

# Lipids in the Wrong Place: Visceral Fat and Nonalcoholic Steatohepatitis

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There is substantial evidence in both animal (1,2) and human models of lipodystrophy (3) that the accumulation of excessive fat in ectopic sites that normally lack adipose tissue, such as the skeletal muscle and the liver, may trigger whole-body insulin resistance. This article will review the evidence showing the condition of fatty liver as an independent and perhaps better indicator of disease than excessive accumulation of visceral adipose tissue.

On the basis of the hypothesis that a fatty liver may increase the risk of cardiovascular disease (CVD), this article will not only focus on the metabolic-related features, but also on the cardiovascular risk factors affecting individuals with fatty liver. The biomarkers of CVD will therefore be analyzed in patients with fatty livers. Finally, epidemiological evidence linking fatty liver to the development of type 2 diabetes and CVD will be given.

## PHENOTYPIC FEATURES OF FATTY LIVERS

### Metabolic features of individuals with insulin impairment: what contributes more, fatty liver or visceral adipose tissue?

Nonalcoholic fatty liver disease (NAFLD) is often found together with insulin resistance syndrome (4,5) and is commonly found in those suffering from visceral adiposity (3,4). Multiple insulin-related metabolic abnormalities in both organs

and tissues have been reported, including impaired insulin-mediated inhibition of hepatic glucose production in livers (4–8), impaired insulin-stimulated glucose metabolism in skeletal muscle tissue (4–9), and impaired insulin-dependent control of lipolysis in adipose tissue (4–10). These metabolic abnormalities are often present along with the increased visceral adipose tissue and a fatty liver. It is very difficult to determine the biological relevance of each anthropometric feature because they are so commonly found together in humans. Recent studies have shown that of these two indicators, i.e., intrahepatic fat content (IHF) and excess visceral adipose tissue, IHF is statistically a more relevant indicator than excess visceral adipose tissue. In insulin-treated type 2 diabetic patients, the IHF content was more closely correlated with the insulin dose and the sensitivity of endogenous (hepatic) glucose production to insulin and better explained the interindividual variation in insulin requirements (11). In addition, when the relationship between peripheral glucose metabolism and fatty liver was explored in healthy nondiabetic humans, the correlation between the IHF content and peripheral insulin resistance was stronger than the correlation with intramyocellular lipid content, visceral fat content, or subcutaneous fat content (12). Stefan et al. (13) recently reported that in the model of the metabolically fit but obese individuals, preserved insulin

sensitivity was more strongly associated with lower IHF content than with other parameters of body adiposity, including intramyocellular lipid content. The authors concluded that ectopic fat in the liver may be more important than visceral fat in the determination of such a beneficial phenotype in obesity. Similar conclusions were also reported in individuals with overt type 2 diabetes (7). In our studies, we also observed that in obese adolescents with fatty liver there was a greater severity of whole-body insulin resistance compared with that of BMI-matched, insulin-resistant, obese adolescents with normal IHF content (14).

### Cardiovascular features of individuals with fatty livers

Several alterations that are linked with the pathogenesis of atherosclerosis were reported in patients with NAFLD, including endothelial dysfunction, alterations of the coronary and carotid arteries, abnormal cardiac energy metabolism, and low-grade inflammation. The arterial endothelium is a target for the atherosclerotic process in the very early stages of the disease. This endothelium response was experimentally modulated in a noninvasive fashion in patients with NAFLD/nonalcoholic steatohepatitis (NASH). Villanova et al. (15) assessed the endothelial response to nitric oxide release induced by the shear stress generated by artery occlusion by means of quantifying the flow-mediated vasodilation of the brachial artery. In NAFLD, the flow-mediated vasodilation was lower than in controls. More interestingly, because the diagnosis of NAFLD was biopsy-based, the defect was more pronounced in those with NASH than in those with just fatty livers. The defect was linked to the endothelium because no differences were observed in flow-independent vasodilation (which is the response to sublingual nitroglycerin, modulating its effect at the level of the arterial smooth muscle). The morphology of coronary vessels was reported in a few studies in patients with NAFLD. Lautamäki et al. (16) reported on data obtained from 55 patients with type 2

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diabetes and coronary artery disease, with and without fatty livers, who underwent classical coronary angiography. The median of the degree of the main stenotic lesion was the same among those with or without fatty livers. The authors also measured myocardial perfusion using positron emission tomography techniques in hyperinsulinemia conditions and demonstrated that those with fatty livers were characterized by a reduced coronary flow reserve. These data support the possible presence of a microvascular dysfunction in these patients even with the lack of major macrovascular alterations.

In the same study, the authors also assessed cardiac insulin sensitivity with respect to glucose metabolism by measuring insulin-stimulated myocardial glucose uptake (with 2-deoxy-2- $^{18}\text{F}$ fluoro-D-glucose) and found that patients with fatty livers had a lower insulin-stimulated myocardial glucose extraction rate compared with those without fatty livers. Using multiple regression analysis, liver fat content was found to be the most significant explanatory variable for myocardial insulin resistance. Patients with both insulin resistance to glucose and fatty livers were also found to have hearts with impaired energy metabolism. The assessment of cardiac energy metabolism was performed noninvasively by means of  $^{31}\text{P}$ -magnetic resonance spectroscopy in young men with newly diagnosed fatty liver. The surrogate marker of cardiac energy metabolism (the phosphocreatine-to-ATP ratio) in these subjects was significantly lower than in a control group of subjects without fatty liver (17). In obese subjects, the homeostatic model assessment (HOMA2-S, a surrogate marker of whole body insulin sensitivity) was the most relevant predictive factor of the phosphocreatine-to-ATP ratio (18). However, this assessment was not detected in patients with fatty livers who also had higher amounts of fat in the epicardial area (17).

An additional feature of insulin resistance is its association with systemic low-grade inflammation. This inflammation has been linked in obese people to macrophage infiltration of adipose tissue. This triggers the activation of inflammatory pathways (19). Liver inflammation can also lead to insulin resistance by promoting the release of cytokines (20). In fact, liver biopsies from 24 subjects who had varying amounts of histologically determined fat (from normal to steatosis) due to NAFLD had mRNA expression

of inflammatory genes such as the monocyte-attracting chemokine *CCL2* (monocyte chemoattractant protein-1), which were over-expressed proportionally to the amount of the hepatic fat content (21). Interestingly, we recently reported an association between CVD mortality and the circulating levels of this chemokine (22). Chronic inflammation of the liver secondary to triglyceride infiltration could increase the production of factors that cause systemic insulin resistance.

### PROGNOSIS OF FATTY LIVER—

All the above described metabolic and cardiovascular features may constitute the typical risk factors for type 2 diabetes and CVD. This is highlighted by the analysis of data generated from a large European population (23). Because the prevalence of NAFLD is rather high (24), there is general concern about the possibility that NAFLD may have a deleterious prognostic impact on these subjects due to the risk of diabetes and CVD rather than to the hepatic outcome.

### Risk of developing type 2 diabetes

It is therefore not surprising that NAFLD may be associated with an increased risk for developing type 2 diabetes. Most of the epidemiological data are based on the use of the surrogate markers of NAFLD such as the liver enzymes, and in particular, alanine aminotransferase (ALT) and  $\gamma$ -glutamyltransferase ( $\gamma\text{GT}$ ). Although these “surrogate measures” of liver fat are far from perfect, there are increasing amounts of epidemiological reports suggesting that NAFLD is associated with an increase in type 2 diabetes incidence. In particular, sustained and nontransient ALT elevations were found to be associated with type 2 diabetes (25). A study was conducted on middle-aged male Japanese workers. The average age of the participants at the onset of the study was 48 years old. Those with established impaired glucose tolerance were excluded from the study based on the performance of oral glucose tolerance test (26). Ultrasound assessments of the livers of the remaining 3,189 workers were taken. The participants were then divided into fatty liver and nonfatty liver groups based on the ultrasounds. The researchers followed both groups for 4 years, checking for the development of diabetes. The age and BMI-adjusted incidence of diabetes was 5.5 (95% CI 3.6–8.5,  $P < 0.001$ ) in the fatty liver group and 4.6 (3.0–6.9,  $P < 0.001$ ) in

the nonfatty liver group. On the basis of these studies, we can conclude that NAFLD is likely to significantly increase the risk of developing diabetes.

### Risk of developing CVDs

Data regarding CVD risks are weak. A nested case-control study was conducted on 137 CVD deaths and 249 control subjects (frequency-matched on age, sex, and examination year; age range 26–85 years) with a 5- to 12-year follow-up. The results suggested that serum  $\gamma\text{GT}$  within its normal range could predict CVD mortality in those aged  $\geq 70$  years but may have limited usefulness for risk assessment in older adults (27). The British Regional Heart Study, a prospective study of 6,997 men aged 40–59 years with no history of CVD (coronary heart disease or stroke) or diabetes drawn from general practices in 24 British towns and followed for up to 24 years, confirmed the same hypothesis—namely that elevated  $\gamma\text{GT}$  was associated with a significant increased risk of stroke, fatal coronary heart disease events, and CVD mortality independent of established CVD risk factors (Framingham score). The authors suggested that  $\gamma\text{GT}$  may be useful as an additional marker for long-term CVD risk (28). ALT levels were also found to be associated with CVD in the Hoorn Study (29). Here, the predictive value of ALT appeared to be independent of traditional risk factors and metabolic syndrome features in a population-based cohort. Unfortunately, no data were found about incident events and quantitative assessment of IHF. In the Diabetes Heart Study, 623 randomly selected participants were evaluated for hepatic steatosis defined as a liver:spleen attenuation ratio of  $> 1.0$  by computed tomography. The study quantified visceral fat, subcutaneous fat; coronary, aortic, and carotid artery calcium by computed tomography; and carotid atherosclerosis by ultrasound. The study found no significant associations between the liver:spleen attenuation ratio and coronary, aortic, or carotid calcium, or carotid intima-media thickness (30). In the Dijon Study, the liver fat of 101 patients with type 2 diabetes was measured using ultrasound.  $^1\text{H}$ -magnetic resonance spectroscopy and carotid intima-media thickness values were calculated. The authors found no significant difference between patients with and without hepatic steatosis for intima-media thickness values (31). It is important to emphasize that this result was in contrast with a previous report by

Targher et al. (32) in a very similar population in which NAFLD was established based on liver biopsies.

There are also studies reporting a higher incidence of major cardiovascular outcomes (33–36) such as nonfatal CVD events (33), death from CVD (34–36), revascularization procedures (35), and all-cause mortality (36) in people with NAFLD. These data were obtained in community-based cohorts (33,36) or nested case-control studies (34,35), in the general population (33,36) or in patients with type 2 diabetes (34,35) in which NAFLD was established using abdominal ultrasonography (33,36), liver biopsy (34,35), and  $\gamma$ GT levels (36).

It can therefore be concluded that there is a growing body of evidence demonstrating an association between CVD and NAFLD. These data may support the belief that CVD may also be a relevant if not the leading cause of death in patients with NAFLD. Additional research is required to draw a definitive conclusion, especially when the accurate segregation of patients with NAFLD into those with and without NASH could be applied to larger studies. Further studies are also needed in order to generate evidence-based recommendations for the treatment of NAFLD and prevention of CVD in patients with NAFLD, as recently suggested by Targher et al. (37).

### Risk of developing cirrhosis

NAFLD/NASH may also represent a significant risk factor for hepatic diseases. In general terms, patients diagnosed with NAFLD have a modest increased risk of death compared with the general population (data generated in Olmsted County, Minnesota, between 1980 and 2000 using the resources of the Rochester Epidemiology Project). The modest increase is associated with older age, impaired fasting glucose, and cirrhosis. Approximately 1 in 30 patients may develop cirrhosis or a liver-related complication. Even if the hepatological risk is low, liver-related death is a leading cause of mortality (38).

An important aspect is related to the different risk of progression to cirrhosis depending on the presence of a histopathological finding of pure steatosis and/or NASH. Patients with pure steatosis on liver biopsy probably have the best prognosis within the spectrum of NAFLD, whereas those with steatohepatitis (or fibrosis) have a worse prognosis (39). There are data suggesting that progression to liver

fibrosis may occur only in patients with necrosis and inflammatory infiltration on liver biopsy (40).

It is also important to emphasize that the coexistence of steatosis with other liver diseases, such as hepatitis C virus infection (41), and more interestingly with type 2 diabetes (39), could increase the risk of progression of the liver disease. In fact, in patients with type 2 diabetes, liver-related death is of even greater proportion than the overall causes of mortality (42).

**CONCLUSIONS**—On the basis of the above-discussed findings in the literature, it is possible to conclude that fatty liver is the hepatic component of the metabolic syndrome and is probably a stronger predictor than visceral adipose tissue of abnormal metabolism in insulin-resistant states. An association between a variant of the apolipoprotein C3 gene in individuals with NAFLD of Asian ethnicity (confirmed in a non Asian-Indian population) and insulin resistance and atherogenic dyslipidemia has been recently found (43). An interesting finding related to this gene variant was that one of the more frequent single nucleotide polymorphisms (SNPs) in the studied population was the one that was associated with an increased risk of NAFLD. The importance of the genetic background was more evident in another report where the discovered SNPs associated with NAFLD were not associated with insulin resistance or other classical metabolic symptoms (atherogenic dyslipidemia). These SNPs induced susceptibility to NAFLD alone (44). It is also important to emphasize that the individual's genetic predisposition may also affect excessive triglyceride deposits within the liver and may also be the cause of direct liver damage in the absence of fatty liver. Finally, fatty liver is associated with endothelial dysfunction and with increased expression of mediators of low-grade inflammation. Fatty liver was also reported in association with macrovascular damage and even if its association with diabetes and CVD is rather robust, additional research is required to draw a definitive conclusion.

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