Insulin Resistance: A Metabolic Pathway to Chronic Liver Disease

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Insulin resistance (IR) is the pathophysiological hallmark of nonalcoholic fatty liver disease (NAFLD), one of the most common causes of chronic liver disease in Western countries. We review the definition of IR, the methods for the quantitative assessment of insulin action, the pathophysiology of IR, and the role of IR in the pathogenesis of chronic liver disease. Increased free fatty acid flux from adipose tissue to nonadipose organs, a result of abnormal fat metabolism, leads to hepatic triglyceride accumulation and contributes to impaired glucose metabolism and insulin sensitivity in muscle and in the liver. Several factors secreted or expressed in the adipocyte contribute to the onset of a proinflammatory state, which may be limited to the liver or more extensively expressed throughout the body. IR is the common characteristic of the metabolic syndrome and its related features. It is a systemic disease affecting the nervous system, muscles, pancreas, kidney, heart, and immune system, in addition to the liver. A complex interaction between genes and the environment favors or enhances IR and the phenotypic expression of NAFLD in individual patients. Advanced fibrotic liver disease is associated with multiple features of the metabolic syndrome, and the risk of progressive liver disease should not be underestimated in individuals with metabolic disorders. Finally, the ability of insulin-sensitizing, pharmacological agents to treat NAFLD by reducing IR in the liver (metformin) and in the periphery (thiazolidinediones) are discussed. (HEPATOLOGY 2005;42:987-1000.)

The liver plays a pivotal role in nutrient and hormone metabolism. Metabolic abnormalities are common in liver disease, hence the term *hepatogenous* diabetes, which is used to describe the hyperglycemia that develops in advanced cirrhosis. However, only recently has a direct effect of altered insulin metabolism been postulated in the pathogenesis of liver disease. Abnormalities in insulin action may be involved in the pathogenesis of nonalcoholic fatty liver disease (NAFLD), a condition that ranges from clinically benign fatty liver to its more severe form, nonalcoholic steatohepatitis (NASH). As a result, interest in insulin resistance (IR), its pathogenesis, and its quantitative measurement has increased. The aim of this review is to focus on the general aspects of IR and to summarize the present knowledge of the biochemical and clinical aspects that support the view of a metabolic mechanism involved in the pathogenesis of chronic liver disease mediated by IR.

Insulin Resistance

IR may be defined as a condition in which (1) higher-than-normal insulin concentrations are needed to achieve normal metabolic responses or (2) normal insulin concentrations fail to achieve a normal metabolic response. The first definition dates back to the 1960s, when a quantitative measurement of insulin concentrations was made available; the second was proposed by Kahn,[1] who also differentiated reduced insulin sensitivity (rightward shift of the insulin-response curve) from reduced insulin responsiveness (lower-than-normal maximal response).

One limitation of this definition is a lack of clarity in the metabolic functions used to measure insulin activity. Historically, glucose metabolism has been the measured function of choice, but other metabolic pathways and/or different organs respond to insulin in nonuniform fashion.[2]

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; IR, insulin resistance; T2DM, type 2 diabetes mellitus; IRS, insulin receptor substrate; FFA, free fatty acid; HGO, human glucose output; HSC, hepatic stellate cell; IL-6, interleukin 6.

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Received June 7, 2005; accepted September 10, 2005.

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Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.20920

Potential conflict of interest: Nothing to report.

Table 1. Advantages and Disadvantages of Different Methods for the Quantitative Assessment of Insulin Resistance

Method	Parameter	Advantages	Disadvantages
Euglycemic clamp[3]	– Whole-body insulin sensitivity = $M/IRI_{s\text{-}s}$	Based on solid physiological understanding	Complex, costly, and time-consuming
		Possibility to obtain a dose–response curve	Not useful for epidemiological studies
		When combined with tracers, it gives a comprehensive estimate of insulin effects	Low compliance of patients
		High intralaboratory reproducibility	Interlaboratory reproducibility not proven
Intravenous glucose tolerance test[4]	– Insulin sensitivity = S_I	Relatively easy to perform	Complex mathematical analysis and need for computer support
	– Glucose effectiveness = S_G	Measurement of first- and second-phase insulin secretion	Not applicable to patients with reduced insulin secretion
		High intralaboratory reproducibility	High cost of hormonal measurements
		High intralaboratory reproducibility	Interlaboratory reproducibility has not been proven
OGTT-derived indices[5-8]	– AUC_{BG}/AUC_{IRI}	Based on a test used in clinical practice diagnostic purposes	Based on empirical basis or complex mathematical models
	– $ISI = 10^4/(IRI_{120} \cdot BG_{120})$	Easy to perform	Low intra- and interlaboratory reproductility
	– OGIS = mathematical modeling		
	– $SI = 10^4/\sqrt{(BG_0 \cdot IRI_0) \cdot (BG_{Mean(0\text{-}120)} \cdot IRI_{Mean(0\text{-}120)})}$		
	– $SI = 18.8-(0.271 \cdot BMI)-(0.0052 \cdot IRI_{120}) \cdot (0.27 \cdot BG_{90})$		
Static (fasting) measurements[9-12]	– IRI_0 or $1/IRI_0$	East to quantify	Not applicable to insulin-treated or poorly controlled patients
	– Glucose/Insulin ratio = BG_0/IRI_0	Low cost	Relatively low correlation with the "clamp"
	– $HOMA = IRI_0 \cdot BG_0/22.5$		
	– $QUICKI = 1/(\log(IRI_0)+\log(BG_0))$		
	– $ISI = 10^4/(IRI_0 \cdot BG_0)$		

Abbreviations: IRI, immunoreactive insulin concentration; s-s, steady state; OGTT, oral glucose tolerance test; AUC, area under the curve; ISI, insulin sensitivity index; OGIS, oral glucose insulin sensitivity; SI, sensitivity index; BG, blood glucose concentration; BMI, body mass index; HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index.

Several dynamic[3-8] and static[9-12] methods have been proposed for the quantitative assessment of IR (Table 1). The glucose clamp technique remains the gold standard,[3] and the validity of different methods is usually measured against that of the clamp.[6,7,13-16] Simple indices based on the sole measurement of glucose and insulin—both in the fasting state and in response to an oral glucose tolerance test—provide data that may be confidently used in epidemiological surveys. The reproducibility of static methods depends largely on analytical and day-to-day variability of insulin concentrations, and small changes in insulin produce a large error in the estimate of IR. Nonetheless, these simple indices have greatly expanded our knowledge of insulin-resistant states.

Since the first demonstration of IR in NAFLD,[17] homeostasis model assessment has been used in epidemiological studies to demonstrate the close association between NAFLD and the metabolic syndrome.[18,19] The glucose clamp technique was first used by Comert et al,[20] and subsequent studies showed that the increased IR of NAFLD also includes defective insulin-mediated suppression of lipolysis,[21-23] similar to that observed in type 2 diabetes (T2DM). IR is accompanied by higher total insulin secretion and normal hepatic insulin extraction[24] and predicts the presence of more severe disease.[25] However, it may be present even in lean, nondiabetic patients who do not exhibit the classical features of the metabolic syndrome.[23]

Pathogenesis of Insulin Resistance

The biological action of insulin depends on a cascade of events following the interaction of insulin with its specific receptor (Fig. 1). The insulin receptor is a glycosylated tetramer consisting of two extracellular insulin-binding (alpha) subunits and two beta subunits crossing the cell membrane, potentially expressing tyrosine kinase activity. The gene for the insulin receptor is located on chromosome 19. The receptor is part of a family of receptors, including the insulin-like growth factor 1 receptor, with different affinities for insulin, but sharing common postsignaling intracellular proteins known as insulin receptor substrate (IRS) proteins.

Insulin binding promotes the autophosphorylation of the receptor and subsequent tyrosine phosphorylation of IRS proteins (namely IRS-1 and IRS-2), which initiates a cascade of events finally leading to translocation of a spe-

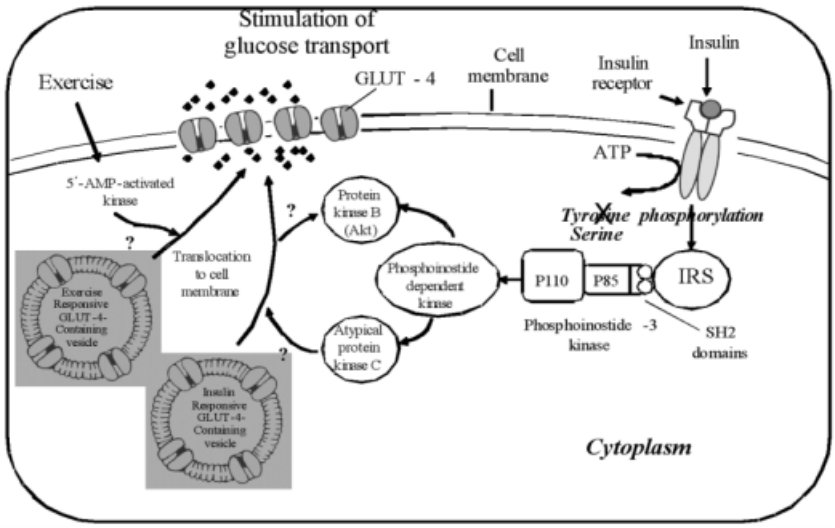

Fig. 1. Schematic of the insulin pathway and glucose receptors in skeletal muscle. The primary defect is phosphorylation of serine rather than tyrosine, which prevents the normal cascade of intracellular signaling for glucose entry into the cell. Note that exercise increases glucose uptake via a mechanism independent of the insulin receptor. GLUT-4, glucose transporter 4; AMP, adenosine monophosphate; ATP, adenosine triphosphate; IRS, insulin receptor substrate.

cific glucose transporter—glucose transporter 4—from its intracellular pool to the cell membrane. Glucose transporter 4 facilitates glucose transport along the concentration gradient from the extracellular space into the cytoplasm.[26] In this sequence of events, the mechanisms responsible for IR may involve either insulin binding or IRS proteins, or finally glucose transporter 4. Exercise stimulates glucose transport by pathways that are independent of phosphoinositide 3 kinase and may involve 5′–adenosine monophosphate–activated kinase.

Abnormalities of cellular glucose uptake appear to result from defects in intracellular signaling. Several factors such as hyperinsulinemia, hyperglycemia, tumor necrosis factor α, free fatty acids (FFAs), ceramide, and transcription factors (*e.g.*, nuclear factor κB) have been implicated in altering insulin signaling in patients with obesity and T2DM.[26,27] The most likely mechanism of IR within the muscle cell is cytokine-induced serine rather than tyrosine phosphylation of IRS-1.[28] It is also now clear that hepatic steatosis itself will cause hepatic IR.[29,30] Although there had been some question as to the primary site of IR in NAFLD (peripheral vs. hepatic), recent data indicate the periphery to be the initial site with hepatic steatosis following and exacerbating the degree of IR[31] (Table 2).

The phenotypic expression of IR is dependent upon a genetically determined trait and environmental conditions. In most cases, IR may be regarded as an energy-sparing mechanism favoring survival during limited food availability or increased energy requirement. IR and subsequent hyperinsulinemia promote energy accumulation as fat and reduces energy expenditure. Trauma, sepsis, surgical stress, and chronic diseases cause IR, as do physiological states (*e.g.*, pregnancy, puberty, physical inactivity, and aging). Overeating and obesity—particularly visceral obesity—are the most important environmental causes of IR. The final balance is the result of the relative contribution of all these factors.

Finally, whatever the cause, IR in concert with hyperinsulinemia favors an increase in fat mass, increased lipolysis, and elevated levels of free fatty acids, further reducing insulin signaling in a dose-dependent manner[32] and increasing both hepatic glucose and lipid production (lipotoxicity).[33]

Metabolic Effects of Insulin Resistance

After an overnight fast (postabsorptive state), most of the total glucose disposal occurs in insulin-independent tissues, approximately 50% in the brain and 25% in the splanchnic area (liver plus gastrointestinal tissues). Glucose utilization, which averages approximately 2.0 mg/kg/min, is precisely matched by the release of glucose

Table 2. Most Likely Progression of Insulin Resistance

- Peripheral insulin resistance
  - Lipolysis not suppressed normally
  - Reactive hyperinsulinemia
- Influx of fatty acids to the liver
- Increased hepatic triglycerides
- Hepatic insulin resistance superimposed on peripheral resistance

from the liver.[34] After the ingestion of a glucose-containing meal, the majority (approximately 80%-85%) of glucose disposal occurs in muscle tissue. The maintenance of normal glucose homeostasis is dependent on three tightly related processes: (1) insulin secretion by pancreatic β cells, (2) stimulation of glucose uptake by splanchnic (liver and gut) and peripheral (primarily muscle) tissues, and (3) suppression of hepatic glucose output. Although fat tissue is responsible for only a small amount of total body glucose disposal (4%-5%), it plays a very important role in the maintenance of total body glucose homeostasis.

Glucose Metabolism and Glucose Disposal. Muscle is the primary site of glucose disposal in humans. After glucose enters a cell, it can either be converted to glycogen (70% of glucose uptake) or enter the glycolytic pathway (90% glucose oxidation and 10% anaerobic glycolysis).

In NAFLD patients, the main site of IR during euglycemic insulin clamp studies is muscle. Because obesity and diabetes are the two most common risk factors for the development of NAFLD, peripheral IR can be partially accounted for by the presence of each or both of them. However, an independent association of NAFLD with IR, as suggested by epidemiological studies,[17] has been confirmed through the use of the clamp technique. In nondiabetic NAFLD patients, glucose disposal is reduced by nearly 50% compared with normal subjects, to an extent similar to that observed in T2DM.[22] The lack of difference in glucose disposal between normal and overweight NAFLD suggests that the defect is not exclusively associated with abnormal glucose regulation and/or excess fat mass. Glucose utilization in muscle progressively deteriorates from fatty liver to NASH,[21] and IR and hyperinsulinemia are not secondary to a reduced hepatic insulin extraction.[24]

When combined with the clamp, indirect calorimetry provides the rate of nonoxidative glucose disposal (*i.e.*, glycogen synthesis) as the difference between whole-body glucose uptake (as determined by the insulin clamp) and glucose oxidation. Insulin-stimulated glycogen synthesis is reduced in insulin-resistant states, including obesity, diabetes, and the combination of obesity and diabetes.[34] Likewise, nonoxidative glucose disposal during insulin clamp is decreased in obese patients with NAFLD, and the defect is more pronounced in subjects with NASH than in those with fatty liver.[21] Glucose disposal is inversely related to the amount of body fat in obese patients,[21] but not in lean patients with NAFLD,[22] in whom insulin sensitivity more closely correlates with visceral adiposity indirectly assessed by waist circumference.[24] The reduced insulin sensitivity in NAFLD is compensated by an enhanced β cell secretion,[24] which is necessary to maintain euglycemia. The relationship between these two parameters is hyperbolic.

The independent association of NAFLD with IR has also been confirmed by studies showing that the presence of fatty liver influences the severity of IR in T2DM and obesity. In T2DM, insulin-stimulated glucose disposal is markedly diminished in relation to fatty liver[35] and correlates with the degree of fat infiltration (measured via CT) and visceral fat (determined via MRI). Intrahepatic lipids, together with intra-myocellular lipids, are increased in T2DM and are negatively associated with insulin sensitivity during a three-step hyperinsulinemic euglycemic clamp.[36] Similarly, impairment in whole-body insulin sensitivity is more pronounced in obese nondiabetic women with high liver fat.[37] Goto et al.[38] found a correlation between insulin sensitivity and liver fat in lean Japanese individuals without liver disease.

In healthy lean elderly subjects, insulin-stimulated muscle glucose metabolism is reduced as compared with young controls and is associated with increased fat accumulation in muscle and liver tissue assessed via ^{1}H nuclear magnetic resonance spectroscopy.[39] In the same group, mitochondrial oxidative and phosphorylation activity is reduced by approximately 40%; mitochondrial abnormalities are also observed in obese patients with fatty liver and NASH.[40]

Hepatic Glucose Output. After an overnight fast, the liver of a healthy individual produces glucose at a rate that meets the needs of the brain. In nondiabetic subjects, basal human glucose output (HGO) shows a large variability, which is mainly dependent upon the amount of lean body mass and the degree of peripheral insulin utilization.[41] Insulin released into the portal vein after glucose or meal ingestion suppresses HGO. Failure of the liver to perceive this signal results in hepatic IR.

The glucose released by the liver can be derived from either glycogenolysis or gluconeogenesis. The HGO-suppressing effect of insulin occurs either directly through the hepatic insulin receptor or indirectly as a consequence of a reduced release of gluconeogenic substrates (*e.g.*, lactate, alanine, glycerol, and free fatty acids), both in muscle and in the liver.

In nondiabetic NAFLD patients, HGO is normal or higher than normal in the basal state. Hyperinsulinemia is a potent inhibitor of HGO, and normal HGO in the face of fasting hyperinsulinemia indicates postabsorptive hepatic IR.[22] Following insulin administration, HGO is less suppressed, confirming a derangement in hepatic insulin sensitivity,[22] which deteriorates with progression from fatty liver to NASH.[21]

NAFLD patients are very similar to patients with well-controlled T2DM, where the presence of fatty liver does

not significantly affect fasting rates of HGO,[35] but slightly decreases insulin-mediated HGO suppression. In regression analysis, the suppression of HGO is negatively correlated with visceral adiposity and with insulin-suppressed plasma FFA, but not with liver fat. In contrast, in nonobese, healthy subjects liver fat, as measured by proton spectroscopy, is an independent determinant of HGO sensitivity to insulin.[42]

Lipolysis and Lipid Oxidation. In addition to muscle and the liver, adipose tissue is the third metabolically relevant site of insulin action. Whereas insulin-stimulated glucose disposal in fat tissue is negligible compared with that in the muscle, regulation of lipolysis with subsequent release of glycerol and FFAs into the bloodstream has major implications for glucose homeostasis. Increased availability and utilization of FFAs contribute to the development of skeletal muscle IR by competitive inhibition of substrate oxidation. Recent ^{1}H nuclear magnetic resonance studies suggest that an increase in intracellular fatty acid metabolites leads to impaired activation of IRS-1 tyrosine phosphorylation, resulting in decreased PI3-kinase activity and decreased glucose transport.[43] FFAs increase HGO, both by stimulating key enzymes and by providing energy for gluconeogenesis, while the glycerol released during triglyceride hydrolysis serves as a gluconeogenic substrate. Hypertriglyceridemia is frequently present in NAFLD, together with increased FFA levels, independent of body weight.[17] When measured as plasma FFA concentration[22] or as rate of lipolysis by means of deuterated glycerol,[21] the insulin-mediated suppression of lipolysis is reduced in NAFLD in response to euglycemic hyperinsulinemia.

The increased availability of lipids enhances lipid oxidation by mass action both in the periphery and in the liver, as demonstrated by higher serum β-hydroxybutyrate levels in subjects with NAFLD.[21] In the setting of a high insulin concentration, hepatic FFA esterification is favored over oxidation until the intracellular long chain acyl coenzyme A concentration level rises sufficiently to offset the inhibitory effect of malonyl-coenzyme A on carnitine palmatoyl transferase. As a result, both fatty acid esterification and oxidation will be enhanced. The ability of insulin to suppress serum FFAs is reduced in normal subjects with as little as 10% liver fat.[42] Similarly, the greater IR in T2DM with fatty liver is characterized by higher values of plasma FFAs during the clamp,[35] and hepatic steatosis is correlated with basal and insulin-stimulated plasma FFAs. Fasting values for plasma FFA are also related to muscle CT attenuation indices, a measure of muscle fat content.[44]

Relationship Between Hepatic Steatosis and Insulin Resistance

The accumulation of lipids in the liver results from an imbalance among the uptake, synthesis, export, and oxidation of fatty acids. Elevated FFA levels during fasting and a reduced suppression of lipolysis after insulin administration are constant findings in NAFLD patients. Both parameters are closely correlated with the amount of fatty infiltration in the liver. Similarly, visceral adiposity is often, but not always, correlated with liver fat and peripheral or hepatic insulin sensitivity.

The specific characteristics of IR in NAFLD can be best defined by studying the small subset of the general NAFLD population who do not share features of the metabolic syndrome.[17] In these patients, IR significantly affects endogenous glucose production, whole-body glucose disposal in its two main components (*i.e.*, glucose oxidation and nonoxidative glucose disposal), lipolysis, and lipid oxidation. The correlation between lipid oxidation and glucose production/disposal suggests that the metabolic defects stem from accelerated lipolysis—the immediate result of IR in adipose tissue—being responsible for increased hepatic FFAs supply and increased lipid oxidation.[23] In keeping with these data, a recent study has shown that the influx of plasma FFAs from adipose tissue to the liver represents the major source of intrahepatic fat (62%–82% of the hepatic triacylglycerols).[45] The contribution of *de novo* lipogenesis, which is less then 5% in healthy subjects, increases to 26% in NAFLD.

Insulin stimulates the membrane-bound transcription factor SREBP-1c, which activates most genes involved in lipogenesis. In insulin-resistant states, hyperinsulinemia may further induce these transcriptional factors, and the effects of insulin may be hardly dissected from those of IR. Hyperinsulinemia *per se* may trigger hepatic fat deposition, as empirically demonstrated by the hepatic steatosis occurring under the capsule of livers in patients undergoing peritoneal dialysis,[46] where insulin is routinely added to dialysate. These observations suggest a primary role of dysfunctional lipid metabolism in the onset and persistence of NAFLD, as part of a more generalized event referred to as lipotoxicity (Table 3),[47,48] leading to ectopic lipid accumulation.

From an anatomical perspective, mesenteric adipocytes might be a crucial source of fatty acids entering portal circulation. Even though FFAs released from the splanchnic bed account for only about 10% of the FFAs reaching the liver,[49] mesenteric adipocytes are less responsive to the antilipolytic action of insulin. Recent studies using selective catheterization of the hepatic vein have concluded that the major source of liver fat is not the

Table 3. Metabolic Alterations Associated With Hepatic Steatosis in Different Populations

Liver Fat Assessment	Study Design	Study Subjects	Metabolic Alterations	Reference
Liver biopsy	Clamp, $^2H_2$ glucose	NAFLD/T2DM/CON	↓ Glucose disposal ↓ Suppression of HGO ↓ Suppression of plasma FFAs	Marchesini et al.[22]
Liver biopsy	FSIGTT	NAFLD/CON	↓ Insulin sensitivity = Hepatic insulin extraction ↑ β-Cell secretion	Pagano et al.[24]
Liver biopsy	2-step clamp, $^2H_2$ glucose, $^2H_5$ glycerol, calorimetry	FL/NASH/CON	↓ Nonoxidative glucose disposal (NASH < FL < CONT) ↓ Suppression of HGO (NASH < FL) ↓ Suppression of lipolysis (NASH < FL)	Sanyal et al.[21]
CT scan	Clamp, $^2H_2$ glucose	T2DM/T2DM + FL	↓ Glucose disposal ↑ Fasting plasma FFA ↓ Suppression of plasma FFAs	Kelley et al.[35]
Proton spectroscopy	Clamp, $^2H_2$ glucose	Healthy	↓ Suppression of HGO ↓ Suppression of plasma FFAs	Seppala-Lindroos et al.[42]
CT scan	Clamp	Healthy	↓ Insulin clearance rate	Goto et al.[38]
Proton spectroscopy	Clamp	Obese post-GDM	↓ Glucose disposal ↑ Fasting plasma triglycerides	Tiikkainen et al.[37]

Abbreviations: NAFLD, nonalcoholic fatty liver disease; FL, fatty liver; T2DM, type 2 diabetes mellitus; CT, computed tomography; GDM, gestational diabetes; HGO, hepatic glucose output; FFA, free fatty acid; CON, controls; FSIGTT, frequently sampled intravenous glucose tolerance test.

visceral depot, but rather upper body nonsplanchnic subcutaneous tissue.[50] Nevertheless, visceral fat and fatty liver represent special depots of adiposity related to the pathogenesis of IR and may be part of an overall axis of central adiposity.[51]

Organ-specific deposition of fat is a strong predictor of hyperinsulinemia and/or insulin resistance. Analogous to fat in the liver, increased intramyocellular triglyceride content as assessed via muscle biopsy[52] or magnetic resonance imaging/computed tomography[53,54] closely correlates with muscle IR and is a better predictor of impaired insulin action than visceral adiposity.[55] The importance of ectopic fat deposition in the pathogenesis of IR is further confirmed in inherited or HIV-related forms of lipodystrophy, where the selective loss of subcutaneous and visceral fat are associated with steatosis, hypertriglyceridemia, and severe IR.[56,57] The sequence of events leading to ectopic accumulation of triglycerides has been referred to as the *overflow hypothesis*,[58] according to which IR is the result of the inability of the adipose organ to expand to accommodate excess calories. Once the capacity of the fat cell to store triglycerides is exceeded, fat overflows to other tissues (muscle and liver), where the intracellular triglyceride metabolism interferes with insulin signaling, glucose transport/phosphorylation, and glycogen synthesis in muscle and augments hepatic gluconeogenesis.

It is also now recognized that the hepatitis C virus (HCV) may contribute to both steatosis and IR, thus explaining the high prevalence of T2DM and steatosis observed in these patients. These aspects, at present largely unexplained, will not be analyzed in the present review.

Cross-Talk Between Liver and Peripheral Tissues

The complex network that regulates energy homeostasis implies a tightly regulated cross-talk between liver, muscle, and adipose tissue and the central nervous system. Recent studies have focused on the crucial role of adipose tissue, which is able to integrate signals from other organs and respond by secreting proteins termed *adipocytokines*.[59] In NAFLD, as well as in different metabolic diseases, the release of adipocytokines is altered. Because these adipocyte-specific proteins share energy-regulating and immune-regulating functions, a dysfunctional cross-talk between adipose tissue and the liver results in metabolic and inflammatory abnormalities.

Adiponectin. Adiponectin (Acrp 30 or AdipoQ) is expressed only in adipocytes. Low circulating levels of adiponectin have been linked to several components of the metabolic syndrome, including visceral adiposity, hyperlipidemia, and IR/T2DM,[49] and adiponectin administration reverses IR and exerts anti-inflammatory actions.[34] In the liver, it increases the sensitivity of insulin to inhibit gluconeogenesis[60] and regulates hepatic FFA metabolism *in vivo* via suppression of lipogenesis and activation of FFA oxidation.[61]

Available evidence from cross sectional studies suggests that in humans adiponectin levels are tightly associated with the amount of centrally located fat. In healthy adults, a relationship is present between adiponectin deficiency and increased liver fat, and both are independent determinants of serum insulin.[62] Adiponectin levels correlate negatively with liver fat and hepatic IR in diabetic patients and in their healthy relatives.[63] In subjects with mild obe-

sity, hypoadiponectinemia predicts the presence of hepatic steatosis on ultrasound.[64] In a cohort of NAFLD patients with a low prevalence of type 2 diabetes, low levels of circulating adiponectin were related to the amount of hepatic fat content and to increased hepatic insulin sensitivity, suggesting that hypoadiponectinemia in NAFLD is part of a metabolic disturbance characterized by ectopic fat accumulation in the central compartment.[65]

Adiponectin has anti-inflammatory properties in the liver, and low adiponectin levels might increase liver cell necrosis and disease progression. In healthy subjects, low adiponectin levels are significantly associated with increased concentrations of liver enzymes (namely, aminotransferase and gamma-glutamyltransferase), in keeping with a possible contribution of adiponectin in maintaining liver integrity.[66] Similarly, low adiponectin levels are associated with increased fat content and more extensive necroinflammation in NAFLD patients.[67] Finally, in KK Ay-obese mice, pretreatment with adiponectin reduces mortality, aminotransferase elevation, and apoptosis induced by D-galactosamine/lipopolysaccharide.[68] The administration of recombinant adiponectin to ob/ob mice alleviates hepatomegaly and steatosis and attenuates inflammation in alcoholic and nonalcoholic fatty liver diseases.[69] These therapeutic effects are likely the result of the ability of adiponectin to enhance hepatic fatty acid oxidation and to reduce fatty acid synthesis, HGO and tumor necrosis factor α (TNF-α).

Adiponectin may influence liver fibrosis. After carbon tetrachloride injections, adiponectin knockout mice develop more extensive liver fibrosis than wild-type mice.[70] In wild-type mice, injection of adenovirus-producing adiponectin prevents liver fibrosis induced by carbon tetrachloride, possibly via suppression of transforming growth factor-β1, one of the cytokines that stimulate hepatic stellate cells (HSCs) to produce fibrosis. Adiponectin overexpression in activated HSCs reduces proliferation and increases apoptosis, suggesting that adiponectin may act to reverse HSC activation or to maintain HSC quiescence. [71]

Adiponectin activity strongly depends upon the metabolic milieu and the inflammatory pattern, and adiponectin concentrations in the plasma and liver are not always correlated. In NASH, the messenger RNA expression of adiponectin receptors (AdipoRII) in the liver is negatively correlated with the histological grade of fibrosis but not with serum or hepatic adiponectin.[72] Similarly, no correlation is observed between circulating adiponectin levels and liver adiponectin expression.

In patients with advanced liver fibrosis, adiponectin levels are normal or even higher than normal.[73] This phenomenon has been related to reduced hepatic extraction.

Tumor Necrosis Factor α. TNF-α is an adipocyte-derived cytokine that plays a central role in IR. TNF-α impairs insulin signaling by inhibiting the function of IRS-1 through serine phosphorylation.[74] Circulating TNF-α levels are increased in obese [75] and diabetic subjects,[76] even if the correlation between IR and plasma TNF-α levels is weak.

Interestingly, although the three-dimensional structure of TNF-α closely resembles that of adiponectin,[77] these two proteins have completely opposite effects. Both *in vivo* and *in vitro* experiments have demonstrated that adiponectin and TNF-α suppress each other's production and also antagonize each other's action in their target tissues.[78] Overproduction of TNF-α in liver tissue has been proposed to play a key role in the pathogenesis of fatty liver.[79] Fatty liver disease in *ob/ob* mice is significantly improved by inhibition of hepatic TNF-α production or infusion of anti–TNF-α neutralizing antibodies.[80] Treatment with adiponectin significantly inhibits lipopolysaccharide-induced TNF-α production in cultured macrophages.[81] TNF-α stimulates leptin secretion in cultured adipocytes and in obese mice, and, as a feedback loop, leptin administration to rats decreases TNF-α expression.[82,83]

Leptin. Leptin is produced by adipose tissue in proportion to adipose tissue mass. It plays a role in modulating food intake and energy expenditure and exerts a proinflammatory influence regulating T cell response, enhancing cytokine production and phagocytosis by macrophages.[84] According to a recent proposal, leptin might also be an antisteatotic hormone, teleologically aimed at preventing FFA entry and fat accumulation in nonadipose tissues.[85]

Chronic leptin treatment improves insulin-stimulated hepatic and peripheral glucose metabolism in severely insulin-resistant lipodystrophic patients, and reduces hepatic and muscle triglyceride content.[56] It also improves hepatic histology in these patients.[86] Leptin is also directly involved in hepatic fibrogenesis through HSC activation.[87] Both quiescent and activated HSCs express the Ob-R_L receptor for leptin, which is further expressed during the development of liver fibrosis and cirrhosis.[88] Finally, in animal models of NAFLD, leptin appears to be essential for developing fibrosis in response to chronic liver injury.[89] However, the importance of leptin in human NAFLD has recently been questioned.[90]

Interleukin 6. One third of interleukin 6 (IL-6) in humans is produced by adipose tissue during noninflammatory conditions. It plays an important role in inflam-

mation and the regulation of T cell and B cell function. Visceral adipose tissue produces three times more IL-6 than subcutaneous adipose tissue.[91] Circulating IL-6 is associated with IR in humans[92] and impairs insulin signaling in hepatocytes.[93] In T2DM, IL-6 levels are increased and correlate with the severity of glucose intolerance[94] and with the severity of inflammation, as indicated by the highly sensitive C-reactive protein serum concentration.[95] The close linear relationship between IL-6 and TNF-α secretion from adipose tissue[59] suggests that IL-6 is part of the TNF-α/leptin axis.

Resistin. Resistin is a novel protein found in preadipocytes undergoing differentiation into mature adipocytes.[96] It is a potent proinflammatory agent. In humans with T2DM, plasma resistin levels are elevated and correlate closely with hepatic IR but not muscle IR.[44] Infusion of resistin into normal animals causes severe hepatic IR but has no effect on muscle insulin sensitivity.[97] In diet-induced IR, treatment with an antisense oligodeoxynucleotide directed against resistin messenger RNA reduced the plasma resistin levels and completely reversed hepatic IR.[98]

Angiotensinogen. Angiotensinogen has also been found in adipocytes.[99,100] Nascent data suggest it may be important in NAFLD, because angiotensinogen II antagonists improve liver function tests in patients with NAFLD[100] and attenuate fibrosis in animal models.[101]

Pathogenic Implications

Several mechanisms may account for fatty liver in insulin-resistant states, but factors responsible for the progression from simple fatty liver to NASH remain obscure. Although the search for an additional cause of oxidative stress (the so-called "second hit"[102]) has been elusive, patients at risk for more severe disease are those with several features of the metabolic syndrome, indicating that IR itself might play a role in disease progression. An imbalance in cytokine activity produced by dysfunctional fat cells can provide a link between metabolic and liver disorders, and it has recently been reported that oxidative stress in accumulated fat may cause dysregulated adipocytokine production, thus being part of the "first hit."[103] The excessive production of TNF-α, IL-6, leptin, and FFAs versus the defective production of adiponectin may cause IR and inflammation.

The recent proposal that the insulin resistance syndrome could be an inflammatory disorder appears particularly relevant.[104] As shown in Fig. 2, the balance between proinflammatory and anti-inflammatory cytokines derived from adipocytes and other tissue may determine the development of IR. Nascent data regarding the mechanism of actions and the beneficial effects of peroxisomes

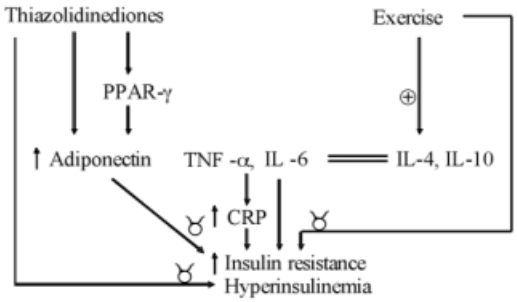

Fig. 2. Schematic showing the relation among cytokines, C-reactive protein, thiazolinediones, IR, and exercise. PPAR-γ, proliferator-activated receptor γ; TNF-α, tumor necrosis factor α; IL, interleukin; CRP, C-reactive protein.

proliferator-activated receptor γ agonists[105,106] and long-chain polyunsaturated fatty acids[107] give credence to the inflammatory nature of IR. The recent report that salicylates or disruption of IKKB reversed obesity and diet-induced IR gives further support to this concept.[108] All insulin-resistant states are characterized by chronic subclinical inflammation.[59] In patients with T2DM and/or obesity, the serum concentration of C-reactive protein, fibrinogen, α_1-acid glycoprotein, amyloid A, sialic acid, and orosomucoid are all increased[109] to a greater extent when fatty liver is also present.[35]

Increased serum ferritin, another acute phase reactant, is a common finding in NAFLD as well as in other insulin-resistant states. When iron stores are not increased, ferritin levels predict the development of diabetes in epidemiological studies[110] and are strongly associated with the degree of liver damage in NAFLD.[25]

To further strengthen this concept, the insulin-sensitizing agents of the thiazolidinedione class, currently being tested for the treatment of NASH, also have anti-inflammatory properties. Thiazolidinediones inhibit resistin and TNF-α gene expression in adipocytes and decrease their circulating levels,[111,112] whereas they stimulate adiponectin gene expression in adipocytes and increase plasma adiponectin concentration.[48] In rats with liver fibrosis induced by dimethylnitrosamine or carbon tetrachloride, administration of pioglitazone or rosiglitazone reduces hepatic extracellular matrix deposition and activation of hepatic stellate cells.[113]

Clinical Aspects of Insulin Resistance in Relation to Metabolic Liver Disease

IR is present in a variety of clinical conditions, most of which have NAFLD as an associated disease. The term *metabolic syndrome*[114] has been coined to indicate a cluster of diseases, strictly correlated with each other, having IR as the common pathogenic determinant, and carrying a

Table 4. Criteria for the Definition of the Metabolic Syndrome According to the Most Commonly Quoted Proposals

WHO Proposal[114]	NCEP Proposal[118]	IDF Proposal[120]
Altered glucose regulation (impaired fasting glucose, impaired glucose tolerance, T2DM) or Insulin resistance (clamp measurement: glucose uptake below lowest quartile for background population under study) + two or more of the following criteria: a. impaired glucose tolerance (2 h glucose during OGTT: 110-125 mg/dL) b. insulin resistance (see above) c. arterial pressure ≥140/90 mm Hg or pharmacologically treated d. dyslipidemia (triglycerides ≥150 mg/dL or fibrate-treated or HDL cholesterol <35 mg/dL [M], <39 [F]) e. central obesity (waist-to-hip ratio >0.9 [M], >0.85 [F] or BMI ≥30) f. microalbuminuria (albumin excretion rate ≥20 μg/min or albumin/creatinine ≥20 μg/g)	Three or more of the following criteria: a. waist circumference >102 cm (M), >88 (F) b. arterial pressure ≥130/85 mm Hg or pharmacologically treated c. triglyceride levels ≥150 mg/dL or fibrate-treated d. HDL cholesterol levels <40 mg/dL (M), <50 (F) e. blood glucose ≥110 mg/dL or treated for diabetes	Abdominal obesity (waist circumference >94 cm [M], >80 [F] for Caucasian, with ethnicity specific values for other groups) + two or more of the following criteria: a. arterial pressure ≥130/85 mm Hg or pharmacologically treated b. triglyceride levels ≥150 mg/dL or fibrate-treated c. HDL cholesterol levels <40 mg/dL (M), <50 (F) d. blood glucose ≥100 mg/dL or treated for diabetes

Abbreviations: T2DM, type 2 diabetes mellitus; WHO, World Health Organization; NCEP, National Cholesterol Education Program; IDF, International Diabetes Foundation; OGTT, oral glucose tolerance test; M, male; F, female.

high risk of cardiovascular disease.[115,116] Regulatory agencies and scientific societies have proposed different criteria to define the metabolic syndrome, all including data on obesity—namely visceral obesity, glucose regulation, dyslipidemia, and blood pressure.[114,117-120] The most commonly quoted proposals are given in Table 4. The Adult Treatment Panel III proposal produced from the National Cholesterol Education Program,[118] which defined the metabolic syndrome as a combination of five clinical and biochemical risk factors, is easily determined in individual patients using routine examination and biochemistry. It is also the basis for the most recent proposal of the International Diabetes Federation,[120] which focuses on abdominal obesity as a necessary feature. Other clinical conditions not included in the definition are strictly associated with IR—namely elevated uric acid, reduced fibrinolysis, endothelial dysfunction, and fatty liver, all of which might also be included. The metabolic syndrome also now has its own ICD9 code carrying the diagnosis of dysmetabolic syndrome X.

Since it was first described, NAFLD has been consistently associated with obesity (60%-95%), diabetes (28%-55%), and dyslipidemia (27%-92%), and less clearly with raised arterial pressure.[121] The presence of any one of these conditions may be evidence of IR. Obesity—more specifically central obesity—characterized by abdominal fat accumulation is definitely associated with both IR and NAFLD. In the general population, obesity increases the risk of steatosis independent of alcohol intake.[122] In severe obesity, the prevalence of liver abnormalities is nearly the rule.[123] Body mass index is an independent predictor of the degree of fat infiltration of the liver,[124] and the likelihood of developing NASH increases with the severity of obesity: about 15%-20% of morbidly obese subjects have NASH.[125] The simple measurement of waist girth proposed by the World Health Organization to categorize visceral obesity[126] may be used as a guide to determine insulin sensitivity.[127]

T2DM is the disease most commonly related to IR. Whereas overt disease also depends on decreased insulin secretion, impaired insulin sensitivity plays a primary role in the altered glucose regulation that heralds the onset of disease.[128] At any level of obesity, T2DM significantly increases the prevalence and severity of NAFLD.[129] It has been reported that up to 50% of T2DM patients have fatty liver.[129] Similarly, any category of altered glucose regulation is associated with NAFLD, and even a family history of T2DM increases the risk of NAFLD. In population studies, raised aminotransferase levels are associated with IR and predict the development of T2DM.[130]

Both triglyceride and high-density lipoprotein cholesterol concentrations are regulated by insulin, and hyperinsulinemia with IR is characterized by hypertriglyceridemia and low high-density lipoprotein cholesterol concentrations. Mixed hyperlipidemia and hypertriglyceridemia are the lipid disorders most commonly associated with NAFLD, but the presence of low high-density lipoprotein cholesterol doubles the risk of NAFLD.[131]

Finally, hypertension has been associated with IR.[132] The correlation of NAFLD with hypertension has been less clearly defined. When present, hypertension is usually associated with obesity and T2DM. However, nonobese

Table 5. Clinical Trials Treating Insulin Resistance in NAFLD

Author	n	Duration	Daily Dose	Outcome Improved
Metformin				
Marchesini et al.[135,*]	14	4 mo	1,500 mg	ALT, liver volume, Insulin sensitivity
Uygun et al.[136,†]	34	6 mo	1,500 mg	ALT, AST
Nair et al.[137]	15	1 yr	20 mg/kg	ALT, AST (transiently), insulin sensitivity histology (?)
Bugianesi et al.[138,‡]	55	1 yr	2,000 mg	ALT, AST, IRI_s histology in metformin arm
Thiazolidinediones				
Troglitazone				
Caldwell et al.[158]	10	Up to 6 mo	40 mg	ALT, AST
Pioglitazone				
Promrat et al.[106]	18	48 wk	30 mg	ALT, histology
Sanyal et al.[141,§]	20	6 mo	30 mg	Insulin sensitivity, histology
Shadid and Jensen[142]	5	4 mo	30 mg	ALT, AST
Rosiglitazone				
Neuschwander-Tetri et al.[105]	30	48 wk	8 mg	ALT, AST, GGT, histology, insulin sensitivity
Tiikkainen et al.[31,‖]	20	16 wk	8 mg	Hepatic fat, PPAR-γ, LPL, and adiponectin in fat tissue

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gammaglutamyltransferase; PPAR-γ, peroxisome proliferator-activated receptor γ agonists; LPL, lipoprotein lipase.

*Compared with 6 noncompliant patients.

†Compared with dietary control group.

‡Compared with either vitamin E (800 IU daily) or a prescriptive diet (all subjects received nutritional counseling).

§Compared with pioglitazone used in combination with vitamin E (400 IU daily) vs. vitamin E alone.

‖Compared with metformin (2 g) treatment group, which did not have improvement in the outcomes stated.

hypertensive patients with normal liver enzymes also have an increased prevalence of fatty liver, which is related to IR.[133]

The overall prevalence of metabolic syndrome in NAFLD patients is largely dependent on selection criteria. Most studies excluded patients with diabetes, where the metabolic syndrome is nearly the rule.[134] In a large NAFLD series, prevalence of the metabolic syndrome was higher in females and increased with increasing body mass index from 18% in normal-weight subjects to 67% in obese subjects.[19] The association of obesity with diabetes and/or altered lipid metabolism leads to a multiplicative effect on the final prevalence of metabolic syndrome, and significantly increases the risk of more severe stages of liver disease.[19]

Summary

Perhaps the strongest evidence supporting the importance of IR in the pathophysiology of NAFLD are the nascent data provided by recent clinical trials. Table 5 displays clinical strategies designed to improve insulin sensitivity in the liver[135-138] (metformin) and in the periphery[31,106,139-142] (thiazolidinediones) in patients with NAFLD. Although none of these studies had a double-blind randomized design, which is limiting,[143] the results certainly warrant additional carefully performed studies with these agents.

In closing, two additional points warrant emphasis. First, this review has focused on the effect of IR on the liver. However, IR and its associated metabolic syndrome is a systemic disease affecting (likely via mitochondrial injury[40]) the nervous system,[144] muscles,[39] pancreas,[145] kidney,[146] heart,[115,147] and immune system.[148] Second, NAFLD is a disease of our generation. NAFLD has ex-

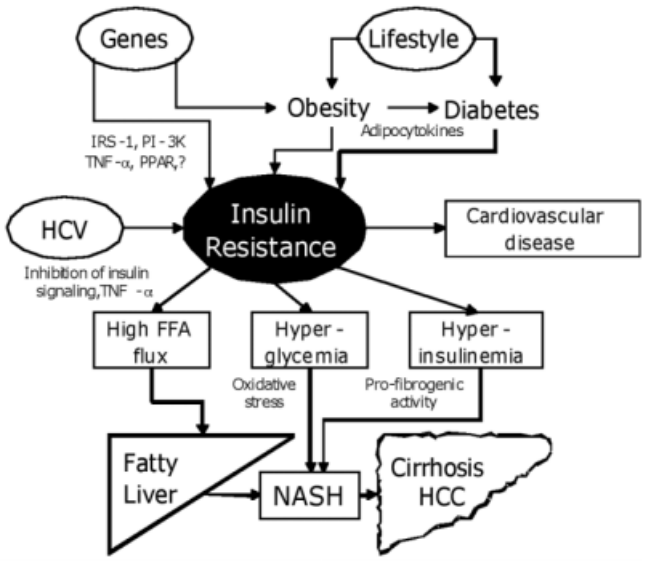

Fig. 3. Schematic showing the interaction of genes, lifestyle, and virus C in the pathogenesis of NAFLD and in disease progression. Note that cardiovascular disease may also result via the metabolic syndrome. IRS-1, insulin receptor substrate 1; TNF-α, tumor necrosis factor α; PPAR, proliferator-activated receptor; HCV, hepatitis C virus; FFA, free fatty acid; NASH, nonalcoholic steatohepatitis; HCC, hepatocellular carcinoma; PI-3K, phosphotidyl-inosytol 3 kinase.

ploded onto the clinical landscape over the past 25 years. Its pathophysiology is complex (Fig. 3), and the available data suggest that environmental factors such as diet, exercise, and/or environmental toxins are likely important. The fact that the prevalence of NAFLD varies among different racial groups[149] and that there are variable rates of disease progression within individuals with similar risk factors[150] strongly suggests that fat genes also play a role in both prevalence and natural history, as indicated by recent studies.[151-153] However, determining the genetics of IR in NAFLD is not easy.[154,155] There are over 400 gene interactions involved in fat storage,[156] and even more involved in insulin action.[157] A recent study suggests that abnormalities in at least 23 genes may be involved in NAFLD.[157] However, as is the case for other common complex metabolic diseases, there is likely to be an interaction between the environment and genetics that will determine the influence of IR on the phenotypic expression of NAFLD in any individual patient.

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