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Obesity Comorbidities

Non-alcoholic fatty liver disease is associated with cardiovascular disease risk markers

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Summary
Recognition of the link between non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) has boosted research in this area. The main objective of this paper is to review the literature on NAFLD in the context of CVD, focussing on underlying mechanisms and treatment. Besides excessive fatty acid influx, etiologic factors may include components of the metabolic syndrome, cytokines and mitochondrial dysfunction. NAFLD is associated with both hepatic and systemic insulin resistance. In the case of NAFLD, the liver overproduces several atherogenic factors, notably inflammatory cytokines, glucose, lipoproteins and coagulation factors, and factors increasing blood pressure. Intervention studies on diet and laparoscopic surgery revealed improvements of hepatic fat content and CVD risk profile. Pharmacological approaches with potential benefit have been developed as well, but effects are often confounded by weight change. NAFLD is associated with an increased CVD risk profile (and hepatic risk). In order to improve CVD risk profile, prevention and treatment of NAFLD seem advisable. However, well-designed intervention studies, randomized clinical trials and long-term follow-up studies are scarce.

Keywords: Cardiovascular disease, hepatic fat.

Introduction
The global increase of overweight and obesity is alarming (1), as obesity is a risk factor for many diseases including cardiovascular disease (CVD) (2). Obesity has the highest CVD risk when fat is located in the abdominal region (3).

In the case of obesity accompanied by insulin resistance, triglycerides (TGs) are often excessively stored ectopically, i.e. in organs and muscles rather than in adipocytes. When TGs accumulate within hepatocytes (HCs; Fig. 1), a pathological condition usually referred to as fatty liver disease (FLD) will develop. FLD includes a wide spectrum, which can broadly be divided into steatosis and steatohepatitis (4). Non-alcoholic fatty liver disease (NAFLD) is used to describe FLD in a person who drinks no or little alcohol prior to diagnosis. In the literature, the amount of ethanol allowed for the diagnosis of NAFLD varies greatly but is maximally 20 g d⁻¹ for women and 30 g d⁻¹ for men. The prevalence of NAFLD in the general adult western population is relatively high, i.e. 20% (5,6); whereas, the prevalence of total FLD, including both NAFLD and alcoholic FLD, is approximately 30% (5). In obese non-diabetic western adults, the prevalence of NAFLD ranges from 80.4% to 97.9% (7,8).

Although there is a hepatic risk for patients with NAFLD, notably cirrhosis (9) and hepatocarcinoma (10), the CVD risk for patients with NAFLD may be higher (10). Few studies have revealed evidence of an association between NAFLD and early CVD markers (11), CVD events (12,13) and CVD mortality (10). Follow-up of patients with
NAFLD showed a higher incidence of CVD compared with controls (10,13). A study by Hamaguchi et al. revealed that NAFLD was an independent predictor, even stronger than the metabolic syndrome, for first time CVD events (13).

The recognition of the CVD risk of NAFLD has boosted research in this area during the recent years. We have reviewed the literature on NAFLD in the context of CVD risk profile markers. In this paper, after a short description of the aetiology of NAFLD, we provide an overview of the cardiovascular risk of NAFLD. Finally, the potential need for prevention and treatment of NAFLD in order to improve CVD risk profile is addressed.

Aetiology

The aetiology of the hepatic lipid imbalance underlying the pathophysiology of NAFLD has been increasingly unravelled. Besides excessive non-esterified fatty acid flux, mediating factors may include: (i) components of the metabolic syndrome (14,15); (ii) cytokines (16–24) and (iii) mitochondrial dysfunction (25) (Fig. 1). A study on the incidence of NAFLD suggested that the metabolic syndrome precedes NAFLD (14), with insulin resistance as a cornerstone (15). Animal models, reviewed by Diehl et al. (16), revealed involvement of the cytokine tumour necrosis factor-alpha (TNF-α) and its possible antagonist adiponectin (17) in the pathogenesis of NAFLD. This is reinforced by cross-sectional studies showing changes in mRNA of both TNF-α receptors (increase) and adiponectin receptors (decrease) in NAFLD (18–20). TNF-α, recently reviewed by Ryden and Arner, may have numerous mediating actions, among others increasing insulin resistance and inhibiting fatty acid oxidation (22). Several animal studies on adiponectin, recently reviewed by Lafontan and Viguerie, suggest opposite actions of adiponectin on energy metabolism, i.e. increasing insulin sensitivity and stimulation of fatty acid oxidation (23). Additionally, adiponectin might contribute to inhibition

### Figure 1
Model on the cardiovascular risk of non-alcoholic fatty liver disease. ↑, increase; ↓, decrease; ≠, dependent on pancreatic β-cell function; b, may decrease in advanced NAFLD; Adipo-R1, adiponectin receptor 1; Adipo-R2, adiponectin receptor 2; AGEs, advanced glycation endproducts; GLUT-4, glucose transporter 4; HDL-R, high-density lipoprotein receptor; I-R, insulin receptor; LDL-R, low-density lipoprotein receptor; NEFA-FF, non-esterified fatty acid flip-flopping; NEFA-TP, non-esterified fatty acid transport protein; SAA, serum amyloid A; sTNF-R2, soluble tumour necrosis factor-alpha receptor 2; TNF-R1; tumour necrosis factor-alpha receptor 1.

<table>
<thead>
<tr>
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<th>Insulin uptake and resistance</th>
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<tr>
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<td>VLDL-apo-B↑, VLDL-TG↑, LDL size↓, NEFA, HDL↓</td>
<td>Glucose↑, AGEs↑</td>
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<td></td>
<td></td>
<td>Lypolysis↑, GLUT-4↑, glycogen↑</td>
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<tr>
<th>Inflammation markers</th>
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</thead>
<tbody>
<tr>
<td>a.o. CRP↑, SAA↑</td>
<td>a.o. TNF-α↑, IL-6↑</td>
<td>a.o. Fibrinogen↑, Factor VII↑, PAI-1↑</td>
<td>AST↑, ALT↑, GGT↑</td>
<td>a.o. Antithrombin III↑, Protein C↑, Protein S↑</td>
</tr>
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<table>
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<tr>
<th>Anti-heamostatic factors</th>
<th>Anti-inflammatory factors</th>
<th>Adipogenesis and lipogenesis</th>
<th>Mitochondrial dysfunction</th>
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</tr>
</thead>
<tbody>
<tr>
<td>a.o. CRP↑, SAA↑</td>
<td>a.o. TNF-α↑, IL-6↑</td>
<td>a.o. Fibrinogen↑, Factor VII↑, PAI-1↑</td>
<td>a.o. CRP↑, SAA↑</td>
<td>a.o. Antithrombin III↑, Protein C↑, Protein S↑</td>
</tr>
</tbody>
</table>

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of lipogenesis (24). Polymorphisms in the gene encoding adiponectin receptor 1 are associated with the presence of high hepatic fat content (and insulin resistance) (21). Mitochondrial dysfunction (in non-alcoholic steatohepatitis [NASH]), recently reviewed by Begriche et al., can be caused by oxidative stress, and may result in TG accumulation and eventually cell death, i.e. necrosis (25).

**Fatty liver-derived cardiovascular disease risk factors**

The liver secretes numerous CVD risk factors, notably cytokines, glucose, lipoproteins, coagulation factors and factors increasing blood pressure. In the case of NAFLD, production of several of these risk factors is altered (Fig. 1).

**Inflammation**

In both patients with non-alcoholic steatosis (NAS) and NASH, analysis of liver biopsies revealed hepatic distribution (mRNA) of the inflammatory cytokine TNF-α with its receptors (18,20) and the anti-inflammatory adipocytokine adiponectin with its receptors (20). As suggested by animal models, the increased amount of fatty acids present in the case of NAFLD may mediate hepatic production of TNF-α, causing increased levels of systemic TNF-α (26). Upon HC damage activated liver-specific macrophages ‘Kupffer Cells’ will secrete more cytokines into the blood, among others TNF-α (18) and interleukin 6 (IL-6) (16,27,28). TNF-α and IL-6 are considered to induce hepatic production of the acute phase protein ‘C-reactive protein’ (CRP) (29).

Hepatic expression of TNF-α mRNA is significantly higher in patients with NAS compared with NAS (18,20), and hepatic expression of adiponectin mRNA is significantly lower in patients with NAS compared with NAS (20). Systemic TNF-α concentration is significantly elevated in both patients with NAS and NASH (19). Both hepatic IL-6 mRNA and systemic IL-6 concentration are elevated in patients with NAS and the highest in NASH (28). Elevated CRP is present in 25% of controls compared with 60% of NAFLD patients (P = 0.003) (30). Fasting adiponectin concentration predicts hepatic fat content (31,32) and is significantly lower in both patients with NAS and compared with controls (19,32) for both men and women (19). Fasting adiponectin is inversely correlated with hepatic fat content in healthy non-diabetic subjects (31,33) but non-significantly in patients with type 2 diabetes mellitus (n = 10) (31). TNF-α, IL-6 and CRP may contribute to the inflammatory CVD milieu, predisposing to atherosclerosis and CVD (34–36). On the contrary, studies in both humans and animals (23,37) have revealed both anti-inflammatory (23) and antithrombotic (37) properties of adiponectin, enabling a protective association between adiponectin and CVD (38).

In addition to a direct predisposition to atherosclerosis, cytokines may have an indirect effect as well, as cytokines may mediate insulin resistance (Fig. 1). Animal models (22,39) and both *in vitro* (40) and *in vivo* (41) human studies provided evidence that not fat accumulation itself but fat-derived cytokines play a role in (obesity-related) insulin resistance. The pleiotropic cytokine TNF-α interferes with the hepatic insulin receptor and the intrahepatocellular insulin signalling cascade (22,40), causing both hepatic and systemic insulin resistance (22,40). Additionally, administration of human recombinant TNF-α in human cancer patients resulted in increased levels of very low-density lipoprotein (VLDL) and TG and decreased levels of high-density lipoprotein (HDL) (41), which are features of diabetic dyslipidaemia (42).

**Hyperglycaemia and diabetic dyslipidaemia**

In the healthy situation, insulin stimulates hepatic and peripheral glucose uptake and suppresses hepatic glucose production. In patients with NAFLD, hepatic glucose uptake may be less effectively stimulated by insulin, contributing to elevated plasma glucose concentrations (40,43). Moreover, hepatic glucose production might be less effectively suppressed by insulin (40) but not in non-diabetic NAFLD patients (43,44).

Hepatic fat content correlates positively with fasting glucose (45), glucose levels after an oral glucose tolerance test (46), fasting C-peptide (45), fasting insulin (45) and insulin resistance by homeostatic model assessment (31). In the general population, the odds ratio of NAFLD compared with normal liver is 9.1 for hyperglycaemia (6). Additionally, the odds ratios of NAFLD, compared with normal liver, increase with increasing insulin quartile (4.2, 5.9 and 20.0 for the second, third and fourth quartile respectively) and insulin resistance by homeostatic model assessment quartile (2.3, 4.4 and 16.7 for the second, third and fourth quartile respectively) (6). Plasma glucose and its advanced glycation endproducts are considered atherogenic (47,48) and predispose to CVD (49).

In the healthy situation, insulin suppresses hepatic production and secretion of VLDL (50). In patients with NAFLD, VLDL secretion is less effectively suppressed by insulin, causing increased systemic VLDL-TG concentrations (31). Hepatic fat content correlates positively with VLDL1-TG and VLDL1-apolipoprotein-B secretion rates (31). Besides the presence of NAFLD, the altered hepatic lipid composition present in the case of NAFLD (51,52) might play a role in both altered VLDL secretion rates and altered lipoprotein composition. An *in vitro* study suggested that the presence of different types of fatty acids in the liver results in both different VLDL-apolipoprotein-B secretion rates and lipoprotein composition (53).
In patients with NAFLD, VLDL concentration is increased, VLDL particles are larger, small dense LDL particles predominate, whereas large HDL particle concentration is decreased (54). Hepatic fat content correlates inversely with fasting HDL cholesterol concentration (45). In the general population, the odds ratios of NAFLD compared with normal liver are 6.3 for low-HDL cholesterol concentration and 3.5 for hypertriglyceridaemia (6). Systemic lipids, i.e. TGs (48,55) and cholesterol (HDL excluded) (47,55), have been considered atherogenic and predispose to CVD (48,55).

Coagulation

Many coagulation factors are synthesized by HCs (56,57). The limited data on the association between obesity-independent markers of NAFLD, among others coagulation factors, has recently been reviewed by Kotronen and Yki-Jarvinen (45). In patients with NAFLD, the liver overproduces several factors (Fig. 1), of which plasminogen activator inhibitor-1 has direct atherogenic effects (47). However, many factors (fibrinogen, protein C and protein S) are increased in NAS but tend to be lower in NASH (58). This may suggest that initially increased factors will decrease while NAS progresses to advanced NASH. In advanced liver disease, bleeding problems are well known to occur (56,57).

Blood pressure

HCs produce angiotensinogen (59,60), a precursor of angiotensin II. Upon HC damage activated Hepatic Stellate Cells even synthesize and secrete mature angiotensin II (61). Angiotensin II is a major pro-atherogenic and vasoconstrictive peptide/neurotransmitter (47,61), considered to predispose to elevated blood pressure (59–61) and possibly CVD (62). Both systolic (only in females) and diastolic blood pressures correlate with hepatic fat content (45). In the general population, univariate odds ratios of NAFLD, compared with normal liver, are 2.0 for systolic hypertension and 1.7 for diastolic hypertension (6).

Prevention and treatment

As obesity (1), including childhood obesity (63), has been increasing in the general population, an earlier peak prevalence of NAFLD and an increased CVD risk profile may be expected in the future, delineating the need for prevention.

Once NAFLD is present, treatment seems advisable to improve CVD risk profile and hepatic risk. Prevention and treatment trials of NAFLD should focus on etiologic factors, notably systemic non-esterified fatty acid influx, components of the metabolic syndrome, cytokines and mitochondrial dysfunction (Fig. 1). Several studies have investigated the effect of lowering hepatic fat content on improving CVD risk profile markers; however, no longitudinal studies on lowering CVD events have been performed yet.

For an overview on diagnosis modalities for NAFLD, the reader is referred to recent review papers (45,64). As serum levels of transferases are of limited use (45,64,65), the intervention studies included in Table 1 have been limited to studies with a diagnosis by histology or imaging.

Dietary (plus exercise) and laparoscopic surgery interventions (3–15 months) revealed that overall loss of adipose tissue, determined by a decreased body mass index (BMI) (2.6–18.2 BMI points), promotes loss of hepatic fat content and results in an improved CVD risk profile (66–70). This suggests that weight loss should be pursued in patients with NAFLD.

Patients unable to lose weight or non-overweight patients might benefit from drug treatment. However, no medication is currently licensed for NAFLD treatment even though some have shown potential benefit. Additionally, as weight loss is associated with an improvement of NAFLD (66–70), statistical adjustment for the amount of weight change as potential confounder (Table 1) should be considered when determining true effects of pharmacological interventions. For an overview on potential treatment modalities, the reader is referred to recent review papers (45,64).

Some of the most often prescribed medicine are based on improving CVD risk profile (components of the metabolic syndrome) by acting on hepatic lipid metabolism, e.g. statins and fibrates (71,72) and by acting on hepatic glucose metabolism, e.g. metformin (73). Although often prescribed, studies on statins (74), fibrates (75) and metformin (76,77) in the case of NAFLD are few, often accompanied by weight loss and inconclusive. Studies on the angiotensin II receptor antagonist losartan revealed beneficial histological results (74,78).

As the possible antagonists TNF-α and adiponectin (17) may be involved in both the aetiology of NAFLD (16–24) and the worsening of CVD risk profile (23,34–38), TNF-α-lowering in NAFLD might be beneficial to improve both NAFLD and CVD risk profile, as suggested by studies on pentoxifylline (74,79).

Conclusion

The NAFLD is associated with an increased CVD risk profile (and hepatic risk). In order to improve CVD risk profile, prevention and treatment of NAFLD seem advisable. However, well-designed intervention studies, randomized clinical trials and long-term follow-up studies are scarce.

Conflict of Interest Statement

No conflict of interest was declared.
Table 1 Selection of intervention studies on NAFLD and cardiovascular disease risk markers, limited to studies with a diagnosis by histology or imaging

<table>
<thead>
<tr>
<th>Studied treatment modality</th>
<th>Patients</th>
<th>n</th>
<th>References</th>
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<tr>
<td><strong>Before–after studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate WL (2.6 BMI points) by diet</td>
<td>T2DM, obese*</td>
<td>8</td>
<td>1–3 months</td>
</tr>
<tr>
<td>Moderate WL (2.7 BMI points) by diet</td>
<td>HFC &gt; 5%, GDM</td>
<td>11</td>
<td>3–6 months</td>
</tr>
<tr>
<td>Moderate WL (2.7 BMI points) by diet</td>
<td>HFC &lt; 5%, GDM</td>
<td>12</td>
<td>3–6 months</td>
</tr>
<tr>
<td>Severe WL (10.4 BMI points) by diet</td>
<td>Morbidly obese*</td>
<td>41</td>
<td>9 months</td>
</tr>
<tr>
<td>Severe WL (17 BMI points) by laparoscopic surgery</td>
<td>NAFLD</td>
<td>70</td>
<td>15 months</td>
</tr>
<tr>
<td>Atorvastatin (10 mg)</td>
<td>NASH, ↑lipids</td>
<td>10</td>
<td>9 months</td>
</tr>
<tr>
<td>Metformin (maximum 2 g)</td>
<td>NASH, ↑ALT</td>
<td>17</td>
<td>12 months</td>
</tr>
<tr>
<td>Losartan (50 mg)</td>
<td>NASH, ↑hypertension</td>
<td>12</td>
<td>9 months</td>
</tr>
<tr>
<td>Pentoxifylline (2 x 400 mg)</td>
<td>NASH, ↑T2DM</td>
<td>13</td>
<td>9 months</td>
</tr>
<tr>
<td>Pentoxifylline (3 x 400 mg)</td>
<td>NASH, ↑ALT</td>
<td>9</td>
<td>12 months</td>
</tr>
<tr>
<td><strong>Non-randomized controlled trials</strong></td>
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<td></td>
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</tr>
<tr>
<td>Moderate WL (3 BMI points) by diet/exercise vs. no diet/exercise</td>
<td>NAFLD, AFLD*</td>
<td>25</td>
<td>3 months</td>
</tr>
<tr>
<td>Ursodeoxycholic acid (13–15 mg kg⁻¹) vs. clofibrate (2 x 1 g)</td>
<td>NASH, ↑Cholelithiasis</td>
<td>40</td>
<td>12 months</td>
</tr>
<tr>
<td>n-3 polyunsaturated fatty acids ethyl ester (1 g) vs. no n-3 polyunsaturated fatty acids ethyl ester</td>
<td>NAFLD*</td>
<td>56</td>
<td>6–12 months</td>
</tr>
<tr>
<td><strong>Randomized clinical trials</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Roziglitazone (2 x 4 mg) vs. metformin (2 x 1 g)</td>
<td>T2DM</td>
<td>20</td>
<td>4 months</td>
</tr>
</tbody>
</table>

*Ethanol intake unreported or higher than allowed for NAFLD.

**P ≤ 0.1.

↑, significant increase and/or significantly inferior; ↓, significant decrease and/or significantly superior; AFLD, alcoholic fatty liver disease; ALT, alanine aminotransferase; BMI, body mass index; GDM, gestational diabetes mellitus; HFC, hepatic fat content; n, amount of subjects who had both pre- and post-measurements; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; T2DM, type 2 diabetes mellitus; TGs, triglycerides; WL, weight loss.

Acknowledgement

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