

Review article

Insulin resistance and its consequences in chronic hepatitis C

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Abstract

Chronic hepatitis C (CHC) is generally a slowly progressive disease, but some factors associated with rapid progression have been identified. Hepatitis C virus (HCV) may contribute to a broad spectrum of metabolic disturbances – namely, steatosis, insulin resistance (IR), increased prevalence of impaired glucose tolerance, type 2 diabetes mellitus (T2DM), lipid metabolism abnormalities and atherosclerosis.

HCV can directly or indirectly cause both IR and steatosis, but it is still not resolved whether this viral impact bears the same prognostic value as the metabolic counterparts. As the population exposed to HCV ages, the morbidity due to this disease is increasing. The rising epidemic of obesity contributes to higher prevalence of IR and T2DM. Our understanding of the mutual association between both disease states continues to grow, but is still far from complete. This review briefly discusses the most probable mechanisms involved in IR development in the course of CHC. Molecular mechanisms for the direct and indirect influence of HCV on intracellular insulin signaling are described. Subsequently, the consequences of IR/T2DM for disease progression and management are summarized.

Key words: chronic hepatitis C, insulin resistance, liver, steatosis, adipokine, fibrosis, steatosis.

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Introduction

Chronic hepatitis C (CHC) and obesity are two major rising epidemics which present tough challenges both for clinicians and healthcare systems in terms of diagnostic, therapeutic and economic implications [1]. Obesity, especially central or visceral obesity, expressed as increased waist circumference, is definitely the feature most commonly associated with insulin resistance (IR) and metabolic syndrome (MS). Recent data seem to underestimate a prominent role for visceral and hepatic fat [nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH)] as a pathogenic driver in the etiology of IR. However, when exploring the relationship between MS, central obesity and CHC, the relative contribution of metabolic IR and NAFLD on one hand, and hepatitis C virus (HCV)-induced IR

and hepatic steatosis on the other hand, should be appreciated. HCV can directly or indirectly cause both IR and steatosis, but it is still not resolved whether this viral impact bears the same prognostic value as the metabolic counterparts [2]. As the population exposed to hepatitis C ages, the morbidity due to this disease is increasing. The rising epidemic of obesity contributes to higher prevalence of IR and type 2 diabetes mellitus (T2DM). Our understanding of the mutual association between both disease states continues to grow, but is still far from complete. This review briefly discusses the most probable mechanisms involved in IR development in the course of CHC. Molecular mechanisms for the direct and indirect HCV influence on intracellular insulin signaling are described. Subsequently, the consequences of IR/T2DM for disease progression are summarized [3].

IR is typically manifested as both decreased insulin-mediated glucose uptake at the level of adipose tissue and skeletal muscle tissue (peripheral IR), and as insufficient inhibition of hepatic glucose release (hepatic IR). Both types of IR may occur simultaneously, but their intensity can be different [4]. IR is defined as a condition when normal insulin levels are not sufficient to achieve a normal metabolic response, or a condition when higher-than-normal insulin concentrations are necessary to achieve a suitable metabolic response. This definition does not allow one to indicate the type of tissue where insulin activity is measured. IR involves multiple sites: (i) the muscle, where it reduces glucose uptake and utilization, (ii) adipose tissue, where lipolysis is not adequately suppressed by insulin, with subsequent release of glycerol and free fatty acids into the bloodstream, and (iii) the liver, where IR is characterized by the overproduction of glucose despite fasting hyperinsulinaemia. Irrespective of which might have been the primary site of IR (muscle, fat or liver), all these metabolic abnormalities contribute finally to the onset of overt diabetes [5].

Evidence that hepatitis C virus causes insulin resistance

CHC should be considered not only as viral but also as a special type of metabolic disease. CHC is associated with lipid metabolism abnormalities leading to hepatic steatosis, impaired glucose metabolism leading to IR and T2DM and is related to an increased risk of carotid atherosclerosis [6]. Several experimental, epidemiological and clinical studies have confirmed the unquestioned participation of HCV in glucose metabolism perturbation [7]. The relationship between CHC and IR was first described by Allison *et al.* [8], who revealed that the prevalence of T2DM in cirrhotic CHC patients was significantly higher than in those with cirrhosis resulting from conditions other than CHC. Cross-sectional studies comparing the prevalence of T2DM in CHC patients with those with chronic liver disease of other origin (including chronic hepatitis B

[CHB]) or with human immunodeficiency virus infection, indicated higher prevalence of T2DM in CHC [7]. Conversely, the prevalence of HCV infection in diabetic patients is markedly higher than in the general population, ranging from 5% up to 12% (34-45). An analysis which included 9841 subjects, aged 20 years or more, revealed three-fold higher risk of T2DM development in CHC patients [9]. These observations have been confirmed both in general population-based [10] and longitudinal studies [11]. HCV potentiates the risk of developing abnormalities in glucose metabolism in patients with other risk factors [11], suggesting that HCV infection accelerates T2DM onset in susceptible patients. The patients with high risk for T2DM and CHC were 11 times more likely to develop T2DM than those without HCV infection [11]. HCV increases the incidence of T2DM after liver [12] and kidney transplantation, impacting on liver fibrosis progression [13] and cardiovascular events [14]. In a recent meta-analysis of 10 studies, the pooled risk ratio for post-kidney transplantation T2DM reached 2 [15].

A relationship between CHC and glucose abnormalities at the early prediabetes stages such as impaired glucose tolerance (IGT) or IR was also found (Fig. 1) [16-18]. Hui *et al.* measured fasting serum insulin, C-peptide and HOMA-IR levels in 121 CHC patients and 137 healthy volunteers. All three variables were significantly up-regulated in CHC patients [17]. This finding was confirmed in a study which included 600 patients (500 with CHC and 100 with CHB) where the prevalence of T2DM was 7.6%. IR was observed in 32.4% out of 462 CHC patients without diabetes. The study comparing CHB and CHC confirmed significantly more frequent IR prevalence in the latter (35% vs. 5%) [19]. IR is observed in CHC patients in very early stages of the infection before there is a marked extent of fibrosis [17-19]. Downregulation of insulin sensitivity rather than impaired pancreatic islet cell function is suggested to be an essential pathophysiological factor contributing to the appearance of abnormal glucose homeostasis in CHC [17]. HCV appears to induce IR via mechanisms operating in the liver and at the periphery. In CHC patients without any features of MS and without advanced fibrosis, endogenous glucose production was high-normal in the basal state [20]. During a hyperinsulinemic clamp, the production of endogenous glucose was 3.5 times higher in comparison with the control group, while muscle glucose uptake was unsettled, ranging from the lower end of normal to severely impaired. The action of insulin for the process of lipolysis was not impaired [20]. However, another study found peripheral IR to be exacerbated but adipose and hepatic IR to be unchanged

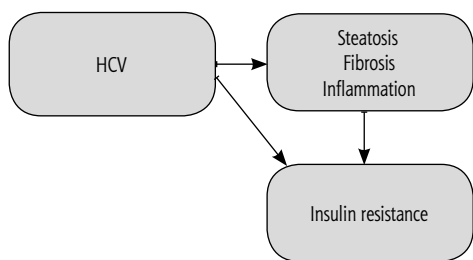


Fig. 1. Pathogenesis of insulin resistance in chronic hepatitis C

in CHC patients [21]. Muscle IR was positively related to viral load and subcutaneous fat and was again independent from viral genotype and hepatic steatosis grade [21]. The association between viral load and IR was also observed in other studies. The study by Mouchari *et al.* showed IR to be related to genotype 1 or 4, but again it was associated with high serum viral load [19]. However, the study by Vanni *et al.* showed both peripheral and hepatic IR to be independent of viral genotype and hepatic steatosis [20]. The discrepancy in the obtained results may result from the fact that blood viral load is not stable but fluctuates during the course of the disease.

The association between T2DM and HCV genotypes also is still controversial [22-24]. The most recent, large-scale study addressing the relationship between IR and viral clearance in genotype-1, -2 and -3 patients revealed that IR was more common in patients with genotype 1 in comparison with those infected with genotype 2/3 [25].

The fact of high importance is that viral clearance after successful antiviral therapy leads to improvement of insulin sensitivity and reduces occurrence in glucose metabolism abnormalities across follow-up [26-28]. These findings represent irrefutable evidence for a causal relationship between HCV and impaired glucose metabolism. The study carried out on 89 Japanese CHC patients showed that efficient antiviral therapy improved HOMA-IR results and intrahepatic expression of IRS-1 and IRS-2 [28]. The results were also confirmed in a group of 181 CHC patients infected with genotype 4 [29]. Romero-Gómez *et al.* assessed the influence of SVR on the incidence of IGT and T2DM in 1059 CHC patients treated with pegylated IFN- α 2b and RBV [26]. SVR reduced the incidence of T2DM or IGT by half during a post-treatment follow-up of 27 months (range 9.3-67 months). Also the retrospective cohort study, which encompassed 2842 CHC patients, carried out by Arase *et al.* showed a two-thirds reduction in the risk of T2DM development after a mean follow-up of 6.4 years [27]. However, the study by Giordanino *et al.* in a group of 202 CHC patients with median follow-up of 8 years (range 5-16 years) showed contradictory results and did not reveal any improvement in insulin sensitivity in CHC patients with SVR [30]. Additionally, the above-mentioned study by Thompson *et al.* showed that viral clearance resulted in IR down-regulation in patients infected with genotype 1, but not in those with genotype 2/3 [25]. These results suggest a causal relationship between IR and HCV genotype 1.

To summarize, HCV evokes IR in the early stage of infection and therefore increases the risk of onset

of T2DM in predisposed individuals. Some studies have indicated that IR is associated with viral load and observed more often in genotype 1 or 4 infection. Moreover, successful antiviral therapy ameliorates insulin sensitivity. All these results point to a direct viral influence on IR independent of BMI and visceral adiposity and suggest that HCV itself may promote and exacerbate IR [3]. The assessment of IR allows one to evaluate the impact of HCV on glucose metabolism before overt T2DM occurs [17]. Nevertheless, the fine details of these mechanisms are not fully resolved and are sometimes speculative.

Molecular mechanisms of insulin resistance

Insulin is the most potent anabolic agent, which enhances the storage and synthesis of lipids, carbohydrates and proteins, and blocks their breakdown and release into the blood stream [31]. The biological action of insulin depends on a cascade of events following the interaction of insulin with its receptor on the cell membrane. The insulin receptor is a heterodimeric complex consisting of two extracellular α -subunits that bind insulin and two transmembrane β -subunits with tyrosine kinase activity. Insulin binding promotes the receptor autophosphorylation and the subsequent tyrosine phosphorylation of several insulin receptor substrates (IRS) (namely IRS-1 and IRS-2), which initiate a cascade of multifaceted events. Key transducers of insulin-mediated glucose regulation are phosphatidylinositol 3-kinase (PI3K) and the protein kinase Akt [32]. These actions are manifested via insulin's action on a complex network of intracellular pathways in adipocytes, muscles and hepatocytes. The final result of this activation is translocation of glucose transporter 4 (GLUT-4) from the intracellular pool to the cell membrane, facilitating glucose transport into the cytoplasm [3]. In IR, there is impairment of insulin receptor binding and phosphorylation of IRS-1 and -2 in the muscle and the liver, and a dramatic decrease in PI3K activity and glucose uptake [32,33]. The most likely mechanism of IR within the muscle is serine rather than tyrosine phosphorylation of IRS-1, and similar events occurring in hepatocytes are likely to mediate IR within the liver. Some additional factors influence insulin signaling, and their activation may lead to IR. Protein tyrosine phosphatases (PTP) (especially PTP1B) [34] may dephosphorylate tyrosine residues on IRS, and phosphatidylinositol phosphate (PIP) phosphatases may dephosphorylate PIPs at position 5' (SH2-containing PIP 5'-phosphatase 2, or SHIP2) or 3' (phosphatase and tensin homolog, or PTEN) [35], attenuating insulin signaling. The suppressors of cytokine signaling (SOCS)

potentiate ubiquitin-mediated IRS degradation [36]. IRS may also be inactivated due to phosphorylation at serine residues by kinases, such as mTOR substrate p70 ribosomal S6 kinase (p70S6K), protein kinase C and c-Jun N-terminal kinase (JNK) [37]. Additionally, the above-mentioned mechanisms interrupting insulin signaling may be provoked by some acquired factors encompassing hyperinsulinemia, hyperglycemia, circulating free fatty acids, high levels of tumor necrosis factor- α (TNF- α), IL-6, nuclear factor kappa B (NF- κ B) and adipokines [38,39].

Molecular mechanisms of HCV-induced insulin resistance

IR development in CHC results from the interaction between various host, viral and environmental factors, contributing to enhanced endogenous glucose production. The first study, carried out on fresh liver samples obtained from 42 nonobese, nondiabetic CHC patients and 10 uninfected controls, matched for age and BMI incubated *ex vivo* with insulin [40], revealed marked inhibition of the ability of IRS-1 to associate with the insulin receptor and thus reduced tyrosine phosphorylation (and hence decreased activation) of IRS-1. Impaired action of IRS-1 contributes to subsequent defective PI3K and Akt phosphorylation. Surprisingly, the remaining pathway of insulin signaling (Ras/MAPK pathway) was not impaired. These data point to a direct, post-receptor interaction between HCV and the insulin signaling pathway. Since the PI3K/Akt pathway is critical for the insulin-mediated inhibition of gluconeogenesis in the liver, the authors concluded that the observed defect may lead to increased glucose synthesis in CHC [2]. The direct action of the virus on the insulin signaling pathway has been suggested in experimental models, although various mechanisms have been implicated [40,41]. At the molecular level, upregulation of SOCS, downregulation of peroxisome proliferator activated receptor (PPAR), increased proteasome activator 28 γ (PA28 γ) level, activation of the mTOR pathway and intense oxidative stress are suggested mechanisms implicated in disturbances in the insulin signaling pathway. All of them may be present in a genotype-specific manner [42].

HCV core protein alone stimulates increased proteasome-mediated degradation of IRS-1, mediated by activation of a member of the SOCS family [42-45]. A HCV genotype specific mechanism of impairment of insulin signaling was observed, since expression of the HCV genotype 3 core protein led to downregulation of PPAR γ and upregulation of SOCS-7, while the core protein of genotype 1 activated mTOR and in-

duced phosphorylation of IRS-1 at inhibitory serine residues [42]. Subsequent work suggested that PPAR γ may directly control the SOCS-7 level in cells expressing the HCV genotype 3 core [46]. It was later shown that the activation of SOCS family members may be a mechanism common to all major HCV genotypes [47], including genotype 1, since the variant originally associated with mTOR activation was shown to be infrequent among known isolates. Intrahepatic SOCS activation (at both the mRNA and protein levels) has been reported in several human studies, with the level of activation being correlated with obesity [48] or hepatic IR [20].

PPARs, which belong to the nuclear receptor superfamily, play a pivotal role in HCV-induced IR. Their action is determined by heterodimerization with receptor X for retinoids (RXR) [49,50]. The PPAR-RXR complex initiates gene transcription after being bound with a ligand such as eicosanoids, unsaturated free fatty acids, and very low-density lipoprotein, which change its conformation and facilitate binding to DNA at PPAR response elements [51]. PPAR α and PPAR γ alongside RXR belong to the main nuclear receptors expressed in the liver, controlling glucose and lipid metabolism, influencing cellular differentiation and proliferation as well as regulating the inflammatory process. In the course of CHC, PPAR α gene expression in the liver was decreased by 86% [52]. The study by Gottardi *et al.* showed that PPAR γ liver expression was significantly lower in patients with genotype 3 infection compared to those with genotype 1 [52]. PPAR α -mediated regulation of lipid metabolism is closely related to adiponectin [53]. Adiponectin levels seem to be lower in CHC. Accumulation of triglycerides in hepatocytes contributes to the loss of adiponectin receptors in the liver, and together with low serum adiponectin concentration leads to systemic IR [54]. Additionally, PPAR γ enhances adiponectin production. As PPAR γ is markedly reduced in CHC, there is another obvious pathway for IR and hepatic steatosis [54].

HCV core protein may also affect the insulin signaling via activation of the proteasome activator 28 γ (PA28 γ) [45]. Transgenic mice expressing the HCV core develop IR [43], which is reversible through the targeted deletion of PA28 γ , suggesting that HCV may induce IR through a PA28 γ -dependent pathway [45]. Activation of the PA28 γ -dependent pathway by HCV core protein leads to suppression of IRS-1 tyrosine phosphorylation and IRS-2 expression and TNF- α promoter activation [46]. Moreover, PA28 γ is involved in the development and progression of steatosis and occurrence of HCC [55]. HCV core protein inhibits insulin-mediated FoxO1 translocation from the nucleus to

the cytoplasm and subsequently reduces accumulation of FoxA2 in the nucleus [56]. FoxO1 and FoxA2 belong to specific Forkhead box transcriptional regulators which modulate hepatic glucose metabolism via the PI3K/Akt signaling pathway [57].

Protein phosphatase 2A (PP2A) and NF- κ B are other agents engaged in HCV-induced IR. HCV NS5A may induce IR via overexpression of PP2A [58]. PP2A, which dephosphorylates and inactivates Akt [58,59], is significantly upregulated in CHC patients [60]. *In vitro*, PP2A overexpression results from enhanced HCV-related endoplasmic reticulum (ER) stress [61]. However, the intrahepatic level of PP2A did not correlate with HOMA-IR [58]. In another study, the HCV core protein alone or in the presence of other viral proteins increased the serine phosphorylation of IRS-1, an effect that was abolished by inhibiting the JNK signaling pathway [62]. JNK inhibitors also restored the hepatocyte glucose uptake reduced by the HCV core expression. Thus, JNK may contribute to HCV-induced IR, as suggested also by recent data in chronic hepatitis C patients [63]. HCV NS5A potentiates the NF- κ B-mediated increase in proinflammatory cytokines (i.e. IL-6, TNF- α), contributing to mitochondrial reactive oxygen species (ROS) production [64-66]. Also, NS3-induced oxidative stress may activate NF- κ B, potentiating the inflammatory process and IR [67].

All the above-mentioned mechanisms imply a direct effect of viral products on the intracellular insulin signaling pathway. However, CHC is a chronic inflammatory state, in which synthesis of pro-inflammatory agents is enhanced. The close interaction between hepatocytes and various immune cells such as Kupffer cells, macrophages, lymphocytes and dendritic cells may be of importance when inflammatory circumstances appear. In the study by Vanni *et al.* [20], hepatic IR was associated with enhanced hepatic expression of IL-18. IL-18-mediated IR may result from inhibition of adiponectin expression in adipocytes [68] and activation of SOCS-3 in the adipose tissue [69]. Also the activation of JNK by the HCV core [62] may occur via pro-inflammatory cytokines, such as TNF- α . The role of TNF- α in inducing IR in HCV-infected persons is debated. The IR in transgenic mice expressing the HCV core, associated with high serum concentrations of TNF- α , can be reverted by anti-TNF- α antibodies [43], implying that HCV core-expressing hepatocytes secrete TNF- α , which may then induce IR via serine phosphorylation of IRS-1. This provokes reduction of GLUT2/GLUT4 gene expression [70], resulting in decreased glucose uptake into hepatocytes and adipocytes [71]. In CHC patients, circulating TNF- α levels are increased [72-75], and may be related

to IR independently of the fibrosis stage [76]. However, in a controlled study [77], serum levels of TNF- α and IL-6 in 154 non-diabetic CHC patients were found to be significantly higher compared to 75 matched uninfected controls. Unfortunately, there was no correlation between TNF- α serum concentration and IR. In contrast, another study carried out in a group of 161 CHC patients found serum TNF- α levels to be related to hepatic steatosis grade and HOMA-IR values, whereas serum adiponectin concentration was inversely associated with HOMA-IR values, serum TNF- α levels and steatosis grade, independently of HCV genotype and gender [78]. Summarized information on HCV proteins affecting insulin sensitivity pathways are shown in Table 1.

Another study revealed that serum levels of adiponectin and leptin levels were significantly associated with IR, but not with HCV infection itself. This observation suggests virus-specific IR in CHC to be a cytokine-independent effect [77]. The role of new adipokines in regulation of insulin sensitivity in CHC also cannot be excluded. However, the data are very limited. Vaspin was found to improve insulin sensitivity and glucose tolerance and down-regulate TNF- α synthesis [79-81]. In CHC serum vaspin levels significantly decreased in patients without or with no advanced fibrosis and increased in cases of advanced fibrosis [16,82]. This suggests vaspin to be a compensatory mechanism switching in CHC-associated insulin resistance. However, there was no relationship between vaspin levels and viral load and HOMA-IR values [82]. The next new adipokine is visfatin, which is significantly increased in CHC, and inversely associated with inflammatory activity [16]. On one hand visfatin exerts an insulin-like effect, enhancing phosphorylation of IRS-1 and expression of PPAR γ [83], but on the other hand it potentiates expression of some adhesion molecules, IL-6 and TNF- α [75,84]. Chemerin is another new adipokine, the concentration of which is markedly altered in CHC, but is not associated with HOMA-IR values and viral load [16]. However, considering chemerin's ability to inhibit TNF- α and IL-6

Table 1. HCV proteins' effects on insulin sensitivity

NS5A	Core protein
overexpression of PP2A	↑ degradation of IRS-1
NF- κ B-mediated ↑ IL-6	↓ PPAR γ
NF- κ B-mediated ↑ TNF- α	↓ PPAR α
↑ ROS	↑ SOCS-7
↑ IL-18	activation of mTOR
↑ SOCS-3	activation of PA28 γ
	activation of JNK

synthesis and to ameliorate IRS phosphorylation, to improve adipocytes' insulin sensitivity and to stimulate adiponectin synthesis [81,85,86], it may be suggested to be another player implicated in regulation of IR in CHC. Retinol binding protein (RBP)-4 is a member of the lipocalin family, and is synthesized predominantly in hepatocytes (80%) and adipocytes (20%) [87]. It is implicated in steatogenesis, and the link has been made between insulin resistance, T2DM and elevated serum and adipose RBP-4 concentration [88-90]. The study by Petta *et al.* [91] showed serum RBP-4 to be independently linked to steatosis in linear regression in CHC patients. On logistic regression, RBP-4 was independently associated with moderate to severe steatosis. However, another study by Kukla *et al.* did not confirm these observations in CHC [92]. Further investigations involving a larger number of patients are required to better determine the exact role of novel adipokines in CHC.

It is well known that oxidative stress and overproduction of ROS are closely associated with metabolic abnormalities and progression of chronic liver diseases. CHC as a chronic inflammatory process and metabolic disease is characterized by exacerbated oxidative stress. HCV core protein can evoke oxidative stress in hepatocytes by increased ROS production and levels of lipid peroxidation products and reduced mitochondrial glutathione reserve [93-96]. The data regarding the contribution of oxidative stress to HCV-induced IR is contradictory. The study by Oliveira *et al.* carried out in 52 genotype non-3 infected CHC patients found oxidative stress to be related to IR but not steatosis [97]. In contrast, in the Vidali *et al.*'s study oxidative stress which was observed in 61% out of 107 genotype non-3 CHC patients, was associated with steatosis, but not with IR. Moreover, steatosis was also related to IR and fibrosis. Based on these results, the authors concluded that in genotype non-3 CHC oxidative stress and IR induce and potentiate hepatic steatosis, which in turn exacerbates both IR and oxidative stress and accelerates fibrosis progression [98].

Mechanisms of HCV-induced steatosis

Since lipids are essential in the HCV life cycle, they must be accumulated in a sufficient amount in infected hepatocytes. Hepatic steatosis apart from inflammation and fibrosis is another characteristic histological feature of CHC. It is observed in 42-73% of patients. Its prevalence is two-fold higher compared to CHB [99-101], and it is up-regulated by concomitant metabolic factors.

The suggestion of a direct HCV influence on lipid accumulation in the liver may be supported by three lines of evidence: 1) steatosis is more frequent and severe in patients with genotype 3 [102,103], pointing to the presence of specific sequences across the genotype 3 genome contributing to the storage of lipid droplets in hepatocytes; 2) viral load is associated with steatosis grade [102,103]; 3) efficient antiviral therapy leads to a smaller steatosis extent [102,104,105]. These observations are more obvious in patients with genotype 3 infection. In patients infected with the genotype non-3 virus, steatosis is more likely to be associated with metabolic abnormalities [103,105]. However, in the case of recurrence of HCV after the end of antiviral therapy, reappearance of steatosis occurs in patients in whom it had been resolved during treatment.

Although in some circumstances such as IR the uptake of fatty acids by hepatocytes is enhanced, there are well-evidenced experimental studies showing the HCV core protein to be sufficient in evoking hepatic steatosis by triglycerides accumulation [16]. The most creative seems to be genotype 3. A study of primary infection in chimpanzees revealed that HCV activates genes involved in lipid metabolism via the sterol regulatory element binding protein (SREBP), which is responsible for the transactivation of enzymes involved in the synthesis of fatty acids and cholesterol. The predominant activating effect was exerted by core and NS2 and NS4B viral proteins. Additionally, the HCV core protein activates the DNA-binding domain of retinoid receptor α , which regulates lipid synthesis [16]. Alongside activation of transcription factors involved in *de novo* lipogenesis, such as retinoid X receptor alpha [106] and SREBP-1c [107-110], inadequate lipoprotein secretion seems to be an essential step to trigger neutral fat accumulation, in keeping with the evidence that serum levels of apolipoprotein B and cholesterol are reduced in CHC patients in whom steatosis later responds to antiviral therapy [111]. Furthermore, the disappearance of steatosis in patients who respond to therapy is paralleled by the normalization of cholesterol and apolipoprotein B levels [111,112]. HCV core protein inhibits microsomal triglyceride transfer protein (MTP) activity, which in turn decreases assembly and secretion of apolipoprotein B-containing VLDL [113]. Interestingly, intrahepatic levels of *MTP* mRNA are reduced in CHC patients, especially those with steatosis and/or genotype 3 [114]. Indeed, the severity of hepatic steatosis in patients with CHC is similar to that observed in hypo-betalipoproteinemia [113]. Impairment of fatty acid oxidation resulting from HCV infection may also add to the fatty liver. Transfection of hepatoma cells with the HCV core protein results in

reduced expression of PPAR α , which is responsible for fatty acid degradation [108]. PPAR α mRNA is markedly decreased in the liver of CHC patients [52,115], especially in those with genotype 3 infection [52]. Moreover, carnitine palmitoyl acyl-CoA transferase 1A, a target gene of PPAR α responsible for mediating long-chain fatty acid transport across the mitochondrial membrane, is reduced by HCV both *in vitro* [116] and in CHC patients' livers [117]. The HCV sequence responsible for the fatty accumulation is not definitively known. Probably, phenylalanine at position 164 of the core sequence of genotype 3a, which is replaced by a tyrosine in all other genotypes, is responsible for enhancement of fatty acid synthesis [108] and accumulation of large lipid droplets in hepatocytes [118]. The recent data suggest a pivotal role of micro-RNA (miRNA) in the pathogenesis of steatosis and HCV replication. Especially mir122, the most abundant liver miRNA, was found to accelerate steatosis development by up-regulation of lipogenesis and by enhancing HCV replication [119].

Relationship between insulin resistance and steatosis in HCV infection

The relationship between IR and HCV infection is complex and bidirectional. HCV induces steatosis, and the latter could also induce and exacerbate IR. Many mechanisms accounting for HCV-related steatosis can also induce IR. In addition to the inflammatory process, HCV proteins may evoke and potentiate IR and oxidative stress [120].

Steatosis has been related to up-regulated ROS production, which promotes lipid peroxidation and enhances hepatic stellate cell activation. In the case of steatosis, increased production of some proinflammatory and profibrotic cytokines is observed. Noteworthy is that steatosis makes the liver more susceptible to TNF- α -mediated inflammation, liver injury, and apoptosis [121], accelerating fibrosis progression in consequence [122].

On the other hand, patients with the highest grades of steatosis do not necessarily present with the highest levels of IR, and vice versa. In HCV genotype 3 infection, IR levels are comparable in patients with versus those without steatosis [123]. Studies have shown that HOMA-IR levels are higher in patients with genotypes 1 and 4 [19], and that patients with genotype 3 are those in whom HOMA-IR levels are the lowest [17]. In a study from Greece, HOMA-IR levels were comparable across viral genotypes [124], and these results suggest that the severe steatosis observed in genotype 3 may not result in increased IR. It is noteworthy

that SREBP-1c not only influences production of enzymes regulating lipogenesis and reducing fatty acid β -oxidation, but is a protein interfering in intracellular insulin signaling. In mice overexpressing SREBP-1c, enhanced systemic IR and hepatic steatosis were observed. Inhibition of SOCS-1 and -3 in this model normalized the levels of SREBP-1c and ameliorated both hepatic steatosis and insulin sensitivity [125]. Oxidative damage induced by the HCV core protein may simultaneously induce steatosis and disturb insulin signaling in the hepatocyte. These observations explain why inflammation in the liver, which may contribute to IR via the mechanisms mentioned above, was positively associated with steatosis grade in some studies [104,126].

Influence on cardiovascular morbidity and mortality

The complex interaction between HCV and the MS, oxidative stress, the inflammatory process, steatosis and disturbances in adipokine profile provokes the question whether this interference may translate into an increase of cardiovascular morbidity and mortality in CHC. Convincing evidence that HCV infection promotes IR and T2DM development amplifies this suspicion. Since HCV infection is a well-known factor stimulating the inflammatory response, it is believed to lead to cardiovascular disease development by several effector mechanisms, including up-regulation of intracellular adhesion molecules, expression of anti-endothelium antibodies, and induction of oxidative stress and IR [6,127,128]. Recently, the relationships between HCV infection, atherosclerosis, and the inflammatory response have been precisely investigated. However, the data concerning the relationship between CHC and atherosclerosis is highly equivocal [6,129-131]. There are also some data pointing to a possible association between HCV infection and coronary artery disease [131-133] and stroke [134].

In the Heart and Soul Study [135], CHC patients were found to have higher TNF- α serum concentration and an increased risk of cardiac failure and death compared to those without HCV infection. Mostafa *et al.* [136] found a greater intima-media thickness (IMT) in CHC patients than controls. Oliveira *et al.* [137] compared non-obese, non-diabetic CHC patients with healthy controls and reported an intermediate cardiovascular risk, as measured by the Framingham score, a raised concentration of IL-6 and TNF- α , and a higher ratio of pro-inflammatory/anti-inflammatory cytokines (TNF- α /IL-10 and IL-6/IL-10) in CHC patients [137]. Another study found the cytokine imbalance – TNF- α /

adiponectin ratio – to be associated with development of IR in CHC patients [78]. Moreover, higher concentrations of intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and oxidized low-density lipoproteins have been observed in CHC patients [127,128,138]. The detection of HCV RNA sequences and intermediate replicative forms within carotid plaques suggests local pro-atherogenic action of HCV [138,139]. However, the presence of HCV RNA within carotid plaques may be due to the known interaction between HCV and LDL, and an active role of HCV in atherogenesis should be further investigated [133].

As for NAFLD [140-142], HCV-related steatosis may be suggested as a cardiometabolic risk factor [128]. CHC patients demonstrated a higher prevalence of carotid atherosclerosis than both healthy controls and NAFLD patients [131]. It is noteworthy that it occurred at a younger age, particularly in the presence of hepatic steatosis, and viral load was found to be independently associated with intima media thickness [131]. HCV viral load and HCV-related steatosis have been hypothesized to favor the development of atherosclerosis (OR 5.21 and 12.98, respectively) independently of known risk factors such as hypercholesterolemia, smoking, hypertension and inflammatory cytokines [131]. Somewhat the study by Targher *et al.* showed different results. Atherosclerosis assessed by carotid IMT was higher in CHC patients than in healthy controls, but less significantly than in NAFLD patients [143].

Another interesting study, on CHC Egyptian patients, did not find age- and sex-adjusted mean carotid IMT or the proportion of individuals with carotid pla-

que to be different between CHC patients and healthy controls. IMT was independently associated with classical atherogenic risk factors, namely LDL cholesterol and systolic blood pressure [136]. However, when these factors appeared, CHC patients had higher IMT, suggesting a direct effect of viral infection on plaque development. HCV infection is a risk factor for earlier and facilitated occurrence of atherosclerosis via viral load and steatosis, which modulate atherogenic factors such as inflammation and dysmetabolic milieu.

Conclusions and implications for management

HCV influences various metabolic aspects (Fig. 2) [16].

Several data suggest that HCV is able to alter intrahepatic insulin signaling through various mechanisms, including direct interference of the virus with the intracellular insulin cascade or functional impairment, e.g. via increased levels of proinflammatory cytokines or through oxidative stress. In chronic hepatitis C, IR is mainly impaired in the liver, but a variable degree of peripheral IR can coexist in the same individual, possibly mediated by superimposed factors such as hepatic steatosis. Furthermore, the metabolic disturbances caused by HCV *per se* can interact with the degree of liver inflammation and fibrosis and with the classical risk factors for T2DM, further aggravating IR. In fact, the increased prevalence and incidence of T2DM carried by HCV is consistently linked to predisposing conditions. This suggests that HCV infection has the potential to trigger the phenotypic expression of metabolic derangements on a genetically determined [144,145], environmentally induced, susceptible basis. IR should be actively sought in patients with HCV also for the implications in management. If IR is present, a potential role of pharmacological therapy can be envisaged.

However, initial results with pioglitazone [146-150] or metformin [151] are contradictory. Our main efforts should rather be directed at promoting healthful dietary practices and physical activity as a cultural norm.

Aforementioned data indicate that HCV may disturb intrahepatic insulin signaling due to different mechanisms, including a direct influence of the virus on the intracellular insulin pathway or indirectly through induction of proinflammatory cytokines and enhancement of oxidative stress. Oxidative stress and metabolic disturbances caused by HCV facilitate hepatic steatosis progression, which accelerates fibrosis progression, potentiates inflammatory activity and, with the traditional risk factors for T2DM, further aggravates IR. Higher prevalence of IR and T2DM in

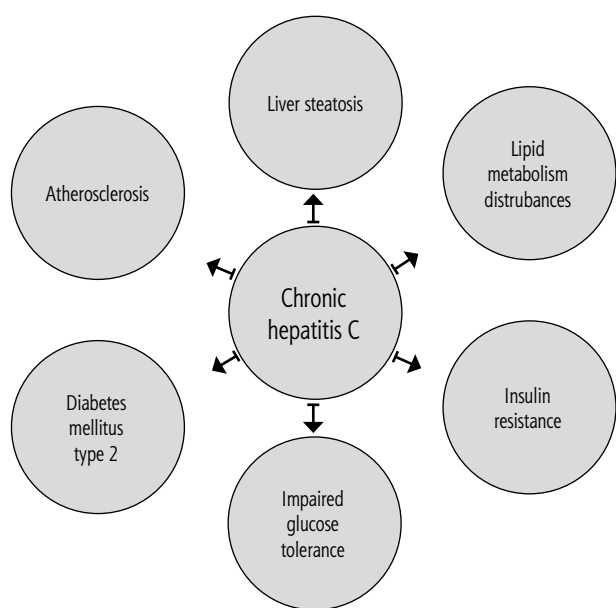


Fig. 2. Metabolic aspects of chronic hepatitis C

CHC is consistently linked to predisposing circumstances.

The metabolic abnormalities observed in CHC exert a pivotal impact on the morbidity and mortality of infected patients due to higher frequency of hepatocellular carcinoma, accelerated progression of liver fibrosis, and a reduced virological response to standard of care (SOC) treatment with ribavirin and pegylated interferon. HCV infection is a risk factor for earlier and facilitated occurrence of atherosclerosis. Increased risk and prevalence of cardiovascular events in CHC point to additional studies which should focus on the exact role of HCV in atherogenesis.

Disclosure

Authors report no conflict of interest.

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