

Insulin resistance in chronic hepatitis C: mechanisms and clinical relevance

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Summary

Insulin resistance is the main clinical and pathogenetic feature of the metabolic syndrome, one of the major health problems worldwide. Chronic liver diseases may induce insulin resistance. The hepatic manifestation of the metabolic syndrome is nonalcoholic fatty liver disease. Insulin promotes the storage of energy in the fed state by stimulation of glycogen synthesis, lipogenesis, suppression of gluconeogenesis and VLDL formation. Epidemiological studies have shown that chronic hepatitis C induces insulin resistance. Insulin resistance in chronic hepatitis C is associated with progression of liver fibrosis, resistance to antiviral therapy and development of hepatocellular carcinoma.

Here we review the major findings from epidemiological studies from 1994 to the present which have resulted in our current knowledge of insulin resistance in chronic hepatitis C. We further summarise the preliminary pathogenetic models that explain the development of hepatitis C virus-induced insulin resistance. Finally, we draw conclusions for the clinical management of these patients.

Key words: hepatitis C virus; insulin resistance; diabetes; steatosis; pathophysiology

Introduction

Hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease. According to recent WHO estimates the worldwide prevalence of HCV is 2.2%, affecting approximately 130 million people worldwide [1]. Chronic hepatitis C is a multifaceted disease associated with numerous clinical manifestations such as mixed cryoglobulinaemia, porphyria, membranoproliferative glomerulonephritis, B-cell lymphoma, insulin resistance and diabetes mellitus [2].

HCV infection is usually clinically inapparent; acute or fulminant hepatitis are uncommon. Up to 85% of patients infected with HCV develop chronic hepatitis C (CHC) and are at risk for fibrosis progression. Approx. 20–30% of CHC patients will develop cirrhosis of the liver within approximately 20–40 years [3]. Once cirrhosis is established the rate of HCC development is 1–4% per year [4]. The highly variable natural course of HCV infection depends on both viral and host factors. Age, male gender, alcohol consumption and also insulin resistance are defined risk factors for a progressive course of chronic hepatitis C.

The current standard treatment of CHC is pegylated interferon- α (pegIFN α) combined with

ribavirin. The duration of treatment depends on the HCV genotype [5]. Successful treatment is defined by a so-called “sustained virologic response (SVR)”, determined by undetectable HCV-RNA in the patients’ serum six months after the end of treatment. With the current treatment some 50% of patients can be cured of HCV depending on HCV genotype (40–50% of patients infected with genotype 1, 60% with geno-

List of abbreviations

| | |
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| CHC | Chronic hepatitis C |
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C virus |
| HOMA-IR | Homeostasis model assessment of IR |
| IR | Insulin resistance |
| NAFLD | Nonalcoholic fatty liver disease |
| NASH | Nonalcoholic steatohepatitis |
| PPAR γ | Peroxisome proliferator-activated receptor γ |
| PP2A | Protein phosphatase 2 A |
| SVR | Sustained virologic response |
| T2DM | Type 2 diabetes mellitus |
| VLDL | Very low density lipoprotein |

type 4 and 80% with genotypes 2 or 3) [6–8]. At present no vaccines against HCV are available.

Insulin resistance (IR) is believed to represent one of the central clinical features of the so-called “metabolic syndrome”, a subsumption of risk factors associated with the incidence of cardiovascular disease and diabetes. IR is further thought to represent the major pathogenetic factor for type 2 diabetes mellitus (T2DM), although recent data suggest a compensatory gain of betacell mass and function in obese patients with regular betacell function [9].

The WHO 1999 definition of the metabolic syndrome has been widely applied in clinical and epidemiological research. The definition is based on the occurrence of T2DM or impaired glucose tolerance or IR in a patient, together with two of the following conditions: hypertension, raised triglycerides, low HDL cholesterol, obesity or

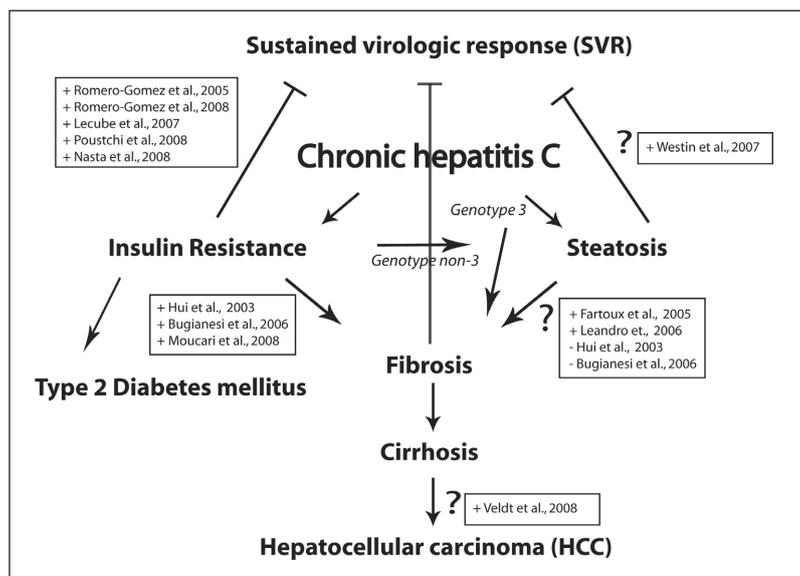
microalbuminuria [10]. According to the recently revised criteria of the International Diabetes Federation (IDF), which were based on recommendations from a consensus workshop of the IDF Epidemiology Task Force, the metabolic syndrome is diagnosed in a person with central obesity meeting two of the following four additional criteria: elevated serum triglycerides, reduced HDL cholesterol, raised arterial blood pressure and raised fasting glucose levels [10]. IR also causes hepatic disease: it is the major pathogenetic factor for nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome. NAFLD is a clinicopathological entity describing hepatic lipid accumulation causing chronic liver disease in the absence of alcohol intake. NAFLD ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) [11].

Insulin resistance is defined as a condition in which higher insulin concentrations are needed to achieve normal glucose metabolism, or in which normal insulin concentrations fail to achieve normal glucose metabolism [12]. The gold standard for the assessment of insulin resistance is the euglycaemic hyperinsulinaemic clamp technique. Another more practicable and also well-accepted method of measuring systemic insulin resistance is the HOMA-IR (homeostasis model assessment of insulin resistance). It is calculated by the following formula: fasting glucose (mmol/L) x fasting insulin (mIU/L) / 22.5 [13]. There is no established method of measuring exclusively insulin responsiveness of the liver, pancreas, muscle or fat in a patient with systemic IR.

In the last decade the HCV virus has been shown to induce IR itself and thereby to promote hepatic inflammation and fibrosis [14–16] and a lower SVR rate [17–21] (see fig. 1). A better understanding of the pathogenetic mechanisms underlying IR development in CHC is required as a basis for the development of drugs for the treatment of IR in CHC, with the aim of preventing progression of fibrosis and improving the response to therapy with pegIFN α and ribavirin.

Figure 1

Consequences of insulin resistance in chronic hepatitis C. Insulin resistance in chronic hepatitis C is a risk factor for fibrosis progression and impairs antiviral treatment response. Steatosis in genotype 3 infection is probably independent of insulin resistance and may not influence fibrosis progression or response to therapy. (→, positive association, ←, negative association/inhibition)



Epidemiology linking insulin resistance to chronic hepatitis C

Abnormalities of carbohydrate metabolism, including hyperinsulinaemia and insulin resistance, are established complications of advanced cirrhosis independent of the aetiology of the underlying chronic liver disease. A link between infection with HCV and an increased risk of type 2 diabetes mellitus (T2DM) or insulin resistance (IR) in noncirrhotic patients has been known for the last 15 years. In a 1994 retrospective study in 100 cirrhotic patients listed for transplantation Allison et al. reported that the prevalence of T2DM was higher in patients with HCV-associated cirrhosis than in cirrhotics with other underlying liver diseases [22]. An increased prevalence of T2DM in CHC was con-

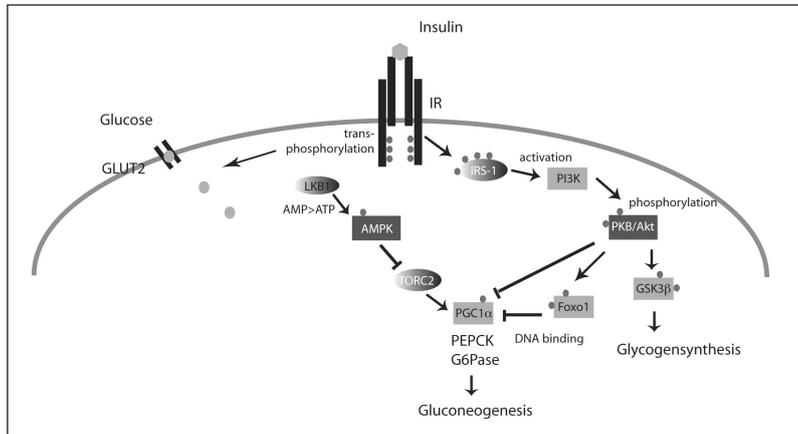
firmed in a study in 1117 noncirrhotic patients with chronic viral hepatitis [23]. T2DM was present in 21% of HCV-infected patients, but only in 12% of HBV-infected patients, a difference that was highly significant. Furthermore, the authors found an increased prevalence of HCV antibodies in a cohort of 594 diabetics (4.2%) compared to a cohort of 377 patients with thyroid disease (1.6%) [23]. In the meantime, the association between HCV and diabetes has been confirmed by several groups [24–28].

In 2003, Hui et al. linked insulin resistance (IR), the primary pathogenetic factor of T2DM, to HCV. The authors compared fasting serum insulin, C peptide, and HOMA-IR levels between

Figure 2

The insulin signalling cascade. Insulin binds to the hepatic insulin receptor and activates the insulin signalling cascade, resulting in activation of glycogen synthesis from glucose and inhibition of gluconeogenesis from amino acids.

(→, positive association, ←, negative association/inhibition) Abbreviations: AMPK, AMP activated protein kinase, Foxo1, forkhead transcription factor 1, GLUT2, glucose transporter 2, G6Pase, glucose-6-phosphatase, GSK3β, glycogen synthase kinase 3β, IR, insulin receptor, IRS-1, insulin receptor substrate 1, PEPCK, phosphoenolpyruvate-carboxykinase, PGC1α, peroxisome proliferator-activated receptor 1α, PI3K, phosphatidylinositol-3-kinase, PKB/Akt, protein kinase B, TORC2, transducer of regulated CREB activity 2.



121 CHC patients without relevant hepatic fibrosis and 137 healthy volunteers matched by sex, body mass index, and waist-to-hip ratio. All three parameters were significantly higher in patients with CHC. Additionally, by showing that IR was an independent predictor of progression of fibrosis, they provided evidence that IR plays a role in the pathogenesis of disease progression in CHC [14]. A more recent analysis proved the association of HCV infection with IR in patients with (32.4%) and without (15 %) metabolic syndrome. IR was associated in particular with genotypes 1

and 4 and high serum HCV RNA levels. Furthermore, the association of IR with fibrosis was confirmed and was independent of steatosis [16]. The association of CHC with IR was subsequently confirmed in several studies [16, 29, 30]. The observation that successful antiviral treatment (SVR) improved IR supports a direct causal role of HCV in IR development [17, 18].

IR also appears to increase the risk of developing HCC. In a large cohort of 541 patients with CHC and advanced fibrosis, 85 (16%) of whom had T2DM, Veldt et al. observed a significantly higher 5-year occurrence of HCC in the diabetes group (11.4% vs 5.0% [$P = 0.013$]) [31].

A factor of clinical importance is the negative impact of IR on response to antiviral treatment. It has been observed in a number of studies that IR interferes with the standard antiviral treatment, pegIFNα and ribavirin. In 2005, Romero-Gomez analysed IR in 159 patients with HCV undergoing antiviral treatment. In addition to fibrosis stage and genotype, the authors found that IR was an independent predictor of SVR: 60.5% of genotype 1 patients with normal HOMA-IR (<2) achieved SVR, but only 20% of patients with pathological HOMA-IR (>4) [17]. Interestingly, HOMA-IR and fasting glucose improved in patients who could be cured of CHC (17, 18). Meanwhile these findings have been confirmed by several other studies [19–21, 32]. It has been suggested that clinical application of pretreatment HOMA-IR made it possible to predict treatment outcome and determine treatment regimens [33].

These associations of insulin resistance in CHC are summarised in figure 1.

Pathophysiology of insulin resistance in chronic hepatitis C

Development of IR and T2DM involves highly complex systemic mechanisms that have not yet been conclusively described.

Virus-induced insulin resistance is a more restricted scenario, since it is primarily limited to the organs and tissues infected by the virus. In the case of hepatitis C, the presence of viral particles in the liver is regarded as the origin of the development of insulin resistance. Viral proteins themselves or host cell adaptive mechanisms could interfere with the insulin signalling pathway in hepatocytes (for details see fig. 2), or the chronic inflammatory response in the liver could induce IR indirectly through the induction of cytokines that could induce IR in the liver but also systemically. Evidence for both pathogenetic mechanisms has been provided in recent years.

Aytug et al. studied phosphorylation of different proteins within the insulin signalling cascade *in vitro* [34]. Liver biopsy tissue from 42 patients with CHC was compared with tissue from 10 patients with other chronic liver disease. The au-

thors reported normal phosphorylation of the insulin receptor in both groups, but the insulin signalling cascade was inhibited downstream of the receptor in the HCV biopsies. Phosphorylation of insulin receptor substrate 1 (IRS1), the association of IRS1 with p85PI3K and phosphorylation of PKB/Akt were all decreased in HCV biopsies. The mechanism whereby the HCV virus induces hypophosphorylation of these proteins remained uninvestigated [34].

In a recently published study of liver biopsies from 55 patients with CHC compared to 5 patients with healthy liver, we reported that the insulin signalling cascade is inhibited at the level of PKB/Akt phosphorylation in CHC *in vitro* and *in vivo* [35]. The mechanism involves HCV-induced hepatic upregulation of protein phosphatase 2 A (PP2A) [36, 37]. We have shown that in human hepatoma cells in culture and in liver tissue of HCV transgenic mice, upregulation of PP2A interferes with insulin signalling by hypophosphorylation of PKB/Akt and GSK3β, while IRS1-phosphoryla-

tion and IRS1-interaction with p85PI3K were intact. Interestingly, PP2A also interfered with phosphorylation of AMPK [35]. AMPK is another important kinase that regulates energy homeostasis in response to changes in the energy state of a cell [38–40]. The antidiabetic drug metformin lowers glucose levels through the inhibition of gluconeogenesis by phosphorylation of AMPK [41]. Thus PP2A interferes with glucose homeostasis regulation by interfering with two independent signalling pathways.

Indirect interference of viral proteins with the insulin signalling pathway has been further described by Kawaguchi et al., who demonstrated *in vitro* that the HCV core protein-induced upregulation of SOCS-3 led to inhibition of the signalling cascade by the proteosomal degradation of insulin receptor substrates 1 and 2 (IRS 1 and 2) [42].

Pazienza et al. described genotype-specific mechanisms by which the HCV core protein interferes with the insulin signalling cascade *in vitro*. While the HCV core protein promoted IRS-1 degradation through downregulation of peroxisome proliferator-activated receptor γ (PPAR γ) and upregulation of the suppressor of cytokine signal 7 (SOCS-7) in genotype 3a, the

core protein of genotype 1b induced IRS-1 degradation due to activation of the mammalian target of rapamycin (mTOR) [43].

Interleukin (IL)-6 and tumour necrosis factor α (TNF α) are two of several proinflammatory cytokines associated with IR. Both IL-6 and TNF α are significantly upregulated in patients with chronic hepatitis C [44]. Chronic exposure to the cytokine IL-6 reduced hepatic insulin receptor autophosphorylation and phosphorylation of IRS1 and 2, and resulted in reduced insulin sensitivity in cell culture and mice [45, 46]. Various authors have also suggested that TNF α upregulation in response to viral infection interferes with the insulin signalling cascade and initiates IR development [47–49]. A recent study revealed that HCV infection is associated with increased IR, which was inversely correlated with the serum adiponectin level [50].

To summarise, different mechanisms have been suggested that might, in parallel or genotype-specifically, be responsible for the virus-induced dysregulation of glucose homeostasis leading to IR development in CHC. It remains to elucidate the mechanism that plays the major role and thus define the most promising drug targets.

Steatosis in chronic hepatitis C

While CHC may coexist with nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH) in patients with metabolic syndrome, it has become obvious that the HCV virus can itself induce steatosis, a feature which by analogy has been called virus-associated steatohepatitis (VASH). Different clinical and experimental studies have shown a direct relationship between HCV replication and steatosis [51, 52]. Many studies have analysed the relationship between steatosis and fibrosis, as well as steatosis and IR, to further our understanding of the pathophysiology and consequences of steatosis.

A study by Rubbia-Brandt et al. in 2000 showed that HCV genotype 3 is associated with higher steatosis scores than other genotypes. A significant correlation has been observed between the steatosis score and the titre of intrahepatic HCV RNA in patients infected with genotype 3, but not genotype 1 [51].

Fartoux et al. analysed the relationship between IR, steatosis and fibrosis in 141 patients with noncirrhotic CHC. HOMA-IR was higher in genotype 1- than genotype 3-related steatosis and independent risk factors for steatosis were indeed IR in genotype 1 ($p = 0.001$) and viral load in genotype 3 ($p = 0.003$). In conclusion, this study proposes a model of virus-induced steatosis for CHC genotype 3, but not genotype 1 where steatosis develops due to virus-induced IR [53]. IR, as an early and sensitive parameter of disturbed glucose metabolism, has been further asso-

ciated with fibrosis progression, while steatosis has not [14]. Bugianesi et al. compared IR, steatosis and fibrosis in CHC genotype 3 and NAFLD. Independent predictors of fibrosis in NAFLD were grading of fat, IR and ferritin, while only IR, but not fat, predicted fibrosis in CHC genotype 3 [15]. A recent analysis of risk factors for rapid fibrosis progression in 1263 patients from the Swiss Cohort database linked genotype 3 to fibrosis progression (OR = 1.97, $p < 0.001$). Preliminary data were presented at the 2009 joint annual meeting of the Swiss Society for Infectious Disease [54]. Analysis of steatosis and treatment outcome of the DITTO trial revealed that steatosis in nongenotype 3 patients significantly impaired SVR (65% vs 46%, $p = 0.01$) [55].

Various HCV transgenic mice models provide insights into the pathogenesis of HCV-induced steatosis. Two transgenic mice models that express the full-length HCV genotype 1b genome in the liver developed steatosis with or without inflammation at an advanced age [56, 57]. In mice expressing the HCV core protein of genotype 1b, IR development preceded development of steatosis, suggesting that IR is a cause rather than a consequence of steatosis [47, 58]. It has been shown that HCV interferes with VLDL assembly and secretion by targeting the microsomal triglyceride transfer protein (MTP) in these mice [59, 60]. Another study proposes lipid accumulation through upregulation of sterol regulatory element binding protein 1c (SREBP1c) [61].

Taken together, in CHC patients infected with genotype-non-3 IR appears to induce steatosis and implicates more severe fibrosis and impaired SVR. On the other hand, steatosis alone is

more common in genotype-3 infected patients and seems not to influence disease progression or treatment outcome.

Therapeutic implications

The current knowledge summarised above identifies IR as a risk factor for fibrosis progression, non-response to antiviral therapy and possibly HCC development in patients with CHC. Nevertheless, there is no consensus for specific recommendations regarding diagnostic workup or therapy in patients with CHC and IR. IR development in CHC patients should be assessed regularly by the HOMA-IR, a non-invasive test to define patients at risk. Additionally, other clinical features of the metabolic syndrome such as adiposity, hypertension or dyslipidaemia should be determined.

Because of the risk of fibrosis progression, patients with CHC and IR who fulfil the criteria for antiviral treatment should be strongly advised to undergo antiviral therapy as soon as possible to eliminate the virus. As mentioned, the current guidelines do not advise additional drug treatment, different protocols or a longer duration of treatment to improve the response to standard antiviral therapy in patients with CHC and IR. Side effects are comparable to those in HCV patients with physiological insulin sensitivity.

IR can be influenced by lifestyle modifications and by drugs. It is known that weight loss can improve different clinical features of the metabolic syndrome, including insulin sensitivity. Moreover, in a study with 19 subjects it has been shown that weight loss may also improve fibrosis in CHC patients [62].

In T2DM and recently also in NASH the antidiabetic drugs metformin and thiazolidinediones were used to improve insulin sensitivity. One multicentre study in which non-responders were retreated with pegylated interferon- α , ribavirin and pioglitazone in addition, was terminated as none of the patients showed a virological response at week twelve despite improving insulin sensitivity [63]. Another interim analysis of a study revealed an increase in rapid virologic response in treatment-naïve nondiabetic patients in a regimen of pioglitazone monotherapy for four weeks, followed by pioglitazone added to standard therapy with peginterferon α and ribavirin [64]. Prelimi-

nary data from a second clinical study adding pioglitazone to the standard of care revealed an increase in virological response during therapy but not SVR. These studies were presented at the 2008 Annual Meeting of the American Association for the Study of the Liver [65, 66]. In a recent case report SVR was achieved in a former non-responder after standard of care. The patient had been pretreated with pioglitazone for five months to induce insulin sensitivity, and received standard of care combined with pioglitazone afterwards [67]. Additional studies are needed to verify the advantage of insulin sensitising drugs and approve protocol and dosing. Treatment with metformin or thiazolidinediones in addition to antiviral therapy will probably be a future add-on to antiviral therapy but cannot be recommended yet.

A recent pilot study has shown improved insulin sensitivity assessed by a significantly improved HOMA-IR in patients with CHC who were treated with the angiotensin II receptor blockers telmisartan and olmesartan [68]. Further studies are needed to confirm the use of these drugs to improve insulin sensitivity in nonhypertensive patients with CHC.

In conclusion, IR and the features of the metabolic syndrome should be assessed in patients with CHC, since IR is a risk factor for fibrosis progression. Antiviral treatment of the patients at risk is recommended as soon as possible, while specific pharmaceutical treatment of insulin resistance is not yet established. In this regard, the identification of drug targets in key positions of HCV induced IR development is needed to selectively improve treatment outcome in these patients and thus prevent fibrosis progression.

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