Fatty Liver
A Novel Component of the Metabolic Syndrome

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Abstract—Although the epidemic of obesity has been accompanied by an increase in the prevalence of the metabolic syndrome, not all obese develop the syndrome and even lean individuals can be insulin resistant. Both lean and obese insulin resistant individuals have an excess of fat in the liver which is not attributable to alcohol or other known causes of liver disease, a condition defined as nonalcoholic fatty liver disease (NAFLD) by gastroenterologists. The fatty liver is insulin resistant. Liver fat is highly significantly and linearly correlated with all components of the metabolic syndrome independent of obesity. Overproduction of glucose, VLDL, CRP, and coagulation factors by the fatty liver could contribute to the excess risk of cardiovascular disease associated with the metabolic syndrome and NAFLD. Both of the latter conditions also increase the risk of type 2 diabetes and advanced liver disease. The reason why some deposit fat in the liver whereas others do not is poorly understood. Individuals with a fatty liver are more likely to have excess intraabdominal fat and inflammatory changes in adipose tissue. Intervention studies have shown that liver fat can be decreased by weight loss, PPARγ agonists, and insulin therapy. (Arterioscler Thromb Vasc Biol. 2008;28:27-38.)

Key Words: liver fat ■ metabolic syndrome ■ nonalcoholic liver disease

Epidemiological studies have documented that particularly those obese subjects who are abdominally obese and have other features associated with insulin resistance, such as hypertriglyceridemia, hypertension or hyperglycemia, are at increased risk of cardiovascular disease. This is perhaps the main reason why the original description of an insulin resistance syndrome as well as the current definition of the metabolic syndrome (vide infra) does not include obesity. Although the prevalence of the components of the metabolic syndrome is increased in obesity, not all obese subjects develop the syndrome, and even lean individuals can be insulin resistant. Extreme examples of the latter include most if not all forms of human lipoatrophies. The reason why some individuals are insulin resistant whereas other equally obese are not has long been unresolved.

The liver, once fatty, is insulin resistant and overproduces both glucose and VLDL, leading to hyperglycemia, hypertriglyceridemia, and a low HDL cholesterol concentration. The ensuing discussion is focused on reviewing the possibility that fat accumulation in the liver distinguishes between those who do and do not develop the metabolic syndrome.

Definitions and Diagnostic Methods

The Metabolic Syndrome
The current definition of the metabolic syndrome by the International Diabetes Federation (IDF) includes increased waist circumference and at least 2 of the following: increased fasting serum glucose, triglycerides, the metabolic syndrome...
or blood pressure, or a low HDL cholesterol concentration. This cluster predicts an increased risk of both CVD and type 2 diabetes. For example, in the San Antonio Heart Study (n=2559, follow-up 7.4 years), the risk of CVD was 1.7-fold and that of type 2 diabetes 5.8-fold increased in subjects with as compared with those without the metabolic syndrome, independent of age, gender, ethnic origin, history of CVD and type 2 diabetes, non-HDL cholesterol, smoking, and family history of CHD. Previous metabolic syndrome definitions by the National Cholesterol Education Program (NCEP) - Adult Treatment Panel III (ATPIII) and the World Health Organization (WHO) imparted similar risk of CVD and diabetes in the latter study.

Definition of NAFLD
NAFLD covers a spectrum of liver disease from steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. According to the American Association for the Study of Liver Diseases, NAFLD is defined as fat accumulation in the liver exceeding 5% to 10% by weight, as determined from the percentage of fat-laden hepatocytes by light microscopy. Steatosis attributable to NAFLD is typically macrovesicular rather than microvesicular. Alcohol consumption should not exceed 14 U/wk (20 g/d), and viral (hepatitis B and C), toxic, autoimmune (clearly elevated antinuclear and anti-smooth muscle antibodies), and other causes of steatosis (Wilson disease, hypobetalipoproteinemia) should be excluded.

Quantification of Steatosis
Liver fat content can be most reliably and noninvasively quantitated using proton magnetic resonance spectroscopy ($^1$H-MRS), but this method gives no information of whether steatosis is micro- or macrovesicular, or of inflammation or fibrosis. These features can only be determined by liver biopsy, but this method for diagnosing NAFLD cannot be routinely used because of ethical limitations. Although a liver biopsy is considered the “gold standard” for quantification of liver fat, it is limited by small sample size compared with $^1$H-MRS where routinely 8 to 27 cm$^3$ volumes of the liver are sampled. Other imaging modalities include fast MRI, computed tomography, and ultrasound, but these methods are not quantitative and their sensitivity is limited in adults as well as in children.

Serum ALT correlates with liver fat (Figure 1), even independent of obesity, but when studied over a wide range of liver fat measured with $^1$H-MRS, serum ALT only explains 17% to 19% of its variation. In the latter study, S-ALT was normal in 69% of those who had increased liver fat. Similarly, in the Dallas Heart Study, 79% of the subjects with a fatty liver (liver fat content >5.6%) had normal serum ALT. This implies that a normal S-ALT does not exclude steatosis. Serum AST and $\gamma$GT also correlate with liver fat content independent of obesity, but are even less sensitive than serum ALT. Serum ALT has to be measured in fresh samples, because the activity decreases by freezing and thawing.

Why Is Liver Fat Related to Components of the Metabolic Syndrome?
As shown in Figure 2, all components of the metabolic syndrome correlate with liver fat content, as determined by $^1$H-MRS. Although the prevalence of steatosis increases as
A function of obesity, these relationships remain significant even when adjusted for BMI.

Of other parameters closely related to the metabolic syndrome, although not included in its present criteria, fasting serum insulin correlates closely with liver fat content (Figure 1) independent of age, gender and BMI. Because C-peptide is similarly closely correlated with liver fat (Figure 1), the association between liver fat and fasting serum insulin is not only attributable to diminished hepatic insulin clearance. The fatty liver is resistant to the action of insulin to suppress hepatic glucose production, which results in hyperglycemia and hyperinsulinemia. Thus, both hepatic insulin resistance and impaired insulin clearance are likely to contribute to fasting hyperinsulinemia. In humans, exogenous insulin therapy (70 IU/d for 7 months) decreases liver fat significantly, suggesting that hyperinsulinemia may be a consequence rather than cause of liver fat. However, during insulin therapy, other changes, such as decreases in glucose and free fatty acids, could decrease liver fat. Thus, these data do not rule out the possibility that hyperinsulinemia per se could promote liver fat accumulation. In vitro, hyperinsulinemia per se activates the lipogenic transcription factor SREBP-1c, which is overexpressed at both mRNA and protein levels in the fatty livers of animal models.

**Waist**

As reviewed elsewhere, waist circumference is a better predictor of the risk of CVD than obesity. Waist circumference is associated with NAFLD as determined using computed tomography, liver biopsy, or ultrasound techniques. This association has remained significant in some but not all studies after adjusting for BMI or BMI and other risk factors. In a study of 271 nondiabetic subjects in whom liver fat was measured by proton spectroscopy, waist circumference correlated more closely with liver fat than BMI.

**Figure 2.** Relationships between liver fat (log scale) and components of the metabolic syndrome: (a) waist circumference \([r=0.59, P<0.0001 \text{ for women}; r=0.56, P<0.0001 \text{ for men}]\), (b) fasting plasma glucose \([r=0.32, P<0.0002 \text{ for women}; r=0.22, P=0.026 \text{ for men}]\), (c) serum triglycerides \([r=0.40, P<0.0001 \text{ for women}; r=0.44, P<0.0001 \text{ for men}; r=0.42, P<0.0001 \text{ for both women and men}]\), (d) HDL cholesterol \([r=-0.44, P<0.0001 \text{ for women}; r=-0.31, P=0.002 \text{ for men}]\), (e) systolic blood pressure \([r=0.30, P=0.0004 \text{ for women}; r=0.14, \text{NS for men}]\) and (f) diastolic blood pressure \([r=0.42, P<0.0001 \text{ for women}; r=0.31, P=0.0006 \text{ for men}]\). Symbols as in Figure 1. Reproduced with permission from Kotronen et al.
copy, the correlations between liver fat and BMI, waist circumference, and intraabdominal fat were 0.47, 0.58, and 0.65, respectively (P<0.0001 for all).25 In another study involving 83 type 2 diabetic patients, the correlation between visceral adipose tissue mass and liver fat was 0.57.45 These correlation coefficients suggest that only 30% to 40% of the variation in liver fat can be explained by variation in the size of the intraabdominal depot and variation in the methodology used, and that factors other than intraabdominal fat may regulate liver fat. The possible mechanisms linking intraabdominal and liver fat are discussed below.

Glucose
After an overnight fast, glucose utilization is largely non-insulin-dependent.46 Therefore, the ability of insulin to inhibit glucose production can be predicted to be a key regulator of fasting glyceremia. Once the liver is fatty, the ability of insulin to inhibit hepatic glucose production is impaired, which leads to an increase in the fasting plasma glucose concentration.7,8 This in turn stimulates insulin secretion resulting in mild hyperinsulinemia and lowering of glucose to near-normal levels.8

Hypertriglyceridemia and Low HDL Cholesterol
Insulin normally inhibits the production of VLDL from the liver.47 Once fatty, this action of insulin is impaired whereas VLDL clearance remains unchanged.9 The overproduction of VLDL results in hypertriglyceridemia and a lowering of HDL cholesterol.48

Blood Pressure
Elevated blood pressure is included in the present definition of the metabolic syndrome, but the mechanisms underlying this association are unclear. Possibilities include increased activation of basal sympathetic tone49,50 and renal sodium reabsorption51 by hyperinsulinemia. The latter implies normal sensitivity of insulin activation of sympathetic nervous activity and sodium reabsorption despite resistance to glucose-regulatory effects of insulin.

Other Markers of NAFLD
As summarized in Table 1, several markers of NAFLD, which are obesity-independent, have been identified. Some, such as lipids,52 coagulation factors,53 and CRP54 originate from the liver, whereas others, such as adiponectin, originate exclusively from adipose tissue.55 The relationships between those markers and liver fat are in general not better than those depicted in Figures 1 and 2. There is a need to identify hepatospecific and sensitive markers among the multitude of proteins produced in the liver.

NASH: Definition and Diagnosis
Necessary components for the diagnosis of NASH include, in addition to macrovesicular steatosis, lobular inflammation and hepatocellular ballooning.14 NASH increases the risk of death particularly from CVD,56 but also from liver-related causes56,57 compared with simple steatosis. It is therefore important to identify individuals with NASH from those with simple steatosis. NASH can, however, only be reliably diagnosed using a liver biopsy.57 Only a few obesity-independent circulating markers of NASH have been reported in cross-sectional studies (Table 1). Noninvasive methods to assess progress of fibrosis in NASH are beyond the scope of this review (see Guha et al58 for review).

A combination of several markers may be more useful in the diagnosis of NASH.59,60 In a recent cross-sectional analysis of 733 patients with NAFLD, use of a NAFLD fibrosis score predicted fibrosis correctly in 90% of the patients.69 By applying this score, which included age, hyperglycemia, body mass index, platelet count, albumin, and the AST/ALT ratio, a liver biopsy could be avoided in 75% of the patients.59

Prevalence and Prognosis of NAFLD

Steatosis
NAFLD appears to be the most common cause of elevated liver enzymes. In the NHANES III population-based survey carried out between 1988 and 1994 in the US of 15 676 adults, NAFLD accounted for 70% of elevated liver enzymes.61 The prevalence of a fatty liver of any cause, measured by 1H-MRS in 2349 participants (age 18 to 65) of the Dallas Heart Study, was 33.6%.20 Liver fat greater than 5.6% was considered abnormal, as it corresponded to the 95th percentile of the distribution of liver fat in the subset of subjects (n=345) with normal serum ALT and fasting glucose concentrations, and low alcohol consumption. On the other hand, in the NHANES III, where excess liver was defined based on an abnormal ALT, the prevalence of NAFLD was 5.4%,61 which is much lower than in the Dallas Heart Study.20 The insensitivity of ALT and collection of NHANES data some 15 years before the Dallas Heart Study could contribute to the low prevalence of NAFLD in NHANES III. In an autopsy study of 742 children and adolescents (age 2 to 19 years), the prevalence of fatty liver was 38% in obese and 5% in normal-weight children.62 Liver fat may also depend on ethnicity. Asian-Indian men appear to be more insulin-resistant and have a 2-fold higher liver fat content measured by 1H-MRS than equally obese White men.63 The latter study did not include measurements of fat distribution. Taken together, the prevalence of NAFLD seems to be as common as the metabolic syndrome, which affects approximately one fourth of adults.64

NASH
The true population prevalence of NASH is unknown because a liver biopsy would be required to establish this diagnosis. Of patients referred to the gastroenterologist because of persistent increases in liver enzymes attributable to nonalcoholic causes, NASH has been reported in 50% to 55%.56,65 In 551 severely obese patients (mean BMI 47±9 kg/m2) undergoing antiobesity surgery, liver biopsies showed 86% to have steatosis, 24% mild inflammation or NASH, and 2% cirrhosis.66

Liver Fat Predicts the Metabolic Syndrome, Type 2 Diabetes, CVD, and Liver Disease

The Metabolic Syndrome and Type 2 Diabetes
In a Japanese study including 4401 employees without liver disease or drug treatment (mean age 48 years, BMI 23 kg/m2),
the odds ratios of men and women with NAFLD to develop the metabolic syndrome (ATP III criteria) during the follow-up were 4.0 and 11.2 after adjustment for age, alcohol intake, and changes in body weight67 (Figure 3). Similar data have been reported by others.68,69

The role of steatosis, estimated by using liver enzymes, in the prediction of type 2 diabetes has been examined in 12 prospective studies. As detailed below, in all except 3 of these studies, elevated liver enzymes predicted type 2 diabetes independent of obesity. In a Swedish study of 54-year-old men, serum ALT concentrations predicted a 3.9-fold increase in the risk of type 2 diabetes, which remained significant after adjusting for BMI.70 In Pima Indians, a high serum ALT, but not AST or γGT, predicted a 2.3-fold risk in the progression from normal glucose tolerance to diabetes.71 This association between ALT and incidence of diabetes persisted after adjustment for the percentage of body fat.71 In 906 nondiabetic subjects participating the Insulin Resistance Atherosclerosis Study, both serum AST and ALT increased the risk of future type 2 diabetes independent of BMI.72 In the British Regional Heart Study, in which 7458 nondiabetic men (age 40 to 59 years) were followed for an average 12.8 years, increased serum γGT predicted type 2 diabetes independent of BMI.73 Other studies documenting that increased serum

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**Table 1. Obesity-Independent Circulating Markers of NAFLD and NASH**

<table>
<thead>
<tr>
<th>Markers of NAFLD</th>
<th>NAFLD/Controls*</th>
<th>Subjects' Characteristics**</th>
<th>Liver Fat Methodology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP ↑, HOMA-IR ↑</td>
<td>16/160</td>
<td>Age: 45 years BMI: 26 kg/m²</td>
<td>Histology</td>
<td>139</td>
</tr>
<tr>
<td>hs-CRP ↑, fibrinogen ↑, v-WF ↑, PAI-1 activity ↑</td>
<td>35/65</td>
<td>Age: 41/43 years BMI: 26/24 kg/m²</td>
<td>Ultrasound and CT</td>
<td>42</td>
</tr>
<tr>
<td>Factor VII clotting activity ↑, PAI-1 activity and antigen ↑, t-PA activity ↓</td>
<td>31/33</td>
<td>Age: 38 years BMI: 27/24 kg/m²</td>
<td>Ultrasound</td>
<td>140</td>
</tr>
<tr>
<td>IL-6 ↑, MCP1 ↑, CCL19 ↑</td>
<td>22/30</td>
<td>Age: 48/42 years BMI: 29/23 kg/m²</td>
<td>Histology</td>
<td>141</td>
</tr>
<tr>
<td>Coenzyme Q10 ↓, SOD ↓, catalase ↓, MDA ↑, S-ALT ↑, S-AST ↑, C-peptide ↑, HOMA-IR ↑, S-triglycerides ↑, S-HDL cholesterol ↓</td>
<td>51/30</td>
<td>Age: 36 years BMI: 28 kg/m²</td>
<td>Histology</td>
<td>142</td>
</tr>
<tr>
<td>Adiponectin ↓, HOMA ↑, fS-insulin ↑, S-ALT ↑, S-AST ↑, S-γGT ↑</td>
<td>17/20</td>
<td>Age: 44/42 years BMI: 27 kg/m²</td>
<td>Histology</td>
<td>143</td>
</tr>
<tr>
<td>Adiponectin ↓</td>
<td>242</td>
<td>Age: 46 years BMI: 26–32 kg/m²</td>
<td>¹H-MRS</td>
<td>144</td>
</tr>
<tr>
<td>Adiponectin ↓, HOMA-IR ↑, QUICKI ↓, S-ALT ↑, S-AST ↑, S-γGT ↑, fS-insulin ↑, S-HDL cholesterol ↓, fS-FFA ↑</td>
<td>174/42</td>
<td>Age: 41/43 years BMI: 27/28 kg/m²</td>
<td>Histology and ultrasound</td>
<td>40</td>
</tr>
<tr>
<td>TNFα ↑, adiponectin ↓, S-ALT ↑, fP-glucose ↑, fS-insulin ↑, C-peptide ↑, HOMA-IR ↑</td>
<td>29/82</td>
<td>Age: 42/50 years BMI: 30 kg/m²</td>
<td>Histology</td>
<td>145</td>
</tr>
<tr>
<td>fP-glucose ↑, fS-insulin ↑, C-peptide ↑, fS-triglycerides ↑, fS-HDL cholesterol ↓, S-AST ↑, S-ALT ↑</td>
<td>271</td>
<td>Age: 38 years ¹H-MRS 25</td>
<td>Histology</td>
<td>46</td>
</tr>
<tr>
<td>α₂-Heremans-Schmid glycoprotein/fetuin-A ↑</td>
<td>90</td>
<td>Age: 45 years BMI: 29 kg/m²</td>
<td>Histology</td>
<td>146</td>
</tr>
<tr>
<td>RBP-4 ↑</td>
<td>75</td>
<td>Age: 44 years BMI: 32/29 kg/m²</td>
<td>¹H-MRS</td>
<td>147</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Markers of NASH</th>
<th>NASH/NAFLD/(Control)*</th>
<th>Subjects' Characteristics**</th>
<th>Liver Fat Methodology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP ↑, HOMA-IR ↑</td>
<td>69/16(160)</td>
<td>Age: 45 years BMI: 26 kg/m²</td>
<td>Histology</td>
<td>139</td>
</tr>
<tr>
<td>Plasma cytokeratin-18 fragment ↑</td>
<td>21/8(10)</td>
<td>Age: 53/47/49 years BMI: 33/31/27 kg/m²</td>
<td>Histology</td>
<td>148</td>
</tr>
<tr>
<td>fP-glucose ↑, fP-insulin ↑, IR index ↑, HbA₁c ↑, C-peptide ↑, S-ALT ↑, S-AST ↑, S-γGT ↑, fS-triglycerides ↑, S-ferritin ↑</td>
<td>26/75(4)</td>
<td>Age: 44/40 years BMI: 47/44 kg/m²</td>
<td>Histology</td>
<td>149</td>
</tr>
<tr>
<td>fS-insulin ↑, C-peptide ↑, HOMA-IR ↑, sTNFR2 ↑, adiponectin ↓</td>
<td>80/29</td>
<td>Age: 51/42 years BMI: 31/30 kg/m²</td>
<td>Histology</td>
<td>145</td>
</tr>
<tr>
<td>MCP1 ↑, TNFα ↑</td>
<td>25/22</td>
<td>Age: 45/48 years BMI: 32/29 kg/m²</td>
<td>Histology</td>
<td>141</td>
</tr>
</tbody>
</table>

*No of subjects; **Age and BMI or body wt denote mean values.
SOD indicates superoxide dismutase; MDA, malondialdehyde; QUICKI, quantitative insulin sensitivity check index.
γGT predicts type 2 diabetes independent of obesity include studies in Japanese74 and Korean75 men, Black and White American men and women,76 and in Finnish men and women (age 25 to 64 years).77 Most recently, in the West of Scotland Coronary Prevention Study (WOSCOP), a sustained increase in serum ALT predicted an increased risk of diabetes independent of changes in body weight.78 In contrast to these data, 3 studies reported no independent association between serum ALT concentrations and risk of type 2 diabetes.79–81

CVD and Advanced Liver Disease
In 1439 men and women aged 50 to 75 years participating in the Hoorn Study in the Netherlands, increased serum ALT at baseline increased the 10-year risk of coronary heart disease events even after adjustment for components of the metabolic syndrome and other CVD risk factors (glucose tolerance status, systolic blood pressure, HbA1c, LDL cholesterol, BMI).82 Serum γGT predicted coronary events during a mean follow-up of 15.7 years in 1878 men participating the MONICA/KORA study after adjustment for traditional CVD risk factors.83 Similarly, in a study of 2839 type 2 diabetic outpatients with NAFLD, the risk of CVD was significantly increased after adjustment for all components of the metabolic syndrome.84 In a Swedish study including 129 consecutive patients with biopsy-proven NAFLD followed for 13.7 years, mortality from cardiovascular (15.5% versus 7.5%) and liver-related (2.8% versus 0.2%) causes was significantly increased compared with a matched reference-population.85 During follow-up, 7 (5% of all, 10% of those with NASH) of the patients with NASH at baseline developed end-stage liver disease, including 3 with hepatocellular carcinoma.86 Weight gain and baseline insulin resistance were predictors of liver fibrosis. At follow-up, 80% of the subjects had either impaired or diabetic glucose tolerance.86 NASH but not simple steatosis was associated with reduced survival.

NASH and Cirrhosis
As reviewed by C. Day,85 it is estimated that 12% to 20% of subjects with simple steatosis develop NASH characterized by mild (F1) or moderate (F2) fibrosis. 5% to 15% of these patients progress to NASH with advanced (F3) fibrosis.85 Of patients with NASH and F1–F2 degree of fibrosis, approximately 13% go on to develop cirrhosis over 4 to 5 years.85–88 Obesity and type 2 diabetes are more common in patients with cirrhosis attributable to NAFLD than in patients with cirrhosis attributable to other causes.57 The prognosis of cirrhosis attributable to NASH appears to be better than in

Figure 3. The nonalcoholic fatty liver (NAFL) predicts, as does the metabolic syndrome (MetS), both type 2 diabetes and advanced forms of liver disease including NASH (nonalcoholic steatohepatitis) and cirrhosis, as well cardiovascular disease. The inability to sustain sufficient β-cell function characterizes those who develop overt hyperglycemia in addition to the MetS.138

Figure 4. A schematic representation of how components of the metabolic syndrome relate to fat accumulation in the liver. Please see text for references.
Pathogenesis of the Fatty Liver

The possibility of noninvasively determining hepatic fat content has made possible to relate the fatty liver to components of the metabolic syndrome (Figure 4). However, the mechanisms underlying excess hepatic fat deposition are currently poorly understood, in part because of methodological and ethical limitations to sample human liver tissue, and difficulties to prove causality in human studies.

Sources of Fatty Acids in Hepatic Triglycerides

The fatty acids in hepatic triglycerides are derived from dietary chylomicron remnants, FFAs released from adipose tissue, or from chylomicrons hydrolyzed at a rate in excess of what can be taken up by tissues (spillover), and from de novo lipogenesis.91 Under fasting conditions, hepatic fatty acids originate predominantly from adipose tissue lipolysis.91 The contribution of splanchnic lipolysis to this hepatic FFA delivery averages only 5% to 10% in normal-weight subjects and approximately 30% in viscerally obese men and women.93 Thus, FFAs delivered to the splanchnic bed from sources other than visceral fat provide the majority of hepatic FFA delivery. Postprandially, the contribution of the spillover pathway and uptake of chylomicron remnants increase and can account for up to half of the fatty acids secreted as VLDL-TG.91 De novo lipogenesis accounts for less than 5% in normal subjects postprandially.94 In subjects with a fatty liver, rates of the de novo lipogenesis appear to be significantly elevated.92,95

Gene and Protein Expression in NAFLD

There are no data relating measurements of FFA kinetics across the splanchnic bed to expression of genes or proteins regulating triglyceride storage in the human liver. Only a few studies have examined gene expression in the human liver.96–101 These studies have not found the same genes to be altered in human NAFLD.

Role of Adipose Tissue Inflammation

In obese mice and humans, the number of macrophages is increased and accompanied by increased expression proinflammatory factors such as tumor necrosis factor (TNF)α, MCP-1, and interleukin (IL)-6.102–105 Cinti et al showed that in both obese (db/db) mice and humans, >90% of the macrophages surround dead adipocytes.106 In 2 studies including data on human adipose tissue from lean and obese subjects, the number of macrophages correlated positively with adipocyte size.102,106 On the other hand, the relationship between macrophage gene expression and liver fat persists even after adjusting for obesity.107 Weight loss reduces macrophage infiltration and expression of genes involved in macrophage recruitment.108 Recently, we tested the hypothesis that adipose tissue inflammation characterizes subjects with high liver fat content independent of obesity in weight-matched obese groups differing with respect to liver fat content.109 Adipose tissue from the group with high liver fat content was inflamed as in the study of Cinti et al,106 and thus was characterized by an increased number of dead adipocytes surrounded by macrophages in crown-like structures. These cross-sectional data in humans do not, however, prove cause and effect. In mice, overexpression of CCL2 (MCP1) in adipose tissue leads to macrophage accumulation and steatosis,110 whereas CCR2 (CCL2 receptor) deficiency reduces adipose tissue macrophage content, increases adiponectin expression, and ameliorates hepatic steatosis.111 On the other hand, hepatic activation of NF-κB in mice via overexpression of IκB kinase β (IKK-β) induces insulin resistance in the liver and signs of systemic inflammation (increase in serum IL-6) and insulin resistance in skeletal muscle.112 Similar changes can be induced by a high fat diet.113 Thus, in mice, hepatic insulin resistance can be induced without inducing inflammation in adipose tissue, but whether these data have any relevance for human disease is unknown.

Causes of Liver Fat Accumulation: Lessons From Intervention Studies

Although neither steatosis nor the metabolic syndrome are approved indications for treatment, there is a need to treat NASH. Effects of various interventions are reviewed below mainly because they may give insights into the causes of NAFLD in humans.

Weight Loss

Weight loss does undoubtedly and effectively reduces steatosis.113 This decrease in liver fat is relatively greater114,115 and occurs more rapidly115 than fat loss from other compartments in the body. The impact of weight loss on other changes, especially fibrosis, is still unsettled.116–118

Diet

An increase in especially saturated fat intake parallels the epidemic of obesity.119 Liver fat increases significantly in response to a single mixed meal in normal subjects.120 In cross-sectional studies, a high liver fat content has been related to increased fat, especially saturated fat intake,114,121 and a high glycemic index.122 Studies examining effects of modulation of dietary composition while maintaining caloric intake constant on liver fat in humans are few and of small size. In our hands, feeding overweight nondiabetic women on isocaloric diet containing either 16% fat for 2 weeks decreased liver fat by 20%, whereas a 2-week diet containing 56% of total energy as fat increased liver fat by 35% measured by proton spectroscopy.123 In the latter study, there were no attempts to control for type of fat or carbohydrate. Four weeks of fructose feeding (1.5 g fructose per kg body weight) induced hypertriglyceridemia but did not change liver fat content measured by 1H-MRS.124

Exercise

Liver fat has been shown to be increased independent of obesity and directly measured VO2max in a cross-sectional study.8 On the other hand, in a cross-sectional analysis of 191 apparently healthy individuals, whose habitual physical activity was assessed using a questionnaire, liver fat content was lower in physically active individuals, even after adjust-
ing for age, gender, and BMI.\textsuperscript{125} These cross-sectional data do not, however, prove cause and effect. In a study of 48 overweight subjects, liver fat, measured using $^1$H-MRS, decreased by 0.97 U by 10% weight loss achieved by diet alone (25% calorie restriction for 6 months) and by 0.52 U by the combination of diet (12.5% calorie reduction) and exercise (12.5% increase in energy expenditure).\textsuperscript{126} The decrease in liver fat was not different between these groups,\textsuperscript{126} but the study was not necessarily powered to detect a difference. Thus, it is still unclear whether exercise independent of weight loss decreases liver fat content.

Other Interventions

Regarding treatment of NAFLD, various interventions have been examined in mostly small uncontrolled studies. Data on controlled drug interventions in humans are listed in Table 2. Of the agents tested, PPAR\textgamma agonists have been in several studies shown to decrease liver fat, and in 1 study also inflammation and ballooning necrosis.\textsuperscript{127} Regarding metformin, we found no decrease in liver fat measured with $^1$H-MRS in patients who were treatment naive and did not lose weight.\textsuperscript{128} In another study, liver fat measured histologically decreased significantly by approximately 50% in patients who also lost 2 kg/m\textsuperscript{2} of BMI.\textsuperscript{129} It remains unclear whether metformin decreases liver fat independent of decreases in body weight, as a 2.5 kg/m\textsuperscript{2} loss of BMI decreases liver fat by 49%.\textsuperscript{114} A number of randomized controlled trials using metformin, glitazones, ACE inhibitors, pentoxifylline, fenofibrate, niacin, vitamin E, and PUFA are currently being tested in NAFLD (www.clinicaltrials.gov). In addition, rimonabant, which has been shown to decrease liver fat independent of body fat in mice,\textsuperscript{130} and incretin mimetics\textsuperscript{131,132} are studied in humans. Liver transplantation has been life-saving for some patients with NASH-related cirrhosis, but the rate of recurrence of steatosis is high.\textsuperscript{133} Whether this is because of an inability to change lifestyle or because of genetic predisposition is unclear.

Causes of Liver Fat Accumulation: Acquired or Genetic?

Study of monozygotic twins discordant for obesity have shown that acquired obesity increases liver fat content independent of genetic background.\textsuperscript{121} This does not exclude a role of genetic factors, but few data are currently available. Polymorphisms of the adiponectin receptor 1 promoter region,\textsuperscript{134} usf-1,\textsuperscript{135} and hepatic lipase\textsuperscript{136} have been linked to variation in liver fat content, but in these studies, which are all from the same German cohort, liver fat content was measured in only approximately 100 subjects. In a cohort including 66 Japanese patients with NASH, 36 with simple steatosis, and 100 control subjects, TNF\textalpha promoter polymorphisms were found to be more common in NASH than in simple steatosis.\textsuperscript{137}

### Table 2. Controlled Studies in Humans to Treat Fatty Liver and/or NASH

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Diagnosis</th>
<th>No.</th>
<th>Duration</th>
<th>Change in Liver Fat*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR\textgamma agonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosiglitazone vs placebo</td>
<td>Type 2 diabetes</td>
<td>33</td>
<td>4 mos</td>
<td>$-45%$ ($^1$H-MRS)**</td>
<td>150</td>
</tr>
<tr>
<td>Rosiglitazone vs metformin</td>
<td>Type 2 diabetes</td>
<td>20</td>
<td>4 mos</td>
<td>$-51%$ ($^1$H-MRS)**</td>
<td>128</td>
</tr>
<tr>
<td>Rosiglitazone vs placebo</td>
<td>HAL\textsuperscript{a}</td>
<td>30</td>
<td>6 mos</td>
<td>$-15%$ ($^1$H-MRS)**</td>
<td>151</td>
</tr>
<tr>
<td>Pioglitazone vs placebo</td>
<td>NASH+IGT or type 2 diabetes</td>
<td>55</td>
<td>6 mos</td>
<td>$-54%$ ($^1$H-MRS)**, steatosis grade ↓ (BX)</td>
<td>127</td>
</tr>
<tr>
<td>Cytoprotective agents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCDA\textsuperscript{b} vs placebo combinations</td>
<td>NASH</td>
<td>166</td>
<td>2 years</td>
<td>No change in steatosis (BX)</td>
<td>152</td>
</tr>
<tr>
<td>Vitamin E vs vitamin E+pioglitazone</td>
<td>NASH</td>
<td>20</td>
<td>6 mos</td>
<td>Steatosis grade ↓ (BX) in both groups</td>
<td>153</td>
</tr>
<tr>
<td>UCDA vs UCDA+vitamin E vs placebo</td>
<td>NASH</td>
<td>48</td>
<td>2 years</td>
<td>Steatosis grade ↓ (BX) by UCDA+vitamin E</td>
<td>154</td>
</tr>
</tbody>
</table>

\textsuperscript{a}HAL indicates highly active antiretroviral therapy associated lipodystrophy; \textsuperscript{b}UCDA, ursodeoxycholic acid. *% decrease in liver fat determined by $^1$H-MRS or biopsy (BX). **% change in the PPAR\textgamma agonist group.

### Conclusions

Regardless of the true origins of the metabolic syndrome, be it our unhealthy values of lifestyle or a defect in the brain or adipose tissue or elsewhere, the liver is the biochemical factory responsible for production of many cardiovascular risk factors. As summarized above, increased fat accumulation in the liver is a marker of hepatic insulin resistance and a close correlate of all components of the metabolic syndrome independent of obesity. Epidemiological data have shown the fatty liver to predict, independent of other factors, the metabolic syndrome, type 2 diabetes, and cardiovascular disease. Despite its obvious importance, the pathogenesis of the fatty liver is poorly understood mainly because of ethical limitations in studying the human liver. Adipose tissue inflammation and a fatty liver seem to coexist, but the direction of causality, if any, is unclear. Both liver fat and adipose tissue inflammation can be reduced by weight loss and independent of weight loss by PPAR\textgamma agonists. The impact of diet composition on liver fat and of physical activity on liver fat independent of body mass index warrants further studies. It is also unknown whether and which genetic factors contribute to variation in liver fat content. All of these questions seem important to address, given that the prevalence of severe liver disease associated with the metabolic syndrome is rapidly increasing.\textsuperscript{90}

### Sources of Funding

This work was supported by research grants from the Academy of Finland, the Sigrid Juselius Foundation, and Novo Nordisk Foundation. This work is part of the project “Hepatic and adipose tissue and functions in the metabolic syndrome” (www.hepadip.org), which is...
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Arterioscler Thromb Vasc Biol. 2008;28:27-38; originally published online August 9, 2007;
doi: 10.1161/ATVBAHA.107.147538
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
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