

REVIEW: The Role of Insulin Resistance in Nonalcoholic Fatty Liver Disease

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Context: Insulin resistance is an almost universal finding in non-alcoholic fatty liver disease (NAFLD). This review outlines the evidence linking insulin resistance and NAFLD, explores whether liver fat is a cause or consequence of insulin resistance, and reviews the current evidence for treatment of NAFLD.

Evidence Acquisition: Evidence from epidemiological, experimental, and clinical research studies investigating NAFLD and insulin resistance was reviewed.

Evidence Synthesis: Insulin resistance in NAFLD is characterized by reductions in whole-body, hepatic, and adipose tissue insulin sensitivity. The mechanisms underlying the accumulation of fat in the liver may include excess dietary fat, increased delivery of free fatty acids to the liver, inadequate fatty acid oxidation, and increased *de novo* lipogenesis. Insulin resistance may enhance hepatic fat accu-

mulation by increasing free fatty acid delivery and by the effect of hyperinsulinemia to stimulate anabolic processes. The impact of weight loss, metformin, and thiazolidinediones, all treatments aimed at improving insulin sensitivity, as well as other agents such as vitamin E, have been evaluated in patients with NAFLD and have shown some benefit. However, most intervention studies have been small and uncontrolled.

Conclusion: Insulin resistance is a major feature of NAFLD that, in some patients, can progress to steatohepatitis. Treatments aimed at reducing insulin resistance have had some success, but larger placebo-controlled studies are needed to fully establish the efficacy of these interventions and possibly others in reducing the deleterious effects of fat accumulation in the liver. (*J Clin Endocrinol Metab* 91: 4753–4761, 2006)

NONALCOHOLIC FATTY LIVER disease (NAFLD) is a common hepatic disorder characterized by fat accumulation in the liver (Fig. 1), identical to that seen in alcoholic fatty liver disease, but in patients who do not drink excessive amounts of alcohol. Although simple steatosis (Fig. 1B) is thought to be a benign condition (1, 2), a subset of NAFLD patients develop nonalcoholic steatohepatitis (NASH). Inflammation and evidence of hepatocyte injury on liver biopsy (Fig. 1, C and D) characterize NASH, which has received more attention recently. Ultimately, and of major clinical significance, NASH can progress to fibrosis and cirrhosis (3).

The ectopic accumulation of fat in the liver has been strongly associated with insulin resistance, an almost universal finding in NAFLD (4–10). The underlying mechanisms linking NAFLD and insulin resistance are a major focus of current research. In this review, we examine the evidence that insulin resistance is a key feature of NAFLD and address the various hypotheses regarding the pathophysiology of the disorder.

Epidemiology of NAFLD

The prevalence of NAFLD is high in conditions associated with insulin resistance such as obesity, type 2 diabetes, dys-

lipidemia, and the metabolic syndrome. Although in the general population the prevalence of NAFLD and NASH is approximately 20 and 3%, respectively (11, 12), in obese populations NAFLD may affect up to 75% of subjects (13). In the morbidly obese, steatosis has been found in almost all subjects (14), with NASH being present in 25–70% of these individuals (14, 15).

NAFLD is also very common in the type 2 diabetes population with between 50 and 75% of subjects demonstrating fat in the liver by ultrasound (16–18). NAFLD, as manifest by elevated alanine aminotransferase levels, predicts the future development of diabetes (19). Additionally, the presence of diabetes has been identified as a risk factor for NASH, with one autopsy series showing a 2.6-fold increased risk of steatohepatitis in individuals who were hyperglycemic (20).

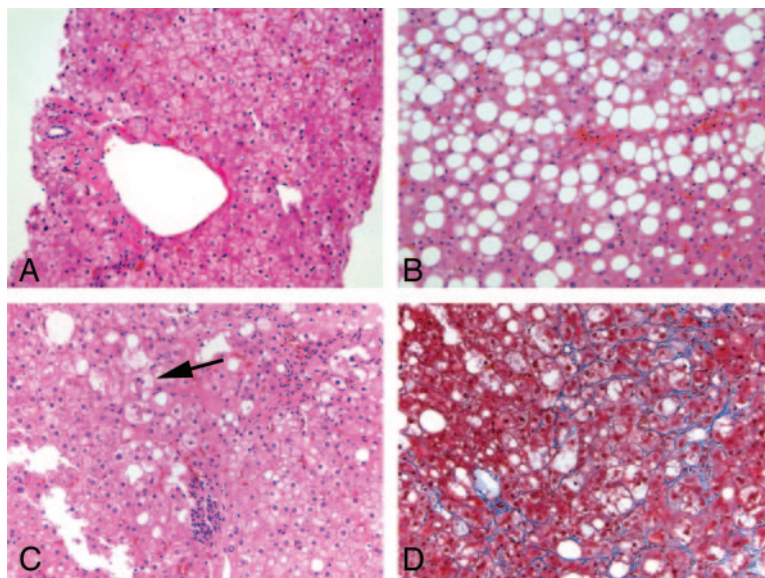
The metabolic syndrome and NAFLD are commonly associated and presence of the metabolic syndrome frequently predicts the future development of NAFLD (21). Forty-eight percent of subjects diagnosed with the metabolic syndrome in one study from China were found to have fatty liver by ultrasound (22). In the same study, fatty liver was diagnosed in 39% of those with a body mass index (BMI) 25 kg/m² or greater, in 41% of those with known diabetes, and in 32% of those with dyslipidemia (22). Conversely, in a large multi-ethnic urban U.S. population studied with magnetic resonance spectroscopy (MRS), the presence of increased hepatic triglycerides relative to low triglyceride content was more frequent in those with obesity (BMI > 30 kg/m²; 67 vs. 33%), diabetes, and/or fasting glucose more than 110 mg/dl (18 vs. 11%), lipid abnormalities (64 vs. 40%), and the metabolic syndrome (30 vs. 8%) (23).

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Abbreviations: BMI, Body mass index; FFA, free fatty acid; IRS, insulin receptor substrate; MRS, magnetic resonance spectroscopy; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SREBP-1c, sterol receptor binding protein 1-c.

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FIG. 1. Hematoxylin and eosin staining of normal liver (A), simple hepatic steatosis (B), and steatohepatitis (C) characterized by steatosis, inflammation, and hepatocyte ballooning (arrow). D, Hepatic fibrosis (blue) is shown by Masson trichrome staining. Photomicrographs were provided courtesy of Dr. Matthew Yeh (Department of Pathology, University of Washington, Seattle, WA).



As the prevalence of obesity in the United States continues to increase, with the 1999–2000 National Health and Nutrition Examination Survey showing that 34% of the adult population is overweight and an additional 30.5% is obese (24), the prevalence of NAFLD and NASH is expected to continue to rise. Furthermore and alarmingly, the increase in obesity in children is now being paralleled by the observation of NASH even in youth (25, 26).

Obesity is certainly the most common factor associated with NAFLD. However, other causes of NAFLD have also been recognized and include medications such as estrogens, tamoxifen, high-dose glucocorticoids, and amiodarone, alterations in nutrition and bowel absorption such as jejunoleal bypass, rapid weight loss, total parenteral nutrition, occupational exposure to hepatotoxins, familial syndromes of severe insulin resistance such as lipodystrophy, and disorders of lipid metabolism such as apolipoprotein B deficiency (27).

Insulin Resistance in NAFLD

NAFLD is strongly associated with both hepatic and adipose tissue insulin resistance (28–30) as well as reduced whole-body insulin sensitivity (28, 29). These studies have documented 45–50% reductions in glucose disposal, a measure of whole-body insulin sensitivity (28, 29), and an impaired ability of insulin to suppress endogenous glucose production, indicative of hepatic insulin resistance (28–30). Additionally, subjects with NAFLD exhibit a defect in insulin suppression of free fatty acids (FFA), in keeping with insulin resistance at the level of the adipocyte (24–26). Compared with control subjects, subjects with NAFLD also demonstrate a blunted inhibition of fatty acid oxidation, reflecting the decreased uptake and use of glucose as a source of fuel (29). These findings suggest the possibility that insulin resistance may be an intrinsic defect in NAFLD, and that diminished insulin responsiveness at the level of the adipocyte may contribute to hepatic steatosis by excess FFA flux to the liver.

Obesity, Adipokines, Inflammation, and NAFLD

Although overall obesity is clearly associated with NAFLD, body fat distribution appears to play an important role in the pathogenesis of NAFLD. Excess intraabdominal fat in particular may be a key determinant in the pathogenesis of NAFLD, via both its strong association with insulin resistance and possibly as a source of FFAs. Intraabdominal fat accumulation is well recognized to be associated with insulin resistance and central adiposity, so that, even in lean individuals, the accumulation of fat in this depot is associated with reduced insulin sensitivity (31). Increased intraabdominal fat deposition is also associated with what are commonly considered to be clinical manifestations associated with insulin resistance, including type 2 diabetes (32, 33), impaired glucose tolerance (34), dyslipidemia (35, 36), and the metabolic syndrome (37). Furthermore, accumulation of intraabdominal fat has also been positively correlated with liver fat (38, 39) and hepatic insulin resistance in both men and women (33).

However, evidence also suggests that liver fat may be associated with insulin resistance independent of intraabdominal fat (30, 38). Lean nondiabetic men with increased liver fat quantified by MRS had both hepatic and adipose tissue insulin resistance, manifest as impaired insulin suppression of glucose production and serum FFAs, when compared with subjects well matched for both BMI and intraabdominal fat, but with low levels of hepatic fat (30). Finally, in women with a history of gestational diabetes, hepatic fat has also been shown to correlate independently of intraabdominal fat with fasting insulin and with features of the metabolic syndrome such as hypertriglyceridemia and hypertension (9).

Although most studies have found intraabdominal fat to be more strongly associated with whole-body insulin resistance, sc fat is also important in NAFLD. Subcutaneous fat makes up the greatest proportion of the total fat mass, and total fat mass may be an important predictor of hepatic

insulin sensitivity. Although intraabdominal fat is more strongly correlated with total glucose disposal during a hyperinsulinemic euglycemic clamp, deep sc fat has also been shown to be associated with total glucose disposal during the low-dose clamp (33). However, in this study, total fat mass was the only independent predictor of hepatic insulin sensitivity in type 2 diabetic subjects (33). Subcutaneous fat, because of its greater overall mass, contributes more than intraabdominal fat to circulating FFAs, although the proximity of intraabdominal fat to the liver could create a portal-peripheral gradient for FFAs entering the liver. Fifty-nine percent of the triacylglycerol that accumulates in the liver in subjects with NAFLD is derived from circulating FFAs (40) and most of this is from nonsplanchnic sources. In lean healthy subjects during the postabsorptive phase, only about 15% of the systemic FFA flux is accounted for by visceral fat, whereas 75% is derived from the upper body and only 10% from the lower extremities (41). In subjects with type 2 diabetes, whereas overall FFA release is higher than in nondiabetic subjects, the relative contributions of upper body, splanchnic, and lower body fractional release did not differ between groups (42).

Interestingly, although excess intraabdominal and overall fat mass are associated with NAFLD, adipose tissue is not required. Humans with generalized lipodystrophy lack both sc and intraabdominal fat, but are characterized by severe insulin resistance and hepatic steatosis (43). The accumulation of ectopic fat may be a result of the severe insulin resistance, lack of adipose tissue-derived hormones such as adiponectin and leptin, or lack of fat as a storage depot, leading to fat storage in the liver. Currently, the exact mechanisms underlying insulin resistance and steatosis in lipodystrophy are not known.

The mechanism(s) whereby increased visceral adiposity is associated with insulin resistance is unclear, but circulating hormones secreted from adipose tissue have been implicated in modulating insulin sensitivity. Adiponectin is one of these adipokines, and we have found it to be associated positively with insulin sensitivity and associated negatively with intraabdominal fat (44). Adiponectin stimulates glucose use and fatty acid oxidation in the liver by activating AMP-activated protein kinase (45). Thus, low levels of adiponectin may play a role in the pathogenesis of NAFLD by decreasing fatty acid oxidation in the liver. Adiponectin levels have been found to be significantly lower in subjects with NAFLD or NASH compared with BMI-matched controls (46), and adiponectin levels have also been found to correlate negatively with hepatic fat (46, 47). Treatment of type 2 diabetic subjects with pioglitazone increases adiponectin levels, and this has been associated with decreases in hepatic fat (48). In this particular study, adiponectin levels were correlated negatively with hepatic fat content and correlated positively with hepatic and peripheral insulin sensitivity both pretreatment and posttreatment (48). Further support for an important role of adiponectin comes from a mouse model of steatohepatitis induced by a high-fat/alcohol diet, in which adiponectin administration alleviated hepatic steatosis and significantly attenuated hepatic inflammation and elevated levels of transaminases by increasing fatty acid oxidation in the liver

and decreasing the activities of enzymes involved in fatty acid synthesis (49).

The inflammatory cytokines IL-6 and TNF α are also secreted from fat and are known to be elevated in obesity and insulin-resistant states (50–52). It is possible that they may be mediators of insulin resistance because plasma levels of both have been correlated negatively with insulin sensitivity (52–56). Furthermore, it has been demonstrated that the plasma levels of these two inflammatory cytokines are increased in subjects with NAFLD (38) and NASH (57, 58) and that peripheral blood monocyte production of TNF α and IL-6 is increased in subjects with NASH (58). A more detailed evaluation of the role of TNF α in NASH has demonstrated overexpression of TNF α mRNA in both the liver and adipose tissue, suggesting that the TNF α system may be of importance in the pathogenesis of NASH (59).

Thus, the pathogenesis of hepatic steatosis is complex with intraabdominal fat, adipokines, and inflammation all appearing to be involved to some extent. However, their relative importance in the accumulation of hepatic fat and development of insulin resistance remains to be resolved.

Potential Mechanisms for Hepatic Fat Accumulation

Fatty acids in the liver come from several different sources: derived from dietary fat, released from adipocytes via lipolysis, and from *de novo* hepatic lipogenesis (Fig. 2). An imbalance of any of the pathways involved in triacylglycerol delivery, synthesis, export, or oxidation could contribute to its accumulation in the liver. Using stable isotope labeling techniques, it has been demonstrated recently that, in subjects with NAFLD on a controlled diet that contained 30% of calories from fat, nearly 60% of the liver triacylglycerol was derived from circulating FFAs, 26% from *de novo* lipogenesis, and 15% from the diet (40). This would suggest that, in the absence of a high-fat diet, overproduction of fatty acids from adipose tissue is the most likely source of excess triglyceride accumulating in the liver. As high-fat diets have been shown to produce fatty liver in both animal (60, 61) and human (62)

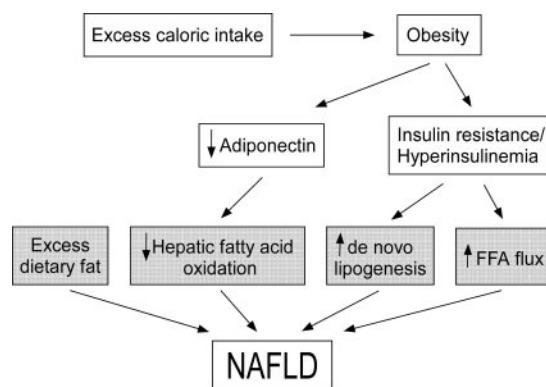


FIG. 2. Diagram of potential sources of and mechanisms for the accumulation of fat in the liver. Excess caloric intake plays a central role directly via excess dietary fat intake and indirectly by leading to obesity and thus contributing to insulin resistance. Obesity may additionally impact fat accumulation in the liver by decreasing adiponectin levels, thus contributing to inadequate fatty acid oxidation. Insulin resistance contributes to NAFLD directly by increasing *de novo* lipogenesis and indirectly by increasing FFA flux to the liver via decreased inhibition of lipolysis.

TABLE 1. Clinical trials targeting insulin resistance in NAFLD and NASH

Ref.	n (male/female)	Age (yr) mean \pm SD (range)	Subjects	NAFLD or NASH
Weight loss				
Park (79)	25 (19/6)	38 \pm 13 (13–61)	Obese	NAFLD by ultrasound
Ueno (80)	25 (13/12)	39 \pm 13 (9–52)	Obese	NAFLD by ultrasound
Clark (117)	16 (8/8)	44 \pm 8 (NR)	Morbidly obese	15/16 NASH
Huang (82)	16 (8/8)	50 \pm 12 (NR)	NASH	NASH
Metformin				
Nair (89)	15 (9/6)	51 \pm 12 (NR)	One with DM	NAFLD or NASH
Marchesini (90)	20 (14/6)	Median 40 (27–59)	Non-DM	NAFLD by ultrasound
Uygun (92)	34 (21/13)	41 \pm 10 (22–64)	Non-DM	NASH
Bugianesi (91)	110 (91/19)	42 \pm 10 (21–68)	Non-DM	NAFLD or NASH
Thiazolidinediones				
Troglitazone				
Caldwell (118)	10 (0/10)	44 \pm 16 (23–69)	One with DM	NASH
Pioglitazone				
Promrat (76)	18 (7/11)	46 \pm 11 (NR)	Non-DM	NASH
Sanyal (75)	20 (10/10)	46 \pm 13 (NR)	Non-DM	NASH
Rosiglitazone				
Neuschwander-Tetri (73)	30 (14/16)	45 (21–68)	BMI > 25, 15 NGT, 7 IGT, 8 DM	NASH

NA, Not assessed; NR, not reported; NS, not significant; DM, diabetes mellitus; IGT, impaired glucose tolerance; NGT, normal glucose tolerance. NASH was diagnosed by liver biopsy.

studies and low-fat/high-carbohydrate diets have been shown to increase *de novo* lipogenesis (63), dietary composition can have a major effect by altering the relative sources of liver triacylglycerol.

In addition to direct effects of diet on hepatic fat accumulation, excess dietary caloric intake can result in obesity and associated insulin resistance. Insulin resistance may then contribute to the development of fatty liver by impairing the ability of insulin to suppress lipolysis, leading to increased delivery of FFAs to the liver (28, 64, 65). This hypothesis is supported by the fact that subjects with NAFLD have been shown to have elevated FFA levels (5, 28, 29) and impaired insulin suppression of lipolysis (28–30). The increased FFAs may then induce hepatic insulin resistance (66). The mechanisms whereby FFAs induce hepatic insulin resistance are unclear but may be mediated by translocation of the PKC- δ isoform from the cytosolic to the membrane compartment resulting in impairment of hepatic insulin receptor substrate (IRS)-associated phosphatidylinositol 3-kinase activity (66).

Increased *de novo* lipogenesis may also contribute to hepatic fat accumulation. Donnelly *et al.* (40) found that elevated *de novo* lipogenesis in the fasting state accounted for 26% of liver triacylglycerol in NAFLD subjects compared with 5% reported in a healthy historical control group. Increased *de novo* lipogenesis may be a result of insulin resistance and the resulting hyperinsulinemia in subjects with NAFLD, as insulin stimulates lipogenic enzymes via sterol receptor binding protein 1-c (SREBP-1c) even in the insulin-resistant state (67). Overexpression of SREBP-1c in transgenic

mice leads to increased lipogenesis and the development of hepatic steatosis (68). Inactivation of the SREBP-1c gene in livers of *ob/ob* mice, a genetic model of leptin deficiency that develops obesity and hepatic steatosis, results in an approximate 50% reduction in liver triglyceride (69). Carbohydrates can also stimulate lipogenesis by activating the carbohydrate response element binding protein leading to transcription of genes involved in glycolysis and lipogenesis, thus resulting in the conversion of excess glucose to fatty acids (70). Although carbohydrate response element binding protein activation may be more important in states of hyperglycemia, it may also contribute to the accumulation of fat in the liver during diets high in carbohydrates.

Hyperinsulinemia may also contribute to triacylglycerol accumulation in the liver by inactivating the forkhead transcription factor Foxa2 (71). Foxa2 promotes fatty acid oxidation in the liver, but is inactivated by phosphorylation by either IRS1 or IRS2 signaling pathways. Because Foxa2 remains sensitive to the actions of insulin in the liver, hyperinsulinemia, even in the fasting state, can result in full suppression of Foxa2, thus decreasing fatty acid oxidation and contributing to fat accumulation (71).

Insulin Resistance in NAFLD—Cause or Consequence?

Subjects with NAFLD display whole-body as well as hepatic insulin resistance, and the relative importance of each of these in the pathogenesis of NAFLD is a matter of debate.

TABLE 1. *Continued*

Study type	Duration	Liver enzymes	Liver fat change and assessment method	Histology
Open	1 yr	Decreased	NA	NA
Open <i>vs.</i> control non-randomized	3 months	Decreased	Decreased; liver biopsy	(n = 25) Steatosis only improved
Gastric bypass cohort study	Average 305 d	Decreased	Decreased; liver biopsy	(n = 16) Improved steatosis, inflammation, ballooning, and fibrosis
Open	1 yr	No significant change	Decreased in seven of 15, steatosis score (NS); liver biopsy	(n = 15) Trend towards improved hepatitis score ($P = 0.06$), total NASH score (NS)
Open-label	48 wk	Decreased at 3 months, no change from baseline at 1 yr	Three of 10 had decreased steatosis; liver biopsy	(n = 10) Two of 10 had improved hepatitis and one of 10 had improved fibrosis
Open-label	4 months	Decreased	NA	NA
Open-label <i>vs.</i> control	6 months	Decreased	Decreased; ultrasound	(n = 23) No significant improvement
Open-label <i>vs.</i> vitamin E or diet	12 months	Decreased	Decreased; liver biopsy	(n = 17) Improved steatosis, necroinflammation, and fibrosis
Open-label	6 months	Decreased	NA	(n = 7) Five of 7 had improved necroinflammation, no improvement fibrosis
Open-label	48 wk	Decreased	Decreased; liver biopsy	(n = 18) Improved steatosis, inflammation, hepatocellular injury, and fibrosis
Open-label, vitamin E <i>vs.</i> vitamin E + pioglitazone	6 months	Decreased	Decreased; liver biopsy	(n = 18) Improved steatosis only combination <i>vs.</i> vitamin E alone
Open-label	48 wk	Decreased	Decreased; liver/spleen ratio by CT	(n = 22) Improved steatosis, ballooning, and pericellular fibrosis

Elegant studies using tissue-specific insulin receptor knockout mice have demonstrated that knockout mice lacking the muscle insulin receptor alone or in combination with loss of the adipocyte insulin receptor have normal glucose levels despite the presence of insulin resistance (72). However, knockout of the insulin receptor in the liver results in both fasting and postprandial hyperglycemia, and the subsequent development of peripheral insulin resistance. These mice also develop hepatic steatosis (72). Although these studies would suggest that hepatic insulin resistance is more important than peripheral muscle and adipose tissue insulin resistance in the pathogenesis of hepatic steatosis, extrapolating rodent studies to humans may not be entirely accurate. The basis for this caution is our finding that the thiazolidinediones, which have been shown in small studies in humans to decrease hepatic fat (73–76), result in fat deposition in the liver of mice on a high-fat diet (Hull, R. L., Z. Shen, S. E. Kahn, unpublished observation). Thus, when considering human studies, whether insulin resistance causes hepatic steatosis or whether the accumulation of fat in the liver is the primary event leading to hepatic and then later peripheral insulin resistance is not clear.

Patients with NAFLD typically are obese and have increased intraabdominal fat that may be responsible for their insulin resistance. As discussed above, peripheral insulin resistance might contribute to steatosis by decreasing insulin suppression of lipolysis and thus increasing delivery of FFAs to the liver and hyperinsulinemia stimulating *de novo* lipogenesis via SREBP-1c. The beneficial effects of insulin-sensitizing agents such as the thiazolidinediones and met-

formin (discussed later) on decreasing hepatic fat support this hypothesis, although direct effects of these agents on the liver cannot be excluded.

An alternative hypothesis is that fat accumulation in the liver may be the primary event leading to hepatic insulin resistance. The early development of hepatic insulin resistance, without changes in insulin-stimulated glucose uptake or body weight, has been observed in a rodent model of high-fat feeding (61). In this model, 3 d on a high-fat diet (59% fat) resulted in a 3-fold elevation in hepatic fatty acyl Co-A. Although FFA levels were elevated in the immediate postprandial period, fasting FFA levels were not different from the control-fed animals (61). When rats were fed 2,4 dinitrophenol, a mitochondrial uncoupler that results in increased energy expenditure and thus increased fat oxidation, the accumulation of fat in the liver was prevented and hepatic insulin sensitivity was improved, although not to the level of the control animals. Although a negative linear relationship between hepatic fat content and hepatic insulin sensitivity was found (61), this does not prove cause and effect. Thus, although these data are intriguing, they do not exclude an effect of elevated postprandial FFAs on hepatic insulin sensitivity or that dinitrophenol may have had direct effects on hepatic insulin sensitivity resulting in decreased hepatic fatty acyl Co-A. Studies in humans with type 2 diabetes before and after a moderately hypocaloric, very-low-fat diet, demonstrated an 81% reduction in intrahepatic lipid content that was associated with improvements in both basal and insulin-stimulated hepatic glucose metabolism, but no significant change in insulin-stimulated peripheral glucose

uptake (77). Thus, changes in dietary fat are able to alter intrahepatic lipid content and are associated with alterations in hepatic insulin sensitivity without significant changes in peripheral insulin sensitivity or fasting FFA levels.

Treatment of Hepatic Steatosis

There is currently no established treatment for NAFLD or NASH, although weight loss and a low-fat diet are recommended. Most treatment studies have focused on subjects with NASH because this entity has the potential to progress to fibrosis and cirrhosis; however, the findings have been limited by variations in treatment endpoints and a paucity of randomized, placebo-controlled trials. Improvements in liver transaminases and histology have been demonstrated with diet and/or weight loss interventions, ursodeoxycholic acid, vitamin E, betaine, fibrates, metformin and the thiazolidinediones (78). The use of approaches such as weight loss, metformin, or thiazolidinediones has been based on the association of NAFLD with obesity and insulin resistance, with the postulate that insulin resistance is a cause rather than a consequence of hepatic steatosis. Table 1 provides a summary of clinical studies in subjects with NAFLD or NASH on the effects of weight loss, metformin, or thiazolidinedione treatment on liver transaminases, liver fat, and histology.

Lifestyle modification

Lifestyle changes, mostly focused on weight loss, have been demonstrated to reduce liver transaminases (79–81) and decrease liver fat content (80). A 1-yr weight loss intervention in 15 subjects with NASH that achieved only an average 3-kg weight loss resulted in an improvement in histology in nine subjects and stable histology in six. Those with improved histology were found to have had greater weight loss, improved liver transaminases, and decreased liver fat (82). Liver fat, measured by MRS, has also been shown to decrease in response to weight loss interventions in obese women (83) and in subjects with type 2 diabetes (77, 84). Although weight loss appears to be beneficial, rapid weight loss after gastropasty has been associated with increased hepatitis despite reductions in steatosis on liver biopsy (85).

The mechanism whereby dietary weight loss results in decreased liver fat is not entirely clear, but decreased dietary fat is likely to be a major factor. Tiikkainen *et al.* (83) found that before weight loss, liver fat was related to the percent of total energy comprised of dietary fat and was not related to the amount of intraabdominal fat. Additionally, changes in liver fat with weight loss did not correlate with changes in intraabdominal fat (83). Others have shown that, when compared with age-, gender-, and BMI-matched controls, subjects with NASH eat a diet richer in saturated fat and poorer in dietary fiber and vitamins E and C, thus suggesting that dietary habits as well as antioxidant activity may be contributing factors to steatohepatitis (6).

Metformin

Metformin is used extensively in the treatment of patients with type 2 diabetes and has been shown in the Diabetes Prevention Program to delay the onset of the disease in subjects with impaired glucose tolerance (86). It reduces plasma glucose

levels primarily by reducing hepatic glucose production through the activation of AMP kinase (87). Activation of this key enzyme also results in decreased lipid synthesis and increased fat oxidation (87). Treatment with metformin for 4 wk in *ob/ob* mice, an obese mouse model that does not synthesize leptin, resulted in resolution of hepatic steatosis (88). Open label human studies have shown promising results. In one, metformin treatment resulted in early decreases in liver enzymes and improved insulin sensitivity in subjects with NAFLD (89). However, after 3 months, liver enzymes gradually increased to pretreatment levels. Another study in subjects with NASH showed improved insulin sensitivity, liver transaminases, and decreased liver volume after 4 months of treatment (90). A recent randomized controlled trial comparing metformin treatment to vitamin E or diet alone in nondiabetic NAFLD subjects found improved transaminases as well as improved histology after 12 months of treatment (91). In contrast, others have failed to show significant improvement in liver histology after 6 months of treatment with metformin (92), although 6 months may have not been a long enough time interval to detect significant changes in histology. Additionally, in a double-blind, randomized study of subjects with type 2 diabetes, 16 wk of treatment with metformin resulted in improved basal hepatic insulin sensitivity, decreased FFAs, and glycosylated hemoglobin, but no significant change in liver transaminases or liver fat quantified by MRS (93). Thus, further randomized, placebo-controlled trials are needed before metformin can be recommended as therapy in NAFLD.

Thiazolidinediones

Thiazolidinediones are insulin-sensitizing agents that have been shown to improve both hepatic as well as whole-body insulin sensitivity (94, 95). The exact mechanism whereby these agents improve insulin sensitivity is not known but may be related, in part, to changes in body fat distribution because they have been shown in humans to increase sc fat while simultaneously not changing or even decreasing intraabdominal fat (95, 96). They are also capable of inducing adipocyte differentiation and, thus, an increase in the number of small adipocytes (97), which, in turn, increases the capacity for lipid storage in fat cells. Treatment with thiazolidinediones results in consistent decreases in FFAs (95, 98, 99), thereby decreasing FFA delivery to the liver. They also increase adiponectin levels (100–103), which may help to increase lipid oxidation of fatty acids in the liver. Additionally, thiazolidinediones have been shown to decrease TNF α (104–106) and the inflammatory marker C-reactive protein (107–109), that may contribute to the development of insulin resistance. Thus, thiazolidinediones would appear to decrease hepatic triacylglycerol content by a number of mechanisms.

Human studies with thiazolidinediones in subjects with NASH have demonstrated improvements in liver function tests (73–76, 110), as well as an improvement in steatosis and the histological score (73, 75, 76). Work in individuals with type 2 diabetes has shown that rosiglitazone leads to a 40% decrease in hepatic triglyceride content (98). These observations suggest that the thiazolidinediones may be beneficial in their effects on the liver in individuals with hepatic steatosis, but larger studies are needed.

Other agents

In addition to insulin-sensitizing agents, lipid-lowering medications and antioxidant and cytoprotective therapies have been tried in NAFLD and NASH. Small uncontrolled studies have shown benefits with statins (111, 112), omega-3-fatty acids (111) and fibrates (113, 114). Antioxidant therapy has been pursued as oxidative stress has been postulated to be one of the factors that may contribute to hepatocyte damage, fibrosis, and cirrhosis in NASH. Vitamin E has received the most attention, but so far studies have produced varying results (115). Ursodeoxycholic acid has been used as a potential cytoprotective agent in subjects with NASH, and although small, mostly uncontrolled, studies showed variable benefit (115), a large randomized placebo-controlled study with liver biopsy at 2 yr demonstrated improvement but no difference between the active drug and the placebo groups (116).

Conclusions and Future Directions

NAFLD is a common liver disorder that is strongly associated with insulin resistance and type 2 diabetes. With the increase in the number of individuals who are overweight or obese, this condition will only increase in prevalence. The mechanisms underlying the development of NAFLD are not completely understood but likely involve a combination of increased FFAs and possibly decreased lipid oxidation in the liver as a result of insulin resistance. Dietary fat can play a major role in the development of NAFLD, and it is possible that hepatic steatosis caused by consumption of excess dietary fat may contribute to the hepatic insulin resistance observed in this condition. Because most treatment studies have been small and placebo treatment has frequently resulted in significant improvements in outcome measures, new treatment recommendations await large placebo-controlled randomized studies.

Future research should focus on clarifying the relationship between hepatic and peripheral insulin resistance and the development of hepatic steatosis. Additionally, information is needed to determine the environmental and genetic factors that predispose some patients to develop NASH, especially as diet may play a key role in the development of NAFLD and perhaps in the progression from NAFLD to NASH. In terms of medical therapy, large, placebo-controlled studies are currently underway to investigate the therapeutic benefits of insulin-sensitizing medications and vitamin E. Investigation of other treatment options such as lipid-lowering medications, antioxidants, or cytoprotective agents should continue. The results of these studies are eagerly awaited to provide better recommendations for treatment of this condition.

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