

The Metabolically Benign and Malignant Fatty Liver

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The epidemics of the major metabolic diseases type 2 diabetes and cardiovascular disease are increasing worldwide and much effort is being undertaken to understand the pathogenesis of these conditions. Both type 2 diabetes and cardiovascular disease share insulin resistance as a common and important risk factor in their natural history. It is now widely accepted that organ cross talk harbors many critical clues that help to better understand the pathogenesis of insulin resistance. In this respect, several studies recently showed that not the increase in body fat mass per se, but the accumulation of fat in the visceral cavity and particularly in the liver, which are conditions commonly accompanied by inflammatory processes, are responsible for the genesis of insulin resistance (1–4).

NONALCOHOLIC FATTY LIVER DISEASE

Prevalence, pathogenesis, and progression. The prevalence of nonalcoholic fatty liver disease (NAFLD) has increased worldwide, affecting more than 25% of adults. In obese people and in patients with type 2 diabetes, the prevalence has increased to more than 70%. In obese children, NAFLD is found in 23–53% (5).

Several mechanisms lead to fatty liver. First, expansion and inflammation of adipose tissue results in adipose insulin resistance and increased lipolysis and thereby in an elevated flux of free fatty acids into the liver. Second, an impaired hepatic fatty acid oxidation and a decrease in proteins inducing lipid oxidation, e.g., adiponectin, results in further accumulation of fat within the liver. Third, increased hepatic de novo lipogenesis driven by hyperinsulinemia and increased carbohydrate intake elevate the hepatic fat content. Fourth, impaired VLDL secretion exacerbates this process. This stage is often referred to as benign steatosis. The overwhelming of lipid oxidation capacity eventually occurs resulting in the generation of reactive oxidative species (ROS), gut-derived signals (e.g., bacterial endotoxins, short-chain fatty acids), inflammatory cytokines, and an imbalanced release of adipokines that then may result in the advance of this condition toward more severe stages such as steatohepatitis, fibrosis, and cirrhosis (5).

It is interesting that in the natural history of NAFLD about 60% of the subjects have no change in their liver

histology parameters during a follow-up period of 3.5 to 11 years and 13% even have an improvement. On the other hand, within the same period of time, 28% of the subjects have a progression to liver damage as steatohepatitis, fibrosis, or cirrhosis (5). This has resulted in the fields of gastroenterology and hepatology that the terms “benign” and “malignant” are being used to separate these hepatic consequences of NAFLD.

What justifies the modified use of these terms when it comes to metabolic diseases? It is the emerging observation that NAFLD without any liver-specific consequences is often already strongly associated with metabolic alterations, most importantly with insulin resistance, which plays an important role in the pathophysiology of dyslipidemia, type 2 diabetes, and cardiovascular disease. As most of the people with fatty liver do not progress to advanced stages of liver diseases, yet are confronted with metabolic diseases that involve hepatic signaling processes, this article focuses on the early stages. Because fatty liver in animals and humans can also be found without these metabolic disorders, which is another focus of this article, the terms “metabolically benign” and “metabolically malignant” are being used to carefully describe these two interesting conditions.

NAFLD as a risk factor for type 2 diabetes and cardiovascular disease. Mainly driven by epidemiological studies, evidence accumulated that fatty liver, measured by ultrasonography or estimated from elevated blood markers of fatty liver and hepatic inflammation, is not only cross-sectionally associated with insulin resistance independent of measures of adiposity in adults and children, but also predicts incident type 2 diabetes and cardiovascular disease (6–8). These findings indicate that fatty liver is directly involved in the pathogenesis of these diseases. However, it may be that it was not possible to precisely account for the exceptionally strong relationship of fatty liver with visceral adiposity in these studies. Hence, the true pathogenic factor in subjects with fatty liver may be visceral obesity. Furthermore, fatty liver simply may be a consequence of elevated levels of insulin and glucose, which induce de novo hepatic lipogenesis, and of circulating free fatty acids, all of which are found in insulin resistance. If this was the only explanation for the observed relationships, then fatty liver can serve as a very good marker of the elevated risk, e.g., in the prediabetic state, but specifically targeting fat accumulation in the liver is not a promising way when it comes to the prevention or treatment of type 2 diabetes or cardiovascular disease.

Fatty liver as a cause of insulin resistance
Hepatic insulin resistance and hepatic inflammation. However, data emerged indicating that fatty liver may indeed be an important cause of insulin resistance. In hepatic steatosis, free fatty acid and triacylglycerol (TAG) metabolites (fatty acyl-CoA, diacylglycerol [DAG], ceramides) accumulate. In particular, DAG activates protein kinase C (PKC) isoforms, which were shown to phosphorylate

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serine residues in insulin receptor substrate (IRS)-1/2, thereby inhibiting insulin signal transduction and ultimately increasing hepatic glucose production (9). Furthermore, fatty acids can induce intracellular inflammation by stimulating the nuclear factor- κ B (NF- κ B) or by generating endoplasmic reticulum (ER) and oxidative stress, mediated by activation of c-Jun N-terminal kinase (JNK) 1. In this aspect, the saturated fatty acid palmitate plays a major role. It was found to inhibit hepatocyte insulin signaling via JNK activation. Interestingly, this appears to be mediated by increased β -oxidation of palmitate resulting in increased electron flux in the mitochondrial respiratory chain and increased ROS production (10), which is known to induce insulin resistance (11). Thus, high mitochondrial oxidative phosphorylation, fueled by palmitate metabolism, may induce ROS production and thereby insulin resistance. Moreover, predominantly saturated fatty acids, ligands of the membrane-bound Toll-like receptor (TLR) 2 and 4, are thought to induce insulin resistance and inflammation virtually by the same intracellular mechanisms without being converted to fatty acyl-CoAs (2).

Interestingly, recently data emerged showing that excessive mitochondrial free cholesterol may be a major cause for the progression of steatosis to more severe forms of liver disease (12). Furthermore, impaired hepatic protein metabolism is involved in the pathogenesis of hepatic inflammation. In this respect particularly metabolism of methionine, which is required for availability of the antioxidant glutathione and is a precursor of s-adenosylmethionine, the key methyl donor for phosphatidylcholine synthesis that is required for the export of VLDL triglycerides, is important (13). In addition, lipopolysaccharides and other proinflammatory molecules such as flagellin, peptidoglycan, or lipoproteins are increasingly found in the circulation as a result of altered gut microbial composition and bind to TLRs (2) (Fig. 1). Thus, the close interaction of immune cells with the metabolically active hepatocytes is thought to trigger local, but also systemic subclinical inflammation.

Hepatokines. Important support for a role of fatty liver in the early stages of the pathogenesis of insulin resistance then came from studies showing that mechanisms of fatty liver-induced metabolic diseases may differ from those of expanded adipose tissue mass. The new concept that we and other investigators propose is that the fatty liver releases factors in the circulation, similarly to the expanded and inflamed adipose tissue (adipokines), which can be called hepatokines, and that they have direct effects on target tissues. In this aspect we carefully studied the protein fetuin-A, which is exclusively secreted from the liver. Two decades ago fetuin-A was identified as a strong inhibitor of insulin signaling (14). We and other investigators recently showed that fetuin-A strongly induces inflammatory cytokine expression in monocytes and in mouse and human adipose tissue, and that it decreases the expression of the insulin-sensitizing and anti-inflammatory adipokine adiponectin (15,16). Furthermore, fetuin-A was found to be upregulated in fatty liver in mice and in humans (17). Finally, variants in the fetuin-A gene (*AHSG*) and circulating levels of fetuin-A predict both incident type 2 diabetes (18) and cardiovascular disease (19) independently from other well-established risk-markers. Importantly, using the Mendelian randomization approach allowed us to show that these relationships are most probably causative (20). This supports an important and independent role of fatty liver in the pathogenesis of these diseases.

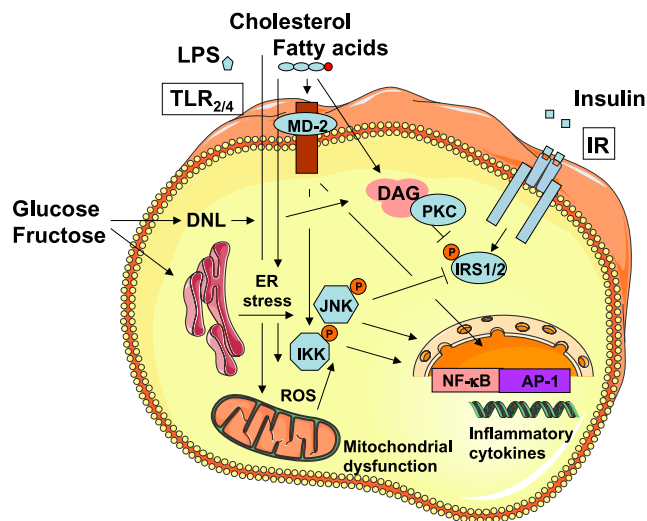


FIG. 1. Pathways involved in inflammation and metabolism in human fatty liver disease. Abundant levels of glucose, fructose, and free fatty acids directly, and fatty acids and lipopolysaccharides (LPSs) via TLR2 and 4 signaling, induce ER stress. Fatty acids and free cholesterol are also thought to induce mitochondrial dysfunction and increase ROS production. This results in the activation of inflammatory pathways involving JNK and IKK, which then induce the transcription of inflammatory cytokines and consequently play a role in the inhibition of insulin signaling via IRS1 and 2. By increasing hepatic de novo lipogenesis (DNL), fatty acids, glucose, and fructose increase the DAG pool which, through activation of PKCs, also impairs insulin signaling. IR, insulin receptor. P, phosphorylation. MD-2, myeloid differentiation protein-2.

In addition, sex hormone-binding globulin (SHBG), which was also shown to be exclusively released from the liver and was found to be causally involved in the protection from diabetes (21), was found to be suppressed in fatty liver in animals during increased glucose and fructose-induced de novo lipogenesis and in humans (22).

More recently, Misu et al. (23) identified selenoprotein P, which is primarily produced by the liver and serves as a selenium supply protein, to be upregulated in the liver of patients with type 2 diabetes and to be associated with insulin resistance. Furthermore, they could show that glucose and palmitate upregulate while insulin downregulates selenoprotein P mRNA expression in cultured hepatocytes. Moreover, they provided proof that selenoprotein P induces insulin resistance in vitro and in animals (23). Thus, there is strong support for the hepatokines fetuin-A, SHBG, and selenoprotein P to play an important role in the pathogenesis of insulin resistance and subclinical inflammation. Finally, studies showing that liver fat content, much stronger than visceral fat mass, determines insulin sensitivity in humans (3,4), support a direct and major role of fatty liver in the pathogenesis of insulin resistance. As a result, there are now worldwide efforts in the scientific field aimed at studying the impact of fat accumulation in the liver as a cause for metabolic diseases.

Dissociation between fatty liver and insulin resistance TAG synthesis and breakdown. However, this may not be easy in the first instance. Mechanisms that are involved in the development of impaired metabolic signaling and more severe hepatic stages of NAFLD are not thought to be associated with increased TAG storage but increased availability of hepatic fatty acyl-CoAs (24). Proof of this concept first emerged from animal data suggesting that under certain conditions, dissociation between fatty liver and insulin resistance is present. The liver-specific

acyl:CoA:diacylglycerol acyltransferase 2 (*Dgat2*) transgenic mouse had a fivefold increase in liver TAG content compared with controls, however, it did not develop whole-body or hepatic insulin resistance (25). DGAT2 catalyzes the final step and therefore the rate-limiting reaction of TAG biosynthesis. It covalently joins a fatty acyl-CoA and a sn-1,2-DAG molecule to form TAGs. This suggests that DGAT2 is responsible for incorporating endogenously synthesized monounsaturated fatty acids into TAGs. Consequently, DGAT2 may protect from fatty acid signaling and fatty acid toxicity. This hypothesis is supported by the finding that on a high-fat diet, activation of JNK and NF- κ B in *Dgat2* transgenic mice was not increased compared with controls (25).

Further support that TAGs represent a safe harbor for fatty acyl-CoAs was provided from the adipose triglyceride lipase (*Atgl*)-deficient mouse model. Under a high caloric diet, large amounts of lipids accumulated in several tissues, including the liver, in these animals. However, compared with controls, these mice remained insulin sensitive (26). In addition to the activity of lipases, the form of package of the lipids in the liver is important. In this respect, the deficiency of perilipin, adipose differentiation-related protein, tail interacting protein of 47 kD (PAT) proteins was found to result in a large increase in hepatic lipid droplet (LD) size and a reduction in LD number. This was accompanied by a 2.5-fold increase in the lipolytic rate with an increase of the ATGL at the LD surface and by increased insulin resistance, as indicated by a decrease in insulin-stimulated Akt phosphorylation (27).

Fatty acid pattern. In hepatocytes, other mechanisms also protect from lipotoxicity. Mice deficient for the elongation of long-chain fatty acids-6 (*Elovl6*) gene developed obesity and hepatic steatosis, but not insulin resistance, hyperinsulinemia, or hyperglycemia under a high-fat, high-sucrose diet (28). ELOVL6, which is induced by sterol regulatory element-binding protein-1c, catalyzes the conversion of the saturated fatty acid palmitate (C 16:0) to stearate (C 18:0) as well as the monounsaturated fatty acid palmitoleate (C 16:1n-7) to vaccinate (C 18:1n-7), thus regulating the tissue fatty acid composition. In this animal model, the expression of peroxisome proliferator-activated receptor (PPAR)- α and PPAR- α target genes, which induce fatty acid oxidation, was reduced. Mechanisms involved in this process resulting in hepatic steatosis are not known. Interestingly, in the *Elovl6*-deficient mice, hepatic inflammatory processes were not altered. However, liver-specific decrease in the activity of the DAG-PKC ϵ pathway was found. As expected, hepatic levels of the fatty acid palmitoleate, which protect from insulin resistance, also were elevated in the *Elovl6*-deficient mice (28).

In addition, stearoyl-CoA-desaturase (SCD) 1, which converts saturated fatty acids to less harmful monounsaturated fatty acids, is another interesting candidate involved in the protection from lipotoxicity. While data in the literature about the role of SCD1 in this process are not consistent, there is increasing evidence that SCD1, which is involved in the channeling of toxic fatty acids into triglyceride pools, may prevent lipotoxicity (29). In agreement with this hypothesis in animals, SCD1 deficiency under a methionine- and choline-deficient diet was found to be associated with less hepatic TAG accumulation compared with wild-type animals, however with increased hepatocellular apoptosis and steatohepatitis (30).

Inflammation. What about lipid-induced hepatic inflammation? It was shown that NF- κ B transcriptional targets

are activated in the liver by obesity and a high-fat diet, resulting in a chronic state of subacute inflammation and insulin resistance. Inhibition of NF- κ B activation under a high-fat diet, through expression of the dominant inhibitory I κ B α , then still resulted in hepatic steatosis, however this was not accompanied by insulin resistance (31). Similar results were found by partial suppression of hepatic NF- κ B activation via inactivation of the NF- κ B essential modulator gene NEMO (32). These findings indicate that under conditions of inhibited NF- κ B activation, fatty liver does not result in hepatic inflammation and insulin resistance. Thus, inhibition of hepatic inflammatory signaling may be a promising therapeutic tool to prevent and treat metabolic consequences of fatty liver as hepatic insulin resistance. However, caution is needed when doing this since recent advances in this field helped to understand in more detail the complex system of hepatic cytokine signaling. In this aspect, it was shown that mice with hepatic deficiency of interleukin-6 receptor a (IL-6Ra) unexpectedly developed insulin resistance in liver, skeletal muscle, and white adipose tissue (33).

In addition, and again unexpectedly, ablation of JNK1 in hepatocytes resulted in glucose intolerance, insulin resistance, and hepatic steatosis (34). Therefore, more knowledge needs to be gathered prior to regulating different proposed proinflammatory signaling pathways in humans.

In this respect, Kupffer cells represent interesting targets. Kupffer cells deriving from circulating monocytes are involved in several hepatic processes including presentation of antigens during viral infections of the liver, the removal of particulate material from the portal circulation, the production of nitric oxide, and the generation of several cytokines. Recently, Kupffer cell depletion by using clodronate liposomes or gadolinium chloride in mice reduced hepatic steatosis, putatively mediated by upregulation of hepatic lipid oxidation, involving decreased proinflammatory tumor necrosis factor (TNF)- α and IL-1 β signaling (35,36). In addition, Kupffer cell depletion was

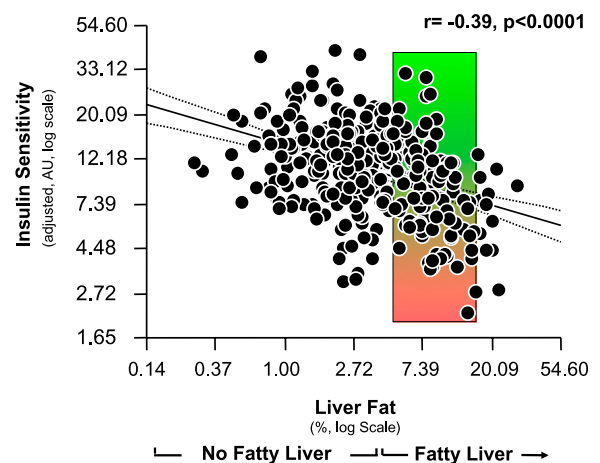


FIG. 2. Relationship between liver fat and insulin sensitivity in humans. There is a strong, negative relationship between liver fat content measured by 1 HMR spectroscopy and insulin sensitivity estimated from the OGTT (as proposed by Matsuda and DeFronzo $[10,000/\sqrt{(\text{mean insulin} \times \text{mean glucose})} \times (\text{fasting insulin} \times \text{fasting glucose})]$) after adjustment for sex, age, and total body and visceral fat mass, here shown in 337 individuals (327 without diabetes and 10 with newly diagnosed type 2 diabetes; regression line and 95% CI). However for a very similar liver fat content, individuals can be identified who are relatively insulin sensitive (green field) and insulin resistant (red field). AU, arbitrary units.

associated with improvement of hepatic insulin sensitivity in mice and with reduced inflammatory signaling, even without reducing hepatic steatosis (37). As a possible major mechanisms by which Kupffer cells are involved in the genesis of hepatic inflammation and of hepatic stellate cell activation and thus hepatic fibrosis, recently bacterial and DNA-induced TLR9 signaling and increase in IL-1 β was identified (38). This finding again supports the important role of hepatic inflammation as a regulator of hepatic lipid signaling.

When focusing on metabolic effects in the liver, the role of adipose tissue inflammation needs to be taken into account. This is not only supported by the large body of evidence regarding the regulation of adiponectin production and its effects on hepatic steatosis and inflammation (39), but also by the evidence that adipose tissue JNK1 activation induces hepatic insulin resistance, possibly by increasing IL-6 secretion (40). Interestingly, in the aforementioned study, JNK1 deficiency in myeloid cells or in hematopoietic cells resulted in reduced production of TNF- α and IL-6 from macrophages, however not in protection from high-fat diet-induced insulin resistance. In contrast, hematopoietic JNK1 (41), inhibitor of κ B kinase (IKK- β) (42), and TLR4 (43) deletion in mice in other studies was associated with prevention of hepatic and adipose tissue insulin resistance. These data highlight the important role of the interaction of adipocytes and hepatocytes with macrophages in the development of obesity-induced insulin resistance.

Human studies. In contrast to animals, information about the existence of ectopic fat accumulation in the liver,

which is not accompanied by insulin resistance, dyslipidemia, or systemic subclinical inflammation in humans, is scarce. However, data from human studies about increased ectopic storage of fat in the skeletal myocyte, which is not accompanied by insulin resistance, support the concept that TAGs represent a safe harbor for fat. Kelley (44) elegantly showed that increase in physical activity does not necessarily change the amount of intramyocellular lipid content but reduces the LD size, and this correlates with an increase in insulin sensitivity. It was hypothesized that the smaller LD size increases the surface area in relation to volume, possibly resulting in higher rates of triglyceride turnover. Furthermore, a mobile skeletal myocyte TAG pool with small, metabolically flexible LDs may help to maintain low concentrations of long-chain fatty acyl-CoAs, DAG, and ceramide, and high insulin sensitivity in skeletal muscle (44,45).

To study whether such dissociation also exists between fatty liver and insulin resistance (1) it has to be established that with increasing liver fat content, insulin sensitivity deteriorates, and that this relationship is independent of other major determinants of these phenotypes such as age, sex, and, in particular, visceral adiposity; 2) subjects need to be identified who remain insulin sensitive, although fatty liver is present; and 3) preferably genetic variability may explain this dissociation.

We performed these analyses with data from 337 subjects who participated in the Tübingen Lifestyle Intervention Program (TULIP) and who underwent measurement of total body and visceral fat by magnetic resonance (MR) tomography and liver and intramyocellular fat content by

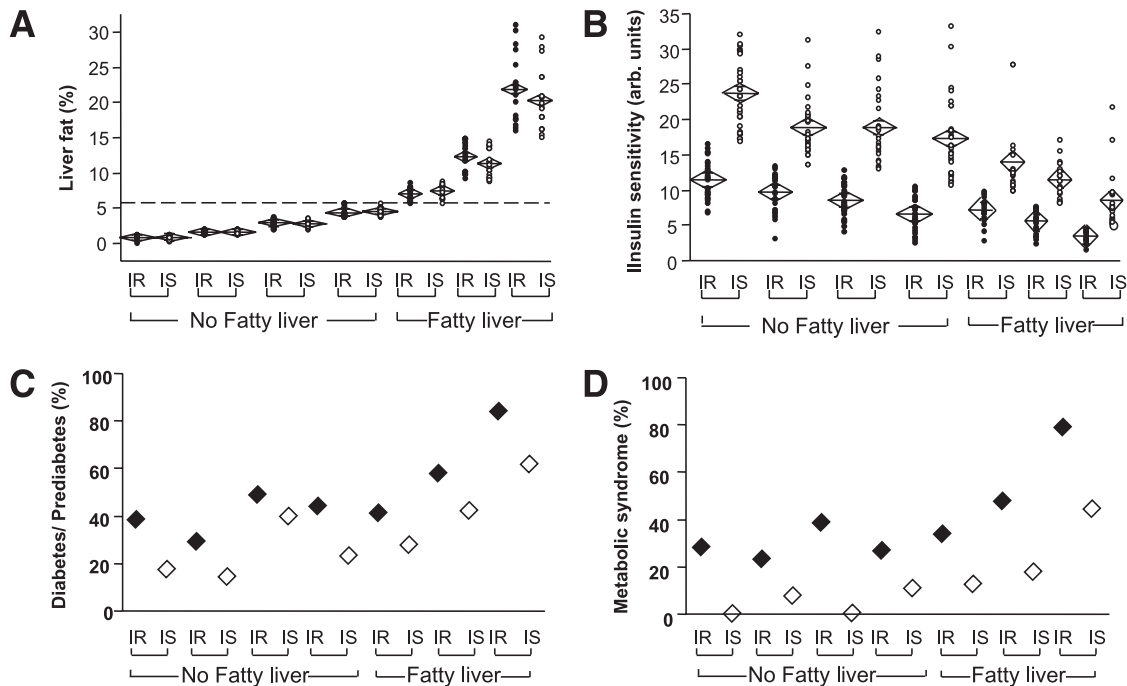


FIG. 3. Relationships of subgroups of individuals based on liver fat and the median insulin sensitivity with liver fat, insulin sensitivity, prediabetes, and the metabolic syndrome. We measured total body and visceral fat in 337 subjects by MR tomography, and liver and intramyocellular fat by ¹HMR spectroscopy. Insulin sensitivity was estimated from the OGTT (as proposed by Matsuda and DeFronzo [$10,000/\sqrt{(\text{mean insulin} \times \text{mean glucose}) \times (\text{fasting insulin} \times \text{fasting glucose})}$]). Participants were first divided into seven groups: quartiles of liver in subjects without fatty liver (liver fat <5.56%, $n = 225$) and tertiles of liver fat in subjects with fatty liver (liver fat $\geq 5.56\%$, $n = 112$). Each group was then divided by the median insulin sensitivity in an insulin sensitive (IS) and an insulin resistant (IR) subgroup. Within each of the seven groups, the subgroups did not differ in liver fat (all $P \geq 0.30$) (A). However, insulin sensitivity (B) was lower and the prevalences of prediabetes (impaired fasting glycemia and/or impaired glucose tolerance) or newly diagnosed diabetes (C) and the metabolic syndrome (National Cholesterol Education Program Adult Treatment Panel III criteria) (D) were higher in the IR compared with the IS subgroups (all $P \leq 0.0001$). Arb., arbitrary.

¹HMR spectroscopy. Indeed, we found a strong negative correlation of liver fat with insulin sensitivity estimated from the oral glucose tolerance test (OGTT) (Fig. 2), even after adjustment for sex, age, and total body and visceral fat mass. In addition, subjects could be identified who remained insulin sensitive while fatty liver was present (Fig. 2). To investigate these relationships in more detail, first we divided the individuals based on liver fat content into seven groups. Each group was then divided by the median insulin sensitivity in insulin sensitive and insulin resistant subgroups. Within each of the seven groups, the subgroups did not differ in liver fat (all $P \geq 0.30$). However, insulin sensitivity was lower, and the prevalences of the metabolic syndrome and prediabetes or newly diagnosed diabetes were higher in the insulin resistant compared with the insulin sensitive subgroups (all $P \leq 0.0001$) (Fig. 3A–D). Interestingly, among individuals with fatty liver, 37% did not have the metabolic syndrome, prediabetes, or diabetes. Furthermore, between the subgroups with fatty liver, the parameters of age, sex, and total body, visceral, or intramyocellular fat content were not different. In addition, circulating adiponectin and IL-6 markers of adipose tissue inflammation in the resting and

fasting state, which are associated with insulin sensitivity independent of visceral fat and liver fat, were not different. However, circulating plasminogen activator inhibitor-1, a putative marker of hepatic inflammation (46), and levels of the hepatokine fetuin-A were higher in the insulin resistant subgroups.

Also in agreement with the animal studies, a single nucleotide polymorphism (SNP) in *DGAT2* in humans was found to be associated with fatty liver but not with insulin resistance (47). Furthermore, such dissociation was detected in patients with familial hypobetalipoproteinemia (48). So far, mechanisms explaining this relationship are still unknown. The strongest evidence that hepatic TAG synthesis may represent a mechanism involved in the detoxification of hepatic lipids came from genomewide association studies. By far, the largest genetic impact on fatty liver in humans was found to be provided by a SNP (rs738409) in the patatin-like phospholipase 3 gene (*PNPLA3*), which is also known as adiponutrin. While the SNP was strongly associated with elevated liver fat, it was not associated with insulin resistance, dyslipidemia, or subclinical inflammation in adults (49–51). Consequently, for a similar liver fat content, the fatty liver-associated G

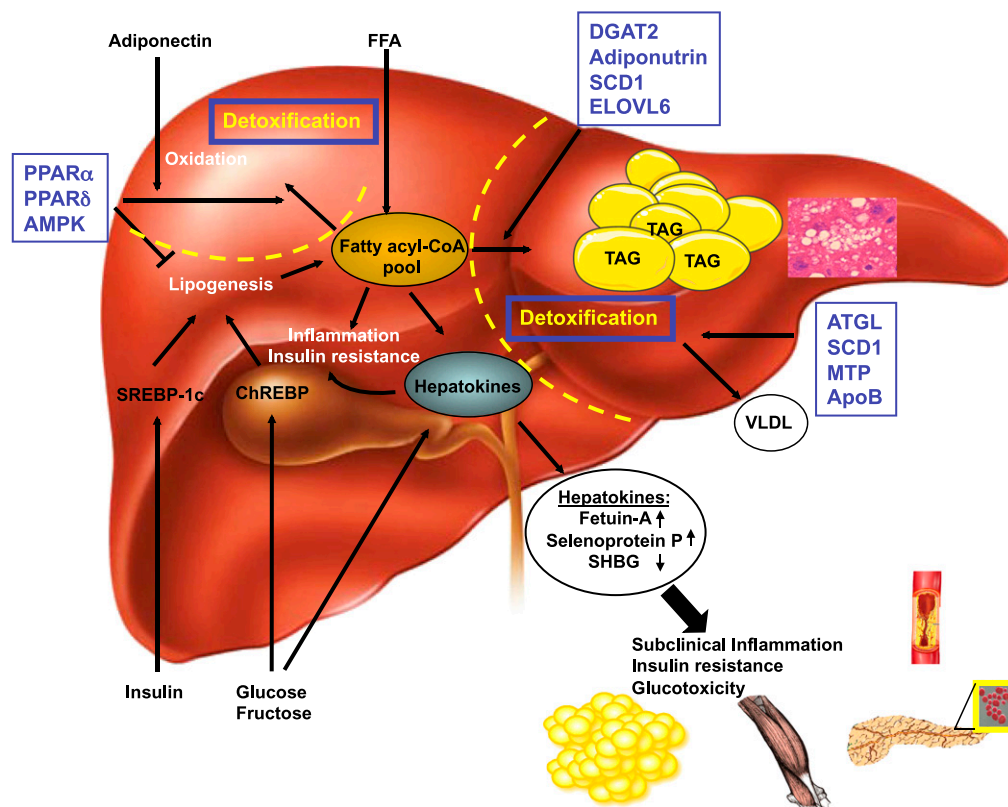


FIG. 4. Cause and metabolic consequences of fatty liver. Hyperglycemia and hyperinsulinemia induce hepatic de novo lipogenesis via carbohydrate response element-binding protein (ChREBP) and sterol regulatory element-binding protein (SREBP)-1c, respectively, thereby increasing the hepatic pool of fatty acyl-CoAs. This pool is also increased by augmented delivery of free fatty acids (FFAs) either through the diet or lipolysis in adipose tissue. Fatty acyl-CoAs are assembled to TAGs that remain in the liver or are secreted in the form of VLDLs. The latter pathway is regulated by several factors, among them the two enzymes SCD1 and DGAT2 as well as the microsomal transfer protein (MTP) and the availability of apolipoprotein B (ApoB). ELOVL6 and PNPLA3/adiponutrin are also involved in the process of hepatic TAG storage, while specific mechanisms of action are not fully understood. Finally, a low activity of the ATGL also results in storage of fatty acyl-CoAs in the form of TAGs, thereby contributing to the detoxification of hepatic lipids. This process can also be accelerated by hepatic oxidation of fatty acyl-CoAs involving the transcription factors PPAR- α and - δ . In addition, the AMP-activated kinase (AMPK) is involved. The adipokine adiponectin stimulates FA oxidation via AMPK activation and PPAR- α induction. AMPK is also involved in the suppression of lipogenesis. When these mechanisms of detoxification are overwhelmed or not active, lipotoxicity prevails resulting in hepatic inflammation and insulin resistance. Via dysregulation of secreted hepatokines (e.g., fetuin-A, SHBG, selenoprotein P), increased glucose production and dyslipidemia, fatty liver then also induces systemic subclinical inflammation, whole-body insulin resistance, hyperglycemia, and ultimately the manifestation of type 2 diabetes and cardiovascular disease.

allele should be protective of metabolic consequences. This hypothesis was supported in humans when increased insulin sensitivity *in vivo* and absence of upregulation of hepatic TNF- α gene expression in human liver biopsies with increasing TAG content was found in carriers of the G allele (50). If so, then according to the current concept, G allele carriers also may be protected from more advanced stages of hepatic diseases. However, in about 600 human liver biopsy samples, the G allele not only associated with hepatic steatosis but also with indices of steatohepatitis, fibrosis, and cirrhosis (52). Again, in the same population, the G allele was not associated with an increased but with a decreased risk of type 2 diabetes and with lower serum lipid levels and blood pressure.

What is known about general mechanisms of PNPLA3/adiponutrin action? PNPLA3 may have lipogenic functions in adipose tissue and most probably also in the liver, thereby increasing hepatic TAG synthesis. Furthermore, PNPLA3/adiponutrin has transacylase functions and uses DAG as an acyl acceptor. Therefore, increased activity of the protein may also result in depletion of the hepatic DAG and the fatty acyl-CoA content, both of which are involved in hepatic insulin resistance. Based on the recent findings that carbohydrates and fatty acids increase PNPLA3 protein levels in liver cells (53), PNPLA3 may rather have a more important role in lipid remodeling than lipid catabolism. The further investigation of the impact of the rs738409 SNP in *PNPLA3* on the different zones of the hepatic acinus, as already shown for steatosis (52), will help to clarify the role of PNPLA3 in hepatic functions and ultimately may help to unravel mechanisms involved in the putative dissociation of hepatic metabolic signaling and processes related to liver damage.

CONCLUSION

Fatty liver may have an important role in the pathogenesis of the major epidemically spreading metabolic diseases: type 2 diabetes and cardiovascular disease. This hypothesis is supported by previous findings about the role of hepatic fat accumulation in the promotion of a metabolically malignant condition that is characterized by dyslipidemia and increased hepatic glucose production. Furthermore, it is supported by the recent data showing that fatty liver-derived proteins—hepatokines—are directly involved in the pathogenesis of both diseases. However, recent *in vitro* animal and human data unexpectedly revealed that under certain conditions fatty liver is not accompanied by such adverse events but is associated with a metabolically benign state. Mechanisms explaining these novel findings are effective hepatic TAG synthesis, lipid desaturation, and inhibition of lipid-induced inflammatory signaling. In particular, hepatic TAG synthesis is now recognized as an adaptive process in situations when TAG precursors are abundant and allows storage of lipids in their least toxic form. However, when these compensatory mechanisms are overwhelmed, fatty acids induce damage to cells resulting in impairment of metabolism (Fig. 4). Hence, targeting mechanisms involved in a safe cellular storage of lipids may provide promising approaches to prevent and treat type 2 diabetes and cardiovascular disease besides also inducing lipid oxidation and inhibiting inflammatory signaling. Furthermore, there is exciting emerging data indicating that hepatic metabolic signaling and mechanisms involved in the process of hepatic steatohepatitis, fibrosis, and cirrhosis may differ. Thus, future research in

this field, including studies applying a translational approach to investigate the impact of novel identified hepatic mechanisms *in vitro* and in animals and their role in humans, may be able to teach us about yet unknown mechanisms involved in the pathogenesis of metabolic diseases.

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N.S. and H.-U.H. wrote the manuscript. N.S. researched the data.

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