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REVIEW ARTICLE

Fat in the liver and insulin resistance

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Abstract
Insulin resistance in humans is not always accompanied by obesity, since severe insulin resistance also characterizes patients lacking subcutaneous fat such as those with HAART- (highly-active antiretroviral therapy)-associated lipodystrophy. Both obese and lipodystrophic patients, however, have an increase in the amount of fat hidden in the liver. Liver fat content can be accurately quantified non-invasively by proton magnetic resonance spectroscopy. It is closely correlated with fasting insulin concentrations and direct measures of hepatic insulin sensitivity while the amount of subcutaneous adipose tissue is not. An increase in liver fat content has been shown to predict type 2 diabetes, independently of other cardiovascular risk factors. This is easily explained by the fact that the liver, once fatty, overproduces most of the known cardiovascular risk factors such as very low density lipoprotein (VLDL), glucose, C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), fibrinogen and coagulation factors. The causes of inter-individual variation in liver fat content, independent of obesity, are largely unknown but could involve differences in signals from adipose tissue such as in the amount of adiponectin produced and differences in fat intake. Adiponectin deficiency characterizes both lipodystrophic and obese insulin-resistant individuals, and serum levels correlate with liver fat content. Liver fat content can be decreased by weight loss and by a low as compared to a high fat diet. In addition, treatment of both lipodystrophic and type 2 diabetic patients with peroxisome proliferators activator receptor-γ (PPARγ) agonists, but not metformin, decreases liver fat and markedly increases adiponectin levels. The fatty liver may help to explain why some but not all obese individuals are insulin resistant and why even lean individuals may be insulin resistant, and thereby at risk of developing type 2 diabetes and cardiovascular disease.

Key words: *Glucose, hypertriglyceridemia, magnetic resonance imaging, proton spectroscopy*

Introduction

Insulin resistance both precedes and predicts type 2 diabetes and increases, even in the absence of diabetes, the risk of cardiovascular disease (1). Insulin resistance is often considered synonymous with obesity, although lean subjects can be insulin resistant, and the degree of insulin resistance varies considerably amongst equally obese subjects (Figure 1). Severe insulin resistance can also be observed in the absence of subcutaneous fat in lipoatrophic patients (2). However, both lean and obese and lipoatrophic insulin-resistant individuals have an increased amount of fat in the form of triglycerides in insulin-sensitive tissues, especially in the liver and skeletal muscles. This fat appears to be the most proximal correlate of insulin resistance. Although fat accumulation in muscle is associated with insulin resistance (3), skeletal muscles do not, unlike the liver, overproduce cardiovascular risk factors once their fat content is increased. This is in contrast to the fatty liver which overproduces multiple cardiovascular risk factors such as glucose, very low density lipoprotein (VLDL) (4), plasminogen activator inhibitor-1 (PAI-1) (5), coagulation factors (6) and C-reactive protein (CRP) (7). The ensuing discussion is focused on reviewing the role of liver fat as the common denominator for insulin resistance associated with both too little (lipoatrophy) and too much (obesity) subcutaneous fat. In the ensuing discussion, liver fat refers to fat in the liver which cannot be attributed to known causes of liver disease such as alcohol, viruses, autoimmune disease, drugs or toxins (8). Indeed, as will be discussed, 70% of elevated liver enzymes are due to 'non-alcoholic fatty liver disease', a condition

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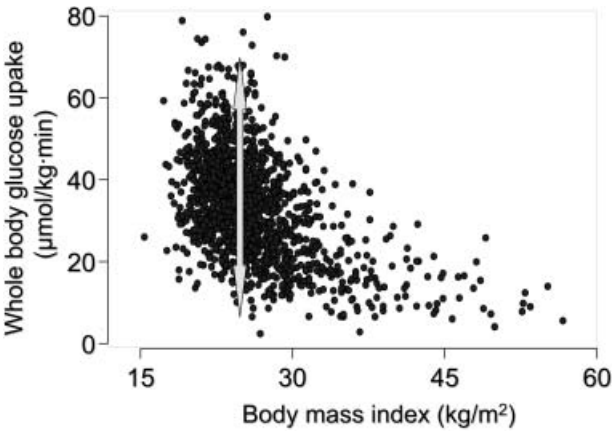

Figure 1. The relationship between body mass index (BMI) and whole body insulin sensitivity, measured using the euglycemic clamp technique, in 1,394 healthy non-diabetic European men and women whose data have been included in the European Group for Insulin resistance (EGIR) database. The arrow depicts the range of variation in whole body insulin sensitivity at a BMI of 28 kg/m^2. Data used by permission from the EGIR.

frequently if not invariably accompanied by insulin resistance.

Insulin resistance and lipoatrophy

Animal models

Genetically engineered animal models have documented that lack of subcutaneous and visceral fat is associated with severe insulin resistance and fat accumulation in insulin sensitive tissues (see (9) for review). As an example, expression of a dominant-negative protein, termed A-ZIP/F in adipose tissue prevents DNA binding of B-ZIP transcription factors of C/EBP and Jun families (10,11). These mice, named A-ZIP/F-1, have no white adipose tissue subcutaneously or elsewhere, but have severe hepatosteatosis, diabetes, and elevated glucose, insulin, triglyceride and free fatty acid (FFA) concentrations (Figure 2). Transplantation of wild-type adipose tissue into these fatless mice reverses hyperglycemia, decreases insulin concentrations, corrects insulin signaling defects and normalizes fat content in the liver and muscle (12). This implies that fat accumulation in insulin-sensitive tissues indeed can cause insulin resistance, although the stored triglyceride itself is inert.

Human lipodystrophies

The most common condition characterized by atrophy of subcutaneous fat in humans is that developing as a side effect of highly-active antiretroviral therapy (HAART) in patients with HIV infection (13). These drugs have dramatically decreased mortality from AIDS (14). However, up to 50% of the patients receiving HAART develop signs of lipodystrophy, which is characterized by loss of subcutaneous fat and accumulation of fat intra-abdominally, and severe insulin resistance (13). Patients with HAART-associated lipodystrophy as well as those with rarer forms of human lipoatrophies have an excess of fat in the liver (2). The amount of fat in the liver correlates closely with fasting insulin concentrations (4). Thus, insulin resistance can be observed, as in the mouse models, in the absence of subcutaneous but not liver fat.

Key messages

- The fatty liver (non-alcoholic fatty liver disease) is as common as the metabolic syndrome.
- Fat accumulation in the liver predisposes to type 2 diabetes.
- The liver, once fatty, overproduces almost all cardiovascular risk factors.

Insulin resistance and obesity

Insulin resistance is not synonymous with obesity, i.e. an increase in body mass index, predominantly because of an increase in subcutaneous fat mass. This is illustrated by the large variation in insulin sensitivity at a given body mass index (Figure 1). A fatty liver has long been known to be more common in non-obese than in obese subjects, but the relationship between obesity and liver fat is weak (15–17). Liver fat is, however, closely correlated with markers (4) and direct measures (15,18) of

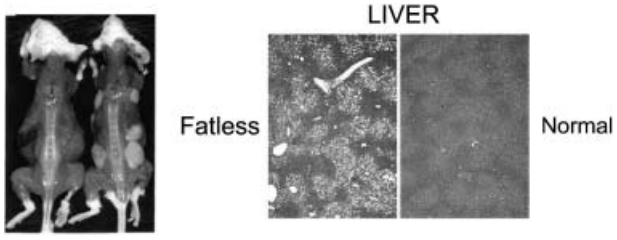

Figure 2. An example of a lipoatrophic mouse model (11). These mice (mouse on the left in the panel on the left) are unable to store fat subcutaneously but store excessive amounts of fat in the liver. The liver becomes severely insulin resistant. Transplantation of normal adipose tissue subcutaneously (mouse on the right in the panel on the left) removes excess fat and normalizes insulin sensitivity in the liver. Reproduced with permission from (11).

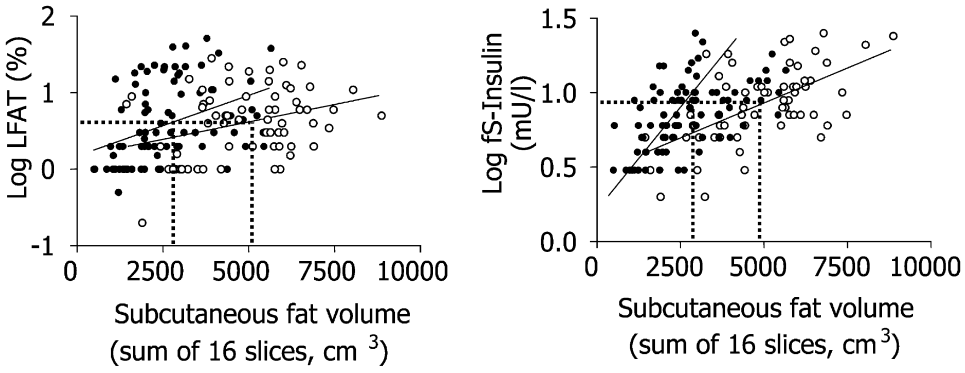

Figure 3. The relationship between the amount of subcutaneous fat measured with magnetic resonance imaging and percent liver fat (LFAT, panel on the left) and fasting serum insulin (fS-insulin, panel on the right) in 132 non-diabetic healthy men and women. Reproduced with permission from (4).

insulin resistance. This is true for both men and women, although women have more subcutaneous and less visceral fat than men (19). If the amount of subcutaneous fat is plotted against liver fat or fasting insulin, men and women fall on their own regression lines (Figure 3). However, if either liver fat or intra-abdominal fat are plotted against fasting insulin, men and women fall on the same regression line (Figure 4). These data imply that it is not the visible subcutaneous fat but rather that hidden in the liver that is the most proximal correlate of insulin resistance.

Diagnosis of a fatty liver associated with insulin resistance

Quantification of liver fat

To determine whether inter-individual differences in liver fat contribute to 'the insulin resistance syndrome', liver fat needs to be quantified accurately and other known causes of liver fat excluded. The most accurate non-invasive method to quantitate the amount of liver triglyceride is by using magnetic resonance proton spectroscopy (20). This method does not provide histological information on the nature of the fat deposits (micro- or macrovesicular steatosis), degree of inflammation or of the presence of fibrosis but liver fat measured with this technique correlates closely with that measured chemically by liver biopsy, and with computerized tomography (CT) (18,21). Ultrasound and CT techniques are widely available but are not as sensitive or quantitative as proton spectroscopy (21–23).

Figure 5 depicts the relationship between liver fat and serum alanine aminotransferase (ALT) in apparently healthy subjects. The correlation coefficient between serum ALT and liver fat measured by proton spectroscopy was 0.52 for women and 0.55 for men (4). Serum aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) were not as well correlated as S-ALT. The intercepts, but not the slopes, of the regression lines relating percent liver fat to S-ALT differed between men and women (Figure 5). This is consistent with a lower reference range of S-ALT for women as compared to men, and probably reflects gender differences in liver size.

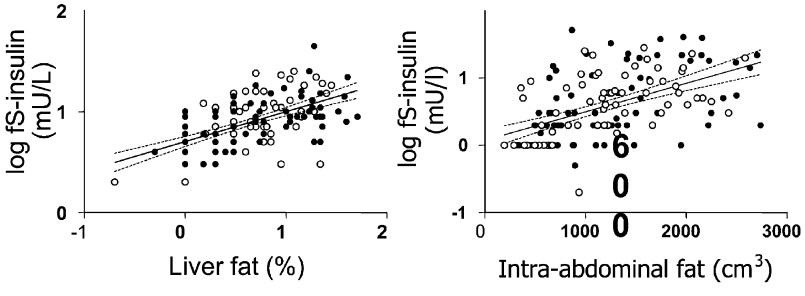

Figure 4. The relationship between the amount of liver fat and fasting serum insulin (fS-insulin, panel on the left) and between intra-abdominal fat and serum fasting insulin (panel on the right) in 132 non-diabetic healthy men and women. Reproduced with permission from (4).

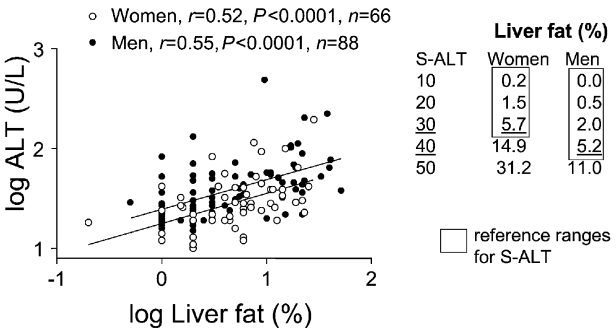

Figure 5. The relationship between liver fat measured with magnetic resonance spectroscopy and serum alanine aminotransferase activity (S-ALT) in 132 non-diabetic healthy men and women. Reproduced with permission from (4).

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of elevated liver enzymes

Triglycerides may accumulate in hepatocytes either as small or large lipid deposits (micro- or macrovesicular steatosis) (24). Microvesicular steatosis is seen in a variety of conditions in which there is either an inherited or acquired defect in β-oxidation of fatty acids such as mitochondrial cytopathies and disorders of ureagenesis, the acute fatty liver of pregnancy and Reye's syndrome (25). Causes of macrovesicular steatosis include non-alcoholic fatty liver disease (NAFLD (*vide infra*), alcohol and other causes such as total parenteral nutrition, protein-calorie malnutrition and jejuno-ileal bypass. NAFLD is a term describing a large spectrum of conditions ranging from fat alone to fat plus inflammation, fat plus ballooning degeneration and non-alcoholic steatohepatitis (NASH) (26). By definition, NAFLD can only be diagnosed in patients who do not consume significant amounts of alcohol (27). NAFLD is commonly, if not invariably, associated with features of insulin resistance and may indeed be part of, or the cause of the insulin resistance syndrome. Patients with NAFLD should not have clinical or laboratory evidence of autoimmune, viral or drug- or toxin-induced liver disease or of inborn errors of metabolism (27). NASH cannot be distinguished from other types of NAFLD without examining liver histology.

In the Third National Health and Nutrition Examination Survey (NHANES III), which is a population-based sample of 13,500 U.S. adults aged 17 to 74 years, NAFLD, when defined as an abnormal AST, ALT or GGT value without evidence for hepatitis B or C, increased transferrin saturation ($>50\%$) or excessive alcohol intake (<2 drinks for women and <3 drinks for men) was the most common cause (69% of all causes) of elevated liver enzymes (Figure 6) (28).

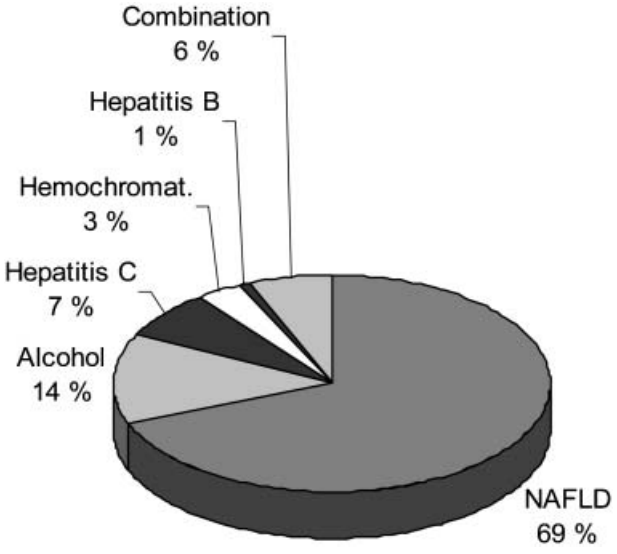

Figure 6. Causes of elevated liver function tests (serum alanine or aspartate aminotransferase above the upper limit of normal) according to the Third National Health and Nutrition Examination Survey (1988–94) including 15,676 adults aged 17 years and over in the United States. NAFLD=non-alcoholic fatty liver disease. Reproduced with permission from (28).

Why and how does fat accumulate in the liver?

Excess FFA flux to the liver could be the consequence of increased FFA delivery to the liver from peripheral or visceral depots, or FFA could originate from postprandial lipolysis of chylomicrons. In the liver, excess triglyceride deposition could be the consequence of impaired hepatic FFA oxidation or enhanced FFA extraction and triglyceride synthesis. Impaired release of VLDL could also contribute. Human data regarding the quantitative importance of these pathways to excessive hepatic triglyceride storage are not available.

Visceral fat

Truncal, or abdominal obesity is the form of obesity which is most likely associated with hepatic steatosis (29). According to the portal hypothesis, visceral adipose tissue releases excess FFA to the portal vein and exposes the liver to high FFA concentrations (30). That this view is indeed based on circumstantial rather than solid scientific evidence has been emphasized (30). The hypothesis has only recently been directly tested in man. Nielsen et al. measured systemic, splanchnic and leg FFA kinetics by combining catheterization and stable isotope techniques in men and women with varying degrees of obesity (31). Only approximately 5% and 20% of portal vein FFA originated from visceral fat in lean and obese subjects (32). The relative amount of portal vein FFA derived from visceral fat was much

less than that derived from subcutaneous fat (32). Thus, after an overnight fast, peripheral subcutaneous tissue seems to be the major source of FFA entering the liver. There are no data on FFA kinetics in relation to liver fat content in individuals with lipoatrophy.

Adiponectin-dependent mechanisms, inflammation in adipose tissue

A key question is what might enhance lipogenesis in obesity and whether any common mechanisms underlie liver fat accumulation in obesity and lipoatrophy. One possible mechanism is adiponectin deficiency, which characterizes both obesity (33) and lipoatrophy (34). Adiponectin is an adipokine exclusively produced by adipocytes, which decreases hepatic lipogenesis, and increases FFA oxidation and hepatic insulin sensitivity in mice (35). Treatment with recombinant adiponectin of mice with non-alcoholic fatty liver ameliorates hepatomegaly, steatosis, and decreases alanine aminotransferase activities (36). In humans, in several studies in lipoatrophic and obese subjects, obesity-independent correlations between adiponectin concentrations and hepatic insulin sensitivity and fat content have been observed (34,37–40). The exact causes of adiponectin deficiency in obese and lipoatrophic adipose tissue are unclear. Tumor necrosis factor-α (TNFα) is a cytokine which has recently been suggested to originate exclusively from macrophages in adipose tissue (41). The number of macrophages in adipose tissue is increased in both obese (41) and lipoatrophic (34,42) patients. TNFα gene expression is increased in obesity (43) and lipoatrophy (44), and TNFα downregulates adiponectin production in adipocytes (45). Its gene expression is inversely related to that of adiponectin in human adipose tissue (43). Thus, adipose tissue inflammation may be one factor which increases liver fat content. This does not exclude adiponectin-independent mechanisms from contributing to liver fat content. Also, adiponectin circulates in serum as a hexamer of low molecular weight and a larger multimeric structure (46). In mice, the full-length protein is active in the liver (46). The ratio of the higher molecular weight to total adiponectin seems to be better correlated with changes in insulin sensitivity than changes in the low molecular weight form during PPARγ agonist treatment in humans (46). However, neither data in mice nor correlations in humans prove causality. Since adiponectin is not available for clinical testing, it is at present unclear which form of adiponectin is active in which tissue in humans.

Adiponectin-independent mechanisms

Regarding adiponectin-independent mechanisms of hepatic fat accumulation, in rats, short-term (3 days) high-fat feeding increases hepatic triglyceride and fatty acyl-CoA content 3-fold without changing visceral or muscle fat content or body weight, or serum adiponectin concentrations (47). The liver of the high-fat fed rats was insulin resistant *in vivo*, and *in vitro* insulin resistance was characterized by normal insulin receptor but blunted insulin receptor substrate-1 (IRS-1) and IRS-2 tyrosine phosphorylation, and impaired insulin activation of protein kinase B isoform 2 (AKT2), glycogen synthase and inactivation of glycogen synthase kinase-3 (GSK3) (47). In this model hepatic insulin resistance could be abrogated by preventing hepatic fat accumulation using a mitochondrial uncoupler to increase hepatic fat oxidation (47). In humans, high as compared to low fat feeding has recently been shown to change liver fat content and serum fasting insulin concentrations without any changes in the sizes of intra-abdominal or subcutaneous fat depots or serum adiponectin concentrations (76). The molecular mechanisms of insulin resistance in the fatty liver in humans have as yet not been characterized. Data obtained using the mouse liver are of questionable significance when it comes to regulation of liver fat. For example, although PPARγ agonism has been shown in multiple studies to decrease liver fat content in humans (*vide infra*), these agents increase liver fat in lipoatrophic and obese animals (48,49). Figure 8 summarizes factors shown to regulate liver fat content or hepatic insulin sensitivity in humans.

Clinical significance of the fatty liver

The fatty liver is becoming a common concern for both diabetologists and hepatologists. This is because liver fat predicts, as discussed below, type 2 diabetes independently of common confounders, and cirrhosis especially in patients with the insulin resistance syndrome.

Liver fat predicts type 2 diabetes

In the NHANES III survey, adults with NAFLD, as compared to those without, were twice as likely to have type 2 diabetes after adjustment for age, gender, race and body mass index (50). Similar data were reported earlier from the Hispanic Health and Nutrition Examination Survey (1982–84). In this survey, data from 2,999 men and women aged 20–74 years representative of the Mexican American

population were analyzed. Six percent of men and 2% of women had an elevated ALT (43 IU/liter). The odds ratio for diabetes as a predictor of elevated ALT was 4.1 after adjustment for age and sex, and decreased to 3.0 after adjustment for age, sex, body mass index and alcohol consumption (51). These cross-sectional data have been confirmed in seven prospective studies. Elevated liver enzymes including serum glutamic pyruvic transaminase (52), gamma-glutamyltransferase (53–56) and alanine aminotransferase activities (57,58) have been shown to predict type 2 diabetes. In all studies, liver enzymes increased the risk of type 2 diabetes independent of major confounding factors such as body mass index, family history of diabetes, alcohol consumption and physical activity.

Type 2 diabetic patients have an increased risk of liver disease

Since NAFLD and insulin resistance coexist, insulin resistance precedes type 2 diabetes, and NAFLD increases the risk of cirrhosis, it is not unexpected that type 2 diabetic patients have an increased risk of cirrhosis (59). In the latter study involving 7,148 type 2 patients, the standardized mortality of liver cirrhosis was 2.5-fold increased, while the risk of death from cardiovascular disease was only 1.3-fold increased. Men with type 2 diabetes also have been reported to have a 2-fold risk of acute and chronic liver disease and hepatocellular carcinoma compared to men without type 2 diabetes (60,61).

Liver fat predicts insulin requirements during insulin therapy in type 2 diabetic patients

Inhibition of hepatic glucose production both between and during meals is a key action of insulin. During insulin therapy, inhibition of hepatic glucose production lowers plasma glucose concentrations, which counteracts (because of a diminished mass action effect of glucose to promote its own utilization) insulin stimulation of glucose utilization (62–64). The net result is an unchanged rate of glucose utilization, despite improved insulin sensitivity in patients with type 2 diabetes. This implies that the sensitivity of hepatic glucose production to insulin is a major determinant of insulin requirements. This has been documented in type 2 diabetic patients treated with basal insulin and metformin (18). Hepatic insulin sensitivity was closely correlated with liver fat content and with the daily insulin dose (18).

The fatty liver as a target for insulin-sensitizing therapy

A reduction in liver fat would seem an attractive target for anti-diabetic drugs as well as therapies aimed at preventing the development of NASH and cirrhosis. Weight loss is effective in reducing liver fat (65). Even moderate weight loss (8%) decreases liver fat by approximately 50%, irrespective of baseline liver fat content (66). The thiazolidinediones (TZDs) rosiglitazone and pioglitazone are peroxisome proliferators activator receptor-γ (PPARγ) agonists, which have recently been shown in mechanistic studies in humans to decrease liver fat content significantly and on average by the same amount (50%) as moderate weight loss (37,67–69). TZDs induce dramatic increases in serum adiponectin concentrations (37,70,71), which in turn correlate with changes in liver fat independent of body mass index. An example of effects of 16 weeks of TZD treatment on liver fat in patients with type 2 diabetes is shown in Figure 7. TZD treatment has also been shown to decrease liver fat and fasting serum insulin concentrations in non-diabetic patients with lipodystrophy (72). Two uncontrolled 48-week studies have examined whether patients with NASH benefit from pioglitazone or rosiglitazone treatment (73,74). In both studies, after the 48-week treatment period, roughly 50% of patients with NASH at baseline no longer had histological changes

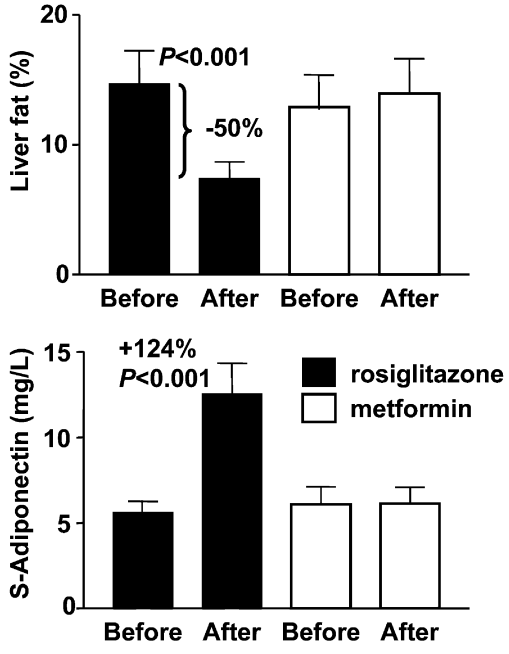

Figure 7. Effects of 4 months of rosiglitazone or metformin treatment on percent liver fat (upper panel) and serum adiponectin concentrations (lower panel) in type 2 diabetic patients. Reproduced with permission from (37).

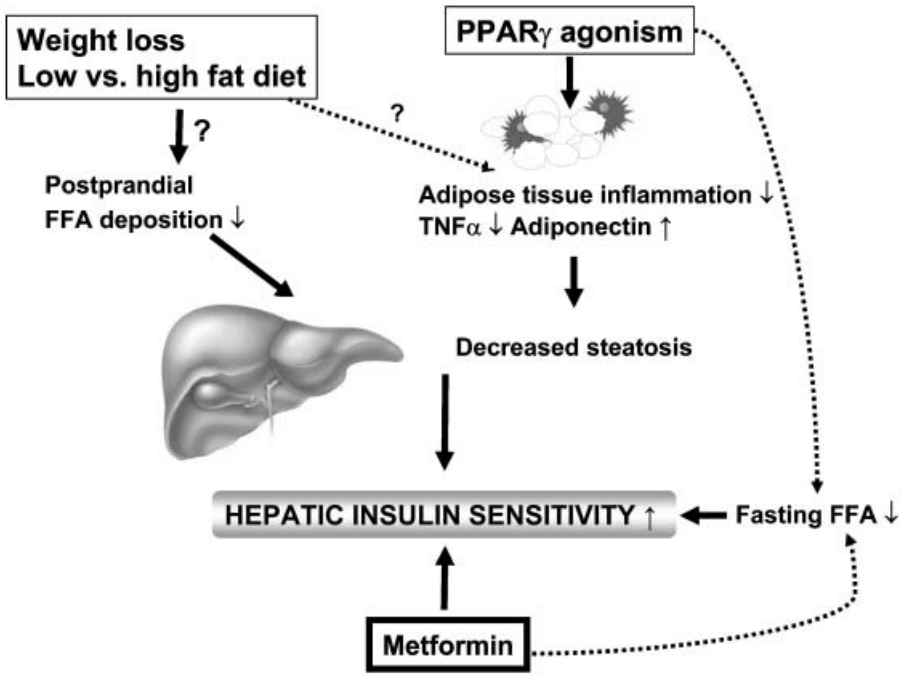

Figure 8. Regulators of liver fat content in humans. Liver fat content can be decreased by weight loss, and by lowering of dietary fat content and PPARγ agonism. While the exact mechanisms underlying the beneficial effects of weight loss and dietary fat content on liver fat are uncertain, at least one of the mechanisms via which PPARγ agonists could reduce hepatic fat content is by increasing adiponectin expression in adipose tissue. Metformin appears to increase hepatic insulin sensitivity without changing liver fat content.

consistent with this diagnosis. Results from controlled studies addressing effects of TZDs on the natural course on NASH and also on cardiovascular disease are awaited with interest (75).

Conclusions

To conclude, liver fat content is increased in both obese and lipoatrophic patients. Once fatty, the liver overproduces multiple cardiovascular risk factors such as VLDL, CRP, anti-fibrinolytic and coagulation factors. Hepatic insulin resistance also increases the amount of endogenous and exogenous insulin needed to maintain normal glucose levels. A fatty liver increases the risk of developing type 2 diabetes, and type 2 diabetes increases the risk of NAFLD, NASH and cirrhosis. Weight loss and insulin sensitizers reduce liver fat content. A major challenge is to define the mechanisms and the acquired and genetic causes underlying fat accumulation in humans.

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