mutation with NAFLD severity [155,156]. The function of the gene seems to be directly related to TG metabolism, whereas mutations have not been associated with insulin resistance or glucose intolerance. The PNPLA3 mutation is increasingly recognised as a modifier of NAFLD [152] in terms of disease severity and the risk on NAFLD-related HCC [157], whilst the link of this mutation with CVD currently remains elusive.

Another non-synonymous SNP, TM6SF2 has also been associated with NAFLD and fibrosis [158]. Mutation of this gene probably results in retention of TG and cholesterol in the liver [159], predisposing to NAFLD and fibrosis. Paradoxically, this mutation results in a reduction of VLDL secretion and improvement of serum TG, while insulin sensitivity was unaltered [160,161]. Therefor this mutation would be cardioprotective rather than deleterious for the CV system, recently called the “Catch-22” paradigm [162].

Other SNP's have been associated with NAFLD [163] and whilst biologically plausible, their association still warrants further validation [152] and the link with CVD is largely unknown.

Other potential mechanisms

Sarcopenia (loss of muscle mass and muscle performance) [164] and sarcopenic obesity, which links sarcopenia with obesity [165] are associated with increased CVD [166,167]. The pathophysiology is rather complex with involvement of joint risk factors/mechanisms [165,168]. Importantly, also NAFLD could be associated with sarcopenia in three recent papers [169–171]. One of them observed a direct association with sarcopenia and increased pulse-wave velocity, a parameter for subclinical CVD [171].

NAFLD and vitamin D deficiency are often associated [172]. Vitamin D deficiency has proven to be a causal and independent CVRF [173]. Whether NAFLD causes hypovitaminosis D and so contributes to CVD has to be proven. We estimate that it's most probably an association rather than a causal link.

Decreased levels of serum albumin are classically seen in advanced liver disease (i.e. high grade fibrosis). Albumin has known inotropic effects [174] and low levels of albumin are linked to higher CVD and mortality [175]. However long-term analysis failed to show decrease in serum albumin levels in patients with NAFLD [176]. As a result, albumin doesn't seem to mediate CVD in patients with NAFLD without advanced liver disease.

Microvesicles are small vesicles formed out of cellular plasma by budding, excretion or after apoptosis and may contain lipids, proteins, RNAs and microRNAs. Initially regarded as cellular debris, they are now recognised as paracrine signals. Circulating microvesicles are increased in liver diseases. These microvesicles can originate from other organs and cause

liver damage. The liver itself can, however, also be a source of microvesicles, illustrated by the presence of liver-derived procoagulant microvesicles reported in acute liver failure [177]. Potential effects of liver-related microvesicles on the CV system and CVD have been reported in a mouse model with NASH and atherosclerosis [178]. In line with this potential role of microvesicles, NAFLD has very recently shown to have an unique profile of circulating microRNAs. This profile was associated with increased CV risk when the alterations were compared to known disease pathways in *in silico* analysis [179].

CLINICAL IMPLICATIONS

Current guidelines

In the current published recommendations, both on the clinical management of the patients with NAFLD and *a fortiori* on the design of therapeutic trials for NAFLD, the link with CVD disease is not a major issue [180–182]. In the clinical guidelines, the overt link of NAFLD with the MetS translates into instructions to screen NAFLD patients for associated CVRFs and to treat these according to their proper guidelines [15]. In the joined AASLD-AGA guidelines, these instructions are only found in the section for the treatment of associated dyslipidaemias, so screening for CVRFs is not even a specific topic [181]. In the EASL position paper of 2010, CVD is recognised as an extrahepatic manifestation of NAFLD. Screening for CVRFs is a separate recommendation and is hence considered a standard element in the approach of a NAFLD patient [180]. New EASL guidelines are expected to be presented in 2016. The subsequent sections propose and discuss a pragmatic approach based on the current knowledge.

Screening

Screening for NAFLD in patients with CVRFs and CVD

As NAFLD is contributing to the development of CVD, one might consider screening for NAFLD in patients with CVRFs, in patients with subclinical CVD, or in patients with clinical CVD. The first question is what to screen for? As discussed previously, data suggesting that the risk of CVD, and especially clinical CVD, seems to be confined to patients with NASH, have recently been challenged by data suggesting that fibrosis is the most important predictor of future CV events in both NAFL and NASH patients [4,19]. Nevertheless, detailed analysis of the data, including recent recent paired-biopsy studies [17,18] and the aforementioned consideration concering methodology and the dynamic nature of both NAFLD and its related conditions like CVD and MetS, still support the concept of necro-

inflammation as the driver of disease progression. This also explains why patietns with NASH and some degree of fibrosis are currently the target population in most of the clinical trials [182]. Therefore, screening should ideally address both the aspects of steatohepatitis and fibrosis

The second question is which screening technique to use. For the diagnosis of steatosis, ultrasound is an ideal screening tool because it is safe and cheap and has a high accuracy if steatosis >30% [183]. and Liver enzymes are cheap, but lack sensitivity and specificity [9,176]. Other diagnostic techniques are more expensive, less easily accessible and/or invasive, so currently not suitable for screening purposes. Biomarker research hasn't provided a sufficiently accurate and validated alternative [184]. Screening must therefore currently rely on a combination of clinical (metabolic) assessment, serum liver enzymes and ultrasound. Screening for fibrosis can be done by liver stiffness measurement, clinicobiochemical parameters and scores, or by a combination of both [184] but these approaches still lack validation in large populations at risk.

Thirdly, the group of patients to screen for NAFLD should be defined. Patients that already present with overt CVD represent a well-delineated group. Screening for NAFLD will hardly add to the overall cost of their treatment. In several countries, some high-risk patients, especially diabetics, undergo regular medical check-ups including screening for CVD [185]. Also in this population, adding screening for NAFLD/NASH by liver enzymes and ultrasound would only minimally impact on overall cost. For other NAFLD risk groups, especially patients with obesity or MetS, baseline-screening guidelines exist (but stringent follow-up programs are less systematically organised) and frequently include liver enzymes and ultrasound. The third category of patients, in whom risk factors for CVD have been identified (e.g. smoking, positive familial history, dyslipidaemia) constitute a large group, in whom liver tests are frequently checked, but abdominal ultrasound isn't part of their routine work-up.

This leads to another problem that hampers screening for NAFLD, namely the unawareness of the relevance of NASH and related conditions (CVD, T2DM, MetS) in the physicians taking care of these patients, with, as a consequence, underdiagnosis of NAFLD [2,3]. Awareness of physicians for the presence of NASH and a correct interpretation of tests that are already performed would probably result in a considerably higher detection rate without any additional cost. Moreover, a Canadian cost-effectiveness study supports cost-effectiveness of non-invasive screening for NASH and advanced fibrosis in a high-risk obese or diabetic population [186].

Formal recommendation cannot be given based on the current evidence, but we suggest to screen for NAFLD and NASH by ultrasound and liver enzymes in patients with overt CVD or in patients with increased CV risk (e.g. SCORE or HeartSCORE estimated 10 year risk ≥5% [187]) and if positive, to subsequently assess fibrosis by a combination of a clinicobichemical score and a liver stiffness measurement [184].

The need for screening, although defendable, is further challenged by the limited treatment options for NASH. Lifestyle modification improves NASH as well CV risk profile [188,189], but whether both are just associated because of a beneficial impact on the different metabolic risk factors that are shared by both conditions, or whether the improvement in NASH severity contributes to an improvement of the risk of a true CVD event, is an unresolved issue. The fact that NASH contributes to CVD logically leads to the hypothesis that improvement in NASH specifically contributes to an improvement in CVD. This, however, remains to be proven, which isn't easy, as similar to pathophysiology, the specific contribution of NASH improvement to the overall improvement in CV risk is difficult to dissect from the beneficial contribution of other factors. The answer to this question remains nevertheless crucial and properly designed studied are highly warranted.

Screening for CVD in patients with NAFLD

The inverse screening question is if we need to screen for CVD in patients with NAFLD. Current recommendation just recommend to screen for classical CVRFs [15]. As presence of subclinical CVD is a well-established risk factor for subsequent clinical CVD [190] and NASH impacts on atherosclerosis, the question rises whether a NASH patient shouldn't be screened for the presence of subclinical CVD. If present, the consequence could be that the patient should undergo more sophisticated examinations to look for clinically significant CV abnormalities and a more aggressive treatment of the CVRFs and lesions. The tool to screen for subclinical CVD is to be discussed and depends on local availability and expertise. Carotid ultrasound including cIMT measurement is one of the most widely used and validated [187,191]. Coronary artery calcium score, ankle-brachial index and flow-mediated vasodilatation are valuable alternatives [187]. Besides, the population to be screened isn't defined, but as the risk seems to be low in NAFL, limiting the screening to NASH seems appropriate. Anyhow, no data on cost-effectiveness are available to date to support this strategy but studies should be encouraged given the potential clinical relevance. A pragmatic approach to screen for CVD in NAFLD is proposed in Fig. 3.

Pharmacological treatment

The question is becoming even more relevant, as drugs specifically aiming at treating NAFLD/NASH are currently being developed, some of them entering phase 3 clinical trials. In the current recommendations for NASH trial design, CVD is poorly touched upon, except for the recommendation that, as most NASH patients die from CVD and treatment is presumably long-term or even life-long, the cardiac safety profile of the drug should be thoroughly monitored [182]. There is hence, besides the monitoring of CVRFs and registariton of CVD events, no specific assessment of the impact of the treatment of NASH on the CV outcome of the patients.

Cardiovascular safety is of course an issue in all studies, and most include also impact on metabolic and CVRFs as one of the secondary endpoints. Effects on hard clinical CV endpoints would take many years and appropriately powered studies to show a benefit of the drug compared to placebo and is hence not realistic in this setting. One wonders, however, why a test for subclinical CVD has not been incorporated in the design of these studies as a secondary endpoint. As well as NASH histology is an accepted surrogate endpoint for clinical benefit in terms of liver disease, measurement of the impact on markers of subclinical CVD (such as cIMT, FMD) are accepted surrogate endpoints for clinical benefit in terms of CVD [190].

If a drug improving steatohepatitis could also show to have a beneficial effect on a parameter of subclinical CVD (and hence predictive of future CV clinical events), this would greatly enhance its utility in clinical practice as this substantially increases the likelihood that the drug results in a significant survival benefit. We would therefore strongly recommend to add the impact of a candidate drug on a marker of subclinical CVD as a co-primary or secondary endpoint in clinical trials for NASH and to ascertain that studies are sufficiently powered to assess this aspect of potential treatment benefit.

Another aspect, besides demonstrating beneficial effects on cardiometabolic risk profile, or on a parameter of subclinical disease, is the treatment indication. The goal of treatment is to improve NASH rather than just improving fibrosis. Since NASH is believed to be the motor of disease progression, resolution of NASH without worsening of fibrosis is the preferred primary endpoint [182,192]. The target population consists of patients with moderate-severe steatohepatitis and significant fibrosis (≥ F2).

Studies on the impact of glycaemic control on long-term outcome, however, failed to show a reduction in CV mortality despite good glycaemic control. This has been attributed to the so-called “metabolic memory”, denoting the persistence of endothelial dysfunction despite

correction of glucose levels. Only patients with short duration of DM, low baseline HbA_{1c} and no history of a CVD event benefitted (in terms of improved survival) from good glycaemic control, suggesting that early intervention is needed to improve survival and once vascular damage has been installed, improved metabolic control does hardly impact on long-term survival.

This concept challenges the restriction of treatment indication to patients with significant fibrosis, as it suggests that NASH patients should be treated early and hence regardless of fibrosis degree. The shift from a purely liver-centered approach to a holistic approach would therefore substantially impact on the number of patients to be treated, but also on the potential overall survival benefit that could be obtained.

A final consideration in this ongoing debate is the relationship between the improvement in NASH and the improvement in cardiometabolic profile and endothelial dysfunction. The causal role of NASH in both the development of DM and CVD doesn't necessarily mean that improvement of NAFLD automatically results in improvement of DM and CVD. A unilateral relationship is difficult to demonstrate in a clinical setting, where several processes are interrelated and patients are heterogeneous. Sub-analyses of the current and future trials, looking into the kinetics of changes in liver and cardiometabolic parameters, into differences between responders and non-responders and into relationships between the magnitude of the improvement of NASH and the observed changes in cardiometabolic risk, might help answering this question.

Interestingly, although beyond the scope of this review, pharmacological treatment of CVRFs and CVD can also positively impact on NAFLD. Statins have no proven benefit on liver histology in randomized controlled trials, but were shown to reduce progression to cirrhosis and cirrhosis decompensation [193], to reduce incident HCC [194] and to improve hepatic endothelial function [49], fibrogenesis [195] and angiogenesis [196] . Sartans reduce fibrosis

in animal models [197] and aspirin use was associated with lower indices of fibrosis in the US adult population [198] and reduced steatohepatitis in an animal model [199]. These and other data further highlight the complex reciprocal interactions of NAFLD and CVD and the need for a multidisciplinary approach.

CONCLUSIONS

CVD remains the most important cause of death in patients with NAFLD. Clinical evidence for a hepato-cardiovascular axis, in which NAFLD is an independent risk factor for subclinical and clinical CVD is supported by evidence from fundamental and clinical research unravelling the mechanisms by which NAFLD causally influences on endothelial dysfunction and the development of atherosclerosis and other CV lesions. The current liver-centered approach of NAFLD should therefore shift to a more holistic approach, including specific assessment of CVD that shouldn't be limited to the treatment of concomitant CVRFs, but should ideally include parameters of subclinical CVD. This not only applies to routine clinical practice, but *a fortiori* to clinical trials, as the ultimate goal of therapy is to improve patient's survival. As metabolic memory negatively impacts on potential treatment-induced survival benefit, early treatment of NASH might be warranted, challenging the currently defined target population for clinical trials in NASH.

LEGENDS TO THE FIGURES

Figure 1: Complex interplay of NAFLD and cardiovascular disease

The liver is centrally positioned in the metabolic syndrome, where NAFLD can be considered as the consequence of mechanism driven by the other components of the metabolic syndrome. However, reciprocal crosstalk exists, wherein the liver may actually drive diabetes mellitus or cardiovascular disease. These synergetic effects become more complex and create a vicious circle.

NAFLD, non-alcoholic fatty liver disease

Figure 2: Summary of potential pathophysiological mechanism responsible for increased CVD in NAFLD

Non-alcoholic fatty liver disease (NAFLD) drives multiple mechanisms that ultimately lead to cardiovascular disease. These mechanisms are summarised in this figure. Genetic background, adipose tissue and the gut all contribute, in part via the liver (direct effects also exists but are not within the scope of this review). The details about these mechanisms are described in the text. Structural alterations of the cardiovascular system are marked in red.

ANGPTL, angiopoetin like proteins; FetA, Fetuin-A; FGF21, fibroblast growth factor 21; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; HDL, high-density lipoproteins; HMGB-1, high mobility group box 1; hsCRP, high sensitivity C-reactive protein; IL-1β, interleukin 1β; IL-6, interleukin 6; M1/M2, macrophage phenotype 1/2 ratio; OxLDL, oxidized low-density lipoprotein; PAI-1, plasminogen activator inhibitor 1; PNPLA3, patatin-like phospholipase domain containing protein 3; sdLDL, small dense low-density lipoproteins; SeP, selenoprotein P; SNP, single nucleotide polymorphism; TG, triglycerides; TM6SF2, transmembrane 6 superfamiliy member 2; TMA, trimethylamine; TMAO, trimetylamine-N-

838 Oxide; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor; VLDL, very
839 low-density lipoproteins.
840
841
842 **Figure 3: Proposed algorithm to assess CVD in patients with NAFLD**
843 Since patients with non-alcoholic fatty liver disease (NAFLD) are at high risk to develop
844 cardiovascular disease (CVD), there is a rationale to screen for CVD. Screening seems to be
845 more appropriate and cost-effective in patients with NASH or clinical significant fibrosis,
846 though must not be restrained to them solely. In case of sufficient clinical arguments,
847 amongst which the presence of diabetes mellitus is of major importance, additional screening
848 can be advocated. Negative assessments do not waive adequate follow-up and lifestyle
849 interventions. Re-assessments can be proposed at 2-3 years interval or based on
850 symptomatic CVD.
851
852 CACS, coronary artery calcium score; cIMT, carotid intima media thickness; CVD,
853 cardiovascular disease; ECG, electrocardiogram; FMD, flow mediated dilatation; NAFL, non-
854 alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic
855 steatohepatitis; PWV, pulse wave velocity.
856

LEGEND TO THE TABLE

Table 1: Potential pathophysiological mechanism responsible for increased CVD in NAFLD

Non-alcoholic fatty liver disease (NAFLD) drives multiple mechanisms that ultimately may lead to cardiovascular disease. This table summarises the potential mechanisms by which the liver can influence CVD. Only mechanisms directly involved are incorporated in the table, e.g. adipokines are not included. See the text for more details about the tabulated mechanisms.

ADMA, asymmetric dimethyl arginine; ANGPTL, angiopoetin like proteins; CCL3, Chemokine (C-C motif) ligand 3; cIMT, carotid intima media thickness; CAD, coronary artery disease; CVD, cardiovascular disease; EPC, endothelial progenitor cells; FGF21, fibroblast growth factor 21; FMO, flavin monoxygenase; HDL, high-density lipoproteins; HMGB-1, high mobility group box 1; hsCRP, high sensitivity C-reactive protein; IL-1β, interleukin 1β; IL-6, interleukin 6; LDL, low-density lipoproteins; LV, left ventricle; M1/M2, macrophage phenotype 1/2 ratio; NAFLD, non-alcoholic fatty liver disease; NAFL, non-alcoholic fatty liver; NASH, non-alcholic steatohepatitis; OxLDL, oxidized low-density lipoprotein; PAI-1, plasminogen activator inhibitor 1; PNPLA3, patatin-like phospholipase domain containing protein 3; PWV, pulse wave velocity; sdLDL, small dense low-density lipoproteins; SeP, selenoprotein P; sICAM, soluble intercellular adhesion molecule-1; TGF-β, transforming growth factor β; TM6SF2, transmembrane 6 superfamiliy member 2; TMA, trimethylamine; TMAO, trimetylamine-N-Oxide; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor; VLDL, very low-density lipoproteins.

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REFERENCES

[1] Charlton MR, Burns JM, Pedersen R a., Watt KD, Heimbach JK, Dierkhising R a. Frequency and outcomes of liver transplantation for nonalcoholic steatohepatitis in the United States. Gastroenterology 2011;141:1249–53. doi:10.1053/j.gastro.2011.06.061.

[2] Said A, Gagovic V, Malecki K, Givens ML, Nieto FJ. Primary care practitioners survey of non-alcoholic fatty liver disease. Ann Hepatol 2013;12:758–65.

[3] Bergqvist C-J, Skoien R, Horsfall L, Clouston a D, Jonsson JR, Powell EE. Awareness and opinions of non-alcoholic fatty liver disease by hospital specialists. Intern Med J 2013;43:247–53. doi:10.1111/j.1445-5994.2012.02848.x.

[4] **Ekstedt M, Hagström H**, Nasr P, Fredrikson M, Stål P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015;61:1547–54. doi:10.1002/hep.27368.

[5] Satapathy SK, Sanyal AJ. Epidemiology and Natural History of Nonalcoholic Fatty Liver Disease. Semin Liver Dis 2015;35:221–35. doi:10.1055/s-0035-1562943.

[6] Söderberg C, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology 2010;51:595–602. doi:10.1002/hep.23314.

[7] Lazo M, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, et al. Non-alcoholic fatty liver disease and mortality among US adults: prospective cohort study. BMJ 2011;343:d6891. doi:10.1136/bmj.d6891.

[8] Stepanova M, Younossi ZM. Independent association between nonalcoholic fatty liver disease and cardiovascular disease in the US population. Clin Gastroenterol Hepatol 2012;10:646–50. doi:10.1016/j.cgh.2011.12.039.

[9] Verma S, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). Liver Int 2013;33:1398–405. doi:10.1111/liv.12226.

[10] Kotronen A, Yki-Järvinen H. Fatty liver: a novel component of the metabolic syndrome. Arterioscler Thromb Vasc Biol 2008;28:27–38. doi:10.1161/ATVBAHA.107.147538.

[11] Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: a precursor of the metabolic syndrome. Dig Liver Dis 2015;47:181–90. doi:10.1016/j.dld.2014.09.020.

[12] Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010;52:1836–46. doi:10.1002/hep.24001.

[13] Heilbronn L, Smith SR, Ravussin E. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. Int J Obes Relat Metab Disord 2004;28 Suppl 4:S12–21. doi:10.1038/sj.ijo.0802853.

[14] Kleiner DE, Brunt EM. Nonalcoholic fatty liver disease: pathologic patterns and biopsy evaluation in clinical research. Semin Liver Dis 2012;32:3–13. doi:10.1055/s-0032-1306421.

[15] Nascimbeni F, Pais R, Bellentani S, Day CP, Ratziu V, Loria P, et al. From NAFLD in clinical practice to answers from guidelines. J Hepatol 2013;59:859–71. doi:10.1016/j.jhep.2013.05.044.

[16] Adams LA, Ratziu V. Non-alcoholic fatty liver - perhaps not so benign. J Hepatol 2015;62:1002–4. doi:10.1016/j.jhep.2015.02.005.

[17] Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol 2015;13:643–54.e1–9; quiz e39–40. doi:10.1016/j.cgh.2014.04.014.

[18] McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol 2015;62:1148–55. doi:10.1016/j.jhep.2014.11.034.

[19] Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology 2015;149:389–97.e10. doi:10.1053/j.gastro.2015.04.043.

[20] Ekstedt M, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865–73. doi:10.1002/hep.21327.

[21] Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med 2011;43:617–49. doi:10.3109/07853890.2010.518623.

[22] Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet (London, England) 2015;385:956–65. doi:10.1016/S0140-6736(14)61933-4.

[23] Ratziu V, Harrison S, Francque S, Bedossa P, Lehert P, Serfaty L, et al. Elafibranor, an Agonist of the Peroxisome Proliferator-Activated Receptor-α and -δ, Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening. Gastroenterology 2016. doi:10.1053/j.gastro.2016.01.038.

[24] Argo CK, Northup PG, Al-Osaimi AMS, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. J Hepatol 2009;51:371–9. doi:10.1016/j.jhep.2009.03.019.

[25] Ying I, Saposnik G, Vermeulen MJ, Leung A, Ray JG. Nonalcoholic fatty liver disease and acute ischemic stroke. Epidemiology 2011;22:129–30. doi:10.1097/EDE.0b013e3181feb50a.

[26] Boddi M, Tarquini R, Chiostri M, Marra F, Valente S, Giglioli C, et al. Nonalcoholic fatty liver in nondiabetic patients with acute coronary syndromes. Eur J Clin Invest 2013;43:429–38. doi:10.1111/eci.12065.

[27] Villanova N, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, et al. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. Hepatology 2005;42:473–80. doi:10.1002/hep.20781.

[28] Long MT, Wang N, Larson MG, Mitchell GF, Palmisano J, Vasan RS, et al. Nonalcoholic fatty liver disease and vascular function: cross-sectional analysis in the Framingham heart study. Arterioscler Thromb Vasc Biol 2015;35:1284–91. doi:10.1161/ATVBAHA.114.305200.

[29] Kaess BM, Rong J, Larson MG, Hamburg NM, Vita JA, Levy D, et al. Aortic stiffness, blood pressure progression, and incident hypertension. JAMA 2012;308:875–81. doi:10.1001/2012.jama.10503.

[30] Madan SA, John F, Pyrsopoulos N, Pitchumoni CS. Nonalcoholic fatty liver disease and carotid artery atherosclerosis in children and adults: a meta-analysis. Eur J Gastroenterol Hepatol 2015;27:1237–48. doi:10.1097/MEG.0000000000000429.

[31] Bonci E, Chiesa C, Versacci P, Anania C, Silvestri L, Pacifico L. Association of Nonalcoholic Fatty Liver Disease with Subclinical Cardiovascular Changes: A Systematic Review and Meta-Analysis. Biomed Res Int 2015;2015:213737. doi:10.1155/2015/213737.

[32] VanWagner LB, Wilcox JE, Colangelo LA, Lloyd-Jones DM, Carr JJ, Lima JA, et al. Association of nonalcoholic fatty liver disease with subclinical myocardial remodeling and dysfunction: A population-based study. Hepatology 2015;62:773–83. doi:10.1002/hep.27869.

[33] Bhatia LS, Curzen NP, Calder PC, Byrne CD. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? Eur Heart J 2012;33:1190–200. doi:10.1093/eurheartj/ehr453.

[34] Oni ET, Agatston AS, Blaha MJ, Fialkow J, Cury R, Sposito A, et al. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver; should we care? Atherosclerosis 2013;230:258–67. doi:10.1016/j.atherosclerosis.2013.07.052.

[35] Luo J, Xu L, Li J, Zhao S. Nonalcoholic fatty liver disease as a potential risk factor of cardiovascular disease. Eur J Gastroenterol Hepatol 2015;27:193–9. doi:10.1097/MEG.0000000000000254.

[36] Ballestri S, Lonardo A, Bonapace S, Byrne CD, Loria P, Targher G. Risk of cardiovascular, cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease. World J Gastroenterol 2014;20:1724–45. doi:10.3748/wjg.v20.i7.1724.

[37] Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol 2015;62:S47–64. doi:10.1016/j.jhep.2014.12.012.

[38] **Francque S, Laleman W**, Verbeke L, Van Steenkiste C, Casteleyn C, Kwanten W, et al. Increased intrahepatic resistance in severe steatosis: endothelial dysfunction, vasoconstrictor overproduction and altered microvascular architecture. Lab Invest 2012;92:1428–39. doi:10.1038/labinvest.2012.103.

[39] Farrell GC, Teoh NC, McCuskey RS. Hepatic microcirculation in fatty liver disease. Anat Rec (Hoboken) 2008;291:684–92. doi:10.1002/ar.20715.

[40] Coulon S, Legry V, Heindryckx F, Van Steenkiste C, Casteleyn C, Olievier K, et al. Role of vascular endothelial growth factor in the pathophysiology of nonalcoholic steatohepatitis in two rodent models. Hepatology 2013;57:1793–805. doi:10.1002/hep.26219.

[41] Francque S, Verrijken A, Mertens I, Hubens G, Van Marck E, Pelckmans P, et al. Noncirrhotic human nonalcoholic fatty liver disease induces portal hypertension in relation to the histological degree of steatosis. Eur J Gastroenterol Hepatol 2010;22:1449–57. doi:10.1097/MEG.0b013e32833f14a1.

[42] Gkaliagkousi E, Douma S. The pathogenesis of arterial stiffness and its prognostic value in essential hypertension and cardiovascular diseases. Hippokratia 2009;13:70–5.

[43] Zang S, Ma X, Zhuang Z, Liu J, Bian D, Xun Y, et al. Increased ratio of neutrophil elastase to α1-antitrypsin is closely associated with liver inflammation in patients with nonalcoholic steatohepatitis. Clin Exp Pharmacol Physiol 2016;43:13–21. doi:10.1111/1440-1681.12499.

[44] Sunbul M, Agirbasli M, Durmus E, Kivrak T, Akin H, Aydin Y, et al. Arterial stiffness in patients with non-alcoholic fatty liver disease is related to fibrosis stage and epicardial adipose tissue thickness. Atherosclerosis 2014;237:490–3. doi:10.1016/j.atherosclerosis.2014.10.004.

[45] Vanhoutte PM. Endothelial dysfunction: the first step toward coronary arteriosclerosis. Circ J 2009;73:595–601. doi:JST.JSTAGE/circj/CJ-08-1169 [pii].

[46] Iwakiri Y, Shah V, Rockey DC. Vascular pathobiology in chronic liver disease and cirrhosis

- current status and future directions. J Hepatol 2014;61:912–24. doi:10.1016/j.jhep.2014.05.047.

[47] Maslak E, Gregorius A, Chlopicki S. Liver sinusoidal endothelial cells (LSECs) function and NAFLD; NO-based therapy targeted to the liver. Pharmacol Rep 2015;67:689–94. doi:10.1016/j.pharep.2015.04.010.

[48] **Pasarín M, Abraldes** JG, Rodríguez-Vilarrupla A, La Mura V, García-Pagán JC, Bosch J. Insulin resistance and liver microcirculation in a rat model of early NAFLD. J Hepatol 2011;55:1095–102. doi:10.1016/j.jhep.2011.01.053.

[49] Pasarín M, La Mura V, Gracia-Sancho J, García-Calderó H, Rodríguez-Vilarrupla A, García-Pagán JC, et al. Sinusoidal endothelial dysfunction precedes inflammation and fibrosis in a model of NAFLD. PLoS One 2012;7:e32785. doi:10.1371/journal.pone.0032785.

[50] Kasumov T, Edmison JM, Dasarathy S, Bennett C, Lopez R, Kalhan SC. Plasma levels of asymmetric dimethylarginine in patients with biopsy-proven nonalcoholic fatty liver disease. Metabolism 2011;60:776–81. doi:10.1016/j.metabol.2010.07.027.

[51] Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation 2007;115:1285–95. doi:10.1161/CIRCULATIONAHA.106.652859.

[52] Dogru T, Genc H, Tapan S, Aslan F, Ercin CN, Ors F, et al. Plasma fetuin-A is associated with endothelial dysfunction and subclinical atherosclerosis in subjects with nonalcoholic fatty liver disease. Clin Endocrinol (Oxf) 2013;78:712–7. doi:10.1111/j.1365-2265.2012.04460.x.

[53] Elsheikh E, Younoszai Z, Otgonsuren M, Hunt S, Raybuck B, Younossi ZM. Markers of endothelial dysfunction in patients with non-alcoholic fatty liver disease and coronary artery disease. J Gastroenterol Hepatol 2014;29:1528–34. doi:10.1111/jgh.12549.

[54] Bruyndonckx L, Hoymans VY, Van Craenenbroeck AH, Vissers DK, Vrints CJ, Ramet J, et al. Assessment of endothelial dysfunction in childhood obesity and clinical use. Oxid Med Cell Longev 2013;2013:174782. doi:10.1155/2013/174782.

[55] Chiang C-H, Huang P-H, Chung F-P, Chen Z-Y, Leu H-B, Huang C-C, et al. Decreased circulating endothelial progenitor cell levels and function in patients with nonalcoholic fatty liver disease. PLoS One 2012;7:e31799. doi:10.1371/journal.pone.0031799.

[56] Gulsen M, Yesilova Z, Bagci S, Uygun A, Ozcan A, Ercin CN, et al. Elevated plasma homocysteine concentrations as a predictor of steatohepatitis in patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2005;20:1448–55. doi:10.1111/j.1440-1746.2005.03891.x.

[57] Bravo E, Palleschi S, Aspichueta P, Buqué X, Rossi B, Cano A, et al. High fat diet-induced non alcoholic fatty liver disease in rats is associated with hyperhomocysteinemia caused by down regulation of the transsulphuration pathway. Lipids Health Dis 2011;10:60. doi:10.1186/1476-511X-10-60.

[58] de Carvalho SCR, Muniz MTC, Siqueira MDV, Siqueira ERF, Gomes AV, Silva KA, et al. Plasmatic higher levels of homocysteine in non-alcoholic fatty liver disease (NAFLD). Nutr J 2013;12:37. doi:10.1186/1475-2891-12-37.

[59] Franco Brochado MJ, Domenici FA, Candolo Martinelli A de L, Zucoloto S, de Carvalho da Cunha SF, Vannucchi H. Methylenetetrahydrofolate reductase gene polymorphism and serum homocysteine levels in nonalcoholic fatty liver disease. Ann Nutr Metab 2013;63:193–9. doi:10.1159/000353139.

[60] **Pastore A, Alisi A,** di Giovamberardino G, Crudele A, Ceccarelli S, Panera N, et al. Plasma levels of homocysteine and cysteine increased in pediatric NAFLD and strongly correlated with severity of liver damage. Int J Mol Sci 2014;15:21202–14.

1078 doi:10.3390/ijms151121202.
1079 [61] Hirsch S, Poniachick J, Avendaño M, Csendes A, Burdiles P, Smok G, et al. Serum folate and
1080 homocysteine levels in obese females with non-alcoholic fatty liver. Nutrition
1081 2005;21:137–41. doi:10.1016/j.nut.2004.03.022.
1082 [62] Kalhan SC, Edmison J, Marczewski S, Dasarathy S, Gruca LL, Bennett C, et al. Methionine
1083 and protein metabolism in non-alcoholic steatohepatitis: evidence for lower rate of
1084 transmethylation of methionine. Clin Sci (Lond) 2011;121:179–89.
1085 doi:10.1042/CS20110060.
1086 [63] Polyzos S a, Kountouras J, Patsiaoura K, Katsiki E, Zafeiriadou E, Deretzi G, et al. Serum
1087 homocysteine levels in patients with nonalcoholic fatty liver disease. Ann Hepatol
1088 2012;11:68–76.
1089 [64] Dahlhoff C, Desmarchelier C, Sailer M, Fürst RW, Haag A, Ulbrich SE, et al. Hepatic
1090 methionine homeostasis is conserved in C57BL/6N mice on high-fat diet despite major
1091 changes in hepatic one-carbon metabolism. PLoS One 2013;8:e57387.
1092 doi:10.1371/journal.pone.0057387.
1093 [65] Pacana T, Cazanave S, Verdianelli A, Patel V, Min H-K, Mirshahi F, et al. Dysregulated
1094 Hepatic Methionine Metabolism Drives Homocysteine Elevation in Diet-Induced
1095 Nonalcoholic Fatty Liver Disease. PLoS One 2015;10:e0136822.
1096 doi:10.1371/journal.pone.0136822.
1097 [66] Distrutti E, Mencarelli A, Santucci L, Renga B, Orlandi S, Donini A, et al. The methionine
1098 connection: homocysteine and hydrogen sulfide exert opposite effects on hepatic
1099 microcirculation in rats. Hepatology 2008;47:659–67. doi:10.1002/hep.22037.
1100 [67] Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease.
1101 Nutr J 2015;14:6. doi:10.1186/1475-2891-14-6.
1102 [68] Santilli F, Davì G, Patrono C. Homocysteine, methylenetetrahydrofolate reductase, folate
1103 status and atherothrombosis: A mechanistic and clinical perspective. Vascul Pharmacol
1104 2016;78:1–9. doi:10.1016/j.vph.2015.06.009.
1105 [69] Leach N V, Dronca E, Vesa SC, Sampelean DP, Craciun EC, Lupsor M, et al. Serum
1106 homocysteine levels, oxidative stress and cardiovascular risk in non-alcoholic
1107 steatohepatitis. Eur J Intern Med 2014;25:762–7. doi:10.1016/j.ejim.2014.09.007.
1108 [70] Musso G, Gambino R, De Michieli F, Biroli G, Fagà E, Pagano G, et al. Association of liver
1109 disease with postprandial large intestinal triglyceride-rich lipoprotein accumulation and
1110 pro/antioxidant imbalance in normolipidemic non-alcoholic steatohepatitis. Ann Med
1111 2008;40:383–94. doi:10.1080/07853890801946515.
1112 [71] Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic
1113 and dietary correlates in patients with nonalcoholic steatohepatitis. Am J Gastroenterol
1114 2004;99:1497–502. doi:10.1111/j.1572-0241.2004.30159.x.
1115 [72] Madan K, Bhardwaj P, Thareja S, Gupta SD, Saraya A. Oxidant stress and antioxidant
1116 status among patients with nonalcoholic fatty liver disease (NAFLD). J Clin Gastroenterol
1117 2006;40:930–5. doi:10.1097/01.mcg.0000212608.59090.08.
1118 [73] Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease.
1119 Arterioscler Thromb Vasc Biol 2005;25:29–38.
1120 doi:10.1161/01.ATV.0000150649.39934.13.
1121 [74] Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic
1122 steatohepatitis: the central role of nontriglyceride fatty acid metabolites. Hepatology
1123 2010;52:774–88. doi:10.1002/hep.23719.
1124 [75] Alkhouri N, Tamimi TA-R, Yerian L, Lopez R, Zein NN, Feldstein AE. The inflamed liver and
1125 atherosclerosis: a link between histologic severity of nonalcoholic fatty liver disease and

increased cardiovascular risk. Dig Dis Sci 2010;55:2644–50. doi:10.1007/s10620-009-1075-y.

[76] Ouweneel AB, Van Eck M. Lipoproteins as modulators of atherothrombosis: From endothelial function to primary and secondary coagulation. Vascul Pharmacol 2015. doi:10.1016/j.vph.2015.10.009.

[77] Sonmez A, Nikolic D, Dogru T, Ercin CN, Genc H, Cesur M, et al. Low- and high-density lipoprotein subclasses in subjects with nonalcoholic fatty liver disease. J Clin Lipidol 2015;9:576–82. doi:10.1016/j.jacl.2015.03.010.

[78] Siddiqui MS, Fuchs M, Idowu MO, Luketic VA, Boyett S, Sargeant C, et al. Severity of nonalcoholic fatty liver disease and progression to cirrhosis are associated with atherogenic lipoprotein profile. Clin Gastroenterol Hepatol 2015;13:1000–8.e3. doi:10.1016/j.cgh.2014.10.008.

[79] Roche HM, Gibney MJ. The impact of postprandial lipemia in accelerating atherothrombosis. J Cardiovasc Risk 2000;7:317–24.

[80] Cassader M, Gambino R, Musso G, Depetris N, Mecca F, Cavallo-Perin P, et al. Postprandial triglyceride-rich lipoprotein metabolism and insulin sensitivity in nonalcoholic steatohepatitis patients. Lipids 2001;36:1117–24. doi:10.1007/s11745-001-0822-5.

[81] Musso G, Gambino R, De Michieli F, Durazzo M, Pagano G, Cassader M. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: Possible pathogenetic role in NASH. Hepatology 2008;47:1167–77. doi:10.1002/hep.22142.

[82] Gill RM, Belt P, Wilson L, Bass NM, Ferrell LD. Centrizonal arteries and microvessels in nonalcoholic steatohepatitis. Am J Surg Pathol 2011;35:1400–4. doi:10.1097/PAS.0b013e3182254283.

[83] Coulon S, Francque S, Colle I, Verrijken A, Blomme B, Heindryckx F, et al. Evaluation of inflammatory and angiogenic factors in patients with non-alcoholic fatty liver disease. Cytokine 2012;59:442–9. doi:10.1016/j.cyto.2012.05.001.

[84] Yilmaz Y, Yonal O, Kurt R, Alahdab YO, Ozdogan O, Celikel CA, et al. Circulating levels of vascular endothelial growth factor A and its soluble receptor in patients with biopsy-proven nonalcoholic fatty liver disease. Arch Med Res 2011;42:38–43. doi:10.1016/j.arcmed.2010.12.001.

[85] Tarantino G, Conca P, Pasanisi F, Ariello M, Mastrolia M, Arena A, et al. Could inflammatory markers help diagnose nonalcoholic steatohepatitis? Eur J Gastroenterol Hepatol 2009;21:504–11. doi:10.1097/MEG.0b013e3283229b40.

[86] Cayón A, Crespo J, Guerra AR, Pons-Romero F. [Gene expression in obese patients with non-alcoholic steatohepatitis]. Rev Española Enfermedades Dig Organo Of La Soc Española Patol Dig 2008;100:212–8.

[87] Khurana R, Simons M, Martin JF, Zachary IC. Role of angiogenesis in cardiovascular disease: a critical appraisal. Circulation 2005;112:1813–24. doi:10.1161/CIRCULATIONAHA.105.535294.

[88] **Ylä-Herttuala S, Rissanen TT**, Vajanto I, Hartikainen J. Vascular endothelial growth factors: biology and current status of clinical applications in cardiovascular medicine. J Am Coll Cardiol 2007;49:1015–26. doi:10.1016/j.jacc.2006.09.053.

[89] Chen R, Hou W, Zhang Q, Kang R, Fan X-G, Tang D. Emerging role of high-mobility group box 1 (HMGB1) in liver diseases. Mol Med 2013;19:357–66. doi:10.2119/molmed.2013.00099.

[90] **Li L, Chen L, Hu L**, Liu Y, Sun H-Y, Tang J, et al. Nuclear factor high-mobility group box1 mediating the activation of Toll-like receptor 4 signaling in hepatocytes in the early stage of nonalcoholic fatty liver disease in mice. Hepatology 2011;54:1620–30.

doi:10.1002/hep.24552.

[91] **Andrassy M, Volz HC**, Maack B, Schuessler A, Gitsioudis G, Hofmann N, et al. HMGB1 is associated with atherosclerotic plaque composition and burden in patients with stable coronary artery disease. PLoS One 2012;7:e52081. doi:10.1371/journal.pone.0052081.

[92] Tripodi A, Mannucci PM. The coagulopathy of chronic liver disease. N Engl J Med 2011;365:147–56. doi:10.1056/NEJMra1011170.

[93] Loeffen R, Spronk HMH, ten Cate H. The impact of blood coagulability on atherosclerosis and cardiovascular disease. J Thromb Haemost 2012;10:1207–16. doi:10.1111/j.1538-7836.2012.04782.x.

[94] Kotronen A, Joutsi-Korhonen L, Sevastianova K, Bergholm R, Hakkarainen A, Pietiläinen KH, et al. Increased coagulation factor VIII, IX, XI and XII activities in non-alcoholic fatty liver disease. Liver Int 2011;31:176–83. doi:10.1111/j.1478-3231.2010.02375.x.

[95] Alessi M-C, Bastelica D, Mavri A, Morange P, Berthet B, Grino M, et al. Plasma PAI-1 levels are more strongly related to liver steatosis than to adipose tissue accumulation. Arterioscler Thromb Vasc Biol 2003;23:1262–8. doi:10.1161/01.ATV.0000077401.36885.BB.

[96] Barbato A, Iacone R, Tarantino G, Russo O, Sorrentino P, Avallone S, et al. Relationships of PAI-1 levels to central obesity and liver steatosis in a sample of adult male population in southern Italy. Intern Emerg Med 2009;4:315–23. doi:10.1007/s11739-009-0240-9.

[97] Sookoian S, Castaño GO, Burgueño AL, Rosselli MS, Gianotti TF, Mallardi P, et al. Circulating levels and hepatic expression of molecular mediators of atherosclerosis in nonalcoholic fatty liver disease. Atherosclerosis 2010;209:585–91. doi:10.1016/j.atherosclerosis.2009.10.011.

[98] **Verrijken A, Francque S**, Mertens I, Prawitt J, Caron S, Hubens G, et al. Prothrombotic factors in histologically proven nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2014;59:121–9. doi:10.1002/hep.26510.

[99] Northup PG, Argo CK, Shah N, Caldwell SH. Hypercoagulation and thrombophilia in nonalcoholic fatty liver disease: mechanisms, human evidence, therapeutic implications, and preventive implications. Semin Liver Dis 2012;32:39–48. doi:10.1055/s-0032-1306425.

[100] Tripodi A, Fracanzani AL, Primignani M, Chantarangkul V, Clerici M, Mannucci PM, et al. Procoagulant imbalance in patients with non-alcoholic fatty liver disease. J Hepatol 2014;61:148–54. doi:10.1016/j.jhep.2014.03.013.

[101] Toth A, Papp J, Rabai M, Kenyeres P, Marton Z, Kesmarky G, et al. The role of hemorheological factors in cardiovascular medicine. Clin Hemorheol Microcirc 2014;56:197–204. doi:10.3233/CH-131685.

[102] Yu K, Zhang M, Li Y, Wang R. Increased whole blood viscosity associated with arterial stiffness in patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2014;29:540–4. doi:10.1111/jgh.12368.

[103] **Zhao H-Y, Li J,** Xu M, Wang T-G, Sun W-W, Chen Y, et al. Elevated whole blood viscosity is associated with insulin resistance and non-alcoholic fatty liver. Clin Endocrinol (Oxf) 2015;83:806–11. doi:10.1111/cen.12776.

[104] Racanelli V, Rehermann B. The liver as an immunological organ. Hepatology 2006;43:S54–62. doi:10.1002/hep.21060.

[105] Stoner L, Lucero AA, Palmer BR, Jones LM, Young JM, Faulkner J. Inflammatory biomarkers for predicting cardiovascular disease. Clin Biochem 2013;46:1353–71. doi:10.1016/j.clinbiochem.2013.05.070.

[106] Kofler S, Nickel T, Weis M. Role of cytokines in cardiovascular diseases: a focus on

endothelial responses to inflammation. Clin Sci (Lond) 2005;108:205–13. doi:10.1042/CS20040174.

[107] Sookoian S, Gianotti TF, Rosselli MS, Burgueño AL, Castaño GO, Pirola CJ. Liver transcriptional profile of atherosclerosis-related genes in human nonalcoholic fatty liver disease. Atherosclerosis 2011;218:378–85. doi:10.1016/j.atherosclerosis.2011.05.014.

[108] Vonghia L, Magrone T, Verrijken A, Michielsen P, Van Gaal L, Jirillo E, et al. Peripheral and Hepatic Vein Cytokine Levels in Correlation with Non-Alcoholic Fatty Liver Disease (NAFLD)-Related Metabolic, Histological, and Haemodynamic Features. PLoS One 2015;10:e0143380. doi:10.1371/journal.pone.0143380.

[109] Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. Am J Gastroenterol 2008;103:1372–9. doi:10.1111/j.1572-0241.2007.01774.x.

[110] Hamirani YS, Katz R, Nasir K, Zeb I, Blaha MJ, Blumenthal RS, et al. Association between inflammatory markers and liver fat: The Multi-Ethnic Study of Atherosclerosis. J Clin Exp Cardiolog 2014;5:1–17. doi:10.4172/2155-9880.1000344.

[111] **du Plessis J**, van Pelt J, Korf H, Mathieu C, van der Schueren B, Lannoo M, et al. Association of Adipose Tissue Inflammation With Histologic Severity of Nonalcoholic Fatty Liver Disease. Gastroenterology 2015;149:635–48.e14. doi:10.1053/j.gastro.2015.05.044.

[112] Yoneda M, Mawatari H, Fujita K, Iida H, Yonemitsu K, Kato S, et al. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. J Gastroenterol 2007;42:573–82. doi:10.1007/s00535-007-2060-x.

[113] **Wolfs MGM, Gruben N**, Rensen SS, Verdam FJ, Greve JW, Driessen A, et al. Determining the association between adipokine expression in multiple tissues and phenotypic features of non-alcoholic fatty liver disease in obesity. Nutr Diabetes 2015;5:e146. doi:10.1038/nutd.2014.43.

[114] Stefan N, Häring H-U. The role of hepatokines in metabolism. Nat Rev Endocrinol 2013;9:144–52. doi:10.1038/nrendo.2012.258.

[115] **Hennige AM, Staiger H**, Wicke C, Machicao F, Fritsche A, Häring H-U, et al. Fetuin-A induces cytokine expression and suppresses adiponectin production. PLoS One 2008;3:e1765. doi:10.1371/journal.pone.0001765.

[116] Haukeland JW, Dahl TB, Yndestad A, Gladhaug IP, Løberg EM, Haaland T, et al. Fetuin A in nonalcoholic fatty liver disease: in vivo and in vitro studies. Eur J Endocrinol 2012;166:503–10. doi:10.1530/EJE-11-0864.

[117] **Kahraman A, Sowa J-P, Schlattjan M**, Sydor S, Pronadl M, Wree A, et al. Fetuin-A mRNA expression is elevated in NASH compared with NAFL patients. Clin Sci (Lond) 2013;125:391–400. doi:10.1042/CS20120542.

[118] Weikert C, Stefan N, Schulze MB, Pischon T, Berger K, Joost H-G, et al. Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. Circulation 2008;118:2555–62. doi:10.1161/CIRCULATIONAHA.108.814418.

[119] **Sato M, Kamada Y, Takeda Y**, Kida S, Ohara Y, Fujii H, et al. Fetuin-A negatively correlates with liver and vascular fibrosis in nonalcoholic fatty liver disease subjects. Liver Int 2015;35:925–35. doi:10.1111/liv.12478.

[120] Kamada Y, Miyoshi E. Value of fetuin-A as a predictor of liver fibrosis in patients with nonalcoholic fatty liver disease. Author's reply. Liver Int 2015;35:2062. doi:10.1111/liv.12833.

[121] Ballestri S, Meschiari E, Baldelli E, Musumeci FE, Romagnoli D, Trenti T, et al. Relationship

of serum fetuin-A levels with coronary atherosclerotic burden and NAFLD in patients undergoing elective coronary angiography. Metab Syndr Relat Disord 2013;11:289–95. doi:10.1089/met.2012.0149.

[122] Jensen MK, Bartz TM, Mukamal KJ, Djoussé L, Kizer JR, Tracy RP, et al. Fetuin-A, type 2 diabetes, and risk of cardiovascular disease in older adults: the cardiovascular health study. Diabetes Care 2013;36:1222–8. doi:10.2337/dc12-1591.

[123] Domouzoglou EM, Naka KK, Vlahos AP, Papafaklis MI, Michalis LK, Tsatsoulis A, et al. Fibroblast growth factors in cardiovascular disease: The emerging role of FGF21. Am J Physiol Heart Circ Physiol 2015;309:H1029–38. doi:10.1152/ajpheart.00527.2015.

[124] **Li H, Fang Q, Gao F,** Fan J, Zhou J, Wang X, et al. Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. J Hepatol 2010;53:934–40. doi:10.1016/j.jhep.2010.05.018.

[125] Dushay J, Chui PC, Gopalakrishnan GS, Varela-Rey M, Crawley M, Fisher FM, et al. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology 2010;139:456–63. doi:10.1053/j.gastro.2010.04.054.

[126] **Shen Y, Ma X,** Zhou J, Pan X, Hao Y, Zhou M, et al. Additive relationship between serum fibroblast growth factor 21 level and coronary artery disease. Cardiovasc Diabetol 2013;12:124. doi:10.1186/1475-2840-12-124.

[127] Choi HY, Hwang SY, Lee CH, Hong HC, Yang SJ, Yoo HJ, et al. Increased selenoprotein p levels in subjects with visceral obesity and nonalcoholic Fatty liver disease. Diabetes Metab J 2013;37:63–71. doi:10.4093/dmj.2013.37.1.63.

[128] Yang SJ, Hwang SY, Choi HY, Yoo HJ, Seo J a., Kim SG, et al. Serum selenoprotein P levels in patients with type 2 diabetes and prediabetes: implications for insulin resistance, inflammation, and atherosclerosis. J Clin Endocrinol Metab 2011;96:E1325–9. doi:10.1210/jc.2011-0620.

[129] Lim J, Park HS, Lee SK, Jang YJ, Lee YJ, Heo Y. Longitudinal Changes in Serum Levels of Angiopoietin-Like Protein 6 and Selenoprotein P After Gastric Bypass Surgery. Obes Surg 2015. doi:10.1007/s11695-015-1808-2.

[130] Katagiri H, Yamada T, Oka Y. Adiposity and cardiovascular disorders: disturbance of the regulatory system consisting of humoral and neuronal signals. Circ Res 2007;101:27–39. doi:10.1161/CIRCRESAHA.107.151621.

[131] Santulli G. Angiopoietin-like proteins: a comprehensive look. Front Endocrinol (Lausanne) 2014;5:4. doi:10.3389/fendo.2014.00004.

[132] Hatsuda S, Shoji T, Shinohara K, Kimoto E, Mori K, Fukumoto S, et al. Association between plasma angiopoietin-like protein 3 and arterial wall thickness in healthy subjects. J Vasc Res 2007;44:61–6. doi:10.1159/000098153.

[133] Adachi H, Fujiwara Y, Kondo T, Nishikawa T, Ogawa R, Matsumura T, et al. Angptl 4 deficiency improves lipid metabolism, suppresses foam cell formation and protects against atherosclerosis. Biochem Biophys Res Commun 2009;379:806–11. doi:10.1016/j.bbrc.2008.12.018.

[134] **Jung CH, Lee WJ**, Lee MJ, Kang YM, Jang JE, Leem J, et al. Association of serum angiopoietin-like protein 2 with carotid intima-media thickness in subjects with type 2 diabetes. Cardiovasc Diabetol 2015;14:35. doi:10.1186/s12933-015-0198-z.

[135] Yilmaz Y, Ulukaya E, Atug O, Dolar E. Serum concentrations of human angiopoietin-like protein 3 in patients with nonalcoholic fatty liver disease: association with insulin resistance. Eur J Gastroenterol Hepatol 2009;21:1247–51. doi:10.1097/MEG.0b013e32832b77ae.

[136] Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is

associated with systemic inflammation in obese humans. Diabetes 2007;56:1010–3. doi:10.2337/db06-1656.

[137] Faber DR, Moll FL, Vink A, van der Waal C, Kalkhoven E, Schipper HS, et al. Adipose tissue quantity and composition contribute to adipokine concentrations in the subclavian vein and the inferior mesenteric vein. Int J Obes (Lond) 2012;36:1078–85. doi:10.1038/ijo.2011.214.

[138] Karbaschian Z, Hosseinzadeh-Attar MJ, Giahi L, Golpaie A, Masoudkabir F, Talebpour M, et al. Portal and systemic levels of visfatin in morbidly obese subjects undergoing bariatric surgery. Endocrine 2013;44:114–8. doi:10.1007/s12020-012-9821-x.

[139] Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013;57:601–9. doi:10.1002/hep.26093.

[140] **Wong VW-S, Tse C-H, Lam TT-Y**, Wong GL-H, Chim AM-L, Chu WC-W, et al. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis--a longitudinal study. PLoS One 2013;8:e62885. doi:10.1371/journal.pone.0062885.

[141] Abu-Shanab A, Quigley EMM. The role of the gut microbiota in nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 2010;7:691–701. doi:10.1038/nrgastro.2010.172.

[142] Aron-Wisnewsky J, Clément K. The gut microbiome, diet, and links to cardiometabolic and chronic disorders. Nat Rev Nephrol 2016;12:169–81. doi:10.1038/nrneph.2015.191.

[143] Brown JM, Hazen SL. The gut microbial endocrine organ: bacterially derived signals driving cardiometabolic diseases. Annu Rev Med 2015;66:343–59. doi:10.1146/annurev-med-060513-093205.

[144] Hui ST, Parks BW, Org E, Norheim F, Che N, Pan C, et al. The genetic architecture of NAFLD among inbred strains of mice. Elife 2015;4:e05607. doi:10.7554/eLife.05607.

[145] Hebbard L, George J. Animal models of nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 2011;8:35–44. doi:10.1038/nrgastro.2010.191.

[146] Spencer MD, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA. Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. Gastroenterology 2011;140:976–86. doi:10.1053/j.gastro.2010.11.049.

[147] Dumas M-E, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci U S A 2006;103:12511–6. doi:10.1073/pnas.0601056103.

[148] Bernsmeier C, Meyer-Gerspach AC, Blaser LS, Jeker L, Steinert RE, Heim MH, et al. Glucose-induced glucagon-like Peptide 1 secretion is deficient in patients with non-alcoholic fatty liver disease. PLoS One 2014;9:e87488. doi:10.1371/journal.pone.0087488.

[149] Carbone F, Montecucco F, Mach F, Pontremoli R, Viazzi F. The liver and the kidney: two critical organs influencing the atherothrombotic risk in metabolic syndrome. Thromb Haemost 2013;110:940–58. doi:10.1160/TH13-06-0499.

[150] Kim J, Samson SL. Cardiovascular effects of incretin therapy in diabetes care. Metab Syndr Relat Disord 2014;12:303–10. doi:10.1089/met.2014.0035.

[151] **Wang Y, Parlevliet ET,** Geerling JJ, van der Tuin SJL, Zhang H, Bieghs V, et al. Exendin-4 decreases liver inflammation and atherosclerosis development simultaneously by reducing macrophage infiltration. Br J Pharmacol 2014;171:723–34. doi:10.1111/bph.12490.

[152] Anstee QM, Day CP. The Genetics of Nonalcoholic Fatty Liver Disease: Spotlight on

PNPLA3 and TM6SF2. Semin Liver Dis 2015;35:270–90. doi:10.1055/s-0035-1562947.

[153] **Tarnoki AD, Tarnoki DL**, Bata P, Littvay L, Osztovits J, Jermendy G, et al. Heritability of non-alcoholic fatty liver disease and association with abnormal vascular parameters: a twin study. Liver Int 2012;32:1287–93. doi:10.1111/j.1478-3231.2012.02823.x.

[154] Loomba R, Schork N, Chen C-H, Bettencourt R, Bhatt A, Ang B, et al. Heritability of Hepatic Fibrosis and Steatosis Based on a Prospective Twin Study. Gastroenterology 2015;149:1784–93. doi:10.1053/j.gastro.2015.08.011.

[155] **Romeo S, Kozlitina J,** Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461–5. doi:10.1038/ng.257.

[156] **Speliotes EK, Yerges-Armstrong LM, Wu J**, Hernaez R, Kim LJ, Palmer CD, et al. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genet 2011;7:e1001324. doi:10.1371/journal.pgen.1001324.

[157] Liu Y-L, Patman GL, Leathart JBS, Piguet A-C, Burt AD, Dufour J-F, et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol 2014;61:75–81. doi:10.1016/j.jhep.2014.02.030.

[158] Liu Y-L, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JBS, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:4309. doi:10.1038/ncomms5309.

[159] **Kozlitina J, Smagris E,** Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46:352–6. doi:10.1038/ng.2901.

[160] **Holmen OL, Zhang H, Fan Y**, Hovelson DH, Schmidt EM, Zhou W, et al. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. Nat Genet 2014;46:345–51. doi:10.1038/ng.2926.

[161] **Zhou Y, Llauradó G**, Orešič M, Hyötyläinen T, Orho-Melander M, Yki-Järvinen H. Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. J Hepatol 2015;62:657–63. doi:10.1016/j.jhep.2014.10.010.

[162] Kahali B, Liu Y-L, Daly AK, Day CP, Anstee QM, Speliotes EK. TM6SF2: catch-22 in the fight against nonalcoholic fatty liver disease and cardiovascular disease? Gastroenterology 2015;148:679–84. doi:10.1053/j.gastro.2015.01.038.

[163] Naik A, Košir R, Rozman D. Genomic aspects of NAFLD pathogenesis. Genomics 2013;102:84–95. doi:10.1016/j.ygeno.2013.03.007.

[164] Cooper C, Fielding R, Visser M, van Loon LJ, Rolland Y, Orwoll E, et al. Tools in the assessment of sarcopenia. Calcif Tissue Int 2013;93:201–10. doi:10.1007/s00223-013-9757-z.

[165] Prado CMM, Wells JCK, Smith SR, Stephan BCM, Siervo M. Sarcopenic obesity: A Critical appraisal of the current evidence. Clin Nutr 2012;31:583–601. doi:10.1016/j.clnu.2012.06.010.

[166] Byeon C-H, Kang K-Y, Kang S-H, Bae E-J. Sarcopenia is associated with Framingham risk score in the Korean population: Korean National Health and Nutrition Examination Survey (KNHANES) 2010-2011. J Geriatr Cardiol 2015;12:366–72. doi:10.11909/j.issn.1671-5411.2015.04.007.

[167] Atkins JL, Wannamethee SG. The effect of sarcopenic obesity on cardiovascular disease and all-cause mortality in older people. Rev Clin Gerontol 2015;25:86–97.

1414 doi:10.1017/S0959259815000076.
1415 [168] Stenholm S, Harris TB, Rantanen T, Visser M, Kritchevsky SB, Ferrucci L. Sarcopenic
1416 obesity: definition, cause and consequences. Curr Opin Clin Nutr Metab Care
1417 2008;11:693–700. doi:10.1097/MCO.0b013e328312c37d.
1418 [169] **Moon JS, Yoon JS,** Won KC, Lee HW. The role of skeletal muscle in development of
1419 nonalcoholic Fatty liver disease. Diabetes Metab J 2013;37:278–85.
1420 doi:10.4093/dmj.2013.37.4.278.
1421 [170] Lee Y-H, Jung KS, Kim SU, Yoon H-J, Yun YJ, Lee B-W, et al. Sarcopaenia is associated with
1422 NAFLD independently of obesity and insulin resistance: Nationwide surveys (KNHANES
1423 2008-2011). J Hepatol 2015;63:486–93. doi:10.1016/j.jhep.2015.02.051.
1424 [171] Hong HC, Hwang SY, Choi HY, Yoo HJ, Seo JA, Kim SG, et al. Relationship between
1425 sarcopenia and nonalcoholic fatty liver disease: the Korean Sarcopenic Obesity Study.
1426 Hepatology 2014;59:1772–8. doi:10.1002/hep.26716.
1427 [172] Kwok RM, Torres DM, Harrison SA. Vitamin D and nonalcoholic fatty liver disease
1428 (NAFLD): is it more than just an association? Hepatology 2013;58:1166–74.
1429 doi:10.1002/hep.26390.
1430 [173] Weyland PG, Grant WB, Howie-Esquivel J. Does sufficient evidence exist to support a
1431 causal association between vitamin D status and cardiovascular disease risk? An
1432 assessment using Hill's criteria for causality. Nutrients 2014;6:3403–30.
1433 doi:10.3390/nu6093403.
1434 [174] Bortoluzzi A, Ceolotto G, Gola E, Sticca A, Bova S, Morando F, et al. Positive cardiac
1435 inotropic effect of albumin infusion in rodents with cirrhosis and ascites: molecular
1436 mechanisms. Hepatology 2013;57:266–76. doi:10.1002/hep.26021.
1437 [175] Schalk BWM, Visser M, Bremmer MA, Penninx BWJH, Bouter LM, Deeg DJH. Change of
1438 serum albumin and risk of cardiovascular disease and all-cause mortality: Longitudinal
1439 Aging Study Amsterdam. Am J Epidemiol 2006;164:969–77. doi:10.1093/aje/kwj312.
1440 [176] Charatcharoenwitthaya P, Lindor KD, Angulo P. The spontaneous course of liver enzymes
1441 and its correlation in nonalcoholic fatty liver disease. Dig Dis Sci 2012;57:1925–31.
1442 doi:10.1007/s10620-012-2098-3.
1443 [177] Lemoinne S, Thabut D, Housset C, Moreau R, Valla D, Boulanger CM, et al. The emerging
1444 roles of microvesicles in liver diseases. Nat Rev Gastroenterol Hepatol 2014;11:350–61.
1445 doi:10.1038/nrgastro.2014.7.
1446 [178] Baron M, Leroyer AS, Majd Z, Lalloyer F, Vallez E, Bantubungi K, et al. PPARα activation
1447 differently affects microparticle content in atherosclerotic lesions and liver of a mouse
1448 model of atherosclerosis and NASH. Atherosclerosis 2011;218:69–76.
1449 doi:10.1016/j.atherosclerosis.2011.03.009.
1450 [179] Pirola CJ, Fernández Gianotti T, Castaño GO, Mallardi P, San Martino J, Mora Gonzalez
1451 Lopez Ledesma M, et al. Circulating microRNA signature in non-alcoholic fatty liver
1452 disease: from serum non-coding RNAs to liver histology and disease pathogenesis. Gut
1453 2015;64:800–12. doi:10.1136/gutjnl-2014-306996.
1454 [180] Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on
1455 NAFLD/NASH based on the EASL 2009 special conference. J Hepatol 2010;53:372–84.
1456 doi:10.1016/j.jhep.2010.04.008.
1457 [181] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and
1458 management of non-alcoholic fatty liver disease: practice Guideline by the American
1459 Association for the Study of Liver Diseases, American College of Gastroenterology, and the
1460 American Gastroenterological Association. Hepatology 2012;55:2005–23.
1461 doi:10.1002/hep.25762.

[182] Sanyal AJ, Friedman SL, McCullough AJ, Dimick-Santos L, American Association for the Study of Liver Diseases, United States Food and Drug Administration. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop. Hepatology 2015;61:1392–405. doi:10.1002/hep.27678.

[183] Ryan CK, Johnson L a., Germin BI, Marcos A. One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. Liver Transpl 2002;8:1114–22. doi:10.1053/jlts.2002.36740.

[184] Castera L. Noninvasive Evaluation of Nonalcoholic Fatty Liver Disease. Semin Liver Dis 2015;35:291–303. doi:10.1055/s-0035-1562948.

[185] Authors/Task Force Members, Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboratio. Eur Heart J 2013;34:3035–87. doi:10.1093/eurheartj/eht108.

[186] Zhang E, Wartelle-Bladou C, Lepanto L, Lachaine J, Cloutier G, Tang A. Cost-utility analysis of nonalcoholic steatohepatitis screening. Eur Radiol 2015;25:3282–94. doi:10.1007/s00330-015-3731-2.

[187] Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by re. Eur Heart J 2012;33:1635–701. doi:10.1093/eurheartj/ehs092.

[188] Nguyen V, George J. Nonalcoholic Fatty Liver Disease Management: Dietary and Lifestyle Modifications. Semin Liver Dis 2015;35:318–37. doi:10.1055/s-0035-1562950.

[189] Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Houston Miller N, Hubbard VS, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2014;129:S76–99. doi:10.1161/01.cir.0000437740.48606.d1.

[190] Halcox JPJ, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, et al. Prognostic value of coronary vascular endothelial dysfunction. Circulation 2002;106:653–8.

[191] O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340:14–22. doi:10.1056/NEJM199901073400103.

[192] Ratziu V, Goodman Z, Sanyal A. Current efforts and trends in the treatment of NASH. J Hepatol 2015;62:S65–75. doi:10.1016/j.jhep.2015.02.041.

[193] Mohanty A, Tate JP, Garcia-Tsao G. Statins Are Associated With a Decreased Risk of Decompensation and Death in Veterans With Hepatitis C-Related Compensated Cirrhosis. Gastroenterology 2016;150:430–40.e1. doi:10.1053/j.gastro.2015.10.007.

[194] **Zhou Y-Y, Zhu G-Q,** Wang Y, Zheng J-N, Ruan L-Y, Cheng Z, et al. Systematic review with network meta-analysis: statins and risk of hepatocellular carcinoma. Oncotarget 2016. doi:10.18632/oncotarget.7832.

[195] Chong L-W, Hsu Y-C, Lee T-F, Lin Y, Chiu Y-T, Yang K-C, et al. Fluvastatin attenuates hepatic steatosis-induced fibrogenesis in rats through inhibiting paracrine effect of hepatocyte on hepatic stellate cells. BMC Gastroenterol 2015;15:22. doi:10.1186/s12876-015-0248-8.

[196] Chang C-C, Wang S-S, Hsieh H-G, Lee W-S, Chuang C-L, Lin H-C, et al. Rosuvastatin improves hepatopulmonary syndrome through inhibition of inflammatory angiogenesis of lung. Clin Sci (Lond) 2015;129:449–60. doi:10.1042/CS20140622.

[197] Shirai Y, Yoshiji H, Noguchi R, Kaji K, Aihara Y, Douhara A, et al. Cross talk between toll-like receptor-4 signaling and angiotensin-II in liver fibrosis development in the rat model of non-alcoholic steatohepatitis. J Gastroenterol Hepatol 2013;28:723–30. doi:10.1111/jgh.12112.

[198] Jiang ZG, Feldbrügge L, Tapper EB, Popov Y, Ghaziani T, Afdhal N, et al. Aspirin use is associated with lower indices of liver fibrosis among adults in the United States. Aliment Pharmacol Ther 2016;43:734–43. doi:10.1111/apt.13515.

[199] Madrigal-Perez VM, García-Rivera A, Rodriguez-Hernandez A, Ceja-Espiritu G, Briseño-Gomez XG, Galvan-Salazar HR, et al. Preclinical analysis of nonsteroidal anti-inflammatory drug usefulness for the simultaneous prevention of steatohepatitis, atherosclerosis and hyperlipidemia. Int J Clin Exp Med 2015;8:22477–83.

[200] **Hallsworth K, Hollingsworth KG**, Thoma C, Jakovljevic D, MacGowan GA, Anstee QM, et al. Cardiac structure and function are altered in adults with non-alcoholic fatty liver disease. J Hepatol 2013;58:757–62. doi:10.1016/j.jhep.2012.11.015.

[201] **Cassidy S, Hallsworth K**, Thoma C, MacGowan GA, Hollingsworth KG, Day CP, et al. Cardiac structure and function are altered in type 2 diabetes and non-alcoholic fatty liver disease and associate with glycemic control. Cardiovasc Diabetol 2015;14:23. doi:10.1186/s12933-015-0187-2.

[202] Goland S, Shimoni S, Zornitzki T, Knobler H, Azoulai O, Lutaty G, et al. Cardiac abnormalities as a new manifestation of nonalcoholic fatty liver disease: echocardiographic and tissue Doppler imaging assessment. J Clin Gastroenterol n.d.;40:949–55. doi:10.1097/01.mcg.0000225668.53673.e6.

[203] Rijzewijk LJ, van der Meer RW, Smit JWA, Diamant M, Bax JJ, Hammer S, et al. Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. J Am Coll Cardiol 2008;52:1793–9. doi:10.1016/j.jacc.2008.07.062.

[204] Granér M, Nyman K, Siren R, Pentikäinen MO, Lundbom J, Hakkarainen A, et al. Ectopic fat depots and left ventricular function in nondiabetic men with nonalcoholic fatty liver disease. Circ Cardiovasc Imaging 2015;8. doi:10.1161/CIRCIMAGING.114.001979.

[205] McGavock J, Szczepaniak LS, Ayers CR, Abdullah SM, See R, Gore MO, et al. The effects of rosiglitazone on myocardial triglyceride content in patients with type 2 diabetes: a randomised, placebo-controlled trial. Diabetes Vasc Dis Res 2012;9:131–7. doi:10.1177/1479164111428628.

[206] Perseghin G, Lattuada G, De Cobelli F, Esposito A, Belloni E, Ntali G, et al. Increased mediastinal fat and impaired left ventricular energy metabolism in young men with newly found fatty liver. Hepatology 2008;47:51–8. doi:10.1002/hep.21983.

[207] Fotbolcu H, Yakar T, Duman D, Karaahmet T, Tigen K, Cevik C, et al. Impairment of the left ventricular systolic and diastolic function in patients with non-alcoholic fatty liver disease. Cardiol J 2010;17:457–63.

[208] Kocabay G, Karabay CY, Colak Y, Oduncu V, Kalayci A, Akgun T, et al. Left atrial deformation parameters in patients with non-alcoholic fatty liver disease: a 2D speckle tracking imaging study. Clin Sci (Lond) 2014;126:297–304. doi:10.1042/CS20130298.

[209] Karabay CY, Kocabay G, Kalayci A, Colak Y, Oduncu V, Akgun T, et al. Impaired left ventricular mechanics in nonalcoholic fatty liver disease: a speckle-tracking echocardiography study. Eur J Gastroenterol Hepatol 2014;26:325–31. doi:10.1097/MEG.0000000000000008.

[210] Targher G, Valbusa F, Bonapace S, Bertolini L, Zenari L, Rodella S, et al. Non-alcoholic fatty liver disease is associated with an increased incidence of atrial fibrillation in patients with type 2 diabetes. PLoS One 2013;8:e57183. doi:10.1371/journal.pone.0057183.

[211] Targher G, Valbusa F, Bonapace S, Bertolini L, Zenari L, Pichiri I, et al. Association of nonalcoholic fatty liver disease with QTc interval in patients with type 2 diabetes. Nutr Metab Cardiovasc Dis 2014;24:663–9. doi:10.1016/j.numecd.2014.01.005.

[212] Mantovani A, Pernigo M, Bergamini C, Bonapace S, Lipari P, Valbusa F, et al. Heart valve calcification in patients with type 2 diabetes and nonalcoholic fatty liver disease. Metabolism 2015;64:879–87. doi:10.1016/j.metabol.2015.04.003.

[213] Bonapace S, Valbusa F, Bertolini L, Pichiri I, Mantovani A, Rossi A, et al. Nonalcoholic fatty liver disease is associated with aortic valve sclerosis in patients with type 2 diabetes mellitus. PLoS One 2014;9:e88371. doi:10.1371/journal.pone.0088371.

[214] **Markus MRP, Baumeister SE**, Stritzke J, Dörr M, Wallaschofski H, Völzke H, et al. Hepatic steatosis is associated with aortic valve sclerosis in the general population: the Study of Health in Pomerania (SHIP). Arterioscler Thromb Vasc Biol 2013;33:1690–5. doi:10.1161/ATVBAHA.112.300556.

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