Nonalcoholic Fatty Liver Disease: Cytokine-Adipokine Interplay and Regulation of Insulin Resistance

SPECIAL REPORTS AND REVIEWS

Nonalcoholic Fatty Liver Disease: Cytokine-Adipokine Interplay and Regulation of Insulin Resistance

HERBERT TILG* and GÖKHAN S. HOTAMISLIGIL‡

*Department of Medicine, Christian Doppler Laboratory for Gut Inflammation and Clinical Division of Gastroenterology and Hepatology, Innsbruck Medical University, Innsbruck, Austria; and ‡Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, Massachusetts

Nonalcoholic fatty liver disease (NAFLD), the major reason for abnormal liver function in the Western world, is associated with obesity and diabetes and is characterized by insulin resistance (IR). IR is regulated by mediators released from cells of the immune system or adipocytes and proinflammatory cytokines such as tumor necrosis factor-α (TNFα). The importance of TNFα in human and animal fatty liver diseases, both caused by genetic manipulation and overnutrition, has been shown convincingly. Furthermore, neutralization of TNFα activity improves IR and fatty liver disease in animals. Adiponectin is a potent TNFα-neutralizing and anti-inflammatory adipokine and in vitro and experimental animal studies have proven the importance of this mediator in counteracting inflammation and IR. Anti-inflammatory effects of adiponectin are exerted both by suppressing TNFα synthesis and by induction of anti-inflammatory cytokines such as interleukin-10 or interleukin-1–receptor antagonist. Therefore, the balance between various mediators, either derived from the immune system or adipose tissue, appears to play an important role in hepatic and systemic insulin action and in the development of fatty liver disease.

The number of obese and overweight individuals has increased dramatically over the past 2 decades. Obesity is associated not only with the development of type 2 diabetes and hypertension, but also has negative effects on liver function. There is now convincing evidence that nonalcoholic fatty liver disease (NAFLD) is a component of the metabolic syndrome.[1,2] NAFLD is a major liver disease throughout the world and is characterized by a broad spectrum of manifestations, ranging from simple steatosis to inflammatory nonalcoholic steatohepatitis (NASH) and cirrhosis in a small percentage of affected individuals. It is estimated that around 1% of the Western population might have NASH.[1,3] However, distinction between NAFLD and NASH is possible only by liver histology and cannot yet be predicted by clinical or laboratory features.[1,3]

The number of patients with NAFLD who have NASH is unclear. This determination is important because inflammation and/or fibrosis dictate the long-term prognosis of this disease. Insulin resistance (IR) has been identified as a crucial pathophysiologic factor in NAFLD.[4,5] However, the mechanistic basis of NAFLD and NASH is incompletely understood.[6,7] Although complex interactions between genetic determinants, nutritional factors, and lifestyle influence IR, it is increasingly recognized that soluble mediators synthesized both from cells of the immune system and by the adipose tissue are critically involved in disease manifestation and progression and even more importantly in regulation of insulin action.

Insulin acts in all cells through binding to its specific receptor and thereby activating a cascade of intracellular signaling events. After binding, the insulin receptor phosphorylates itself and several members of the insulin-receptor substrate (IRS) family. IRS-1 and IRS-2 are the main mediators of insulin signaling in the liver, controlling insulin sensitivity. Therefore, the primary mechanism of IR induced by inflammatory mediators is exerted by interference at this level of signaling.[8] The importance of visceral fat in the pathogenesis of hepatic IR and steatosis has been shown in many animal models including *fa/fa* rats. In these animals with inherited leptin resistance, surgical resection of intra-abdominal fat depots reverses both conditions.[9] The details of insulin receptor signaling pathways are not covered here but can be found in excellent recent reviews.[10,11] This article summarizes the current knowledge, highlighting the inflammatory/cytokine view of this disease, with a detailed discussion on the role of cytokines and adipokines in NAFLD and their contribution to IR.

Cytokines in NAFLD

Identification of the mechanisms that cause and mediate obesity-related fatty liver disease are awaited and progress in the past few years has been substantial.[6,7] Cytokines are critically involved in the physiology of a healthy liver and in the pathophysiology of many acute and chronic liver diseases. These mediators are released by almost all cell types in the liver and play a fundamental role in liver function and regeneration. Cytokines are key mediators of hepatic inflammation, liver cell death, cholestasis, and fibrosis,[12–14] as well as regeneration of the liver after injury.[15,16]

Abbreviations used in this paper: IKKβ, I κ B kinase-β; IL, interleukin; IR, insulin resistance; IRS, insulin-receptor substrate; JNK, c-Jun N-terminal kinase; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor κB; PPAR, peroxisome proliferator activating receptor; ROS, reactive oxygen species; SOCS, suppressors of cytokine signaling; SREBP, sterol regulatory element-binding protein; TNF, tumor necrosis factor; TNFR1, type 1 tumor necrosis factor receptors.

0016-5085/06/$32.00
doi:10.1053/j.gastro.2006.05.054

Production of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor α (TNFα), the 2 prototypic proinflammatory cytokines, is one of the earliest events in many types of liver injury, triggering the production of other cytokines that together recruit inflammatory cells and initiate a healing process in the liver that includes fibrogenesis. TNFα and other cytokines are barely detectable in the healthy normal liver. Antibody-neutralization studies[15] and experiments with mice that lack type 1 TNF receptors (TNFR1)[17] showed a key role for TNFα during liver regeneration after partial hepatectomy. In addition, similar phenomena also are observed in mice with deletion of other critical cytokines such as IL-6, indicating that the action of a complex network of cytokines is contributing to liver regeneration.[16]

Role of Cytokines and Apoptosis in NAFLD

Inflammatory mediators are thought to play a key role in NFALD. Enhanced TNFα expression has been shown in patients with NASH/NAFLD. Crespo et al[18] showed increased expression of TNFα and its type 1 receptor in patients with NASH compared with patients with simple steatosis. Interestingly, more advanced fibrosis also was accompanied by increased TNFα expression.

Apoptosis is a common mechanism of liver injury. Patients with NASH compared with simple steatosis show increased numbers of terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling–positive cells in the liver being accompanied by enhanced expression of caspases 3 and 7, confirming higher rates of apoptosis in NASH.[19] This increased apoptotic response seems to correlate with nuclear factor κB (NF-κB), the master transcription factor of many proinflammatory mediators, and TNFR1 expression, inflammatory activity, and the amount of fibrosis.[19,20] Fas and TNFR1 expression also increase in experimental models of NASH, and Fas ligand and TNFα promote hepatocyte apoptosis and inflammation in animal models of fatty liver disease.[21] Therefore, increased expression of TNFα and an enhanced apoptosis rate in human NASH may appear in parallel and be causally linked to each other.

It has been well established that TNFα also modulates systemic and hepatic insulin sensitivity (see later). Furthermore, certain TNFα polymorphisms are associated with susceptibility for IR, highlighting the importance of this cytokine in the interaction between fat accumulation, insulin action, and inflammation in human beings.[22] Additional studies to assess local cytokine production/expression and to understand detailed mechanisms of action in regulating liver function would be necessary for the development of effective therapeutic interventions.

Which Factors Might Mediate Enhanced Cytokine Production in NASH/NAFLD? Role of Free Fatty Acids in Cytokine Production: The Obesity Perspective

Free fatty acids (FFAs) are important mediators of lipotoxicity. When lipids accumulate in various nonadipose tissues (as in obesity), they may enter nonoxidative pathways leading to cell injury and death. FFAs have been shown to circulate at higher concentrations in patients with NAFLD, and even more importantly their levels correlate with disease severity.[23] Accumulation of FFA affects lysosomal permeabilization, which also is observed after exposure to proinflammatory cytokines such as TNFα. Feldstein et al[24] recently provided evidence regarding the role of FFAs in mediating hepatic lipotoxicity. FFAs show dramatic lipotoxicity in the liver and induce TNFα expression. Liver injury triggers translocation of bax, a proapoptotic Bcl2 family member, to lysosomes and subsequent lysosomal destabilization with release of the cysteine protease cathepsin B (Figure 1). Lysosomal destabilization leads to activation of NF-κB and generation of more TNFα, initiating a vicious cycle. Importantly, genetic or pharmacologic inactivation of cathepsin B protects against the development of hepatic steatosis, liver injury, and IR associated with the metabolic syndrome.[24] In the cytosol, cathepsin B activates cytokine signaling cascades, thereby also facilitating triglyceride accumulation in the liver and aggravating steatosis and inflammation. This also could reflect a pathway where hepatic steatosis develops and worsens under conditions in which inflammation is dominant irrespective of the extent of obesity. For example, TNFα could promote IR by triggering I κ B kinase-β (IKKβ), the upstream activator of NF-κB, and/or other critical intracellular kinases such as c-Jun N-terminal kinase (JNK) activation and consequently block insulin receptor signaling (Figure 2).[8] All these potential loops and mechanisms are supported by the fact that in a murine model of steatohepatitis: (1) antibody-mediated neutralization of TNFα improves liver disease[25] and (2) development of fatty liver disease requires the presence of intact TNFR1 and is dependent on cathepsin B activity.[24] Therefore, evidence is increasing that inflammation in general and proinflammatory cytokines such as TNFα in particular could be involved in the development of NASH and IR at various stages, either earlier or later in the disease process.

Obesity is associated with production of increased inflammatory cytokines, particularly from visceral adipose tissue.[8] Definitive links also have been established between TNFα action and suppression of insulin action in cells, whole animals, and human beings (see later).[8] A high-fat diet causes an increase in soluble and membrane-bound TNFα both in fat, liver, and muscle tissues in experimental animals and human beings, whereas TNFα remains mostly at low levels in serum.[26,27] Aberrant expression also has been observed in muscle, leading to the conclusion that obesity-related increased TNFα expression at this site also contributes to the development of IR.[28]

Furthermore, visceral fat might promote fatty liver disease by the release of FFAs that are delivered directly into the portal vein.[29] In mice, conditions that increase the delivery of FFAs to tissues, including the liver, can induce localized IR.[30] In addition, gene disruption studies in mice have proven that interference with insulin signaling in hepatocytes activates fat-synthesizing enzymes in these cells and results in hepatic steatosis.[31] Therefore, one might conclude that obesity in itself might be sufficient with (or without) enhanced levels of circulating FFAs to activate the cytokine cascade, thereby triggering and regulating IR. In fact, there are experimental models in which animals are strikingly protected against fatty liver disease despite obesity and higher FFA availability.[32]

Early vs Late Disease Stages of Liver Disease and Role of Gut-Derived Flora and Endotoxin

As discussed earlier, increased cytokine production could be the result of metabolic disturbances and caused by

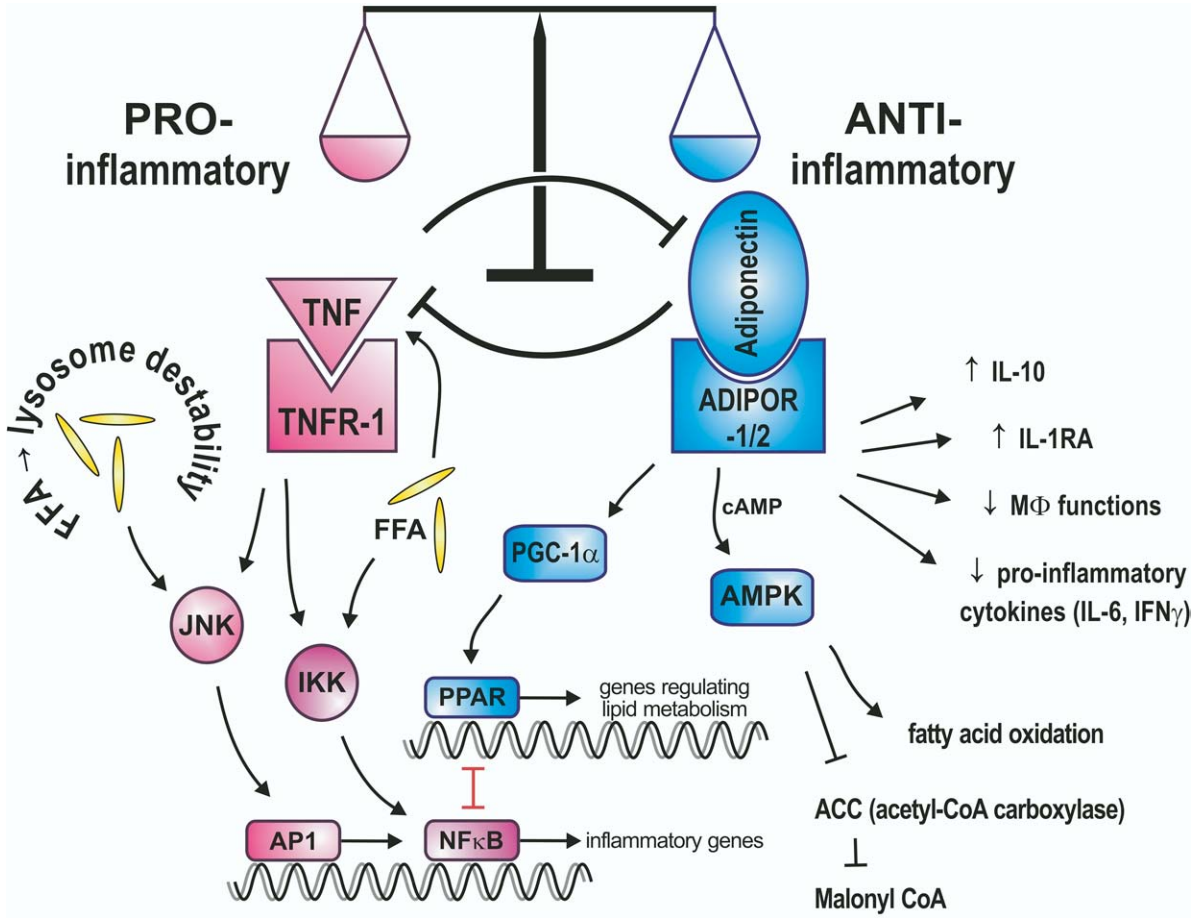

Figure 1. TNFα and adiponectin interplay in various biological systems, particularly in metabolic homeostasis. TNFα, a key proinflammatory cytokine synthesized by many cell types, including adipocytes, is a pleiotropic mediator of the immune system. Adiponectin is the abundant fat cell–derived product with important metabolic and immune functions. These 2 key mediators control the synthesis and activity of each other and thereby allow a balanced physiologic situation. This could be of key importance in diseases associated with IR because the critical balance might be impaired, leading to chronic inflammation. Such an imbalance might directly lead to IR. Besides suppressing TNFα adiponectin has several other anti-inflammatory features (eg, induction of other anti-inflammatory cytokines such as IL-10 and IL-1–receptor antagonist). Adiponectin stimulates glucose use and fatty acid oxidation by activating AMP-activated protein kinase (AMPK). It also increases the transcriptional regulator peroxisome proliferator-activated receptor γ coactivator α (PGC-1α) that is required for adiponectin-induced up-regulation of PPARα. FFAs induce TNFα expression and injury in the liver and lead to lysosomal destabilization with release of cathepsin B. Lysosomal destabilization leads to activation of NF-κB and generation of more TNFα. In addition, FFAs activate the stress/inflammatory kinases JNK and IKKβ. ↑, up-regulation; ↓, suppression.

lipid products such as FFAs. On the other hand, endotoxin and related products mainly derived from the gut also could present an alternative trigger for inflammatory responses. Although this might not generally be the case in early liver disease, it could hold true for advanced stages. Probiotics and manipulation of gut flora has been shown to positively affect liver fat content and inflammation in the *ob/ob* mouse model.[25,33] Data in human beings are rare but a small study suggests a benefit for probiotic treatment in patients with NASH.[34] Endotoxin can be absorbed under normal circumstances from the gastrointestinal tract into the portal vein system and then undergoes clearance by the hepatic reticuloendothelial system.[35,36] In patients with acute and chronic liver disease, an impairment of the reticuloendothelial system and/or the presence of portosystemic shunts may lead, in the absence of sepsis, to an increased release of endotoxin into the systemic circulation.[37] Increased endotoxin levels correlate well with the severity of hepatic insufficiency.[38] Therefore, in advanced stages of NAFLD, gut-derived products might be involved in the activation of cytokine cascades and/or regulation of IR. It will be interesting to explore whether such phenomena also are operative in early disease stages and could be exploited for therapeutic or preventive purposes.

Suppressors of Cytokine Signaling in NAFLD

Intracellular suppressors of cytokine signaling (SOCS) contribute to the negative regulation of many inflammatory mediators. The importance of these negative regulators (ie, anti-inflammatory mediators) is best illustrated by data showing that SOCS-1 (−/−) mice die by 3 weeks of age with inflammation and fatty necrosis of the liver.[39] It has been shown recently that mice with SOCS-1 deficiency in myeloid and lymphoid cells also develop disease, although at 50–250 days of age, with uncontrolled inflammation.[40] These animals also showed liver inflammation with infiltration by $CD4^+$ and

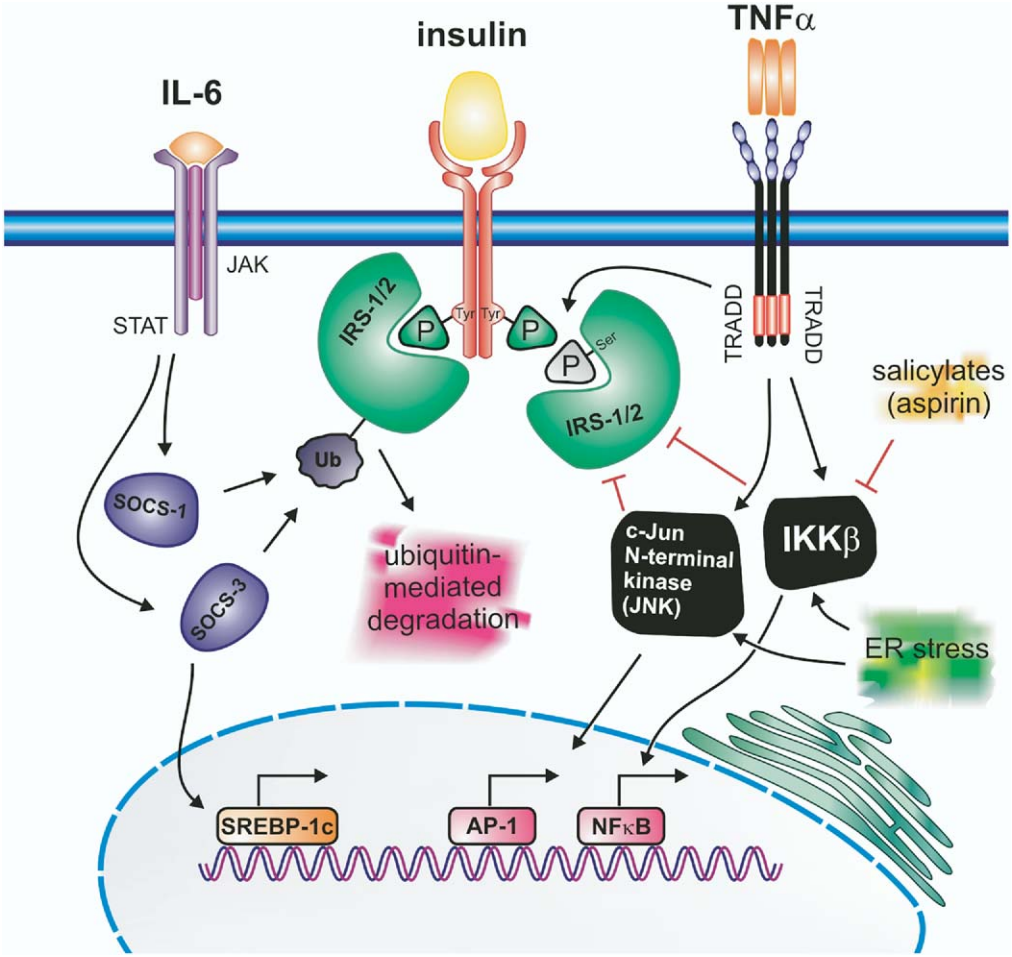

Figure 2. Regulation of NASH and IR: involved mediators and pathways. TNFα and other cytokines such as IL-6 are involved in the generation of IR. Other factors of the immune system such as SOCS-proteins (1, 2, and 7) also are involved in these processes. SOCS-1 and SOCS-3 can link IRSs to ubiquitin-mediated degradation pathways and also to an increase in the key regulator of fatty acid synthesis in liver, the transcription factor SREBP-1c. In recent years, critical intracellular pathways have been identified that are involved in the molecular pathogenesis of IR. Endoplasmic reticulum (ER) stress and various kinases such as JNK and IKKβ, are of critical importance because they not only activate inflammatory pathways but also attenuate insulin signaling. ER stress leads to suppression of insulin-receptor signaling through activation of JNK and the subsequent serine phosphorylation of IRS-1.

$CD8^+$ T cells, and macrophages localized mainly to portal tracts or in isolated foci.

More interestingly, SOCS-1 and SOCS-3 have been shown to block insulin signaling by ubiquitin-mediated degradation of IRS-1 and IRS-2 (Figure 2).[41,42] Several SOCS proteins are regulated by cytokines that could modulate insulin action.[43] For example, SOCS-3 seems to be involved in IL-6–dependent IR in hepatocytes.[44] The overexpression of SOCS-1 and SOCS-3 in liver also causes IR and an increase in the key regulator of fatty acid synthesis in the liver, the transcription factor sterol regulatory element-binding protein (SREBP)-1c. Conversely, inhibition of SOCS-1 and SOCS-3 in obese diabetic mice improves insulin sensitivity, normalizes increased expression of SREBP-1c, and improves hepatic steatosis and hypertriglyceridemia.[45] SOCS-3 haploinsufficiency in mice also produces a similar phenotype of increased insulin sensitivity.[46] Recently, SOCS-7 also has been implicated in the regulation of IR.[47] A similar SOCS-mediated mechanism might be involved in hepatitis C–associated IR because it has been shown that hepatitis C virus core protein up-regulates SOCS-3 and promotes proteosomal degradation of IRS-1 and IRS-2 through ubiquitination.[48] SOCS proteins therefore reflect good candidates as a key link between liver inflammation, steatosis, and IR.

Role of Cytokines in Genetically Obese Rodents

Studies of genetically obese *ob/ob* mice and *fa/fa* rats have provided information about the pathogenesis of obesity-related fatty liver disease. Both of these rodent strains have spontaneous mutations that either diminish production of the appetite-suppressing hormone, leptin (in *ob/ob* mice), or that inactivate the leptin receptor (in *fa/fa* rats).[6,7,49]

Similar to obese human beings, *ob/ob* mice and *fa/fa* rats have IR, hyperglycemia, hyperlipidemia, and fatty livers.[6–8] These rodent models also show several immunologic defects including phagocyte dysfunction and altered cytokine gene transcription including enhanced TNFα expression.[6,7,50] Furthermore, murine leptin deficiency influences production of other cytokines such as IL-12 or IL-15, thereby promoting hepatic CD4-positive natural killer cell depletion in *ob/ob* livers.[51] Therefore, in fatty liver disease, cytokine imbalance and dysregulation might involve not only inflammatory but also other immunoregulatory cytokines.

It has to be mentioned that these animal models might have certain limitations. Obese human beings almost always have high serum levels of leptin.[52] Leptin-deficient *ob/ob* mice show no or only mild chronic steatohepatitis and do not develop fibrosis, which can be overcome by exogenous administration of leptin,[53] suggesting that leptin is one essential mediator of hepatic fibrosis. Noradrenaline administration has been shown to promote hepatic fibrosis by inducing hepatic transforming growth factor β and collagen gene expression in the *ob/ob* mouse.[54] Interestingly, in these studies fibrosis developed despite reduction in proinflammatory cytokine production, further supporting the notion that fibrosis might develop in cer-

tain instances without any inflammation. Despite the shortcomings of several animal models including the *ob/ob* mouse and their limited relation to human NAFLD, they are helpful in studying and elucidating several pathophysiologic aspects of fatty liver diseases.

How to Improve Fatty Liver Disease in Animal Models?

Considering that gut-derived flora and proinflammatory cytokines play a key role in fatty liver diseases, interference at one of these steps might be beneficial. Similarities in the histopathology of alcohol-induced steatohepatitis and obesity-related NASH suggest that common mechanisms may mediate both diseases. Various treatments, at least in experimental animal systems, that inhibit TNFα activity prevent both diseases. Several anti-TNF agents such as anti-TNF antibodies protect against fatty liver diseases.[25,55] It also has been shown that metformin improves liver disease in *ob/ob* mice via suppression of TNFα.[56] Furthermore, peroxisome proliferator activating receptor γ (PPARγ) ligands such as pioglitazone, which can suppress TNFα function, also positively affect fatty liver diseases.[57–59] Li et al[25] treated *ob/ob* mice with either a probiotic (VSL#3, ie, lyophilized bifidobacteria, lactobacilli, and *Streptococcus thermophilus*) or an anti-TNFα antibody for 4 weeks. Treatment with both agents improved liver histology, reduced hepatic total fatty acid content, and decreased enhanced liver function tests. These benefits were paralleled by decreased hepatic expression of TNFα, especially after treatment with the anti-TNFα antibody. Furthermore, these treatments reduced activity of JNK and NF-κB pathways, both of which are downstream of TNFα and promote IR. Therefore, intestinal bacteria might induce endogenous signals that play a role in hepatic IR and NAFLD and suggest that either interfering at this stage or disrupting the proinflammatory cytokine cascades might be beneficial in fatty liver diseases.

Conclusions

There is now compelling evidence that (1) enhanced liver TNFα expression is observed in animal models and human beings with NASH/NAFLD, (2) this cytokine, released by many cells in the body including various cell types in liver and adipocytes, is crucially involved in the pathogenesis of IR (as discussed later), and (3) neutralization of TNFα, at least in experimental animal models, improves IR, hepatic steatosis, and liver inflammation.

Adiponectin, the Key TNFα-Neutralizing Adipocytokine, and Its Role in Inflammation and NAFLD

Adipose tissue and its metabolic products recently have gained great interest. Various products of the fat tissue have been characterized including not only cytokines such as TNFα or IL-6, but also the so-called *adipo(cyto)kines* including leptin, adiponectin, and visfatin.[8] Adiponectin, the predominant protein synthesized by adipocytes, circulates in rather high concentrations and shows a wide spectrum of biological activities.

Adiponectin is secreted predominantly from adipose tissue and shares sequence homology with a family of proteins that show a characteristic NH_2-terminal collagen-like region and a COOH-terminal, complement factor C1q-like globular domain.[60–62] Plasma levels of adiponectin are reduced markedly in visceral obesity and in states of IR such as NASH,[63] atherosclerosis, and type 2 diabetes mellitus.[64–67] Therefore, low adiponectin plasma levels correlate negatively with percentage of body fat, central obesity, and IR. Adiponectin exists in the circulation as a full-length and a putative proteolytic cleavage fragment consisting of the globular C-terminal domain, which might have enhanced activity within high-order complexes. Adiponectin receptors (AdipoR1/2) have been cloned recently. AdipoR1 is expressed primarily in adipose tissue and has a wide distribution throughout the organism; however, AdipoR2 is expressed primarily in the liver.[68] In addition to these molecules, T-cadherin, which shows a broader tissue distribution, also has been identified as a receptor for adiponectin.[69]

Adiponectin Serum Levels in Various Liver Diseases

Adiponectin serum levels are decreased in states of IR. A negative association between serum levels of adiponectin and liver enzyme levels has been shown in healthy subjects.[70] Hui et al[63] convincingly showed that patients with both steatosis and NASH have decreased serum levels of adiponectin. Hypoadiponectinemia might predict severity of liver fibrosis in patients with NASH because its serum levels were correlated negatively with the amount of necroinflammation and fibrosis.[71] This, however, was not observed in another large study in which decreased levels of adiponectin were related to hepatic insulin sensitivity and to the amount of hepatic fat content but not with necroinflammation and fibrosis.[72] We recently observed decreased hepatic adiponectin expression in patients with NASH[73] and observed that in these patients weight loss induced by bariatric surgery increases expression of adiponectin (Wolf, unpublished data). Surprisingly, patients with cirrhotic disease of various origins including fatty liver disease have high levels of adiponectin, probably reflecting one of the body's anti-inflammatory strategies in such situations.[74,75]

Regulation of Adiponectin Expression

Adipose tissue so far has been considered as the major site of endogenous adiponectin production, even though other potential sources such as muscle cells, cardiac myocytes,[76] or endothelial cells also have been reported.[77,78] TNFα suppresses the transcription of adiponectin in 3T3-L1 adipocytes, which might be an underlying mechanism for the lower adiponectin levels in obesity. The best evidence that adiponectin synthesis is correlated negatively with proinflammatory cytokine production came from studies on adiponectin-deficient mice.[62] In these studies, adiponectin (−/−) mice were characterized with high levels of TNFα messenger RNA (mRNA) expression in adipose tissue and high TNFα protein concentrations in plasma.

Adiponectin also is regulated by other inflammatory mediators. IL-6 and dexamethasone suppress adiponectin mRNA expression and protein synthesis in 3T3-L1 adipocytes.[79] Besides these immune mediators, weight loss itself is a potent inducer of adiponectin synthesis.[80] Keller et al[81] recently showed that circulating adiponectin levels are reduced during resting and fasting states and this effect could be reversed by endotoxin injection into human beings. In contrast to proinflammatory cytokines, activation of PPARγ results in induction of adiponectin in adipose tissue.[82] Accordingly, adipose-specific

PPARγ knockout causes IR and decreased circulating levels of adiponectin.[83]

Stimulation of adiponectin and suppression of TNFα also might contribute to the anti-inflammatory and insulin-sensitizing effects of PPARγ ligands (thiazolidinediones). Thiazolidinediones increase fatty acid uptake in peripheral adipose tissue, thus decreasing circulating FFA levels and hepatic triglyceride content, partly via altered adipocytokine release. Initial pilot studies have suggested that thiazolidinediones such as pioglitazone or rosiglitazone might be beneficial in patients with NAFLD.[84,85]

Other sources of adiponectin. Delaigle et al[77] recently presented evidence that skeletal muscle tissue also is able to synthesize adiponectin. Injection of endotoxin into animals caused a 10-fold increase in adiponectin mRNA expression in tibialis anterior muscle. This effect also was reproducible in cultured human myotubes. Recently, cardiac myocytes have been shown to produce adiponectin.[76] We showed that liver might be an additional source of adiponectin.[78] Concanavalin A–induced liver failure resulted in increased production of adiponectin by the liver and cell-specific analysis revealed that most likely primary hepatic sinusoidal endothelial cells contributed to its synthesis.[78]

Anti-Inflammatory Properties of Adiponectin In Vitro

Adiponectin and its role in suppressing TNFα. Initially, an anti-inflammatory effect of adiponectin was described in endothelial cells.[86] In these experiments, adiponectin resulted in inhibition of TNFα-regulated endothelial adhesion molecule expression. Adiponectin inhibits endothelial NF-κB signaling through a 3′,5′-cyclic adenosine monophosphate–dependent pathway[86] and interferes with macrophage function (Figure 1).[87] Treatment of cultured macrophages with adiponectin significantly inhibited TNFα synthesis in response to endotoxin. Adiponectin (−/−) mice show evidence of increased local and systemic TNFα production, suggesting a suppressive effect of adiponectin on TNFα expression and synthesis.[62]

Adiponectin induces its anti-inflammatory properties through induction of other mediators such as IL-10 and IL-1–receptor antagonist in various cell types and suppression of IL-6 and interferon γ (Figure 1).[88–90] Therefore, adiponectin exerts anti-inflammatory effects at several levels, which might be of importance in health and disease to counteract proinflammatory cytokines such as TNFα.

Adiponectin improves experimental fatty liver disease and liver fibrosis. Adiponectin exerts anti-inflammatory effects in various animal models of liver inflammation. Xu et al[91] showed a beneficial effect of adiponectin in both alcoholic and nonalcoholic fatty liver disease in mice. In this study, adiponectin considerably improved hepatomegaly, steatosis, and abnormal alanine aminotransferase activity in *ob/ob* mice. These beneficial effects were paralleled by a decrease in TNFα expression in the liver. Kamada et al[92] used the carbon-tetrachloride liver fibrosis model and showed that adiponectin attenuates liver fibrosis in mice. Hepatic stellate cells express type 1 and 2 adiponectin receptor mRNAs, in which adiponectin inhibits proliferation and migration as well as the expression of TGFβ1, the main activator of extracellular matrix protein synthesis. Adiponectin also protects endotoxin-induced liver injury in another model of fatty liver, namely the KK-A^y obese mice.[93] Galactosamine/endotoxin injury was more pronounced in KK-A^y obese mice compared with lean controls. Pretreatment with adiponectin ameliorated the galactosamine/endotoxin-induced increase of liver enzyme levels and apoptotic and necrotic changes in hepatocytes, resulting in reduced lethality. These adiponectin effects were accompanied by decreased levels of TNFα both systemically and locally in the liver. Sennello et al[94] studied concanavalin A–induced hepatotoxicity in lipodystrophic aP2-nSREBP-1c transgenic mice lacking adipose tissue, *ob/ob* mice, and lean wild-type controls. Protection from hepatotoxicity was observed in *ob/ob* mice (high serum adiponectin levels), but not in lipodystrophic aP2-nSREBP-1c transgenic mice (low serum adiponectin levels), despite low cytokine levels, reduced T-cell activation, and diminished hepatic natural killer T cells in both groups. Administration of adiponectin protected lipodystrophic aP2-nSREBP-1c transgenic mice from hepatotoxicity and protected primary hepatocytes from TNFα-induced cell death. Our recent studies in concanavalin A–induced hepatotoxicity indicated that the protective effect of adiponectin in this model is mediated mainly via induction of IL-10.[78]

Conclusions

Adiponectin is an abundant adipocyte-derived protein with well-established anti-atherogenic, anti-inflammatory, and insulin-sensitizing properties. Both serum levels and hepatic adiponectin expression are decreased in patients with NAFLD. PPARγ ligands have been used recently in the treatment of NAFLD and are able to increase tissue and serum concentrations of adiponectin. Adiponectin might play a role in suppressing inflammation and macrophage activity, and its reduced synthesis as observed in NASH/NAFLD might lead to a imbalance in favor of proinflammatory mediators.

Hepatic IR and Underlying Mechanisms

The first link between obesity, increase in the expression of a proinflammatory cytokine, namely TNFα, and insulin action came from a study more than 13 years ago.[95] These findings led to the concept of inflammation in obesity and showed that adipocytes express TNFα. In these studies, expression of this cytokine in obese animals (*fa/fa* rat and *ob/ob* mouse) has been increased and shown to regulate insulin action.[95] Further evidence supporting a key role for TNFα in IR came from studies published by Uysal et al[96] in which it was shown that mice lacking TNFα or TNF receptors had improved insulin sensitivity in both dietary and genetic (*ob/ob*) models of obesity. These observations were paralleled by similar findings in human beings[97] with increased adipose tissue TNFα expression in obesity and correction of this increased TNFα expression after weight loss.[97,98] Furthermore, both TNFα levels and its soluble receptors were correlated positively with body mass index.[99] Besides TNFα, other cytokines such as IL-6[100] and cytokine-regulated molecules such as C-reactive protein[101] also correlate with obesity and body mass index, further enhancing the concept that obesity and IR are inflammatory conditions.

At a molecular level, exposure of cells to TNFα or increased levels of FFA stimulates inhibitory phosphorylation of IRS-1 on serine residues.[102–104] After this modification, tyrosine phosphorylation of IRS-1 and its ability to associate with the insulin receptor is reduced in response to insulin, thereby inhibiting

downstream signaling and insulin action (Figure 2).[104,105] It was shown that these effects are mediated via TNFR1 in cultured cells[106] and whole animals with genetic obesity.[107] Csehi et al[108] also showed that the death domain of TNFR1 is responsible for the inhibitory effects of TNFα on tyrosine phosphorylation of IRS-1, implicating ceramide generated by an acid sphingomyelinase as a downstream mediator of inhibition of IR signaling.

Role of the IKKβ NF-κB Pathway

In searching for mechanisms involved in cytokine-induced IR, Yuan et al[109] identified the IKKβ pathway as a target for TNFα-induced insulin resistance. Yin et al[103] already showed in 1998 that aspirin and salicylates inhibit the activity of IKKβ. Interestingly, high doses of salicylates (up to 10 g) have been used historically to treat inflammatory conditions such as rheumatic fever and rheumatoid arthritis. High doses of salicylates also were suggested for decreasing high blood glucose concentrations 130 years ago by William Ebstein.[110] Yuan et al[109] showed the following in their work: (1) high doses of salicylates reverse hyerglycemia, hyperinsulinemia, and dyslipidemia in *fa/fa* rats and *ob/ob* mice, (2) overexpression of IKKβ attenuates insulin signaling in cultured cells, (3) IKKβ inhibition by salicylates reverses IR, and (4) heterozygous depletion (IKK$\beta\pm$) protected against the development of IR during high-fat feeding and in *ob/ob* mice. These findings showed the important role of IKKβ, a proximal mediator in NF-κB activation in IR.

Two studies recently have shown the relationship between IKKβ expression in the liver and IR.[111,112] Cai et al[111] created a stage of chronic subacute inflammation in the liver in a transgenic mouse model by selective hepatocellular activation of NF-κB, causing continuous low-level expression of IKKβ. These mice showed a diabetic phenotype with evidence of moderate systemic IR. Interestingly, IR was improved by systemic neutralization of IL-6, thereby suppressing SOCS protein expression in the liver or by oral salicylate therapy in this transgenic model. Hepatic expression of the IκBα super-repressor also reversed both the phenotype of these transgenic mice and mice fed a high-fat diet. Although many pieces of the mechanistic network are still missing, including the pathways through which steatosis activates IKKβ and NF-κB and how IL-6 mediates hepatic IR, it is clear that IKKβ, NF-κB, and IL-6 all contribute to liver insulin action in experimental mice models.

Arkan et al[112] recently presented similar findings in mice lacking either IKKβ in hepatocytes or myeloid cells. Liver-specific deletion of IKKβ resulted in relative insulin sensitivity in the liver when placed on a high-fat diet or intercrossed with the *ob/ob* model of genetic obesity, while developing IR in muscle and fat tissues. In contrast, mice deficient in myeloid IKKβ showed increased insulin sensitivity and were partially protected from IR. Importantly, because circulating cytokines were not measurable, it seems likely that the improvement in insulin sensitivity reflects impaired inflammatory pathway activity within resident myeloid cells such as Kupffer cells in the liver. These data suggest that drugs with an insulin-sensitizing effect also may act by targeting myeloid cells in addition to muscle or fat tissues.

When energy intake exceeds energy expenditure, most of the excess is stored as triglycerides in fat and other insulin-sensitive tissues. Indeed, lipid accumulation in the liver is a hallmark of high-fat diet–induced IR. Although it is not clear how a high-fat diet causes activation of NF-κB and its target genes in liver cells, it is possible that excessive fatty acid oxidation in mitochondria generates peroxidation products[113] that may initiate a signaling cascade that culminates in NF-κB activation. Most recently, generation of ER stress has been discovered as a potential mechanism linking metabolic surplus to activation of stress and inflammatory pathways.[114] Thus, lipid accumulation may lead to macrophage activation as these cells try to clear oxidized lipid deposits through scavenger receptors. Taken together, these studies highlight the role of inflammation, especially through the IKKβ pathway in IR and may offer future therapeutic targets.

JNK

Several serine/threonine kinases are activated by inflammatory stimuli contributing to IR including JNK, IKK, and others. Activation of these kinases takes place in situations in which inflammatory and metabolic pathways are triggered, which is, for example, also seen after activation of Toll-like receptors or endotoxin stimulation. JNK recently has emerged as an important regulator of IR in obesity.[115] The JNK group belongs to the group mitogen-activated protein kinases and controls many cellular functions through regulation of activator protein-1, including c-Jun and JunB. In obesity, JNK activity is increased in the liver, muscle, and fat tissues, and loss of JNK1 prevents the development of IR in both genetic and dietary models of obesity. FFAs activate the stress/inflammatory kinases JNK and IKKβ, SOCS-3, increase TNFα synthesis and decrease synthesis of adiponectin into the medium.[116] Nguyen et al[116] showed that JNK can be activated by FFAs through TNFα-independent mechanisms, activated JNK is a major contributor to FFA-induced cellular IR, and TNFα is an autocrine/paracrine downstream effector of activated JNK that also mediates IR. Therefore, it seems that JNK is regulated by FFAs and is an important proximal mediator in IR.

For limitations of space, we are unable to provide an extensive overview of an important group of key mediators that also act at the interface of lipid metabolism and inflammatory pathways. These molecules include adipocyte/macrophage fatty acid binding proteins,[32,117] the PPAR[118–120] and LXR family of nuclear hormone receptors,[121,122] and the SREBP family of transcription factors.[123,124]

Role of Oxidative Stress and Why Does NASH Evolve in a Liver Loaded With Fat?

One of the final common mediators of this process seems to be oxidative stress caused by generation of reactive oxygen species (ROS) and/or decreased antioxidant defenses.[125] ROS can be generated in the liver through several mechanisms including mitochondria, peroxisomes, cytochrome P-450, reduced nicotinamide adenine dinucleotide oxidase, cyclooxygenase, and lipoxygenase. In both NASH and experimental steatohepatitis, the hepatic expression of CYP2E1 is increased, leading to oxidative stress,[126] and this enhanced expression has been shown to impair insulin signaling.[127] Further supporting the importance of ROS in NAFLD, Xu et al[128] recently showed a key role for the Nrf1 gene in NASH. Mice with liver-specific deletion of Nrf1, a gene mediating activation of oxidative stress-response genes, develop all features of NAFLD including steatosis, apoptosis, necrosis, inflammation, fibrosis, and, finally, liver cancer, highlighting the importance of oxidative stress in this disease.

In patients with NASH at least 40% of mitochondria are structurally abnormal.[125] This abnormality is associated with impaired electron-transport-chain enzyme activity resulting in uncoupling oxidation from phosphorylation leading to further ROS formation.[129] Hepatic gene expression studies in histologically progressive human NASH have shown reduced expression of several genes associated with mitochondrial function,[130] which might lead to the attenuated capacity of the liver to control ROS activity. ROS also increase TNFα expression and can cause additional lipid peroxidation of mitochondrial membranes, further worsening mitochondrial function and eventually cell death.

So, why and who develops NASH? The exact mechanisms by which NAFL develops into steatohepatitis and/or cirrhosis are unknown. From the discussion so far it is clear that besides genetic factors several other aspects have to be taken into consideration including inflammatory signals, cytokines, ROS, adipokines, and triggering factors such as gut-derived flora and calorie intake as an initiating factor in most patients. It is also unknown whether progression toward NASH is independent of IR.

Conclusions

NAFLD has emerged as a major cause of abnormal liver function tests worldwide and is considered an integral part of the metabolic syndrome. Accumulating insights should help clinicians to consider this diagnosis even when patients present without other clinical features of the metabolic syndrome, thereby allowing early identification of this syndrome. Cytokines and adipokines seem to play a major role at various stages of NAFLDs and improvement of understanding in the past few years has clearly led to identification of inflammatory underpinnings of insulin resistance and NAFLD. Recent developments in these areas have been discussed and with a better understanding of the molecular mechanisms, it is anticipated that better treatment of the metabolic syndrome and its hepatic manifestations will be possible in the near future.

References

1. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346:1221–1231.
2. Marchesini G, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. Curr Opin Lipidol 2005;16:421–427.
3. Adams LA, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. CMAJ 2005;172:899–905.
4. Marchesini G, Forlani G. NASH: from liver diseases to metabolic disorders and back to clinical hepatology. Hepatology 2002;35: 497–499.
5. Bugianesi E, Marzocchi R, Villanova N, Marchesini G. Non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH): treatment. Best Pract Res Clin Gastroenterol 2004;18: 1105–1116.
6. Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. N Engl J Med 2000;343:1467–1476.
7. Sanyal AJ. Mechanisms of disease: pathogenesis of nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol 2005;2:46–53.
8. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest 2005;115:1111–1119.
9. Gabriely I, Barzilai N. Surgical removal of visceral adipose tissue: effects on insulin action. Curr Diab Rep 2003;3:201–206.
10. Malbon CC. Insulin signalling: putting the "G-" in protein-protein interactions. Biochem J 2004;380:e11–e12.
11. Pirola L, Johnston AM, Van Obberghen E. Modulation of insulin action. Diabetologia 2004;47:170–184.
12. Mizuhara H, O'Neill E, Seki N, Ogawa T, Kusunoki C, Otsuka K, Satoh S, Niwa M, Senoh H, Fujiwara H. T cell activation-associated hepatic injury: mediation by tumor necrosis factors and protection by interleukin 6. J Exp Med 1994;179:1529–1537.
13. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 2000;275: 2247–2250.
14. McClain CJ, Song Z, Barve SS, Hill DB, Deaciuc I. Recent advances in alcoholic liver disease. IV. Dysregulated cytokine metabolism in alcoholic liver disease. Am J Physiol 2004;287: G497–G502.
15. Akerman P, Cote P, Yang SQ, McClain C, Nelson S, Bagby GJ, Diehl AM. Antibodies to tumor necrosis factor-alpha inhibit liver regeneration after partial hepatectomy. Am J Physiol 1992;263: G579–G585.
16. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, Taub R. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. Science 1996;274: 1379–1383.
17. Yamada Y, Kirillova I, Peschon JJ, Fausto N. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. Proc Natl Acad Sci U S A 1997;94:1441–1446.
18. Crespo J, Cayon A, Fernandez-Gil P, Hernandez-Guerra M, Mayorga M, Dominguez-Diez A, Fernandez-Escalante JC, Pons-Romero F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. Hepatology 2001;34:1158–1163.
19. Feldstein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, Gores GJ. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 2003;125:437–443.
20. Ribeiro PS, Cortez-Pinto H, Sola S, Castro RE, Ramalho RM, Baptista A, Moura MC, Camilo ME, Rodrigues CM. Hepatocyte apoptosis, expression of death receptors, and activation of NF-kappaB in the liver of nonalcoholic and alcoholic steatohepatitis patients. Am J Gastroenterol 2004;99:1708–1717.
21. Feldstein AE, Canbay A, Guicciardi ME, Higuchi H, Bronk SF, Gores GJ. Diet associated hepatic steatosis sensitizes to Fas mediated liver injury in mice. J Hepatol 2003;39:978–983.
22. Valenti L, Fracanzani AL, Dongiovanni P, Santorelli G, Branchi A, Taioli E, Fiorelli G, Fargion S. Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology 2002;122:274–280.
23. Nehra V, Angulo P, Buchman AL, Lindor KD. Nutritional and metabolic considerations in the etiology of nonalcoholic steatohepatitis. Dig Dis Sci 2001;46:2347–2352.
24. Feldstein AE, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, Burgart LJ, Gores GJ. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. Hepatology 2004;40:185–194.
25. Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, Desimone C, Song XY, Diehl AM. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 2003;37:343–350.
26. Xu H, Hirosumi J, Uysal KT, Guler AD, Hotamisligil GS. Exclusive action of transmembrane TNF alpha in adipose tissue leads to reduced adipose mass and local but not systemic insulin resistance. Endocrinology 2002;143:1502–1511.
27. Xu H, Uysal KT, Becherer JD, Arner P, Hotamisligil GS. Altered tumor necrosis factor-alpha (TNF-alpha) processing in adipocytes and increased expression of transmembrane TNF-alpha in obesity. Diabetes 2002;51:1876–1883.

28. Borst SE, Conover CF. High-fat diet induces increased tissue expression of TNF-alpha. Life Sci 2005;77:2156–2165.
29. Katsuki A, Sumida Y, Urakawa H, Gabazza EC, Murashima S, Maruyama N, Morioka K, Nakatani K, Yano Y, Adachi Y. Increased visceral fat and serum levels of triglyceride are associated with insulin resistance in Japanese metabolically obese, normal weight subjects with normal glucose tolerance. Diabetes Care 2003;26:2341–2344.
30. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, Lutz EP, Kako Y, Velez-Carrasco W, Goldberg IJ, Breslow JL, Shulman GI. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A 2001; 98:7522–7527.
31. Tobe K, Suzuki R, Aoyama M, Yamauchi T, Kamon J, Kubota N, Terauchi Y, Matsui J, Akanuma Y, Kimura S, Tanaka J, Abe M, Ohsumi J, Nagai R, Kadowaki T. Increased expression of the sterol regulatory element-binding protein-1 gene in insulin receptor substrate-2(−/−) mouse liver. J Biol Chem 2001;276: 38337–38340.
32. Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, Cao Q, Atsumi G, Malone H, Krishnan B, Minokoshi Y, Kahn BB, Parker RA, Hotamisligil GS. Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. Cell Metab 2005;1:107–119.
33. Solga SF, Diehl AM. Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. J Hepatol 2003; 38:681–687.
34. Loguercio C, Federico A, Tuccillo C, Terracciano F, D'Auria MV, De Simone C, Del Vecchio BC. Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. J Clin Gastroenterol 2005;39:540–543.
35. Fukui H, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. J Hepatol 1991;12:162–169.
36. Guarner C, Soriano G. Bacterial translocation and its consequences in patients with cirrhosis. Eur J Gastroenterol Hepatol 2005;17:27–31.
37. Wiest R, Das S, Cadelina G, Garcia-Tsao G, Milstien S, Groszmann RJ. Bacterial translocation in cirrhotic rats stimulates eNOS-derived NO production and impairs mesenteric vascular contractility. J Clin Invest 1999;104:1223–1233.
38. Rao RK, Seth A, Sheth P. Recent advances in alcoholic liver disease I. Role of intestinal permeability and endotoxemia in alcoholic liver disease. Am J Physiol 2004;286:G881–G884.
39. Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. Nat Rev Immunol 2002;2:410–416.
40. Chong MM, Metcalf D, Jamieson E, Alexander WS, Kay TW. Suppressor of cytokine signaling-1 in T cells and macrophages is critical for preventing lethal inflammation. Blood 2005;106: 1668–1675.
41. Rui L, Yuan M, Frantz D, Shoelson S, White MF. SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J Biol Chem 2002;277:42394–42398.
42. Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, Hotamisligil GS, Van Obberghen E. SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. J Biol Chem 2001;276:47944–47949.
43. Croker BA, Krebs DL, Zhang JG, Wormald S, Willson TA, Stanley EG, Robb L, Greenhalgh CJ, Forster I, Clausen BE, Nicola NA, Metcalf D, Hilton DJ, Roberts AW, Alexander WS. SOCS3 negatively regulates IL-6 signaling in vivo. Nat Immunol 2003;4:540–545.
44. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, Mooney RA. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. J Biol Chem 2003;278:13740–13746.
45. Ueki K, Kondo T, Tseng YH, Kahn CR. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. Proc Natl Acad Sci U S A 2004;101:10422–10427.
46. Howard JK, Cave BJ, Oksanen LJ, Tzameli I, Bjorbaek C, Flier JS. Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. Nat Med 2004; 10:734–738.
47. Banks AS, Li J, McKeag L, Hribal ML, Kashiwada M, Accili D, Rothman PB. Deletion of SOCS7 leads to enhanced insulin action and enlarged islets of Langerhans. J Clin Invest 2005; 115:2462–2471.
48. Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, Taniguchi E, Kumemura H, Hanada S, Maeyama M, Baba S, Koga H, Kumashiro R, Ueno T, Ogata H, Yoshimura A, Sata M. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol 2004;165:1499–1508.
49. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995;269:540–543.
50. Diehl AM. Fatty liver, hypertension, and the metabolic syndrome. Gut 2004;53:923–924.
51. Li Z, Lin H, Yang S, Diehl AM. Murine leptin deficiency alters Kupffer cell production of cytokines that regulate the innate immune system. Gastroenterology 2002;123:1304–1310.
52. Chitturi S, Farrell G, Frost L, Kriketos A, Lin R, Fung C, Liddle C, Samarasinghe D, George J. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? Hepatology 2002;36:403–409.
53. Leclercq IA, Farrell GC, Schriemer R, Robertson GR. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. J Hepatol 2002;37:206–213.
54. Oben JA, Roskams T, Yang S, Lin H, Sinelli N, Li Z, Torbenson M, Thomas SA, Diehl AM. Norepinephrine induces hepatic fibrogenesis in leptin deficient ob/ob mice. Biochem Biophys Res Commun 2003;308:284–292.
55. Yin M, Wheeler MD, Kono H, Bradford BU, Gallucci RM, Luster MI, Thurman RG. Essential role of tumor necrosis factor alpha in alcohol-induced liver injury in mice. Gastroenterology 1999;117: 942–952.
56. Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin reverses fatty liver disease in obese, leptin-deficient mice. Nat Med 2000;6:998–1003.
57. Solomon SS, Mishra SK, Cwik C, Rajanna B, Postlethwaite AE. Pioglitazone and metformin reverse insulin resistance induced by tumor necrosis factor-alpha in liver cells. Horm Metab Res 1997;29:379–382.
58. Tomita K, Azuma T, Kitamura N, Nishida J, Tamiya G, Oka A, Inokuchi S, Nishimura T, Suematsu M, Ishii H. Pioglitazone prevents alcohol-induced fatty liver in rats through up-regulation of c-Met. Gastroenterology 2004;126:873–885.
59. Ohata M, Suzuki H, Sakamoto K, Hashimoto K, Nakajima H, Yamauchi M, Hokkyo K, Yamada H, Toda G. Pioglitazone prevents acute liver injury induced by ethanol and lipopolysaccharide through the suppression of tumor necrosis factor-alpha. Alcohol Clin Exp Res 2004;28:139S–144S.
60. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746–26749.
61. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996;271: 10697–10703.
62. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y,

Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002;8:731–737.
63. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? Hepatology 2004;40:46–54.
64. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;257:79–83.
65. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595–1599.
66. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T, Noda T. Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem 2002;277:25863–25866.
67. Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, Arita Y, Okamoto Y, Shimomura I, Hiraoka H, Nakamura T, Funahashi T, Matsuzawa Y. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003;23:85–89.
68. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003;423:762–769.
69. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proc Natl Acad Sci U S A 2004;101:10308–10313.
70. Lopez-Bermejo A, Botas P, Funahashi T, Delgado E, Kihara S, Ricart W, Fernandez-Real JM. Adiponectin, hepatocellular dysfunction and insulin sensitivity. Clin Endocrinol (Oxf) 2004;60:256–263.
71. Musso G, Gambino R, Biroli G, Carello M, Faga E, Pacini G, De Michieli F, Cassader M, Durazzo M, Rizzetto M, Pagano G. Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic beta-cell dysfunction in nondiabetic nonobese patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2005;100:2438–2446.
72. Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, Gentilcore E, Natale S, Cassader M, Rizzetto M, Pasquali R, Marchesini G. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. J Clin Endocrinol Metab 2005;90:3498–3504.
73. Kaser S, Moschen A, Cayon A, Kaser A, Crespo J, Pons-Romero F, Ebenbichler CF, Patsch JR, Tilg H. Adiponectin and its receptors in non-alcoholic steatohepatitis. Gut 2005;54:117–121.
74. Tietge UJ, Boker KH, Manns MP, Bahr MJ. Elevated circulating adiponectin levels in liver cirrhosis are associated with reduced liver function and altered hepatic hemodynamics. Am J Physiol 2004;287:E82–E89.
75. Kaser S, Moschen A, Kaser A, Ludwiczek O, Ebenbichler CF, Vogel W, Jaschke W, Patsch JR, Tilg H. Circulating adiponectin reflects severity of liver disease but not insulin sensitivity in liver cirrhosis. J Intern Med 2005;258:274–280.
76. Pineiro R, Iglesias MJ, Gallego R, Raghay K, Eiras S, Rubio J, Dieguez C, Gualillo O, Gonzalez-Juanatey JR, Lago F. Adiponectin is synthesized and secreted by human and murine cardiomyocytes. FEBS Lett 2005;579:5163–5169.
77. Delaigle AM, Jonas JC, Bauche IB, Cornu O, Brichard SM. Induction of adiponectin in skeletal muscle by inflammatory cytokines: in vivo and in vitro studies. Endocrinology 2004;145:5589–5597.
78. Wolf AM, Wolf D, Avila MA, Moschen AR, Berasain C, Enrich B, Rumpold H, Tilg H. Up-regulation of the anti-inflammatory adipokine adiponectin in acute liver failure in mice. J Hepatol 2006;44:537–543.
79. Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, Paschke R. Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. Biochem Biophys Res Commun 2003;301:1045–1050.
80. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. Am J Physiol 2003;285:E527–E533.
81. Keller P, Moller K, Krabbe KS, Pedersen BK. Circulating adiponectin levels during human endotoxaemia. Clin Exp Immunol 2003;134:107–110.
82. Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, Shimomura I. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes 2003;52:1655–1663.
83. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. Proc Natl Acad Sci U S A 2003;100:15712–15717.
84. Promrat K, Lutchman G, Uwaifo GI, Freedman RJ, Soza A, Heller T, Doo E, Ghany M, Premkumar A, Park Y, Liang TJ, Yanovski JA, Kleiner DE, Hoofnagle JH. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. Hepatology 2004;39:188–196.
85. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. Hepatology 2003;38:1008–1017.
86. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999;100:2473–2476.
87. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 2000;96:1723–1732.
88. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. Circulation 2004;109:2046–2049.
89. Wulster-Radcliffe MC, Ajuwon KM, Wang J, Christian JA, Spurlock ME. Adiponectin differentially regulates cytokines in porcine macrophages. Biochem Biophys Res Commun 2004;316:924–929.
90. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in

human leukocytes. Biochem Biophys Res Commun 2004;323:630–635.
91. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest 2003;112:91–100.
92. Kamada Y, Tamura S, Kiso S, Matsumoto H, Saji Y, Yoshida Y, Fukui K, Maeda N, Nishizawa H, Nagaretani H, Okamoto Y, Kihara S, Miyagawa J, Shinomura Y, Funahashi T, Matsuzawa Y. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. Gastroenterology 2003;125:1796–1807.
93. Masaki T, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, Yoshimatsu H. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-A^{y} obese mice. Hepatology 2004;40:177–184.
94. Sennello JA, Fayad R, Morris AM, Eckel RH, Asilmaz E, Montez J, Friedman JM, Dinarello CA, Fantuzzi G. Regulation of T cell-mediated hepatic inflammation by adiponectin and leptin. Endocrinology 2005;146:2157–2164.
95. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91.
96. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 1997;389:610–614.
97. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995;95:2111–2119.
98. Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T. Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. J Clin Endocrinol Metab 1998;83:2907–2910.
99. Mantzoros CS, Moschos S, Avramopoulos I, Kaklamani V, Liolios A, Doulgerakis DE, Griveas I, Katsilambros N, Flier JS. Leptin concentrations in relation to body mass index and the tumor necrosis factor-alpha system in humans. J Clin Endocrinol Metab 1997;82:3408–3413.
100. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, Klein S, Coppack SW. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 1997;82:4196–4200.
101. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972–978.
102. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 1996;271:665–668.
103. Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature 1998;396:77–80.
104. Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). J Biol Chem 2000;275:9047–9054.
105. Paz K, Hemi R, LeRoith D, Karasik A, Elhanany E, Kanety H, Zick Y. A molecular basis for insulin resistance. Elevated serine/threonine phosphorylation of IRS-1 and IRS-2 inhibits their binding to the juxtamembrane region of the insulin receptor and impairs their ability to undergo insulin-induced tyrosine phosphorylation. J Biol Chem 1997;272:29911–29918.
106. Sethi JK, Xu H, Uysal KT, Wiesbrock SM, Scheja L, Hotamisligil GS. Characterisation of receptor-specific TNFalpha functions in adipocyte cell lines lacking type 1 and 2 TNF receptors. FEBS Lett 2000;469:77–82.
107. Uysal KT, Wiesbrock SM, Hotamisligil GS. Functional analysis of tumor necrosis factor (TNF) receptors in TNF-alpha-mediated insulin resistance in genetic obesity. Endocrinology 1998;139:4832–4838.
108. Csehi SB, Mathieu S, Seifert U, Lange A, Zweyer M, Wernig A, Adam D. Tumor necrosis factor (TNF) interferes with insulin signaling through the p55 TNF receptor death domain. Biochem Biophys Res Commun 2005;329:397–405.
109. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, Shoelson SE. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 2001;293:1673–1677.
110. Ebstein W. Zur therapie des diabetes mellitus, insbesondere über die anwendung des salicylsauren natron bei demselben. Berl Klin Wochenschrift 2005;13:337–340.
111. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 2005;11:183–190.
112. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M. IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 2005;11:191–198.
113. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 2004;350:664–671.
114. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 2004;306:457–461.
115. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. Nature 2002;420:333–336.
116. Nguyen MT, Satoh H, Favelyukis S, Babendure JL, Imamura T, Sbodio JI, Zalevsky J, Dahiyat BI, Chi NW, Olefsky JM. JNK and tumor necrosis factor-{alpha} mediate free fatty acid-induced insulin resistance in 3T3-L1 adipocytes. J Biol Chem 2005;280:35361–35371.
117. Makowski L, Brittingham KC, Reynolds JM, Suttles J, Hotamisligil GS. The fatty acid-binding protein, aP2, coordinates macrophage cholesterol trafficking and inflammatory activity. Macrophage expression of aP2 impacts peroxisome proliferator-activated receptor gamma and IkappaB kinase activities. J Biol Chem 2005;280:12888–12895.
118. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 1998;391:82–86.
119. Daynes RA, Jones DC. Emerging roles of PPARs in inflammation and immunity. Nat Rev Immunol 2002;2:748–759.
120. Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nat Med 2001;7:48–52.
121. Seo JB, Moon HM, Kim WS, Lee YS, Jeong HW, Yoo EJ, Ham J, Kang H, Park MG, Steffensen KR, Stulnig TM, Gustafsson JA, Park SD, Kim JB. Activated liver X receptors stimulate adipocyte differentiation through induction of peroxisome proliferator-activated receptor gamma expression. Mol Cell Biol 2004;24:3430–3444.
122. Joseph SB, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. Nat Med 2003;9:213–219.
123. Shimomura I, Bashmakov Y, Horton JD. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse

models of diabetes mellitus. J Biol Chem 1999;274:30028–30032.
124. Horton JD, Shimomura I, Ikemoto S, Bashmakov Y, Hammer RE. Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. J Biol Chem 2003; 278:36652–36660.
125. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183–1192.
126. Leclercq IA, Farrell GC, Field J, Bell DR, Gonzalez FJ, Robertson GR. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. J Clin Invest 2000; 105:1067–1075.
127. Schattenberg JM, Wang Y, Singh R, Rigoli RM, Czaja MJ. Hepatocyte CYP2E1 overexpression and steatohepatitis lead to impaired hepatic insulin signaling. J Biol Chem 2005;280:9887–9894.
128. Xu Z, Chen L, Leung L, Yen TS, Lee C, Chan JY. Liver-specific inactivation of the Nrf1 gene in adult mouse leads to nonalcoholic steatohepatitis and hepatic neoplasia. Proc Natl Acad Sci U S A 2005;102:4120–4125.
129. Chavin KD, Yang S, Lin HZ, Chatham J, Chacko VP, Hoek JB, Walajtys-Rode E, Rashid A, Chen CH, Huang CC, Wu TC, Lane MD, Diehl AM. Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. J Biol Chem 1999;274:5692–5700.
130. Sreekumar R, Rosado B, Rasmussen D, Charlton M. Hepatic gene expression in histologically progressive nonalcoholic steatohepatitis. Hepatology 2003;38:244–251.

Received November 18, 2005. Accepted May 11, 2006.

Address requests for reprints to: Herbert Tilg, MD, Department of Medicine, Christian Doppler Laboratory for Gut Inflammation and Clinical Division of Gastroenterology and Hepatology, Innsbruck Medical University, Anichstrasse 35, 6020 Innsbruck, Austria. e-mail: Herbert.Tilg@uibk.ac.at; fax: +43 512-504-67-23374.

Supported by the Christian Doppler Society and a grant from the Austrian Science Foundation (P17447), National Institutes of Health USA (DK52539 and DK64360), and the American Diabetes Association.

The authors gratefully acknowledge the contributions of Dr. Alexander Moschen, Clinical Division of Gastroenterology and Hepatology, Innsbruck Medical University, in the preparation of this article.