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REVIEW ARTICLE

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Hepatitis C virus infection, genotypes and mechanism of insulin resistance

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ABSTRACT

Background: Hepatitis C virus (HCV) infection is a major global public health problem that causes profound metabolic abnormalities, primarily in insulin-sensitive target tissues, notably the phenomenon of steatosis or fatty liver. The route of transmission and genetic mutation of HCV, together with the lack of reliable nation-specific epidemiological data on the distribution of genotypes and sub-genotypes of this RNA virus, provide significant challenges to correct diagnosis and effective treatment.

Issue: HCV-induced insulin resistance in HCV-infected individuals is independent of the occurrence of metabolic syndrome and diabetes mellitus, primarily type 2 diabetes mellitus that causes insulin resistance. Some but not all HCV genotypes exert a steatotic effect. However, the molecular mechanism(s) by which HCV infection causes insulin resistance in insulin target tissues or hepatic steatosis is not elucidated clearly.

Findings: Mechanisms proposed by experimental studies include interference with insulin signaling pathways, upregulation of genes controlling gluconeogenesis, phosphorylation of insulin receptor substrate proteins, and induction and overexpression of inflammatory cytokines that interact closely with host lipid metabolism.

Conclusions: We review HCV genotypes and subtypes, the mechanisms by which HCV infection induces insulin resistance, the virus genotypes and subtypes that are implicated in this and those that are steatotic. We conclude by discussing the proposed mechanisms of steatosis and considering HCV laboratory investigation methods from traditional to current techniques.

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Introduction

Hepatitis C virus (HCV) infection poses a significant public health threat. It is estimated that HCV infects around 80 million people, of which 11 million are children. Some 70 million individuals are chronically infected, resulting in almost 400,000 deaths each year [1]. HCV is the leading cause of liver cancer and the main reason for liver transplantation [2,3]. According to the most recent World Health Organization (WHO) report, in 2015, there were 1.75 million new HCV infections globally [4]. HCV infection is caused by one of the six distinct genotypes but their distribution in many countries remains unknown [5]. The worldwide prevalence of HCV is 2.5%, with the highest proportion of the population infected in Africa (2.9%) followed by the Americas (1.3%) [6].

HCV is the principal cause of acute and chronic liver diseases, affecting more than 200 million people around the world [7]. HCV has a tropism for hepatocytes that it enters via specific receptors, including the cell surface protein CD81, and once inside the hepatocyte, it initiates a lytic cycle by utilizing the intracellular translational machinery required for its replication [7]. Inadequately sterilized needles used for medical treatment, drug misuse or tattooing account for approximately 30% of HCV transmission and the prevalence of HCV is estimated to be 60% among injected drug users. The global eradication of this virus is the ultimate goal to solve health problems associated with infection [2,3,8]. The WHO presented a Global Health Sector Strategy on HCV infection for the 5-year period

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2016–21 [5]. This has an ambitious aim to eliminate viral hepatitis by 2030, diagnosing 90% of infected people and treating 80% of those. The lack of robust country-specific epidemiological data on the distribution of HCV genotypes is the main hurdle to developing an effective diagnosis and treatment strategy to combat HCV infection [6].

HCV infection also impairs the action of the hormone insulin, the tight regulation of which is essential for blood glucose homeostasis. Insulin stimulates glucose uptake by cells; it enhances glycogenesis in the liver and in muscles, and lipogenesis in adipocytes. In contrast, insulin inhibits gluconeogenesis and glycogenolysis in the liver and lipolysis in adipocytes [8,9].

Insulin Biosynthesis

Discovered in Toronto, Canada, in 1921 by Banting and Best, insulin is produced by β-cells of the pancreatic islets [10]. It is a peptide hormone composed of a linear amino acid polymer. Insulin is synthesized from a precursor, pro-insulin, which is a single, long polypeptide with internal disulfide bonds. In its mature secreted form, insulin contains two separate polypeptide chains that are linked together by disulfide bonds. By cleavage, mature insulin and a C-peptide fragment are generated from pro-insulin in the endoplasmic reticulum and then stored in secretory granules [11]. The 31-amino acid C-peptide is secreted into the portal circulation in equimolar concentrations with insulin [8].

Mechanism of Insulin Action: General Considerations

The primary physiological variable that controls insulin release from β-cells is the concentration of blood glucose. Carbohydrate metabolism is regulated by the action and counteraction of insulin and glucagon that predominantly influence the pathway of carbohydrate metabolism. Insulin acts upon the membrane receptor to allow the movement of glucose into tissue cells [9]. It is the principal anabolic hormone in the human body. While most cells are responsive to insulin, its most important effects are mediated by major metabolic organs, including the liver, adipose tissue and muscle [12]. In the liver, insulin acts on hepatocytes in contrasting ways to either inhibit gluconeogenesis or to activate glycogenesis. In doing so, this organ plays a key role in maintaining stable glucose concentrations due to its ability to store glucose as glycogen through glycogenesis and also to degrade glycogen through glycogenolysis, depending on the body's needs. Dramatic insulin resistance, severe glucose intolerance, and a failure of insulin to suppress hepatic glucose production and to regulate hepatic gene expression have been observed in liver-specific insulin receptor knockout mice when these alterations resulted in marked hyperinsulinemia [13].

The continued presence of insulin promotes lipogenesis, resulting in any more excess glucose being converted into fatty acids once hepatic glycogen stores become maximal [9]. In adipose tissue, insulin modulates multiple enzymatic activities to promote fat storage. In order to reduce lipid metabolism in adipocytes, insulin hinders the breakdown of fat by inhibiting intracellular lipase that hydrolyzes triglycerides to release fatty acids. Insulin facilitates entry of glucose into adipocytes, and within these cells, glucose can be used to synthesis glycerol by enhancing extracellular lipase. Finally, the glucose metabolized to glycerol is combined with imported fatty acids to generate triglycerides that are stored in adipocytes [14]. Insulin acts on myocytes to increase glucose import and shifts muscle metabolism to use glucose primarily as an energy source; moreover, any excess glucose imported is stored as glycogen in the presence of insulin. During prolonged fasting or starvation, the glycogen in muscles cannot break down into glucose to provide energy, unlike the glycogen in the liver, due to an absence of glucose-6-phosphatase activity [15–17].

In general terms, insulin is the hormone that facilitates the uptake of glucose from the blood circulation and the conversion of excess glucose into glycogen to be stored in target tissues (liver, kidney and muscle) by inhibiting gluconeogenesis, lipolysis and glycogenolysis. Glucagon is the counter-regulatory hormone produced by α-cells of the pancreatic islets that stimulates glycogenolysis and gluconeogenesis in order to maintain blood glucose homeostasis during prolonged fasting or starvation [18]. However, if the normal physiological functions of insulin are impaired, a high concentration of glucose accumulates in the blood (hyperglycemia) and excess free fatty acids (FFAs) are present in the peripheral circulation due to lipolysis [19].

Insulin Signaling Pathways

Insulin signals through insulin receptor and to a lesser extent through insulin-like growth factor-1 receptor. The hormone is secreted in response to increased blood glucose levels and its binding to

its receptor activates a cell signaling pathway that promotes glucose uptake [20]. Insulin signals to a variety of cellular functions in multiple tissues including: gene transcription; glucose, lipid and protein metabolisms; cell survival; growth control; and apoptosis [21]. Insulin receptor is a large disulfide-linked glycoprotein that spans the cell membrane with its insulin-binding surfaces on the outside of the cell and its tyrosine kinase (TYK) domains on the inside [22]. Insulin receptors comprise 2β and 2α subunits presenting on the surface of target cells (Fig. 1). The α-subunits contain the insulin-binding domain, whereas the β-subunits function as tyrosine-specific protein kinases that undergo autophosphorylation following insulin binding. Autophosphorylation of insulin receptor activates insulin receptor kinase and enables it to phosphorylate insulin receptor substrate (IRS) proteins 1 and 2 [23]. Stimulation of receptor kinase activity induces tyrosine phosphorylation of IRS-1 and IRS-2 that permits them to interact with and engage Src homology (SH)2-containing proteins like phosphatidyl-inositol-3 kinase (PI3K) that lead to multiple signaling pathways required for insulin action, resulting in promotion of glucose uptake, glycogenesis, mitogenesis and gene expression [24]. Insulin receptor signaling starts with the autophosphorylation of key tyrosine residues in the intracellular domain of the insulin receptor, thereby generating phosphotyrosine-docking sites for various proteins containing SH2 or phosphotyrosine-binding domains. Phosphorylation and dephosphorylation of protein tyrosine residues catalyzed by protein TYKs and protein tyrosine phosphatases, respectively, are crucial events for insulin signaling pathways [25].

Insulin signaling is downregulated by internalization of the insulin receptor complex, leading to dissociation and degradation of insulin in the intracellular endosome/lysosome system [26,27]. Insulin signaling pathways involve cascades of phosphorylation by kinases and dephosphorylation by phosphatases [28]. There are short-term and long-term downstream effects of insulin receptor stimulation. For instance, translocation of the glucose transporter (GLUT)4 to the surface of a target cell is a short-term action (Fig. 2), while increased expression of glucokinase and reduced expression of gluconeogenic

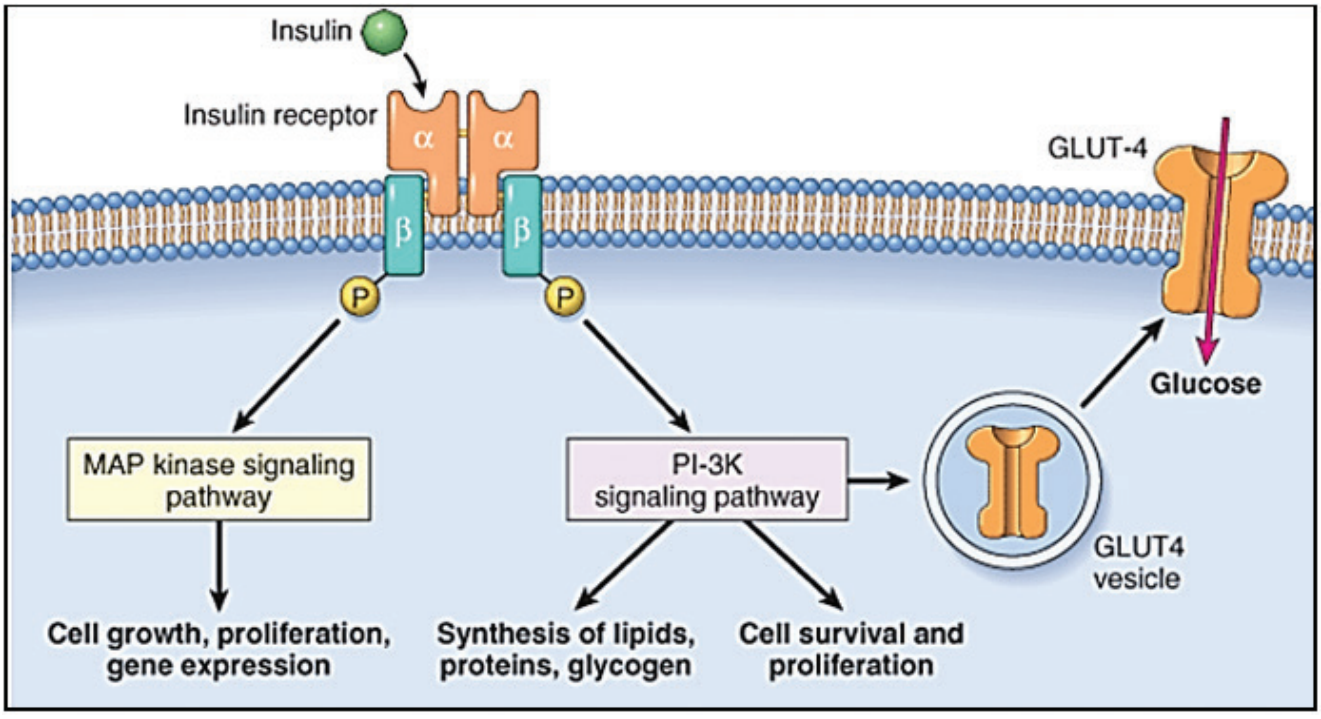

Figure 1. Schematic of insulin signaling pathways. When stimulated by insulin, the insulin receptor recruits and phosphorylates IRS proteins that mediate activation of the PI3K/ TYK signaling pathway and the MAP kinase signaling pathway. The PI3K/TYK pathway mediates metabolic action of insulin including lipogenesis, glycogenesis, and protein synthesis. It also stimulates translocation of glucose transporters (e.g. GLUT4) to the cell surface that is crucial for glucose uptake by insulin-sensitive tissues such as skeletal muscle and fat. The MAP kinase pathway mediates cell growth, proliferation, and regulation of expression of various genes in insulin-responsive cells. Modified from https://usmle.biochemistryformedics.com/mechanism-of-action-of-insulin/.

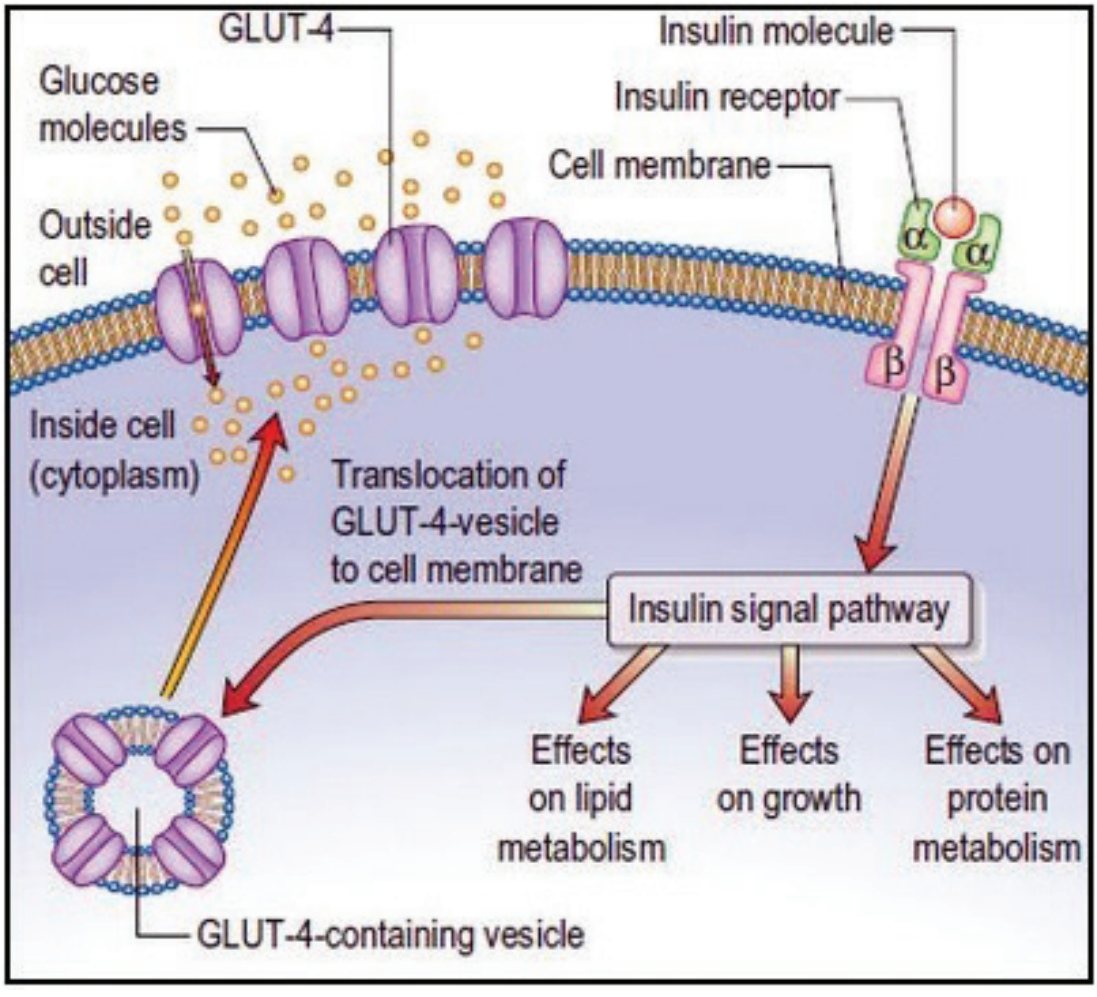

Figure 2. Schematic of downstream effects of insulin signaling pathways. Insulin binding to its receptor stimulates intrinsic TYK activity that triggers receptor autophosphorylation and the recruitment of intracellular signaling molecules including IRS proteins. Together with other adaptor proteins, IRS initiates a complex cascade of phosphorylation and dephosphorylation reactions, promoting the downstream systemic metabolic and mitogenic effects of insulin. Modified from http://www.namrata.co/insulin-biosynthesis-secretion-and-action/.

and ketogenic enzymes in the liver are long-term actions [11,29]. Studies in a mouse model revealed that selective internalization of the activated insulin receptor is achieved by a phosphorylation switch of IRS-1/-2 arranged by an extracellular signal-regulated kinase (ERK)-1 and ERK-2 and Src homology phosphatase (SHP)2. This also indicated that insulin receptor feedback regulation and growth promotion are blocked and insulin action on metabolism is prolonged, while insulin sensitivity is improved on inhibition of SHP2 [30]. Fork headbox (Fox) proteins are a family of transcription factors that plays important roles in regulating the expression of genes involved in cell growth, proliferation and differentiation [31]. FoxO is a major target of insulin signaling that translocates from the nucleus to the cytoplasm following insulin-stimulated phosphorylation. An experimental study conducted in mice indicated that FoxK1 and FoxK2 are downstream targets of insulin action but that they translocate from the cytoplasm to the nucleus following insulin stimulation [32]. The insulin and insulin-like growth factor-1 receptor TYKs mediate the effects of type 2 diabetes mellitus (T2DM) through tyrosine phosphorylation of substrate molecules, such as IRS-1 and IRS-2, leading to activation of two major pathways: the PI3K/TYK pathway and the mitogen-activated protein kinase (MAPK)/ERK pathway (Fig. 1). The PI3K/TYK pathway activates several distinct downstream pathways and is central to most of the metabolic actions of insulin, whereas the MAPK/ERK pathway is more important in the regulation of cell growth [20,32]. Another recent animal model study has suggested that the cytohesin-3 genetic locus is required for full insulin signaling in mammals and may constitute a novel therapeutic target for weight reduction [21].

Insulin signaling regulates carbohydrate (glucose), lipid and energy homeostasis, and a proper and coordinated biological response to insulin in different tissues is ensured by positive and negative modulator actions on different steps of signaling pathways and through the diversity of protein form interactions [20].

Insulin Resistance

Insulin resistance (IR) is the decreased ability of insulin to act effectively on target tissues including liver, skeletal muscle, and adipocytes. It is thought to be a major pathogenic factor implicated in metabolic syndrome and abnormalities like T2DM, obesity and dyslipidemia [7]. Impaired insulin signaling results from mutations in post-translation modifications of the insulin receptor or any of its downstream effector molecules [23]. Inherited defects in the membrane and nuclear receptors or in the pathways that transduce receptor signals are rare genetic disorders that cause severe hormone resistance syndromes characterized by defective hormone action despite the presence of increased hormone levels [33]. Glucose homeostasis reflects a balance between hepatic glucose production and peripheral glucose uptake and utilization, and insulin is the most important regulator of this metabolic equilibrium [8,12]. When insulin regulation of blood glucose levels is impaired, hepatic gluconeogenesis and glycogenolysis are greatly enhanced; glucose uptake in insulin-sensitive tissues (skeletal muscle and fat) is reduced and mobilization of FFAs from fat cells is promoted. However, as the body has a perpetual need for insulin, the β-cells of the pancreas produce insulin continuously and, due to the circulatory concentration of insulin, hyperinsulinemia may develop. As a result of increased hepatic glucose synthesis, diminished uptake of glucose by insulin-sensitive tissues and the ingestion of dietary glucose, blood glucose levels become very high if not reversed early and the cumulative effect of both hyperglycemia and hyperinsulinemia can damage internal organs [8–10,15]. Hyperinsulinemia and insulin resistance are pervasive features of obesity, particularly intra-abdominal obesity. The factors that contribute a molecular link between IR and obesity in insulin-sensitive tissues include FFAs, intracellular lipid accumulation, insulin itself by inducing downregulation of its own receptor, as well as different circulating peptides produced by adipocytes; these include the cytokines interleukin (IL)-6 and tumor necrosis factor (TNF)-α [34]. The underlying molecular mechanisms of IR are not clear; however, alterations in the early steps of insulin signaling, including decreased insulin binding, diminished receptor kinase activity, reduced levels of IRS proteins and their associated PI3K activity in primary target tissues, have been demonstrated in a mouse model to be related to IR [24]. The serine phosphorylation of IRS-1 contributes to peripheral IR by inhibiting insulin signal transduction in a variety of cell types, although the mechanisms of inhibition are difficult to determine due to a large number of potential phosphorylation sites [35].

HCV Genotypes: General Considerations

HCV is a member of the *Hepacivirus* genus of enveloped, positive-sense, single-stranded RNA viruses in the family Flaviviridae. The structure of each virus particle consists of an icosahedral protein coat, embedded in cellular lipid and surrounding a core containing RNA. The viral RNA encodes a single very long protein of more than 3,000 amino acids that is processed into individual proteins using viral and host cellular proteases. Based on sequencing, seven genotypes labeled 1–7 are currently recognized, with a further 67 subtypes, including 1a, 1b, 2a and 2b. A putative eighth genotype was identified recently in Punjab, India. HCV genotyping of infection has therapeutic implications for patient therapy. For instance, genotypes 1 and 4 are more resistant to interferon therapy than are the other genotypes [8,36,37]. The nucleotide sequences of the seven genotype classifications differ from each other by about 30% [38].

Mutation in the viral genome during replication, viral amino acid changes due to nucleotide substitution accumulation, lack of proofreading activity to correct these errors and transmission of mutant viruses across patient populations all lead to the diversification of HCV genotypes and subtypes, which spread throughout the world by various routes of transmission [2,39]. Globally, the most prevalent genotype is HCV-1 (49.1%), followed by HCV-3 (17.9%), HCV-4 (16.8%) and HCV-2 (11.0%) [6].

Geographical diversification of HCV genotypes is less studied. However, it is well established that some genotypes show regional predominance. In developed countries, genotype 1 is the most prevalent and that which is responsible for the majority of infections. Genotype 3 is often found in South Asia, Europe and in the USA among drug users infected with HCV. Genotypes 2 and 6 are the most common in East Asia. Genotype 4 is prevalent

in North Africa and the Middle East. Genotype 5 accounts for less than 1% of HCV infections and mainly appears in Africa, while genotype 7 infection was confirmed in Canada in one patient of central African origin [1,6,8]. In Africa, where around 3.4% of HCV infections are reported to be of mixed genotype, genotype 4 (28.1%) and 1 (26.3%) are the most prevalent genotypes followed by genotype 2 (23.7%), 5 (12.2%) and 3 (6.3%); however, there is no evidence for genotype 6. HCV genotype 1 is highly prevalent in both North America (74.5%) and Europe (64.4%); while subtype 1a predominates in North America, subtype 1b predominates in Europe. Globally, genotype 1 is the most prevalent (49.1%), followed by genotype 3 (17.9%), with genotype 6 being the least prevalent (1.4%) [6]. HCV genotypes 3 and 1 are those isolated most commonly from children, 77.15% and 17.14%, respectively [1]. A study conducted in Egypt revealed HCV genotype 4 is the most prevalent (73.0%), followed by genotype 1 (26.0%) and mixed genotypes (15.7%) [40]. HCV genotypes 1 and 3 are the most common genotypes in Australia, responsible for 50%–55% and 35%–40% of clinical cases, respectively [41]. Identification of HCV genotypes and subtypes remains a priority in order to tailor treatment regimens. Patients with confirmed HCV infection should be tested to determine the infecting genotype.

An experimental study was conducted to measure the gene and protein expression of the suppressor of cytokine signaling 3 (SOCS3) gene and to evaluate the insulin impairment pathway by analysis of IRS1 and phospho-AKT by infecting the human hepatocellular carcinoma cell line with human HCV genotype 1b or 2 [42]. The findings indicated that SOCS3 gene expression was significantly higher in genotype 1b-infected cells compared with those infected with genotype 2. This led to the conclusion that up-regulation of the SOCS3 gene is one of the mechanisms for non-responsiveness to therapy and IR by HCV genotype 1b [42].

Pathogenesis of HCV Infection

HCV is a blood-transmitted virus that enters hepatocytes in which it replicates causing cell necrosis through immune- and metabolic-mediated mechanisms. HCV has evolved a mechanism to avoid recognition by cytolytic T lymphocytes that would otherwise eliminate virus-infected host cells. It is thought either to reduce expression of the major histocompatibility complex or to prevent presentation of the viral peptide on the cell surface [43–45]. In addition to immune-mediated HCV pathogenesis, there are several clinical and metabolic-mediated HCV pathogenesis outcomes including HCV-insulin resistance, oxidative stress, and hepatic steatosis [8,17,44–48]. Without treatment, spontaneous clearance of the virus occurs in 15%–45% of HCV-infected individuals, while the remaining 55%–85% develop chronic infection that leads to fibrosis and cirrhosis. The likelihood of progression to cirrhosis of the liver and hepatocellular carcinoma varies depending on a person's characteristics and behaviors. For instance, the risk is very high in individuals who are co-infected with hepatitis B virus or human immunodeficiency virus (HIV), who misuse alcohol or who are immunosuppressed for any reason. HCV-associated liver disease is the leading cause of death in people living with HIV, accounting for almost half (47%) of the deaths in the USA. After initiation of anti-retroviral therapy, the recovery of $CD4^+$ T cells is impaired in HIV/HCV-co-infected people when compared to HIV-mono-infected counterparts. HIV disease progression is more rapid in HIV/HCV co-infection than for infection with HIV alone [4,5,45]. HCV infection can lead to extrahepatic manifestations; depression (24%), diabetes mellitus (15%) and chronic renal disease (10%) are the three most frequent co-morbidities in HCV-infected individuals [5].

HCV Infection, Hepatic Steatosis and Insulin Resistance

HCV genes play a crucial role in the pathogenesis of infection by interfering with host cellular genes. The mechanism(s) by which HCV genes induce IR is not clear. Through experimental studies, it is known that HCV core protein inhibits insulin signaling and HCV causes impairment of the cellular response to insulin by interfering with serine phosphorylation of insulin receptor-1 [49]. A very recent study has shown that non-structural protein 5a (NS5A) of HCV genotype 3a upregulates serine 473 phosphorylation, suggesting that impairment of the normal insulin protein kinase signaling pathway leads to IR [7]. Chronic HCV infection impairs intrahepatic insulin signaling pathways by increasing the production of TNF-α, phosphorylation of insulin receptors, and overexpression and induction of suppressor of cytokine signaling (SOCS)-3 and -7, respectively [50]. Among patients infected with HCV genotype 4, there is a high prevalence of IR, which manifests early in infection [39]. T2DM is a metabolic disorder that

is caused by IR and chronic HCV infection is often associated with T2DM; however, the precise mechanism for this association is not apparent. HCV infection promotes hepatic gluconeogenesis, the process facilitated by glucagon in the liver during fasting whereupon insulin activity is impaired and upregulates the gene for phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [51].

HCV genotypes 1 and 4 have been identified among the majority of patients chronically infected with HCV and these two genotypes have the greatest association with IR. HCV genotype 1 is strongly associated with IR without any weight gain, as indicated by animal models [52]. HCV genotypes 1 and 3 revealed IR, respectively, among 65% and 57% of patients without cirrhosis and risk factors for metabolic syndrome [53]. IR is identified in more than half of people with chronic HCV infection [54]. Around 32% of non-diabetic patients chronically infected with HCV genotype 1 or 4 develop IR [39]. Individuals infected with HCV genotype 1 or 3 may develop IR and steatosis [53].

Very low-density lipoprotein (VLDL) supplies the tissues of the body with endogenously synthesized triglycerides, primarily of hepatic origin. VLDL is a rich source of triglycerides and triglyceride-glycerol formed from pyruvate through the process of glyceroneogenesis. This is the critical component of triglyceride fatty acid cycling in the body, which contributes to more than half of the triglyceride concentration in individuals with T2DM [55]. During an IR state, such as found in diabetic patients, intact liver insulin signaling drives enhanced lipogenesis while excess circulating FFAs become a dominant inducer of non-suppressible hepatic glucose production [56]. This indicates that a defect in the insulin signaling pathway permits the lipid-promoting effects of insulin to be preserved despite the loss of hepatic glucose production.

HCV produces IR in the liver and other peripheral tissues by mechanisms including production of proinflammatory cytokines like TNF-α, changing host lipid metabolism in hepatocytes (during viral replication, assembly, and release), phosphorylation of IRS-1 and upregulation of gluconeogenic genes [47,57]. TNF-α impairs insulin signaling by inhibiting insulin receptor TYK in the muscle and fat tissues of obese individuals [58,59].

Fatty acids cause a defect in insulin-stimulated glucose transport in skeletal muscle by inhibiting insulin-stimulated tyrosine phosphorylation of IRS-1 and IRS-2 associated with PI3K activity, in addition to certain fatty acid metabolic abnormalities such as mitochondrial fatty acid oxidation and adipocyte fat metabolism defects [33]. A study of selected individuals without any features of metabolic syndrome but confirmed for HCV infection showed that HCV infection *per se* is strongly associated with hepatic IR and total body glucose disposal, brought about mainly by impairing one component of glucose oxidation [60]. A suggested mechanism for hepatic IR is the direct interaction of HCV core protein with the signaling pathway of insulin inside the hepatocyte. The development of IR consequent to HCV infection occurs early and is independent of either body weight, stage of liver disease, or the presence or absence of diabetes [61]. Successful treatment to eradicate HCV improves insulin sensitivity in HCV-infected T2DM patients [62]. A direct steatotic effect of HCV genotype 3 due to accumulation of intracytoplasmic triglyceride-rich droplets induced by overexpression of viral proteins (core protein and NS5A) has been reported in transgenic mice and cell culture models of HCV. The study also revealed host factors, principally associated with IR, are primarily responsible for steatosis developed by HCV genotype 1, whereas steatosis developed in HCV genotype 3-infected individuals is primarily viral-mediated [61]. IR is strongly associated with obesity, primarily intraabdominal obesity [49]. Accumulation of intracellular lipid, receptor downregulation induced by insulin itself, increased FFAs and various circulating peptides (adiponectin, resistin, IL-6) produced by adipocytes are among major factors that provide a molecular link between obesity and IR [46,49]. Inflammatory mediators (TNF-α, IL-1, and IL-6) and adipose-specific cytokines (leptin and resistin) can interfere with insulin receptor signaling at target cells by promoting serine phosphorylation to mediate heterologous inhibition of IRS-1 signaling thereby counter-regulating the insulin response [63]. Experiments on mice suggested that components of the extracellular matrix, including collagen, convert extracellular signals via their cytoplasmic domain and binding to intracellular integrin-binding proteins [64].

IR is strongly associated with fat accumulation in the body. Hepatic steatosis or fatty liver is defined as the intrahepatic fat accumulation of at least 5% of the liver weight that may lead to hepatic metabolic dysfunction, inflammation and non-alcoholic fatty liver disease (NAFLD) [44,65]. There is a high prevalence of HCV-related NAFLD, with genotype 3 identified as the only HCV genotype that induces fatty liver accumulation and causes viral steatosis [66]. NAFLD and HCV are associated with increased

gluconeogenic drive and IR, and steatosis development and chronic HCV infection are clearly linked [67]. Metabolic syndrome accelerates the progression of liver diseases in patients with HCV infection. HCV infection is associated with the development of IR, hepatic steatosis, and T2DM [68]. Metabolic syndrome or IR is highly prevalent in chronically infected HCV patients and associate significantly with HCV genotype 1 [69]. Mitochondrial β-oxidation is impaired by HCV infection, with 24% of patients in one study developing hepatic steatosis [70]. Lipid β-oxidation is attenuated significantly by HCV infections, which leads to low lipid combustion and energy supply as well as to certain metabolic abnormalities including fatty liver or steatosis [71]. HCV infection is associated with an increased rate of glucose abnormality including IR and this leads to negative liver-related outcomes [72]. A strong association between high-fat diets and hepatic and muscle IR has been confirmed in animal models [73]. Lack of hepatic glycogen suggests liver-specific IR. Deletion of the gene encoding the liver iso-form of glycogen synthase generates a knock-out mouse that almost completely lacks hepatic glycogen. In this model, insulin signaling is reduced in the liver, but not muscle, suggesting an organ-specific defect, unlike hepatic steatosis [44,74].

There is a clear link between the development of steatosis and chronic HCV infection [75]. IR is a major feature of NAFLD [44,46,73,76]. Chronic HCV infection is a unique infective/inflammatory model of IR, predominantly in muscle, and HCV genotype 3 is typically associated with hepatic steatosis [77]. An improvement in IR after HCV clearance depends on the genotype of the virus. A sustained virological response is associated with a reduction in homeostasis model assessment-IR in patients with HCV genotype 1 but not either genotypes 2 or 3 that may suggest genotype 1 has a direct effect on the development of IR, independent of host metabolic factors [78]. The prevalence of hepatic steatosis in chronic HCV infection is about 55% due to a combination of viral factors like core proteins and host factors like obesity, IR, diabetes mellitus, and alcohol consumption [79]. Inflammation, T2DM, hypertension, fat accumulation, obesity, and NAFLD are each strongly associated with IR [44,48,49,57,80].

A recent investigation conducted in Italy on 82 HCV RNA-positive patients attending a liver clinic to receive direct-acting anti-viral therapy with no previously known diabetes revealed that 55% had developed T2DM by the end of their treatment; HCV RNA fell below the threshold of detection (<12 IU/ml) and insulin sensitivity increased [81]. A US longitudinal study concluded that suppression of HCV results in improvement of insulin activity [82]. Dysfunction of mitochondria is one mechanism for IR because these organelles are where fatty acids and glucose are used in the production of ATP through the process of oxidative phosphorylation. The suggested mechanism for causing IR is the incomplete burning of fatty acids in mitochondria. This results in the accumulation of triglyceride intermediate products including diacylglyceride, which activates protein kinase C. In turn, this suppresses the insulin signaling pathway through phosphorylation of IRS-1 and IRS-2 that causes IR [83–85]. Within 72 hours of rodents starting a high-fat diet, before obesity develops, hepatic steatosis and IR can be detected [73]. HCV core proteins interfere directly with hepatocyte intracellular insulin signaling or with IRS-1 to induce downstream TYK activity, thereby impairing cellular responsiveness to insulin action [34,86].

HCV infection induces IR probably due to proteasomal degradation of IRS-1 and IRS-2 caused by HCV core protein and as a result of blockage of intracellular insulin signaling caused by TNF-α and SOCS-3 [87]. In a transgenic mouse model, insulin fails to suppress hepatic glucose production in animals overexpressing HCV core protein. More recent studies have indicated that SOCS families play a key role in inducing IR during HCV infection by suppressing the insulin signaling cascade through proteasomal degradation and ubiquitination of IRS-1 and IRS-2 [88]. SOCS proteins are powerful inhibitors of pathways involved in the survival and function of pancreatic β-cells. SOCS-1 and SOCS-3 induce IR by inhibition of tyrosine phosphorylation of IRS proteins. Overexpression of SOCS-3 in the liver by adenoviral-mediated gene transfer significantly decreases tyrosine phosphorylation of both IRS-1 and IRS-2; in contrast, SOCS-1 overexpression preferentially inhibits IRS-2 [89,90]. A Japanese study using a hepatocyte-specific SOCS-3-deficient mouse model showed that SOCS-3 produced by pro-inflammatory cytokines TNF-α and IL-6 induces inflammation-mediated IR in hepatocytes and adipocytes. This suggests that insulin sensitivity is improved in both cell types [91]. SOCS-3 antagonizes insulin-induced IRS-1 tyrosine phosphorylation, indicating that it may play a prominent role in the development of IR [92]. Overexpression of SOCS-3 in adipocytes diminishes IRS-1 levels and

subsequent insulin-stimulated phosphorylation of IRS-1 and IRS-2 leads to decreased insulin-stimulated glucose uptake in adipocytes [93].

Diagnosis of HCV Infection: General Considerations

Direct and indirect methods are both available for diagnosing HCV infection. The indirect method measures antibodies produced against HCV antigen. Antibody detection is indicative of either current infection (anti-HCV IgM) or previous infection (anti-HCV IgG). This is a serological method performed by using immunoassay techniques and is primarily for qualitative screening purposes. The direct method involves the detection of purified HCV antigen by nucleic acid detection techniques [94]. This is performed by nucleic acid amplification or recombinant immunoblot and is quantitative in nature. Methods to quantify HCV RNA include polymerase chain reaction (PCR), real-time PCR, reverse transcriptase PCR, nucleic acid sequence-based amplification and branched DNA assay. Of these, real-time PCR and transcription-based amplification are used most routinely [8,94–96]. In order to tailor anti-HCV therapy, the identification of virus genotypes and differentiation of subtypes is crucial. HCV genotype determination methods that are based only on the analysis of the 5' non-coding region (5'NCR) fail to identify correctly HCV subtypes 1a and 1b in approximately 30% and 10% of cases, respectively. Currently available genotyping methods that are based on reverse hybridization or real-time PCR target not only 5'NCR but also the core- or non-structural 5B (NS5B)-coding regions; consequently, they identify accurately HCV genotypes and differentiate subtypes 1a and 1b in most cases. However, these globally used assays are neither designed to identify mixed genotype/subtype infections nor HCV subtypes other than 1a and 1b. Thus, there is a need for a high-resolution system, such as that predicated on phylogenetic analysis of reads obtained by deep sequencing of a relevant genome region. Sentosa SQ HCV genotyping is a new automated deep sequencing-based assay that performs genotypes 1 to 6 determinations, 1a/1b subtype identification, and genotypes 4, 5 and 6 sub-typing. This compares favorably against Sanger sequencing of the NS5B region, the reference method for HCV genotype determination, except for HCV genotype 2 subtyping [2,97,98].

Conclusion

Many experimental, clinical and epidemiological studies indicate the occurrence of HCV-induced IR in HCV-infected persons. The consensus from this collective research is that a clear link exists between HCV infection and hepatic steatosis or fatty liver in causing IR in a person with HCV or in an individual who develops NAFLD. Nevertheless, the underlying molecular mechanism by which HCV infection induces IR in insulin target tissues is to date only suggested and not confirmed. Ongoing concerns surround the high mutation rate of HCV, the cause of genetic variation and a possible reason for the emergence of new HCV genotypes. Improved country-specific epidemiological data on HCV genotype or subtype responsible for infection is required in order to map genotypes identified as being associated with IR, the pattern of which is known to vary throughout the world.

Conflict of interest

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