The role of insulin resistance in nonalcoholic steatohepatitis and liver disease development – a potential therapeutic target?

Paola Dongiovanni, Raffaela Rametta, Marica Meroni and Luca Valenti

Department of Pathophysiology and Transplantation, Università degli Studi di Milano, and Internal Medicine and Metabolic Diseases, Fondazione IRCCS Ca’ Granda Ospedale Policlinico Milano, Milano, Italy

ABSTRACT
Insulin resistance (IR) is defined by the inability of insulin to exert its metabolic actions, due to impaired activation of intracellular insulin signaling. This condition is caused by genetic defects or by environmental conditions, among which the most common is obesity. Systemic IR determines the development of hepatic fat accumulation, which can progress to nonalcoholic steatohepatitis, cirrhosis and hepatocellular carcinoma, and is a major determinant of liver disease independently of coexisting factors. Therefore, insulin-sensitizing drugs are currently under evaluation to improve steatohepatitis. Indeed, manipulation of nuclear hormone receptors is already under scrutiny for liver disease prevention by amelioration of IR, whereas NOTCH signaling inhibition represents a novel approach. Nevertheless, further research is warranted to better understand the mechanism linking IR to progressive fibrogenesis in the absence of inflammation and to identify novel drug targets.

Introduction
Insulin resistance – fatty liver syndrome

Insulin resistance (IR) is traditionally defined as reduced insulin capability to increase glucose uptake and utilization. It is a complex condition in which the liver, skeletal muscles and white adipose tissue become less sensitive to insulin and its downstream metabolic actions under normal serum glucose concentration. However, hepatic IR is characterized by a dissociation of the effect of insulin on glucose and lipid metabolism, in that hepatic de novo lipogenesis is paradoxically increased. The phenotypic expression of IR is dependent upon genetic defects or environmental triggering conditions, among which the most common is obesity. Indeed, obesity leads to adipose tissue inflammation and adipose IR, with spillover of lipids and ectopic fat accumulation in the muscles and visceral organs, determining alterations in the intracellular pathways regulating the response to insulin binding to its receptor. Altered secretion of adipokines contributes to a proinflammatory state and in turn increases IR.

A major role in the development of systemic IR syndrome is played by hepatocellular fat accumulation, i.e. steatosis. In particular, nonalcoholic fatty liver disease (NAFLD), defined as triglyceride (TG) accumulation in excess of 5% of liver weight in the absence of at risk alcohol drinking, has recently gained attention as a key player in liver disease progression.[1] Paralleling the obesity epidemics, NAFLD has become the leading cause of liver disease in Western countries.[2] Indeed, the prevalence of NAFLD increases with body mass index,[3] reaching 60–70% in obese patients.[4]

Moreover, the risk of developing the progressive form of the disease, presently identified as nonalcoholic steatohepatitis (NASH), is higher in obese subjects.

In susceptible individuals NASH may lead to hepatic complications such as cirrhosis and hepatocellular carcinoma (HCC), the risk being higher in those with more severe IR and type 2 diabetes mellitus (T2DM). Importantly, NAFLD frequently coexists with other liver diseases, related, for example, to alcohol abuse and chronic viral hepatitis, increasing the risk of disease progression. On the other hand, NAFLD also confers increased predisposition to extrahepatic complications of metabolic syndrome (MetS), including T2DM, proatherogenic dyslipidemia and cardiovascular disease.

As a key component of MetS, NAFLD is also closely associated with visceral obesity, and represents the hepatic manifestation of MetS. In this context, T2DM is the consequence of β-cell exhaustion in the setting of IR, and now a common determinant of liver disease.[5] Virtually, the entire spectrum of liver disease is seen in patients with T2DM. This includes abnormal liver
enzymes, NAFLD, cirrhosis, HCC and acute liver failure. The prevalence of NAFLD in T2DM patients is estimated at 34–74% and at 100% in T2DM with obesity.[6] Vice versa, NAFLD may contribute IR. Aminotransferase levels, reflecting hepatic fat content in individuals without viral hepatitis and alcohol abuse, predict T2DM development.[7,8] The mechanism may involve altered release of hepatokines influencing insulin signaling. IR, NAFLD and T2DM are all characterized by pro-atherogenic dyslipidemia, defined by hypertriglyceridemia, low high-density lipoprotein cholesterol concentrations, and the predominance of small dense LDL.[9]

Finally, hypertension has been associated with IR, obesity and T2DM. The high prevalence of NAFLD in non-obese hypertensive patients with normal liver enzymes appears related to increases in IR and body weight. Importantly, T2DM and hypertension are independent risk factors for the progression of liver fibrosis in NAFLD, possibly via activation of the renin–angiotensin–aldosterone axis.[10,11]

This review is aimed to summarize the general aspects of IR, its pathophysiology and the role of IR in the pathogenesis of chronic liver disease, with a special emphasis on the therapeutic implications.

Pathophysiological and molecular features of hepatic insulin resistance

Insulin is involved in mediating the metabolic transition that happens upon refeeding through tight regulation of several pathways. Insulin action is required for maintaining the balance between nutrients intake and storage. In fact, insulin promotes energy storage in adipose tissue, liver and muscle by stimulating lipogenesis, glycogenolysis and protein synthesis and inhibiting lipolysis, glycogenolysis and protein breakdown,[12] and regulates food intake and behavior. MetS and NAFLD are characterized by IR, so that higher levels of insulin are required to counteract hyperglycemia and maintain glucose homeostasis. The pancreatic β-cells compensate the impaired insulin response by increasing insulin production, but eventually in susceptible individuals, β-cells undergo exhaustion, and glucose concentration in the blood rises, leading to impaired glucose tolerance and T2DM.

From a clinical point of view, individuals are generally defined as insulin resistant by their response to an oral or intra venous glucose or insulin challenge.[13] Hyper-insulinemic euglycemic clamp techniques represent the gold standard for IR quantification. During the hyper-insulinemic euglycemic clamp, insulin-resistant subjects show reduced capability to metabolize glucose due to lower peripheral glucose clearance and unblunted hepatic glucose production. The oral glucose tolerance test commonly performed in clinical practice to classify patients according to their glycemic status (normal, impaired glucose tolerant or diabetic). IR can also be estimated from biochemical parameters using several indices. First described in 1985, the homeostasis model assessment-insulin resistance (HOMA-IR) is the most widely used index for the clinical assessment of IR.

IR may be caused directly from altered insulin receptor (INSR) activity, or by alterations in the downstream signaling pathways. The former is rarely determined by loss-of-function mutations of INSR gene, but most frequently reflects reduced expression on the cellular membrane. This is particularly relevant in the liver during NAFLD, and is involved in the pathogenesis of hyperinsulinemia by decreasing insulin clearance.[14]

Genetic and acquired alterations in insulin signaling play a major role in tissue specific IR. Under physiological conditions, insulin binding to INSR on the plasma membrane leads to INSR auto-phosphorylation and the consequent tyrosine phosphorylation of insulin receptor substrates-1 and –2 (IRS1 and IRS2). This event starts a signaling cascade resulting in phosphorylation of Forkhead transcription factor O1 (FOXO1), favoring translocation out of the nucleus and the shutdown of transcription of target genes. Among FOXO1-regulated genes, glucose-6-phosphatases (G6Pc), the rate-limiting enzyme in gluconeogenesis and hepatic glucose release,[15] plays a key role.

On the other hand, insulin induces sterol regulatory element-binding protein-1c (SREBP1c), which enhances transcription of genes required for de novo lipogenesis: Acetyl coenzyme A carboxylase and fatty acid synthase (FAS).[16] As de novo lipogenesis proceeds, the intermediate malonyl coenzyme A accumulates in the cytosol and inhibits carnitine palmitoyltransferase-1, the transporter that shuttles fatty acids into the mitochondria and the rate-limiting enzyme of fatty acids β-oxidation. Newly synthesized TGs are then packaged with apolipoprotein B into very low-density lipoproteins (VLDL) and exported for peripheral use.

In peripheral metabolic tissues, muscle and fat, insulin stimulates dietary glucose uptake by driving the translocation of glucose transporter GLUT4 to the cell surface and promotes fat storage through induction of lipoprotein lipase, which in turns hydrolyzes circulating TG to fatty acids that, once inside the cells, can be reesterified by adipocytes or oxidized for energy by myocytes. At the same time, in order to prevent release of stored fatty acids back to circulation, insulin inhibits lipolysis and induces lipogenesis in adipocytes.

Individuals affected by obesity and T2DM show significant decrease of IRS1-associated tyrosine...
phosphorylation and PI3K activity in skeletal muscle and adipose tissue. Subjects with T2DM show also reduced INSR expression and activity in both muscle and adipocytes. During NAFLD, IR prevents AKT-mediated inactivation of FOXO1 and its downstream targets,[17,18] resulting in deregulation of gluconeogenesis and persistent hepatic glucose output that cause mild hyperglycemia and hyperinsulinemia. Furthermore, oxidative stress associated with NAS induces FOXO1 de-acetylation mediated by SIRT1, resulting in unrestrained hepatic glucose output independently of FOXO1 phosphorylation status, despite improved adipose tissue insulin sensitivity.[19,20] However, it should be noted that overall evidence suggests muscle, adipose tissue and at whole body level, SIRT1 activation improves insulin sensitivity.[20,21]

As previously anticipated, MetS and NAFLD are characterized by "selective" or "dissociated" hepatic IR. In fact, insulin maintains the ability to induce de novo lipogenesis by increasing SREBP1c and preventing β-oxidation. NAFLD patients have 2-fold higher de novo lipogenesis compared to subjects with low liver fat concentration.[22] Several hypotheses have been raised to explain this apparent conundrum; here we will discuss some. Insulin signaling through different isoforms of IRS and AKT may contribute explaining dissociated hepatic IR during MetS. Specifically IRS2 and AKT2 would be involved in de novo lipogenesis,[23,24] and FOXO1 may represent the molecular switch of selective hepatic IR. Indeed, FOXO1 becomes active in the presence of IR and NAFLD, but it induces IRS2 and downregulates inhibitors of AKT.[25,26]

Furthermore, unrestricted FOXO1 activation contributes to dyslipidemia by reducing VLDL secretion, by downregulating MTTP and by inducing APOC3, a circulating inhibitor of lipoprotein lipase. Conversely, FOXO1 silencing by antisense oligonucleotides improved steatosis, IR and dyslipidemia in experimental models.[27] Notably, increased AKT2 activity has been also implicated in the progression of NAFLD to HCC. Indeed, liver-specific deletion of the AKT inhibitor PTEN results in severe steatosis evolving to HCC, and PTEN is frequently mutated during hepatic carcinogenesis.[28]

Recent data indicate that NOTCH-dependent signaling has been shown to play an important role in hepatic lipid metabolism regulation by interfering with insulin signaling. NOTCH receptor engagement mediates cell-fate decisions via interactions among neighboring cells and is involved in liver development and repairing. Recent studies have revealed a pivotal role of NOTCH in gluconeogenesis and lipogenesis regulation. An aberrant activation of this pathway in hepatocytes leads to hyperglycemia and fatty acids accumulation.[29,30] NOTCH regulates glucose and lipid homeostasis mainly through synergy with FOXO1 and AKT. In mouse models, combined haplo-insufficiency for Foxo1 and Notch-1 ameliorated insulin sensitivity in diet-induced IR.[30] Indeed, pharmacological blockade of Notch signaling by γ-secretase inhibitors improved glucose tolerance and IR. Conversely, constitutive activation of Notch-1 in liver induced G6PC expression, exacerbating IR in a FOXO1-dependent manner. Remarkably, NOTCH signaling uncouples gluconeogenesis activation and lipogenesis suppression. Liver-specific constitutive activation of Notch leads to steatosis, stabilizing mammalian target of rapamycin complex 1 (mTORC1) and stimulating SREBP1c-induced lipogenesis. Conversely, Notch ablation prevented steatosis blocking mTORC1 activity. Notably, NOTCH signaling activation is also observed in patients with NAFLD, and is correlated with the severity of IR and liver damage, suggesting that it may be involved in NASH pathogenesis.[31] Furthermore, Notch pathway activation may be involved in the process of fibrogenesis by promoting ductular reaction and neovessel proliferation.[32]

A schematic representation of the main alterations in hepatic insulin signaling discussed in this review is presented in Figure 1.

Another pathway deregulated during MetS is represented by bile acids signaling. Bile acids are increasingly recognized as key regulators of systemic metabolism. Bile acid interactions with the nuclear hormone receptor farnesoid X receptor (FXR) and the membrane receptor G-protein-coupled bile acid receptor 5 regulates the secretion of incretins and fibroblast growth factor 19, lipid metabolism, and energy expenditure, contrasting IR development. Bile acid levels and distribution are altered in T2DM and increased following bariatric procedures, and the bile acid metabolome is also altered in NASH.[33] Thus, modulation of bile acid levels and signaling may be exploited to treat IR and related liver disease.[34] Interestingly, recent studies indicate that the alterations in the bile acid pool during fatty liver and IR may be related to altered hepatic insulin signaling via unrestricted activation of FOXO1.[35,36]

**Insulin resistance in liver diseases development: NAFLD**

Epidemiological data from cross-sectional studies indicate that IR, MetS and T2DM are associated with liver damage severity and fibrosis,[37] independently of liver enzymes.[38] Previously, it was believed that NAFLD progresses to advanced liver disease only in the presence of inflammation; however, a recent meta-analysis of prospective studies on a new data from a large cohort provided novel results indicating that T2DM is associated with more rapid progression of fibrosis, even in the absence of NASH.[10,11]
Underscoring the relevance of these findings, recent data indicate that the severity of fibrosis, and not the presence of NASH, in the major determinant of NAFLD prognosis.\[39,40\] Although the exact mechanism of progressive liver disease in NAFLD is still under definition, current knowledge supports a model whereby development of liver damage is multifactorial, commonly summarized as the multi-hits hypothesis.\[41\] The first hit causes hepatic fat accumulation due to IR. Initially, hepatic fat accumulation results from an increased efflux of non-esterified or free fatty acids (FFA) from the adipose tissue to the liver.\[42\] FFA are stored into the hepatocytes as TG, in order to protect hepatocytes themselves from lipotoxicity.\[42\] Reduction in neutral lipid secretion through VLDL \[43\] and in \(\beta\)-oxidation due to mitochondrial damage are also involved in hepatic fat accumulation.

Excess of intracellular accumulation of lipid metabolites, such as diacylglycerol, ceramides and long chain acyl CoA, has also been implicated as a mediator of IR.\[44,45\] Aberrant accumulation of these bioactive intermediates engages c-Jun-N terminal kinase (JNK) that contributes to IR by phosphorylating IRS1 at serine residues, inhibiting its activity.\[46\] Indeed, accumulation of ceramides and/or diacyl-glycerol triggers the activation of atypical protein kinase C (PKC) isoforms that phosphorylate JNK.\[47,48\] Furthermore, in NASH patients, activation of the JNK pathway, which is involved in induction of programmed cell death by triggering mitochondrial damage in response to inflammation and/or cellular stress, may also contribute directly with the progression of liver damage, as it correlates with hepatocellular death by apoptosis.\[49\] Visceral obesity also contributes to NASH development. Several adipokines are released by adipose tissue, such as adiponectin, leptin, resistin, TNF-\(\alpha\), IL-1\(\beta\) and IL-6. These adipokines downregulate the expression of the glucose transporter GLUT-4 expression via increased serine IRS1 phosphorylation,\[12\] inducing IR.\[50\]

In addition, genetic experiments in mouse models suggest that hepatic IR directly contributed to the progression of liver damage. This has been shown in liver-specific INSR knockout mice, which display severe primary IR and a defect in insulin clearance. These mice develop an age-dependent nodular hyperplasia of the liver, oxidative stress and liver dysfunction with impaired ability to regenerate following partial hepatectomy. Moreover, IRS2-deficient hepatocytes are more susceptible to apoptosis.\[51\] This evidence suggests a causal role of IR in liver disease progression.
Human genetics seems to confirm that IR determines hepatocellular damage and fibrosis progression in NAFLD.[52] Functional common genetic variants of molecules involved in insulin signaling, such as IRS1 and ectonucleotide pyrophosphatase/phosphodiesterase1 (ENPP1) or PC-1 have been associated with the severity of liver damage.[53] Moreover, loss or gain-of-function mutations in these genes increase the risk of IR and T2DM, respectively. Thus, the amelioration of IR might improve the long-term outcomes of NAFLD patients. Since genetic variants are independent of confounders and reverse causation is not an issue, genetic data indicating that loss-of-function variants in the insulin signaling pathways are associated with more severe fibrosis suggest that IR has a causal role in NAFLD progression. This does not rule out that progressive NAFLD may be triggered by other insults in patients and in animal models. Indeed, the most studied mouse model of fibrosing NAFLD is induced by methionine and choline-deficient diet, where altered hepatic lipid metabolism is deranged by nutritional deficiencies instead of IR.[42]

During the last years, genome-wide association studies have revealed that polymorphisms in genes involved in TG remodeling and VLDL secretion play a major role in steatosis and NASH development and liver damage progression, though they have no impact on IR. These data suggest that IR is more a cause of steatosis and progressive liver disease than a consequence of these processes.[37,54,55] The mechanism may involve alteration of hepatic lipid metabolism favoring lipotoxicity or a direct effect on hepatocellular regeneration and the regulation of fibrogenesis.

**Mechanisms of progressive liver injury associated with IR**

The mechanisms underpinning liver damage progression related to IR have been investigated during last years. Excess hepatic FFA results in the generation of toxic lipid metabolites, which cannot be safely disposed of via mitochondrial β-oxidation, but are shifted toward peroxisomal and microsomal oxidation. These pathways produce more reactive oxygen species (ROS), worsening oxidative stress. Lipotoxicity, i.e. cellular injury and death caused by FFA and their metabolites, represents the major mechanism underlying hepatocellular dysfunction leading to the development of hepatitis. Lipotoxicity induces alteration in cellular metabolism leading to organelles injuries; oxidative stress; activation of stress-related signaling pathways, such as atypical PKC isoforms and JNK; and elevated pro-inflammatory cytokines and activation of Kupffer cells, which requires engagement of Toll-like receptor-4 (TLR-4) by intestinal bacterial products entering the portal circulation due to altered intestinal microbiota and increased permeability,[56] and directly by oxidized lipid species.[57] In parallel, excess free cholesterol trafficking to mitochondria leads to glutathione depletion impairing cellular antioxidant machinery and inducing hepatocellular susceptibility to TNFα and FAS.[58]

Lipotoxic injury induces mitochondrial dysfunction and endoplasmic reticulum stress, playing a central role in fat accumulation and ROS generation. Consequently, several self-sustained vicious cycles involving lipid peroxidation, mitochondrial DNA damage, ROS formation, depletion of antioxidants and cytokine release may trigger necroinflammation and fibrogenesis. Indeed, NASH patients had functional and morphological abnormalities in mitochondria,[59] impaired ability to re-synthesize ATP after carbohydrate challenge, and decreased respiratory chain complex activity.[60]

ROS accumulation can also cause endoplasmic reticulum stress and the consequent activation of the compensatory unfolded protein response pathway. Endoplasmic reticulum stress increases the activity of JNK and IKK (inhibitor of nuclear factor-κB – NF-κB – kinase), further impairing insulin signaling,[46] and leading to activation of NADPH, a transcription regulating inflammatory and acute-phase responses in the liver.[61]

The progression of liver damage is marked by accumulation of hepatic fibrosis resulting from deposition of extracellular matrix (ECM) by hepatic stellate cells (HSC). HSC are usually quiescent and reside in the sinusoids adjacent to hepatocytes. Once activated by injury, HSC loose retinoids contained in lipid droplets, proliferate and migrate to the sites of tissue damage, assuming a matrix-producing, contractile phenotype, as well as the ability to produce cytokines and chemokines that perpetuate inflammation and fibrogenesis. The ability of activated HSC to secrete collagen and other components of the ECM is potently stimulated by tissue growth factor-beta, as well as by lipid peroxidation products and connective tissue growth factor (CTGF).

In particular, hyperinsulinemia and hyperglycemia stimulate HSC to produce ECM, fibrosis deposition and CTGF secretion. Interestingly, it has recently been shown that the PNPLA3 I148M variant, the major genetic risk factor for NAFLD/NASH, alters retinol release by HSC, potentially contributing directly to fibrogenesis and carcinogenesis.[55,63]

Following resolution of damage, most activated HSC undergo apoptosis. Senescence of persistent HSC in...
response to chronic inflammation is now thought to contribute to the pathogenesis of HCC in NASH. In animal models of NASH, alterations of intestinal gut microbiota result in TLR-4 activation in Kupffer cells, triggering inflammation and contributing to the onset of senescence-associated secretory phenotype in HSC.[64] Interestingly, epidemiological and pathological evidence indicate that HCC in NAFLD frequently occurs outside cirrhosis.[65] Potential mediators of carcinogenesis include genetic factors and IR-mediated lipotoxicity, deregulation of proinflammatory/anti-inflammatory cytokines, hyperinsulinemia and deregulation of the insulin signaling pathway, which provide a permissive microenvironment for the development of cancer. However, the relative role of such pathways and their interplay with individual genetic background remain to be investigated.[66]

The main mechanisms involved in liver damage progression related to IR are shown in Figure 2.

Impact of insulin sensitizers on liver disease progression

Since IR is a key driver of liver disease progression, insulin-sensitizing drugs are currently undergoing extensive evaluation with regard to safety and ability to improve hepatic inflammation and fibrogenesis, while at the same time reducing the metabolic alterations that occur because of fatty liver. Until recently, the two most widely used classes of molecules are represented by metformin and thiazolidinediones (TZD).

Metformin is an AMPK-activating biguanide that represents the cornerstone of T2DM treatment.[67] It improves IR decreasing hepatic gluconeogenesis; enhances fatty acid oxidation, peripheral and hepatic insulin sensitivity; and induces weight loss. It decreases also intestinal glucose absorption, facilitating skeletal muscle glucose uptake. Steatosis, inflammation and...

Figure 2. Schematic cartoon depicting the pathophysiological role of insulin resistance in the development and progression of liver disease. Multiple parallel hits, including insulin resistance (IR), genetic factors, intestinal microbiota and inflammation account for steatosis development and progression. Fatty liver is characterized by FFA accumulation in lipid droplets resulting from an unbalance between triglycerides acquisition and removal. FFA stored as triglycerides during hepatic steatosis derive from peripheral lipolysis related to adipose tissue IR, followed by de novo lipogenesis (DNL) induced by hyperinsulinemia, and excessive food intake. FFA can be catabolized through β-oxidation and re-esterification to TG and stored as lipid droplets or exported as VLDL. Impaired ability to secrete VLDL and decreased β-oxidation due to mitochondrial damage play a role in hepatic fat accumulation. Long-term injury arising from TG storage and lipotoxicity; (ii) oxidative stress secondary to free radical produced during FFA oxidation; inflammation triggered by endotoxin; cytokine release; and endoplasmic reticulum (ER) stress lead in the end to inflammation (NASH), perpetuation of cellular damage and activation of fibrogenesis. Direct recruitment of Kupffer cells (KC) and other components of the innate immune response occurs with activation of the inflammation and the coordinated release of pro-inflammatory and fibrogenic cytokines. Hepatic stellate cells (HSC) are subsequently activated to produce extra-cellular matrix (ECM) leading to progressive fibrosis, cirrhosis and its complications (e.g., hepatocellular carcinoma (HCC)). Apoptotic bodies and factors produced by senescent cells (senescence-associated secretory phenotype (SASP)) can also influence HSC activity.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study type</th>
<th>N</th>
<th>Therapy (dose per day)</th>
<th>Compared with (dose per day)</th>
<th>Liver disease</th>
<th>Liver enzymes</th>
<th>US steatosis OR (CI 95%)</th>
<th>Histology</th>
<th>Histological steatosis OR (CI 95%)</th>
<th>Lobular inflammation OR (CI 95%)</th>
<th>Ballooning OR (CI 95%)</th>
<th>Fibrosis OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uygun [69]</td>
<td>OL, RCT</td>
<td>36</td>
<td>Met (850 mg)</td>
<td>Diet/exercise</td>
<td>NASH</td>
<td>Improved</td>
<td>NA</td>
<td>Not</td>
<td>Not</td>
<td>Not</td>
<td>Not</td>
<td>Not</td>
</tr>
<tr>
<td>Bugianesi [70]</td>
<td>OL, RCT</td>
<td>110</td>
<td>Met (2000 mg)</td>
<td>Diet/exercise</td>
<td>NA</td>
<td>Improved</td>
<td>NA</td>
<td>NA</td>
<td>Not</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Duseja [71]</td>
<td>OL, RCT</td>
<td>50</td>
<td>Met (500 mg)</td>
<td>Diet</td>
<td>NA</td>
<td>Improved</td>
<td>NA</td>
<td>Not</td>
<td>Not</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Haukeland [72]</td>
<td>OL, RCT</td>
<td>48</td>
<td>Met (2000 mg)</td>
<td>Diet/exercise</td>
<td>NA</td>
<td>Improved</td>
<td>NA</td>
<td>Not</td>
<td>Not</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Garinis [73]</td>
<td>OL, RCT</td>
<td>50</td>
<td>Met (1000 mg)</td>
<td>Diet</td>
<td>NA</td>
<td>Improved</td>
<td>NA</td>
<td>Not</td>
<td>Not</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Sanyal [74]</td>
<td>OL, SA</td>
<td>20</td>
<td>Piogl (30 mg) + Vit E (800 IU)</td>
<td>Diet/exercise</td>
<td>NASH</td>
<td>Improved</td>
<td>NA</td>
<td>Improved</td>
<td>Improved</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Belfort [75]</td>
<td>Blinded,</td>
<td>55</td>
<td>Piogl (45 mg)</td>
<td>Placebo</td>
<td>NASH</td>
<td>Improved</td>
<td>NA</td>
<td>Improved</td>
<td>Improved</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Athial [76]</td>
<td>RCT</td>
<td>74</td>
<td>Piogl (30 mg)</td>
<td>Placebo</td>
<td>NASH</td>
<td>Improved</td>
<td>NA</td>
<td>NA</td>
<td>Not</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Idilman [77]</td>
<td>OL, RCT</td>
<td>74</td>
<td>Rosigl (8 mg)</td>
<td>Met (850 mg) + lifestyle modification</td>
<td>NA</td>
<td>Improved</td>
<td>NA</td>
<td>Improved</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ratziu [78]</td>
<td>Blinded,</td>
<td>63</td>
<td>Rosigl (4 mg)</td>
<td>Placebo</td>
<td>NA</td>
<td>Improved</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Omer [79]</td>
<td>OL, RCT</td>
<td>64</td>
<td>Rosigl (4 mg)</td>
<td>Met (1700 mg)</td>
<td>NA</td>
<td>Improved</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Sanyal [80]</td>
<td>Blinded,</td>
<td>274</td>
<td>Piogl (30 mg)</td>
<td>Placebo and Vit E (800 UI)</td>
<td>NASH</td>
<td>Improved</td>
<td>NA</td>
<td>Improved</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA: not available; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; Met: metformin; OL: open label; Piogl: pioglitazone; RCT: randomized controlled trial; Rosigl: rosiglitazone; SA: single arm; Vit E: vitamin E.

References are reported in the Supplementary material.
fibrosis were improved in NASH patients treated with metformin (2 g/day) for 12 months. Specifically, metformin reduced aminotransferase levels and hepatic expression of TNF-α, which interferes with insulin signaling in hepatocytes. However, subsequent studies and a meta-analysis showed that metformin was not an effective treatment for patients with NASH without T2DM, as it was not superior to placebo for any historical or biochemical outcome. The main randomized controlled studies are reported in Table 1.[68] However, in non-randomized retrospective clinical studies, metformin has been associated with reduced incidence of HCC and mortality in cirrhosis, although the mechanism is not clear. Notably, metformin reduced by about 50% HCC risk even in hepatitis C virus (HCV)-infected patients. Nonetheless, metformin should be used with caution in patients with decompensated cirrhosis, as it can accumulate during liver and renal failure and cause lactic acidosis.

TZD are peroxisome active proliferator receptor (PPAR)γ agonists, which promote hepatic fatty acid oxidation, decrease hepatic lipogenesis and improve insulin sensitivity. PPARγ is a nuclear hormone receptor, acting as transcription factor that controls adipocyte differentiation and the production of adiponectin. Indeed, TZD stimulate FFA storage in subcutaneous adipocytes.[81] TZD include different compounds, such as pioglitazone and rosiglitazone, even if the use of rosiglitazone has been limited as a result of increased cardiovascular risk due to fluid retention. TZD ameliorate insulin sensitivity, steatosis, adiponectin and aminotransferases in NAFLD. The effects on inflammation and fibrosis are variable. However, anti-inflammatory properties may be suggested by a decrease in NF-κB expression and by an increase in klotho and adiponectin levels. In multiple small pilot studies, daily administration of rosiglitazone (8 mg/day for 12 months) and pioglitazone (30 mg/day) improved insulin sensitivity, aminotransferases and liver damage in obese patients with biopsy-proven NASH without T2DM and cirrhosis (Table 1). A recent meta-analysis indicated that high-quality evidence supports the effects of TZD in improving ballooning degeneration and possibly inflammation, but not fibrosis.[82] Furthermore, the beneficial effects of these drugs disappear after treatment interruption suggesting that they should be administrated to patients life-long. In addition, the clinical use of TZD is limited by the occurrence of several adverse effects including weight gain, increased risk of congestive heart failure, osteoporosis and bladder cancer.[83]

The effects of PPARγ agonists on NAFLD may be explained by the ability to target different tissues and pathways. First, PPARγ activation improves IR in peripheral tissues, thus decreasing FFA flux to the liver. Second, PPARγ inhibits activation and proliferation of HSC, thus preventing fibrogenesis. Third, PPARγ displays anti-inflammatory effects in macrophages and in hepatic endothelial cells.[84] Therefore, manipulation of nuclear receptors by potent specific ligands may represent a viable approach to prevent the development and progression of liver disease related to IR.[84]

Lastly, clinical studies are ongoing to evaluate the impact of PPARα/δ ligands on hepatic damage in patients with NASH, as these molecules improved IR and lipid metabolism in preclinical models of MetS and steatohepatitis.[86]

Clearly, further studies are required and several are already ongoing, but modulation of nuclear hormone receptors activity represents a promising approach, possibly to be used in combined regimens for prevention of liver disease progression.

Insulin resistance in the progression of other liver diseases

The role of IR in determining the progression of liver disease is not restricted to “pure” NAFLD. HCV infection affects 1–3% of the world population and is a major cause of chronic liver disease and cirrhosis.[87] HCV directly alters glucose homeostasis and is associated with an increased risk of IR and T2DM, especially in patients at risk due to obesity and aging.[88] IR occurs in early stages of hepatic lesions and it worsens as hepatic fibrosis progresses,[89] but viral eradication decreases IR.[90] Several hypotheses have been proposed to explain HCV induction of IR: inflammatory cytokines, such as TNF-α and IL-6, that induce IR through tyrosine phosphorylation of IRS1; induction of suppressor of cytokines signaling (SOCS3), which promotes proteasomal degradation of IRS1/2; and increased oxidative stress and lipid peroxidation, triggering inflammation and IR.[91]
The severity of IR has been associated with fibrosis progression also in chronic HCV hepatitis.[89] Indeed, serum insulin and HOMA-IR are higher in HCV patients compared to uninfected controls and increase with fibrosis development.[89,92] Thus, IR is a driving force that promotes disease progression in patients infected with HCV.[93] On the other hand, steatosis also accelerates fibrogenesis in chronic HCV hepatitis.[94] In addition, steatosis and IR were associated with lower response rate to interferon based-therapy.[95] The use of insulin sensitizer drugs (TZD or metformin) has therefore been tested with variable success to increase the efficacy of these treatments (the main studies are reported in Table 2). However, the clinical relevance of these findings is vanishing in the new direct antiviral agents era.

The frequency of steatosis and T2DM is lower in patients with chronic hepatitis B (V) than in HCV-infected patients. Although the incidence of HBV infection has decreased since the implementation of hepatitis B vaccination, HBV still represents an important health problem worldwide, and MetS increases liver fibrosis progression and HCC incidence even in this setting.[102,103] IR is also a major risk factor for the progression of alcoholic liver disease (ALD). ALD is a complex disease whose development depends on long-term excessive drinking and other environmental, acquired and inherited factors. It is the major cause of liver failure worldwide. The histological spectrum of ALD includes simple steatosis, steatohepatitis and cirrhosis. However, the majority of individuals who abuse alcohol do not develop cirrhosis.

Importantly, increased adiposity and fasting glucose are independently associated with disease severity in ALD. Conversely, ALD patients have high basal levels of insulin and an impaired insulin signaling. Impaired insulin secretion, tissue response to insulin, non-oxidative glucose disposal and glucagon response may contribute to IR in ALD. The mechanism through which alcohol interferes with insulin signaling is not well understood. Both in vitro and in vivo studies reported an interference of alcohol on insulin signaling, through several mechanisms: direct binding of alcohol to INSR, inhibition of INSR phosphorylation or internalization, and impairment of downstream insulin signaling. Taken together, these alterations lead to disease progression, increasing the risk of advanced disease. However, due to the difficulty to conduct studies in this setting, to date there are no data supporting the clinical use of insulin sensitizers in ALD.[104]

Finally, large epidemiological studies indicate that obesity is associated with higher risk of HCC.[66] Moreover, obesity is an independent predictor of HCC in patients with alcoholic and cryptogenic cirrhosis.[66] Several population studies indicate that T2DM is also a risk factor for HCC.[105] It has been hypothesized that a high concentration of insulin and insulin growth factor 1 in T2DM may have carcinogenic activity.[106] This would suggest that glucose-lowering drugs, such as metformin, could reduce HCC development in at risk patients.[107]

### Conclusions

IR represents the most frequent trigger of NAFLD. Initially, hepatic fat accumulation results from an increased flux of non-esterified or FFA to the liver due to adipose tissue IR. Nonetheless, hyperinsulinemia and diet activate lipogenic transcription factors and induce de novo lipogenesis. Furthermore, fatty liver per se
precipitates hepatic IR promoting metabolic disturbances and cardiovascular damage. IR may be caused directly from altered INSR activity (loss-of-function mutation or reduce cell membrane expression), or by alterations in the downstream signaling pathways. In the presence of impaired insulin signaling the transcription factor FOXO1 is de-phosphorylated, determining unrestrained transcription of genes involved in glucose production. Recently, NOTCH signaling has been implicated in the upregulation of G6Pc expression in a FOXO1-dependent manner during IR, while at the same time stimulating de novo lipogenesis. Thus, IR promotes NASH development and liver damage progression. Hepatic lipotoxicity, oxidative stress, inflammation and cytokines release are important contributing factors. Genetic studies suggest that IR has a causal role in disease progression.

Insulin sensitizing drugs are currently evaluated with regard to safety and ability to improve histological features and inflammation. Recent meta-analyses showed that metformin is an ineffective treatment for patients with NASH without T2DM, whereas TZD are effective in improving ballooning degeneration and possibly inflammation, but not fibrosis, and are burdened by side effects. A novel approach to treat IR and steatosis development is represented by manipulation of nuclear receptors, which control glucose and lipid metabolism, inflammation and fibrogenesis, by potent specific ligands. Nevertheless, further research is warranted to better understand the mechanism linking IR and chronic liver diseases and to identify new therapeutic targets.

Expert commentary

During recent years, the field of IR in liver disease has witnessed important novelties at different levels, from both the pathophysiological and therapeutic point of view.

At the basic research level, the mechanisms underpinning dissociation of hepatic IR during metabolic diseases, i.e. lack of suppression of glucose production by insulin despite heightened drive on lipogenesis, have begun to be unraveled. Indeed, activation of bile acid receptors, as well as of other pathways that converge on FOXO1 and mTOR, in particular the NOTCH pathway, have been shown to modify insulin action promoting hepatic fat accumulation and dyslipidemia. These findings have major implication for understanding both the liver-related complications of nonalcoholic fatty liver disease and cardiovascular disease in strongly associated MetS. Furthermore, they provide attractive new therapeutic targets.

Indeed, accumulating evidence from clinical studies has dampened the hope that currently used insulin sensitizing drugs have major beneficial effects on liver disease progression. Though it may reduce mortality in cirrhosis, metformin did not ameliorate steatohepatitis and fibrosis in individuals without T2DM. On the other hand, pioglitazone improved steatosis with possible minor effect on fibrogenesis, but is burdened by severe side effects. However, great expectations stem from the clinical development of new classes of drugs, which may also regulate IR, such as PPARα/δ and FXR agonists. The first trial in NASH showed that the prototypical compound OCA reduced liver damage, despite an increase in serum cholesterol.

Future studies are still required to understand the mechanisms by which IR determines progression of liver disease, as new evidence points out that the effect may be independent of inflammation, and to develop the aforementioned novel therapeutic approaches.

Five-year view

In the next five years, we expect that at the basic research level, the detailed molecular mechanisms of dissociated hepatic IR during steatosis will be clarified. In particular, the sources and regulation of NOTCH signaling identified, as long as novel therapeutic targets in this pathway.

Furthermore, the impact of IR on liver fibrosis progression will be widely recognized as independent by inflammation, and the cellular mechanisms characterized. This will have a major impact for the clinical management, indication for therapy, and the design and inclusion criteria for clinical trials in metabolic liver diseases. Besides, it will provide a new approach to therapy.

Concerning the ongoing clinical studies, the efficacy of FXR and PPAR α/δ ligands will be clarified. FXR ligands will likely be developed in combinations with statins and possibly anti-fibrotic agents, and the first drug regimens will hopefully be approved by regulatory agencies for the treatment of NASH.

Financial & competing interests disclosure

The authors were supported by the Ricerca Corrente 2015, Fondazione IRCCS Ca’ Granda Ospedale Policlinico Milano, and by the FIR grant, Department of Pathophysiology and Transplantation, Università degli Studi di Milano. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
Key issues

- Insulin resistance is a complex condition in which the liver and peripheral metabolic tissues become less sensitive to insulin.
- Insulin resistance is involved in hepatic steatosis development and plays a causal role in the progression of several liver diseases, representing one of the major drivers of liver disease worldwide.
- Metformin and thiazolidinediones improve insulin sensitivity and their use may be helpful to improve some features of insulin-resistance-related liver damage, although their impact on fibrogenesis is limited and are burdened by side effects.
- Recent findings point to nuclear receptors and NOTCH pathway as novel therapeutic targets for insulin resistance and liver disease treatment. However, further studies are needed to confirm the efficacy of this therapeutic strategy.

References


