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## Fatty liver disease

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*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2011

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Edens, M. A. (2011). *Fatty liver disease: pathophysiology & assessment*. s.n.

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**Chapter 3**

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

*Obes Rev* 2009; 10(4):412-419 \_ Review Paper

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**ABSTRACT**

**Background:** Recognition of the link between non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) has boosted research in this area.

**Main objective:** To review the literature on NAFLD in the context of CVD, focussing on underlying mechanisms and treatment.

**Results:** 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, 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.

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

## **INTRODUCTION**

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

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 (HC in figure I), 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 <sup>4</sup>. Non-alcoholic FLD (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 that is allowed for the diagnosis NAFLD varies greatly, but is maximally 20 g/day for women and 30 g/day for men. The prevalence of NAFLD in the general adult Western population is relatively high, i.e. 20% <sup>5,6</sup>, whereas the prevalence of total FLD, including both NAFLD and alcoholic FLD (AFLD), is approximately 30% <sup>5</sup>. In obese non-diabetic Western adults, the prevalence of NAFLD ranges from 80.4% to 97.9% <sup>7,8</sup>.

Although there is a hepatic risk for patients with NAFLD, notably cirrhosis <sup>9</sup> and hepatocarcinoma <sup>10</sup>, the CVD risk for patients with NAFLD may be higher <sup>10</sup>. Few studies have revealed evidence of an association between NAFLD and early CVD markers <sup>11</sup>, CVD events <sup>12, 13</sup> and CVD mortality <sup>10</sup>. Follow-up of patients with NAFLD showed a higher incidence of CVD compared to controls <sup>10, 13</sup>. A study by Hamaguchi et al. (2007) revealed that NAFLD was an independent predictor, even stronger than the metabolic syndrome, for first time CVD events <sup>13</sup>.

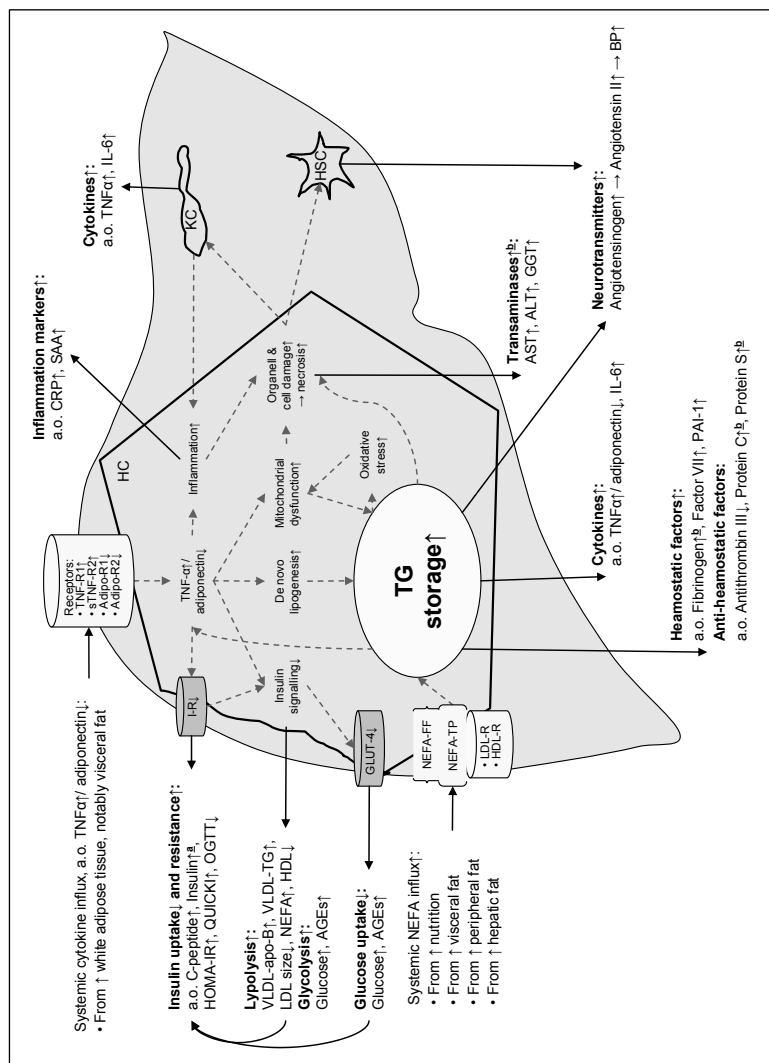
The recognition of the CVD risk of NAFLD has boosted research in this area during the recent years. We have reviewed the literature on NAFLD in the context of CVD risk profile markers. In this paper, after a short description of the etiology of NAFLD, we provide an overview of the cardiovascular risk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

## **ETIOLOGY**

The etiology of the hepatic lipid imbalance underlying the pathophysiology of NAFLD has been increasingly unravelled. Besides excessive non-esterified fatty acid (NEFA) influx, mediating factors may include: i] components of the metabolic syndrome<sup>14,15</sup>, ii] cytokines<sup>16-24</sup>, and iii] mitochondrial dysfunction<sup>25</sup> (figure I). A study on the incidence of NAFLD suggested that the metabolic syndrome precedes NAFLD<sup>14</sup>, with insulin resistance as a cornerstone<sup>15</sup>. Animal models, reviewed by Diehl et al. (2005)<sup>16</sup>, revealed involvement of the cytokine tumour necrosis factor alpha (TNF $\alpha$ ) and its possible antagonist adiponectin<sup>17</sup> in the pathogenesis of NAFLD. This is reinforced by cross-sectional studies showing changes in messenger ribonucleic acid (mRNA) of both TNF $\alpha$  receptors (increase) and adiponectin receptors (decrease) in NAFLD<sup>18-20</sup>. TNF $\alpha$ , recently reviewed by Ryden and Arner (2007), may have numerous mediating actions, amongst others increasing insulin resistance and inhibiting fatty acid oxidation<sup>22</sup>. Several animal studies on adiponectin, recently reviewed by Lafontan and Viguerie (2006), suggest opposite actions of adiponectin on energy metabolism, i.e. increasing insulin sensitivity and stimulation of fatty acid oxidation<sup>23</sup>. Additionally, adiponectin might contribute to inhibition of lipogenesis<sup>24</sup>. Polymorphisms in the gene encoding adiponectin receptor 1 are associated with the presence of high hepatic fat content (and insulin resistance)<sup>21</sup>. Mitochondrial dysfunction (in NASH), recently reviewed by Begriche et al. (2006), can be caused by oxidative stress amongst others, and may result in TG accumulation and eventually cell death, i.e. necrosis<sup>25</sup>.

## FATTY LIVER DERIVED CVD 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 (figure I).



**Figure I.** Model on the cardiovascular risk of non-alcoholic fatty liver disease

Signs,  $\uparrow$  = increase,  $\downarrow$  = decrease.

Abbreviations: <sup>a</sup> dependent on pancreatic  $\beta$ -cell function; <sup>b</sup> may decrease in advanced NAFLD; Adipo-R1, adiponectin receptor 1; Adipo-R2, adiponectin receptor 2; AGEs, advanced glycation endproducts; GLUT-4, glucose transporter 4; HC, Hepatocyte; HDL-R, high density lipoprotein receptor; HSC, Hepatic Stellate Cells; I-R, insulin receptor; KC, Kupfer Cell; 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.

## INFLAMMATION

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

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

In addition to a direct predisposition to atherosclerosis, cytokines may have an indirect effect as well, as cytokines may mediate insulin resistance (figure I). Animal models<sup>22, 39</sup> and both in vitro-<sup>40</sup> and in vivo<sup>41</sup> 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 $\alpha$  interferes with the hepatic insulin receptor and the intra-hepatocellular insulin signalling cascade<sup>22, 40</sup>, causing both hepatic and systemic insulin resistance<sup>22, 40</sup>. Additionally, administration of human recombinant TNF $\alpha$  in human

cancer patients, resulted in increased levels of very low density lipoprotein (VLDL) and TG, and decreased levels of high density lipoprotein (HDL) <sup>41</sup>, which are features of diabetic dyslipidaemia <sup>42</sup>.

#### **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 <sup>40, 43</sup>. Moreover, hepatic glucose production might be less effectively suppressed by insulin <sup>40</sup>, but not in non-diabetic NAFLD patients <sup>43,44</sup>.

Hepatic fat content correlates positively with fasting glucose <sup>45</sup>, glucose levels after an oral glucose tolerance test <sup>46</sup>, fasting C-peptide <sup>45</sup>, fasting insulin <sup>45</sup>, and insulin resistance by homeostatic model assessment (HOMA-<sub>IR</sub>) <sup>31</sup>. In the general population, the odds ratio of NAFLD compared to normal liver is 9.1 for hyperglycaemia <sup>6</sup>. Additionally, the odds ratios of NAFLD, compared to normal liver, increase with increasing insulin quartile (4.2, 5.9 and 20.0; for the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> quartile, respectively) and HOMA-<sub>IR</sub> quartile (2.3, 4.4 and 16.7; for the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> quartile, respectively) <sup>6</sup>. Plasma glucose and its advanced glycation endproducts (AGEs) are considered atherogenic <sup>47,48</sup> and predispose to CVD <sup>49</sup>.

In the healthy situation, insulin suppresses hepatic production and secretion of VLDL <sup>50</sup>. In patients with NAFLD, VLDL secretion is less effectively suppressed by insulin, causing increased systemic VLDL-TG concentrations <sup>31</sup>. Hepatic fat content correlates positively with VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apolipoprotein-B secretion rates <sup>31</sup>. Besides the presence of NAFLD, the altered hepatic lipid composition present in the case of NAFLD <sup>51, 52</sup> might play a role in both altered VLDL secretion rates and altered lipoprotein composition as well. 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 <sup>53</sup>.

In patients with NAFLD, VLDL concentration is increased, VLDL particles are larger, small dense LDL particles predominate, whereas large HDL particle concentration is decreased <sup>54</sup>. Hepatic fat content correlates inversely with fasting HDL cholesterol concentration <sup>45</sup>. In the general population, the odds ratios of NAFLD compared to normal liver are 6.3 for low HDL cholesterol concentration and 3.5 for hypertriglyceridaemia <sup>6</sup>.



Systemic lipids, i.e. TGs<sup>48, 55</sup> and cholesterol (HDL excluded)<sup>47, 55</sup>, have been considered atherogenic and predispose to CVD<sup>48, 55</sup>.

#### **COAGULATION**

Many coagulation factors are synthesised by hepatocytes<sup>56, 57</sup>. The limited data on the association between obesity-independent markers of NAFLD, amongst others coagulation factors, has recently been reviewed by Kotronen and Yki-Jarvinen (2008)<sup>45</sup>. In patients with NAFLD, the liver overproduces several factors (figure I), of which plasminogen activator inhibitor 1 (PAI-1) has direct atherogenic effects<sup>47</sup>. However, many factors (fibrinogen, protein C and protein S) are increased in NAS, but tend to be lower in NASH<sup>58</sup>. 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<sup>56, 57</sup>.

#### **BLOOD PRESSURE**

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

## **PREVENTION AND TREATMENT**

As obesity<sup>1</sup>, including childhood obesity<sup>63</sup>, 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 NEFA influx, components of the metabolic syndrome, cytokines and mitochondrial dysfunction (figure I). 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<sup>45, 64</sup>. As serum levels of transferases are of limited use<sup>45, 64, 65</sup>, the intervention studies included in table I, 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 BMI (2.6 – 18.2 BMI-points), promotes loss of hepatic fat content and results in an improved CVD risk profile<sup>66-70</sup>. 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<sup>66-70</sup>, statistical adjustment for the amount of weight change as potential confounder (table I) 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<sup>45, 64</sup>.

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<sup>71, 72</sup>, and by acting on hepatic glucose metabolism, e.g. metformin<sup>73</sup>. Although often prescribed, studies on statins<sup>74</sup>, fibrates<sup>75</sup> and metformin<sup>76, 77</sup> 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<sup>74, 78</sup>.

As the possible antagonists TNF $\alpha$  and adiponectin<sup>17</sup> may be involved in both the etiology of NAFLD<sup>16-24</sup> and worsening of CVD risk profile<sup>23, 34-38</sup>, TNF $\alpha$ -lowering in NAFLD might be beneficial to improve both NAFLD and CVD risk profile, as suggested by studies on pentoxifylline<sup>74, 79</sup>.

### **CONCLUSION**

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

**Table I.** Selection of intervention studies on NAFLD and CVD risk markers, limited to studies with a diagnosis by histology or imaging

Studied treatment modality	Patients	n	Length	HFC	Tot	BMI	Ref
BEFORE-AFTER STUDIES							
Moderate WL (2.6 BMI-points) by diet	T2DM, obese *	8	1-3 m	↓	na	↓	66
Moderate WL (≈2.7 BMI-points) by diet	HFC>5%, GDM	11	3-6 m	↓	na	↓	67
Severe WL (10.4 BMI-points) by diet	HFC<5%, GDM	12	9 m	↓	-	-	69
Severe WL (17 BMI-points) by L-surgery	Morbidly obese *	41	15 m	↓	↓	↓	70
Atorvastatin (10 mg)	NAFLD	70	9 m	↓	=	=	74
Metformin (maximum 2 g)	NASH, ↑lipids	10	12 m	↓	↓	↓	76
Losartan (50 mg)	NASH, ↑ALT	17	9 m	↓	↓	=	74
Pentoxifylline (2*400 mg)	NASH, hypertension	12	9 m	↓	↓	=	74
Pentoxifylline (3*400 mg)	NASH, T2DM	13	12 m	↓	↓	=#	79
NON-RANDOMISED CONTROLLED TRIALS							
Moderate WL (3 BMI-points) by diet/ exc. v/s. no diet/ exc.	NAFLD, AFLD *	25	3 m	↓	-	↓	68
UDCA (13-15 mg/kg) v/s. clofibrate (2*1 g)	NASH, Cholelithiasis	40	12 m	↓	-	=	75
n-3 PUFA ethyl ester (1 g) v/s. no n-3 PUFA ethyl ester	NASH, ↑TG	56	6-12 m	↓	na	=	80
RANDOMISED CLINICAL TRIALS							
Roziglitazone (2*4 mg) v/s. metformin (2*1 g)	NAFLD *	20	4 m	↓	na	↑	77

Signs: ↑ = significant increase and/ or significantly inferior, ↓ = significant decrease and/ or significantly superior, ‘=’ = no change, - = missing value, \* = ethanol intake unreported or higher than allowed for NAFLD, # = p ≤ 0.10.

Abbreviations: exc. = exercise, GDM = gestational diabetes mellitus, HFC = hepatic fat content, L-surgery = laparoscopic surgery, m = months, n = amount of subjects who had both pre and post measurements, na = not applicable, PUFA = polyunsaturated fatty acids, Ref = reference, Tot. = total NASH score/ activity index, T2DM = type 2 diabetes mellitus, UDCA = ursodeoxycholic acid, v/s. = versus, WL = weight loss.

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