# Peroxisome proliferator-activated receptors and hepatitis C virus

M. Eslam, M. A. Khattab and S. A. Harrison

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***Abstract***: The prevalence of type 2 diabetes mellitus and insulin resistance are higher among people chronically infected with hepatitis C (CHC) when compared with the general population and people with other causes of chronic liver disease. Both insulin resistance and diabetes are associated with adverse outcomes across all stages of CHC, including the liver transplant population. CHC is also associated with the development of hepatic steatosis, a common histological feature present in approximately 55% (32–81%) of cases. There is a complex interrelationship between insulin resistance and hepatic steatosis and both are postulated to aggravate each other. The peroxisome proliferator-activated receptors (PPARs) are nuclear factors involved in the regulation of glucose, lipid homeostasis, inflammatory response, cell differentiation, and cell cycle. The relationship between hepatitis C virus replication and PPARs has been the focus of recent study. Given the availability of potent agonists, PPARs may represent a novel pharmacological target in the treatment of CHC.

***Keywords***: hepatitis C, insulin resistance, liver fibrosis, peroxisome proliferator-activated receptors, pioglitazone, steatosis

## Introduction

Chronic hepatitis C virus (CHC) infects approximately 170 million people worldwide, and is a major cause of mortality and morbidity [WHO, 2009]. The natural history of HCV infection is characterized by a high rate of progression to chronic hepatitis leading to cirrhosis in approximately 20% of cases and possibly to hepatocellular carcinoma [Seeff, 2009]. In addition to progressive liver disease, CHC has been linked to dysregulated energy metabolism to include insulin resistance (IR), type 2 diabetes mellitus (T2DM), hepatic steatosis and increased risk of carotid atherosclerosis [Koike, 2009; Negro and Sanyal, 2009; Romero-Gómez, 2006]. This is due, in part, to a direct interference of HCV with lipid and glucose metabolism and a complex relationship between IR and steatosis in patients with CHC. The mechanisms involved seem to be HCV genotype specific [Moucari *et al.* 2008; Pazienza *et al.* 2007]. Since the peroxisome proliferator-activated receptors (PPARs) are nuclear factors involved in the regulation of glucose and lipid homeostasis, the relationship between HCV replication and protein expression and PPARs has been the focus of recent study. This review first describes the main function of PPARs within the liver, the interaction between PPARs and HCV, and then its potential role in HCV pathophysiology and therapy is discussed.

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## Peroxisome proliferator-activated receptors

PPARs belong to the nuclear receptor superfamily and require heterodimerization with receptor X for retinoids (RXR) in order to function [Desvergne and Wahli, 1999; Bardot *et al.* 1993]. The PPAR:RXR heterodimer, when bound to a ligand, changes conformation and binds to DNA at PPAR response elements, resulting in gene transcription [Bardot *et al.* 1993; Gearing *et al.* 1993]. PPARs play essential roles in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid, protein), and tumorigenesis [Desvergne and Wahli, 1999; Bardot *et al.* 1993].

There are three isotypes of PPAR designated in mammals: PPARα (NR1C1), PPARδ (NR1C2) and PPARγ (NR1C3) [Nuclear Receptors Nomenclature Committee, 1999]. PPARα is activated by ligands termed peroxisome proliferators, which were named for their effects on peroxisomes in rodent livers [Svoboda and Azarnoff, 1966; Hess *et al.* 1965].

PPARα/γ, together with their obligate partner RXR, are the main nuclear receptors expressed in the liver. These receptors contribute to important physiological processes occurring in the liver to include control of lipid and glucose metabolism, inflammatory responses, and cellular differentiation and proliferation. The availability of new nonhepatotoxic ligands made it possible to evaluate certain PPARs as potential new therapeutic targets in liver disease.

## PPARs and insulin resistance and diabetes

PPARs play an essential role in glucose homeostasis. PPARα upregulates glycerol-3-phosphate dehydrogenase, glycerol kinase and glycerol transport proteins, which allows for glucose synthesis during fasting states [Patsouris *et al*. 2004]. PPARα also stimulates pancreatic islet ß cells, increasing fatty acid oxidation and potentiating glucose-stimulated insulin secretion [Lefebvre *et al*. 2006]. In addition, PPARs possess insulin-sensitizing effects which lead to decreased ß-cell workload. This is particularly beneficial for ß-cell survival and prevention of pancreatic degradation.

In a recent randomized controlled trial the diabetic patients from the placebo group demonstrated a progressive decline in homeostatic indices of ß-cell function and an increase in IR was calculated according to the homeostasis model of assessment (HOMA) over 2 years of follow up. These longitudinal changes were attenuated by the PPARα ligand bezafibrate [Tenenbaum *et al*. 2007].

For PPAR*γ,* clinical data demonstrated that the PPAR*γ* agonists (thiazolidinediones such as pioglitazone) improve hyperglycaemia and hyperlipidaemia in obese and diabetic animals through reduction of both peripheral and hepatic IR [Jay and Ren, 2007].

The mechanisms of PPAR*γ*-mediated insulin sensitization are complex and are thought to involve specific effects on fat, skeletal muscle, and liver (Figure 1). Specifically, PPARγs:

1. Increase glucose catabolism and decrease hepatic glucose output [Nagashima *et al*. 2005; Sakamoto *et al*. 2005).
2. Increase GLUT4 expression and translocation in adipocytes [Armoni *et al*. 2003].
3. Lead to adipose remodeling. This phenomenon may explain the 'fatty acid steal' hypothesis [Berthiaume *et al*. 2004].
4. Increase expression of adipocytokines such as adiponectin, which may stimulate fatty acid oxidation in skeletal muscle and liver [Pajvani *et al*. 2004].
5. Increase glucose uptake in skeletal muscle resulting in lower blood glucose levels [Le Brasseur *et al*. 2006].
6. Impact directly on factors involved in lipid and glucose homeostasis.
7. May have insulin-sensitizing effects via their anti-inflammatory activity.

Thus, treatment with PPARγ agonists results in improved insulin sensitivity via diverse mechanisms, both direct and indirect, and at the level of the liver and other extrahepatic tissues.

## PPARs and lipid metabolism

The liver is the central organ responsible for fat metabolism and lipid homeostasis [Everett *et al*. 2000]. It is involved in free fatty acid synthesis, esterification of triacylglycerols and their packaging into very low-density lipoproteins (VLDLs) for exportation during the fed state [Everett *et al*. 2000]. During the fasted state, the liver is responsible for controlling the rates of fatty acid ß-oxidation and ketogenesis. The liver maintains lipid homeostasis by balancing these processes.

PPARα is a key mediator in maintaining this balance [Mandard *et al*. 2004; Nakamura *et al*. 2004; Hertz *et al*. 1995] by acting as a sensor for the level of free fatty acids and modulating the responses of fat-oxidizing tissues [Everett *et al*. 2000].

PPARα is activated by dietary fatty acids and eicosanoids or by specific drugs such as the fibrates. Activation of PPARα results in an increase in the enzymes involved in lipid metabolism and fatty acid ß-oxidation. Loss of expression of the PPARα gene in mice results in hepatic steatosis under conditions of increased fatty acid metabolism in the liver, such as fasting or a high-fat diet [Kersten *et al*. 1999; Lee *et al*. 1995]. Administration of a potent PPARα agonist decreases hepatic steatosis in mice receiving a methionine and choline deficient diet [Nagasawa *et al*. 2006].

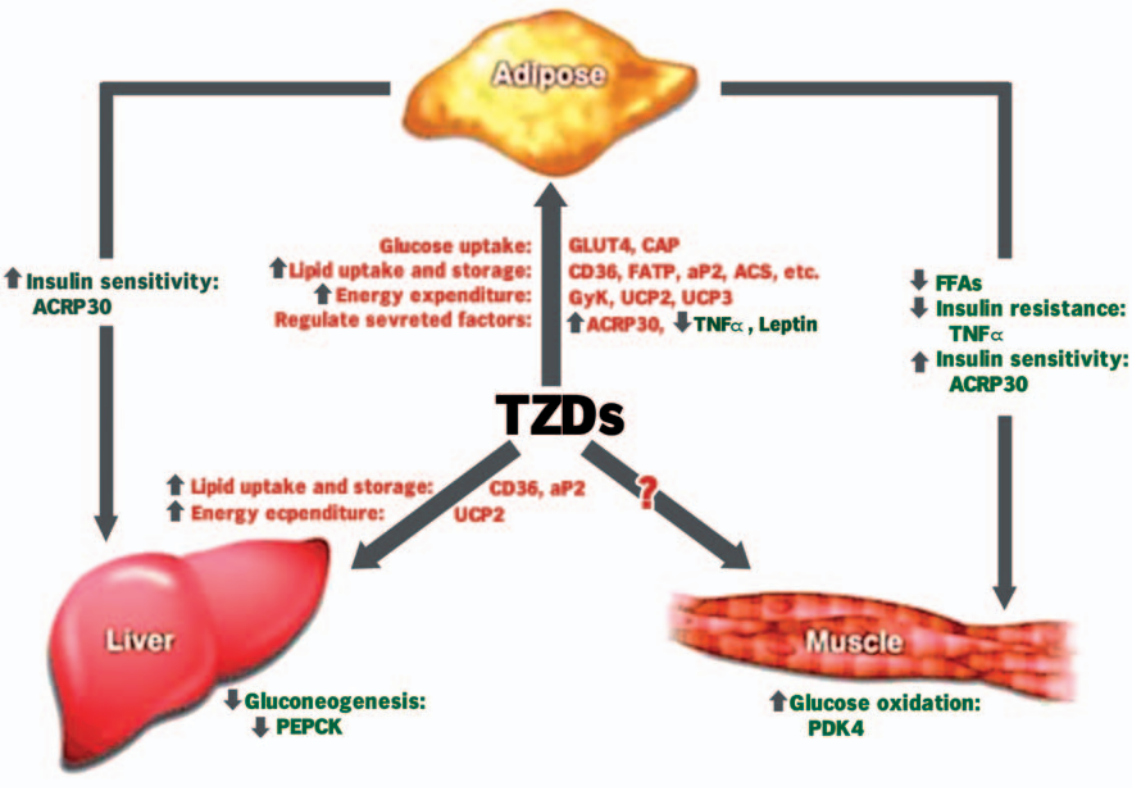


**Figure 1.** The complex mechanisms of peroxisome proliferator-activated receptor-$\gamma$ agonist-mediated insulin sensitization. ACRP30, adipocyte complement related protein of 30 kDa; FATP, FA transport protein; FFA, free fatty acid; GLUT4, insulin-regulated glucose transporter isoform; PDK4, pyruvate dehydrogenase kinase 4; PEPCK, phosphoenolpyruvate carboxykinase; TNF, tumor necrosis factor; TZD, thiazolidinedione; UCP, uncoupling protein 2 and 3.

Intracellular fatty acid concentrations are controlled, in part, by regulation of the fatty acid import and export system. Activation of PPARα directly regulates genes involved in fatty acid uptake [Escher and Wahli, 2000]. Also, both PPARα and PPARγ prevent the efflux of fatty acids by promoting their activation into fatty acyl CoA thioesters by the acyl-CoA synthetase [Hsu *et al.* 2001].

In addition, PPARα activation regulates hepatic triglyceride content by promoting fatty acid oxidation in peroxisomes and in the mitochondria, and reducing the fatty acid pool available to the liver for triglyceride synthesis [Latruffe *et al.* 2000]; enhancing lipoprotein lipase expression [Schoonjans *et al.* 1996]; and inhibiting apolipoprotein C-III in the liver [Staels *et al.* 1995] (Figure 2).

Adiponectin, an adipocyte produced peptide hormone, limits fat accumulation in the liver by a number of mechanisms including activation of PPARα to increase hepatic fatty acid oxidation [Yamauchi *et al.* 2003]. Adiponectin is upregulated by PPARγ, providing a connection between the two isotypes [Neschen *et al.* 2006] (Figure 2).

PPARγ is highly expressed in adipose tissue under two isoforms (PPARγ1 and PPARγ2) generated by the same gene through altered splicing [Fajas *et al.* 1997] and they have a complementary role in lipid homeostasis. PPARγ mediates its effect on lipid homeostasis by dual action: its role in increasing insulin sensitivity, and its effect on adipocytes.

PPARγ plays an important role in control of hepatic steatosis by increasing insulin sensitivity (see above). IR is integral to the development of nonalcoholic fatty liver disease (NAFLD), leading to increased fatty acid flux to the liver and increased hepatic fatty acid synthesis [Yu and Ginsberg, 2005].

Alternatively, PPARγ mediates its effect on adipocytes by promoting fatty acid uptake into adipocytes, adipocyte differentiation [Schoonjans

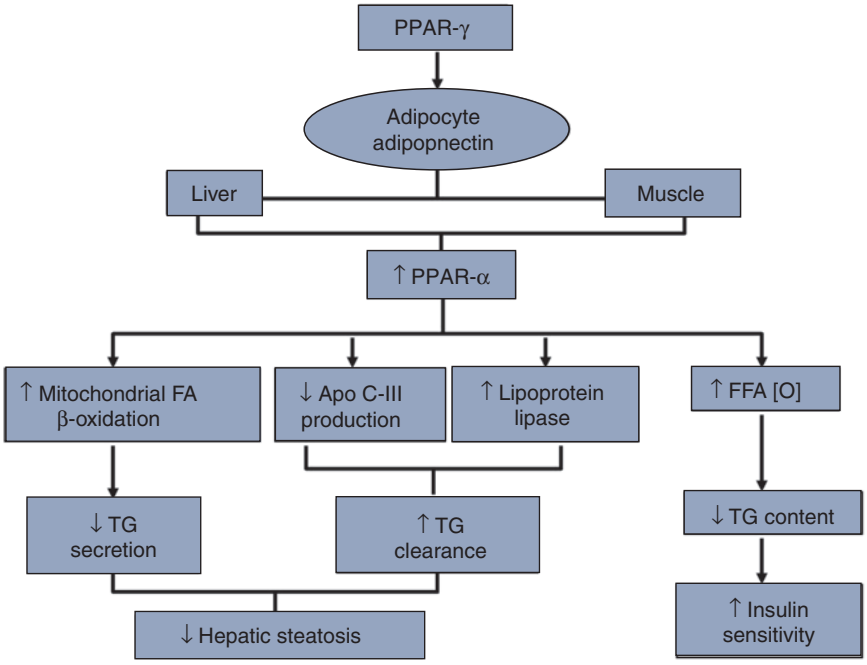


**Figure 2.** The mechanism of peroxisome proliferator-activated receptor-α (PPARα) action in lipid and glucose metabolism and its connection with PPARγ through adiponectin. Apo, apolipoprotein; FA, fatty acid; FFA, free fatty acid; TG, triglyceride.

*et al.* 1997] and by increasing the expression of adipocyte proteins involved in fatty acid uptake [Desvergne and Wahli, 1999], transport [Desvergne and Wahli, 1999] and synthesis [Frohnert *et al.* 1999]. The net effect of these processes is to increase triglyceride storage in adipocytes, reducing delivery of fatty acids to the liver. Patients with dominant negative mutations in PPARγ have NAFLD and metabolic syndrome, while lacking adipose tissue, suggesting increased triglyceride delivery to the liver [Savage *et al.* 2003].

PPARγ is also involved in the induction of uncoupling protein-2, which might decrease hepatic triglyceride accumulation by increasing energy expenditure [Castelein *et al.* 1994].

## PPAR: anti-inflammatory and immunomodulatory properties in the liver

In addition to the lipid-lowering and insulin-sensitizing effects of the PPAR ligands, there are numerous experimental and clinical data in favor of the anti-inflammatory activity of PPARs in the liver.

Evidence has demonstrated the effects of an RXR agonist and a PPARγ agonist on tumor necrosis factor-α (TNF-α) production [Uchimura *et al.* 2001]. PPARγ:RXR activation suppresses nuclear factor kappa B, signal transducers and activators of transcription, activating protein 1 signalling pathways and TNF-α production in Kupffer cells in primary hepatocyte cultures and monocytes/macrophages.

PPARα can also control hepatic inflammation by regulating hemostatic factors, acute-phase response proteins in hepatocytes [Anderson *et al.* 2002], and regulating antioxidant enzyme activities, such as catalase. In addition, PPARα agonists may reduce the oxidative stress [Hashimoto *et al.* 2000].

## Hepatitis C virus and insulin resistance

The prevalence of both IR and T2DM is significantly higher in patients with CHC when compared with other chronic liver diseases [Romero-Gómez *et al.* 2005; Hui *et al.* 2003; Caronia *et al.* 1999; Mason *et al.* 1999; Grimbert *et al.* 1996; Allison *et al.* 1994]. The prevalence of diabetes in patients with chronic hepatitis has ranged from 20% to 50% [Moucari *et al.* 2008; Caronia *et al.* 1999; Grimbert *et al.* 1996]. This is higher than that reported for patients with other chronic liver diseases such as chronic hepatitis B, independent of the stage of liver fibrosis [Romero-Gómez *et al.*

2005; Hui *et al.* 2003; Caronia *et al.* 1999; Mason *et al.* 1999; Grimbert *et al.* 1996; Allison *et al.* 1994].

This association between diabetes and CHC was first reported by Allison and colleagues [Allison *et al.* 1994], who observed that diabetes was significantly more prevalent in patients with hepatitis C-related cirrhosis than those with cirrhosis resulting from conditions other than CHC (50% *versus* 9%). Since then, a number of cross-sectional, case control, and longitudinal studies, performed in both large unselected cohorts and in patients with liver or kidney transplantation, have re-affirmed this association. Hui and colleagues [Hui *et al.* 2003] found that 121 patients with HCV and stage 0 or 1 liver fibrosis had higher HOMA scores compared with 137 healthy volunteers matched by sex, body mass index, and waist-to-hip ratio. This work proved that HCV may induce IR at early stages of liver disease. Another classic way to prove an association between infection and disease comes from the fact that curing HCV results in improvement in the HOMA score and a decreased incidence of T2D after cessation of therapy [Chehadeh *et al.* 2009; Romero-Gómez *et al.* 2008; Kawaguchi *et al.* 2007].

The mechanisms of IR in CHC are an area of intense study, and numerous molecular pathways have been implicated. IR in CHC arises both as a direct consequence of the virus and indirectly as a consequence of lipid accumulation and/or inflammation. HCV seems to lead to IR through interference of intracellular insulin signalling by HCV proteins, mainly the serine phosphorylation of IRS-1 and impairment of the downstream Akt signaling pathway. Also, HCV core protein inhibits PPARα and PPARγ expressed in hepatocytes and adipocytes promoting insulin receptor substrates-1 (IRS-1) degradation and IR. The HCV core protein interferes with *in vitro* insulin signaling by genotype-specific mechanisms [Moucari *et al.* 2008; Pazienza *et al.* 2007]. Moucari and colleagues found genotypes 1 and 4 to be correlated independently with IR [Moucari *et al.* 2008]. The rationale is unclear and may be based on the known differences in treatment response between these groups rather than interactions between viral proteins and host signaling pathways.

While CHC is independently associated with IR and T2DM, the presence of concomitant host-specific risk factors also contributes to both the prevalence and the degree of disturbance of glucose homeostasis in a complex interrelationship between these factors in CHC.

The association between HCV and IR has noteworthy consequences, clinically and conceptually. From the clinical standpoint, IR accelerates fibrogenesis [Hui *et al.* 2003], impairs early and sustained virologic response (SVR) to interferon-based antiviral therapy [Khattab *et al.* 2010; Mizuta *et al.* 2010; Chu *et al.* 2009; Dai *et al.* 2009; Poustchi *et al.* 2008; Conjeevaram *et al.* 2007; Romero-Gómez *et al.* 2005] (Figure 3), and increases the incidence of hepatocellular carcinoma [Pekow *et al.* 2007].

## Hepatitis C virus and fatty liver

The prevalence of steatosis in patients with CHC is reported to be between 40% and 80% depending on the features of the population studied in terms of alcohol consumption, prevalence of overweight/obesity, diabetes and other risk factors of fatty liver [Asselah *et al.* 2005]. However, when all common factors of steatosis have been ruled out, the prevalence of steatosis in CHC remains about 40%. This figure represents an approximately twofold increase compared with the prevalence of steatosis in other common chronic liver diseases such as hepatitis B (20%) [Thomopoulos *et al.* 2006; Rubbia-Brandt *et al.* 2000]. This suggests that hepatitis C virus (HCV) may directly cause fatty liver, at least in subgroups of patients with CHC.

Steatosis appears to be a direct consequence of viral protein expression in genotype 3 infection, suggesting the presence of specific sequences across the genome of genotype 3 that are involved in fat accumulation within hepatocytes. With other genotypes, steatosis is associated with features of the metabolic syndrome to include IR and obesity [Negro and Sanyal, 2009].

This is further supported by two additional observations. First, the severity of steatosis correlates with the level of HCV RNA, both in liver [Rubbia-Brandt *et al.* 2000] and in serum [Adinolfi *et al.* 2001], especially in patients with genotype 3. Second, fatty liver may significantly decrease, if not disappear altogether, when patients are successfully treated with antiviral agents. Again, this is most pronounced in patients infected with genotype 3 virus [Poynard *et al.* 2003; Rubbia-Brandt *et al.* 2000]. Steatosis may persist in most patients

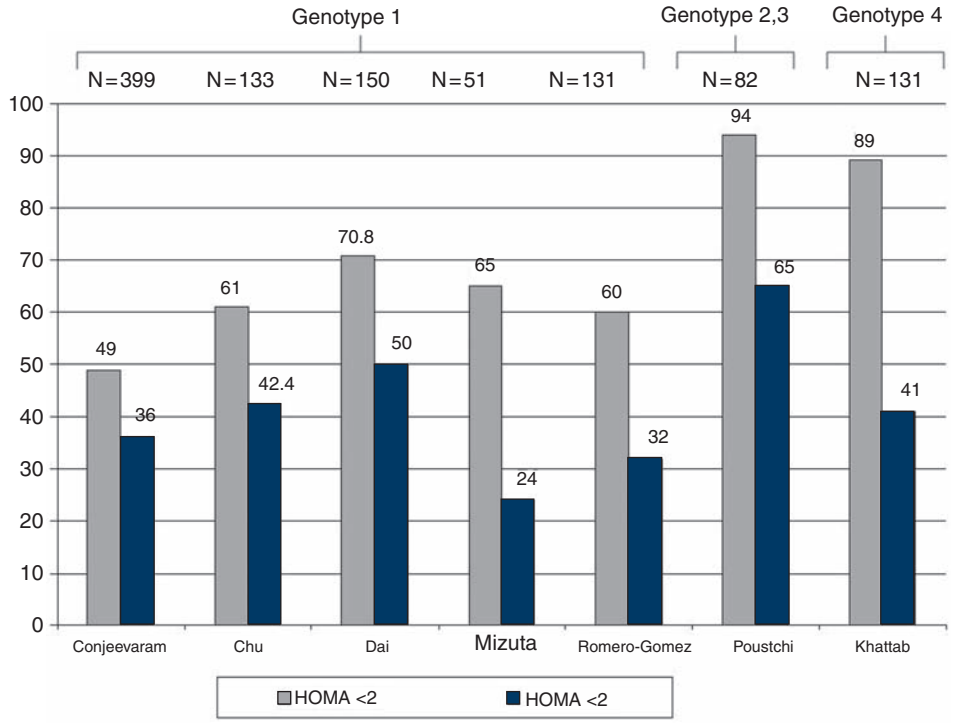


**Figure 3.** Impact of insulin resistance on sustained virologic response (SVR) in patients with chronic hepatitis C virus genotype (1, 2, 3 and 4). HOMA, homeostasis model of assessment.

with non-3 genotypes, even in the case of sustained virologic response [Poynard *et al.* 2003]. A relapse after the end of therapy may result in the reappearance of steatosis in patients in whom it had disappeared while on treatment [Rubbia-Brandt *et al.* 2001].

The composition of fatty acids that are accumulated in the liver of core gene transgenic mice is different from that in fatty liver due to simple obesity. Carbon-18 monounsaturated fatty acids (C18: 1) such as oleic or vaccenic acids are significantly increased. This is also the case in the comparison of liver tissues from patients with CV and those with simple fatty liver due to obesity [Moriya *et al.* 2001].

Recent studies have attempted to explain the potential mechanisms underlying the steatosis often seen in patients with CHC. In general, HCV may interfere with lipid metabolism at three levels: impaired secretion, impaired degradation and increased neolipogenesis.

Impaired secretion of lipoproteins from the infected hepatocyte was the first proposed mechanism of HCV-induced steatosis. Data suggest that HCV core protein may cause hepatic steatosis through inhibition of microsomal triglyceride transfer protein (MTP) activity [Perlemuter *et al.* 2002] because this enzyme plays a key rate-limiting role in very-low-density lipoprotein assembly. This inhibits the secretion of VLDL consisting of apolipoprotein B, cholesterol and triglycerides from the liver. The findings of impaired MTP functioning as a result of HCV core protein expression have more recently been extended to humans. In a study of 58 patients infected with various HCV genotypes, a highly significant ($p = 0.0017$), inverse correlation was found for liver MTP mRNA levels and the degree of hepatic steatosis that was independent of genotype, suggesting an important role for MTP in hepatic steatosis [Mirandola *et al.* 2006].

HCV may also cause steatosis by decreasing fatty acid oxidation through impairment of the expression and transcriptional activity of PPARα which regulates several genes responsible for fatty acid degradation [Cheng *et al.* 2005] (see below).

HCV may induce fatty liver by stimulating *de novo* synthesis of fatty acids. During primary infection in chimpanzees, HCV activates genes

involved in lipid metabolism via upregulation of sterol regulatory element binding protein-1c (SREBP) [Waris *et al.* 2007; Su *et al.* 2002]. SREBP activity is stimulated *in vitro* by several viral proteins, including core [Waris *et al.* 2007] and nonstructural proteins 2 [Oem *et al.* 2008] and 4B [Park *et al.* 2009; Oem *et al.* 2008; Waris *et al.* 2007]. Activation of SREBP and several enzymes involved in lipidogenesis has also been reported in transgenic mice expressing different HCV proteins [Lerat *et al.* 2009; Chang *et al.* 2008]. In addition to activating SREBP, the HCV core protein may also bind to and activate the DNA-binding domain of the retinoid receptor α, a transcriptional regulator that controls many cellular functions including cellular lipid synthesis [Fukasawa *et al.* 2006].

Finally, an additional mechanism leading to fatty liver may be related to the increased prevalence of IR among patients with CHC. IR increases the peripheral release and hepatic uptake of fatty acids, resulting in an accumulation of lipid in the liver [Browning and Horton, 2004]. Although HCV may be associated with extrahepatic IR [Milner *et al.* 2010; Vanni *et al.* 2009], this seems to involve striated muscles rather than the adipose tissue. Thus, there is no evidence as yet that HCV may induce fatty liver via increased lipolysis in adipocytes.

## Insulin resistance and steatosis: the chicken and the egg relationship

Although both IR and steatosis are common findings in patients with CHC, what comes first is not entirely clear. In the absence of HCV infection, much of the cardiovascular and endocrine literature has attributed IR to the presence of excessive triglyceride in organs such as the liver and muscle [den Boer *et al.* 2004; Ryysy *et al.* 2000]. By contrast, accumulating data seem to indicate that rather than causing IR, the presence of visible hepatocyte triglyceride droplets is a consequence of IR, hyperinsulinemia, and the result of excessive flux of free fatty acids through the liver [Monetti *et al.* 2007; Buettner *et al.* 2004].

Moreover, it is still unclear if hepatic steatosis represents the first hit to proinflammatory cytokine production which then leads to the development of IR or vice versa. Recent evidence, discriminating between 'systemic' and 'hepatic' IR, shows that in young, lean, patients with IR there was a low prevalence of hepatic steatosis and no cytokine/adipocytokine changes. This suggests that steatosis and cytokines interact without assuming a primary and independent role in the early stage of IR [Petersen *et al.* 2007]. Alternatively, other authors support the idea that hyperinsulinemia is likely to be the consequence rather than the cause of a fatty liver, as suggested by the fact that fatty liver is associated with both hepatic IR and impaired insulin clearance [Harsha and Bray, 2008; Hickman *et al.* 2004].

In patients with CHC, the HCV virus seems to play an additive role in this complex and reciprocal relationship as evidenced by the accumulating evidence of a direct 'metabolic' effect of the HCV virus on a large number of molecular pathways that lead to hepatic steatosis, IR and metabolic syndrome [Sheikh *et al.* 2008; Eckel *et al.* 2005]. Whereas core proteins from all HCV genotypes appear to influence this relationship, genotype-specific mechanisms may account for these effects, and although selected genotypes (e.g. 3a) may be more efficient in promoting hepatic steatosis, triglyceride droplets may be inert with respect to promoting injury and altered cellular homeostasis. In this case fat accumulation does not in itself precipitate IR and those patients who are IR typically have other causes such as obesity [Hui *et al.* 2003].

Preexisting metabolic conditions such as metabolic syndrome can also contribute to IR and steatosis in patients with HCV. In a study of 271 patients without diabetes, liver fat was four times higher in patients with metabolic syndrome (median 8.2%) compared with those without metabolic syndrome (median 2.0%; $p = 0.0001$), and this difference remained significant when adjusted for age, gender and body mass index (BMI) ($p = 0.011$) [Kotronen *et al.* 2007]. Among patients with metabolic syndrome in this study, liver fat was progressively increased ($p = 0.0001$ for trend) in patients as the number of components of metabolic syndrome increased. Patients with metabolic syndrome also had significantly higher fasting serum insulin and C-peptide concentrations than those without metabolic syndrome independent of age, gender and BMI (fasting serum insulin adjusted $r = 0.39$, $p = 0.0001$; C-peptide adjusted $r = 0.36$, $p = 0.0001$) [Kotronen *et al.* 2007]. Thus, this study suggests that liver fat content is an important component of the metabolic syndrome and increases proportionally with the number of

components of the metabolic syndrome. Moreover, of all measures of IR, fasting serum insulin and C-peptide were the best correlates of liver fat [Kotronen *et al.* 2007]. These findings, taken together, suggest a definite genotypic association between the IR and steatosis/steatohepatitis with HCV as the 'third player'. However, to further refine the nature of this interaction more investigation of the genotypic specificity of the virus–host interaction is needed.

## Interaction between hepatitis C and PPARs

The interaction between HCV products and PPAR expression has been an area of recent focus. Liver inflammation, hepatocyte fat accumulation, and diabetes are three pivotal hallmarks in the natural history of CHC infection which are at least in part controlled by PPARs. Expression of PPARα appears to be impaired with HCV infection [de Gottardi *et al.* 2006; Dharancy *et al.* 2005]. In patients with CHC, expression of the PPARα gene in the liver was reduced by 86% compared with controls, and the expression of its target gene, CPT1A, was coordinately reduced by 85%. Thus, hepatocytes infected with HCV display abnormally low levels of PPARα. Alternatively, PPARγ, RXR and liver X receptor were not different. Similarly, expression of the core protein in hepatoma cells was also found to reduce PPARα levels, but not the expression of the aforementioned receptors, and the induced expression of the CPT1A target gene by fenofibric acid was inhibited in core protein-expressing cells but not control cells [Dharancy *et al.* 2005].

Recent evidence of a direct role for PPARα activation in the pathogenesis of steatosis and HCC has been obtained from studies that combined core gene transgenic mice with PPARα knockout (KO) mice. The results of these experiments found that PPARα KO mice have reduced expression of target genes of PPARα, and have mild steatosis in the liver as expected [Tanaka *et al.* 2008]. Steatosis and hepatocellular carcinoma developed in PPARα intact but not in PPARα heterozygous or PPARα null core gene transgenic mice, indicating that not the presence but the persistent activation of PPARα would be important in hepatocarcinogenesis by HCV core protein. In general, PPARα acts to ameliorate steatosis, but with the presence of mitochondrial dysfunction, which is also provoked by the core protein, the core activated PPARα may exacerbate steatosis. This study showed that expression of the core protein in and of itself was insufficient to cause steatosis, as evidenced by the lack of steatosis in mice heterozygous or homozygous for the deletion of PPARα. A persistent activation of PPARα with 'strong' ligands such as the core protein of HCV could be carcinogenic in humans, although the low-affinity fibrate ligands are not likely associated with human cancers [Tanaka *et al.* 2008].

Others have shown that PPARγ expression is significantly lower in genotype 3 compared with genotype 1 HCV infection and that steatosis is associated with decreased levels of PPARγ in genotype 1 HCV infection [de Gottardi *et al.* 2006]. In this study, there was no significant relationship between the PPARs mRNA levels and liver activity or fibrosis. The presence of steatosis and HCV genotype 3 were both associated with a significant downregulation of PPARs.

## Perspectives for treatment

Although, increasing insulin sensitivity may be a rationale option in patients with CHC, especially those with metabolic syndrome, the modalities for this intervention have not been established yet. The primary data on the use of pioglitazone (PIO) was from a prospective, multicenter study aimed at investigating the efficacy and safety of PIO, 15 mg daily, added to pegylated interferon-2α (Peg-IFN-2α), 180 g every week and ribavirin (RBV) 1000–1200 mg daily combination therapy in retreatment of patients with CHC who were previously nonresponders to a Peg-IFN-α/RBV combination. All patients were IR and had a baseline HOMA > 2 [Overbeck *et al.* 2008]. The study was prematurely terminated as none of the first five patients enrolled into the trial had a sufficient virological response after 12 weeks. The dose of PIO used in this study was quite low, however, and may have impacted on the results [Negro, 2009]. The following emerging data from the four available studies evaluating the use of PIO in addition to the Peg-IFN α/RBV combination is provocative [Khattab *et al.* 2010; Vierling *et al.* 2010; Conjeevaram *et al.* 2008; Elgouhari *et al.* 2008]. In one study of treatment-naïve, non-diabetic, genotype 1 patients, 30 mg/day of PIO was given for 4 weeks as monotherapy and then added for the first 4 weeks of a standard therapy. The authors showed that the triple regimen increased the rate of virological response significantly after 4 weeks of therapy compared with the standard of care. Long-term data from this study is keenly awaited

[Elgouhari *et al.* 2008]. In another randomized, double-blind, placebo-controlled study in genotype 1 patients, adding PIO 30 mg/day simultaneously to the standard of care (i.e. without preceding administration as monotherapy) clearly improved IR, steatosis and increased the on-treatment virological response but failed to increase the SVR [Conjeevaram *et al.* 2008]. The third trial was conducted conclusively in patients infected with HCV genotype 4. The use of PIO 30 mg/day simultaneously with the standard antiviral therapy increased rapid virologic response (RVR) and SVR rates with improvement in all parameters of IR [Khattab *et al.* 2010].

Results from another study were previously presented at the 2010 meeting of the American Association for the Study of Liver Disease. The sequential use of PIO for 16 weeks (30 mg/day × 8 weeks then 45 mg/day × 8 weeks) prior to and concordant with 48 weeks of standard of care therapy revealed improvements in several glycemic variables, but no improvement in RVR or early virologic response (EVR) was seen. Data on SVR were not available [Vierling *et al.* 2010].

The final answer as to whether improving IR with thiazolidinedione therapy such as PIO equates to improved virologic response remains unknown at the current time. It is possible that there may be genotypic variations in response. In addition, genetic and epigenetic influences, such as IL28B single nucleotide polymorphisms, may be associated with IR and despite improvement in IR enhancement in SVR may not be seen. Clinical trials are underway seeking answers to these questions and the data are eagerly anticipated.

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