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Insulin Sensitivity and Variability in Hepatitis C Virus Infection Using Direct Measurement

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Abstract

Background & Aims—Studies investigating insulin resistance (IR) in chronic hepatitis C virus (HCV) infection have used surrogate measures of IR that have limited reliability. We aimed to describe the distribution and risk factors associated with IR and its change over time in HCV using direct measurement.

Methods—One hundred and two non-cirrhotic, non-diabetic, HCV-infected subjects underwent clinical, histologic, and metabolic evaluation, and 27 completed repeat evaluation at 6 months. Insulin-mediated glucose uptake was measured by steady-state plasma glucose (SSPG) concentration during the insulin suppression test.

Results—Three subjects with diabetes were excluded and 95 completed all testing. SSPG ranged from 39 to 328 mg/dL (mean 135 mg/dL) and was stable over time (mean SSPG change -0.3 mg/dL). SSPG was associated with Latino ethnicity (Coef 67, 95%CI 37-96), BMI (Coef 19 per 5 kg/m^2, 95%CI 5-32), ferritin (Coef 1.4 per 10 ng/ml, 95%CI 0.2-2.5), male gender (Coef -48, 95%CI -80 to -16), and HDL (Coef -16, 95%CI -28 to -5 mg/dL). Current tobacco use (Coef 55, 95%CI 19-90), steatosis (Coef -44, 95%CI -86 to -3), and increases in BMI (Coef 30 per 5 kg/m^2, 95%CI 6-53) and triglyceride (Coef 3.5 per 10 mg/dL, 95%CI 0.3-6.7) predicted change in SSPG.

Conclusions—There was a wide spectrum of insulin resistance in our HCV population. Host factors, rather than viral factors, appeared to more greatly influence insulin action and its change in HCV.

Keywords

Diabetes; HCV; Insulin Resistance; Oral Glucose Tolerance Test

Chronic hepatitis C virus (HCV) infection represents a significant public health burden, affecting approximately 4 million Americans.(1) Over the past decade, the findings of several large epidemiologic studies linking HCV with type 2 diabetes mellitus has generated great interest in understanding the pathophysiologic mechanisms underlying this association. To date, a clear cause-and-effect relationship has yet to be established, and our

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understanding of the role of insulin resistance (IR) in the natural history of HCV infection remains limited.

Peripheral IR, a decrease in insulin-mediated glucose disposal, is thought to represent the earliest derangement in glucose metabolism preceding the onset of diabetes.(2) There is a spectrum of insulin sensitivity in the general population, and high degrees of IR are associated with deleterious health consequences, including an increased risk for cardiovascular disease and cancer among others.(3, 4) An increased prevalence of IR has been reported in HCV, and although HCV was shown to be independently associated with IR, the spectrum of insulin sensitivity in the HCV population has yet to be clearly described. (5-9) Evaluation of IR in the HCV population is critical as it has been suggested to promote fibrosis progression(10), decrease responsiveness to interferon-based antiviral therapy(7), and contribute to poor outcomes following liver transplantation.(11)

However, the vast majority of studies evaluating IR in HCV have utilized surrogate estimates of IR, as opposed to direct quantification of insulin-mediated glucose uptake. Indeed, we have shown that surrogate measures of IR, when correlated to the direct measure of insulin-medicated glucose disposal, can be impacted by degrees of obesity and ethnicity. (12) For example, the most commonly used surrogate measure of IR in HCV studies, the Homeostasis Model Assessment (HOMA-IR), has a high misclassification rate and significant within-person variability in the HCV population, and similar limitations have been observed with its use in other populations.(12, 13) Although surrogate measures are practical in large epidemiologic studies, more precise measures of IR are required to adequately describe insulin sensitivity in the HCV population. The validated hyperinsulinemic-euglycemic clamp and insulin suppression test (IST) are considered gold standards for direct physiologic measurement of IR and are highly correlated ($r>0.9$).(14) Recently, small studies utilizing the hyperinsulinemiceuglycemic clamp have shown impairment of peripheral insulin resistance in the setting of HCV infection.(15, 16) Moreover, a recent study employing direct assessment of insulin sensitivity by measurement of steady state plasma glucose concentration (SSPG) during IST demonstrated that treatment of HCV infection reduces insulin resistance.(17)

The goal of this prospective study was to build upon these findings by performing a comprehensive evaluation of IR using direct quantification of resistance to insulin-mediated glucose uptake in the peripheral tissues (mainly muscle) using the IST in the largest cohort of non-diabetic, non-cirrhotic HCV subjects described to date. Our specific aims were to: (i) describe the frequency and distribution of insulin resistance in HCV-infected patients and (ii) determine host and viral factors associated with insulin action and identify those factors predictive of its change over time in the HCV population.

MATERIALS AND METHODS

Study Subjects

One hundred and two non-diabetic patients with chronic HCV infection (detectable HCV viral load) between ages 18-60 were recruited from San Francisco General Hospital (SFGH) and affiliated clinics at the University of California, San Francisco (UCSF) from 2002-2009. Patients with diabetes on the basis of a fasting plasma glucose ≥ 126 mg/dl at screening(18), a history of diabetes, or use of anti-diabetic agents were excluded. Additional exclusion criteria included presence of HBV or HIV infection, liver disease other than HCV, prior HCV treatment, steroid or anabolic therapy, or medical conditions influencing study participation. Patients with clinical, histologic, or known diagnosis of cirrhosis or decompensated liver disease were excluded from the study given the significant alterations in glucose metabolism known to occur in the setting of cirrhosis.(19, 20) This study was

approved by the UCSF Committee on Human Research and subjects provided written informed consent.

Study Procedures

Subjects underwent a medical interview, physical examination, fasting laboratory evaluation, and a liver biopsy at screening. Histologic evaluation was performed by a pathologist blinded to the patient's metabolic profile using the Ludwig-Batts scoring system.(21) Subjects were admitted to the UCSF Clinical and Translational Science Institute-Clinical Research Center (CRC) for study tests.

Metabolic Testing

At baseline, patients underwent a two-day inpatient hospital admission. On day one, a 75-g oral glucose tolerance test (OGTT) was performed after an overnight 12-hour fast. On day two, following another overnight 12-hour fast, each subject underwent the modified 240-minute IST.(12, 22) During this test, new glucose production is inhibited, and similar plasma levels of exogenous insulin are reached in all patients. The steady-state plasma glucose (SSPG) concentration as the result of an identical glucose infusion rate in all patients is a direct measure of insulin-mediated glucose uptake. Higher SSPG levels represent higher degrees of IR. As there is a spectrum of insulin sensitivity in the population, there are no single absolute cutoff values to define IR versus insulin sensitivity. In this study, IR was operationally defined as SSPG >180 mg/L(13), a value that has been shown to significantly increase the risk of developing clinical syndromes associated with IR in prospective studies of healthy, non-diabetic populations.(3, 4) All patients who remained anti-HCV treatment naïve were contacted to return at six months follow-up for a repeat comprehensive evaluation as outlined above. Liver enzymes and HCV viral load were measured within one month of metabolic testing in all subjects.

Laboratory Evaluation

Plasma glucose concentrations were measured by the glucose oxidase method (YSI 2300 STAT-Plus Analyzer, Yellow Springs, OH). Plasma insulin was measured using a single antibody radioimmunoassay without cross reactivity with human proinsulin (Millipore, Billerica, MA).

Statistical Analyses

Descriptive analyses were summarized using mean±SD, median (range), and frequency. Univariable and multivariable stepwise forward selection linear regression modeling was used to evaluate host and viral predictors associated with higher SSPG levels at baseline and change in SSPG from baseline. Statistical significance was assessed at a p-value <0.05 (2-sided) in all models. Similar logistic regression models were used for the outcome of insulin resistance (SSPG>180 mg/L). All analyses were performed using SAS v9.1.3 (SAS Institute, Cary, NC).

RESULTS

Cohort Characteristics

One hundred-two HCV-infected subjects were enrolled. Three subjects with diabetes based on a 2-hour plasma glucose ≥200 mg/dL during the baseline OGTT were subsequently excluded, and 95 subjects completed all baseline metabolic testing. Overall, subjects had a mean age of 48 years, 71% were male, mean BMI was 27 kg/m^2, 44% self-identified as Caucasian, 43% had a family history of diabetes, and 25% reported current alcohol consumption. The mean duration of HCV infection was estimated at 26 years, and 77% had

a history of intravenous drug use as the mode of HCV acquisition. The mean HCV viral load was 5.8±0.7 log IU/mL, 69% were genotype 1, and mean ALT was 93 units/L. Liver biopsy was available in 77 (82%) subjects; 45 (56%) subjects had mild liver fibrosis and 28 (35%) subjects had evidence of steatosis on histology and 24 (86%) of subjects with steatosis had mild steatosis (grade 1).

Twenty-five percent of subjects were insulin-resistant (SSPG>180 mg/dl). In comparing insulin-resistant to insulin-sensitive patients (Table 1), a higher proportion of insulin-resistant subjects were female (42 vs. 22%) and of Latino ethnicity (54 vs. 20%). Insulin-resistant subjects had higher BMI (29 vs. 26 kg/m^2) and rates of family history of diabetes (58 vs. 41%) and metabolic syndrome (42% vs. 9%), but lower levels of tobacco consumption (12 vs. 18 pack-years) and HDL cholesterol (46 vs. 52 mg/dL) compared to insulin-sensitive subjects. Insulin-resistant subjects also had significantly higher levels of fasting glucose (mean 97±11 vs. 91±9 mg/dL), fasting insulin (mean 24±11 vs. 13±5 μIU/mL) and, as expected, baseline SSPG levels (mean 248±40 vs. 99±39 mg/dL). Insulin-resistant patients had higher degrees of inflammation (76 vs. 58%), fibrosis (59 vs. 40%), and steatosis (53 vs. 30%) on liver histology, but these findings did not reach statistical significance. HCV-related factors, including HCV viral load, genotype, and duration of infection were similar among the two groups.

Distribution and Variability of Insulin Sensitivity—In evaluating the distribution of insulin sensitivity, the mean baseline SSPG was 135 mg/dL, ranging broadly from 39 to 328 mg/dL (Figure 1). Twenty-seven of the 72 patients who remained anti-HCV treatment-naïve agreed to undergo follow-up metabolic evaluation at 6-months. The mean SSPG at the second visit was 127 mg/dL, ranging from 35 to 321 mg/dL. Overall, there was little change in insulin sensitivity over time (mean change in SSPG of -0.3, 95%CI -18.8 to 18.3 mg/dL, p=0.98) (Figure 2).

Influence of Host and Viral Factors on Insulin Sensitivity—On univariable analysis, higher levels of SSPG were associated with Latino (Coef 67, 95%CI 32-102) and African-American (Coef 39, 95%CI -2 to 80) race/ethnicity, increasing BMI (Coef 31 per 5 kg/m^2, 95%CI 16-47) and waist circumference (Coef 12, 95%CI 5-20 cm), and the presence of steatosis on liver biopsy (Coef 36, 95%CI 1-71) (Table 2). On the other hand, higher education level (Coef -44, 95%CI -72 to -16) and current tobacco consumption (Coef -33, 95%CI -64 to -2) were associated with lower SSPG levels. Although they did not reach statistical significance, SSPG levels were also positively associated with a family history of diabetes, HCV genotype 3 (vs. 1), and higher degrees of inflammation and fibrosis on liver histology, but negatively associated with male sex and current alcohol use.

On multivariable regression analysis, independent predictors of higher SSPG were Latino race/ethnicity (vs. Caucasians, Coef 67, 95%CI 37-96), increasing BMI (Coef 19 per 5 kg/m^2, 95%CI 5-32), and increasing ferritin levels (Coef 1.4 per 10 ng/mL, 95%CI 0.2-2.5) (Table 3). Male gender (Coef -48, 95%CI -80 to -16) and higher HDL levels (Coef -16, 95%CI -28 to -5 mg/dL) were negatively associated with SSPG. Adjusting for age or family history of diabetes did not significantly alter the coefficients. The estimated effect of HCV viral factors, including HCV genotype 1 (vs. non-1, Coef -11, 95%CI -41 to 19), HCV duration of infection (Coef -7.1 per 10 years, 95%CI -19.0 to 4.7), and HCV viral load (Coef -6.6 per 1 $\log_{10}$IU/mL, 95%CI -24.4 to 11.2) were all too small to be important and were not statistically significant.

When using the cutoff of SSPG>180 mg/dL, on univariable analysis, Latino race/ethnicity (vs. Caucasian, OR 6.7, 95%CI 2.0-22.2, p=0.0019) and higher BMI (OR 1.7 per 5 kg/m^2, 95%CI 1.0-2.9, p=0.036) were found to be significantly associated with IR. In addition,

male gender (OR 0.41, 95%CI 0.15-1.09, p=0.074) and HDL levels (OR 0.65 per 10 mg/dL, 95%CI 0.43-1.0, p=0.050) were negatively associated with IR, and the presence of steatosis (OR 2.6, 95%CI 0.9-7.9, p=0.086) was associated with a nearly three-fold increased odds of IR, though these findings did not reach statistical significance. On multivariable analysis, IR was positively associated with African American and Latino race/ethnicity and BMI, and negatively associated with male sex and higher HDL levels (Table 3). Adjusting for age and family history of diabetes did not substantially alter the observed odds ratios.

Predictors of Change in SSPG Over 6 Months—When evaluating the impact of baseline factors on change in SSPG levels over 6 months, univariable analysis showed that higher baseline inflammation (grade ≥2) on liver biopsy (vs <2, Coef 73, 95%CI 2-145, p=0.045) was associated with an increase in SSPG over time, whereas Latino race/ethnicity (Coef -66, 95%CI -121 to -10, p=0.021) and the presence of steatosis on liver biopsy (Coef -31, 95%CI -66 to 4, p=0.087) at baseline were associated with a decrease in SSPG over time. On multivariable analysis, when controlling for the presence of IR at baseline, current tobacco use (Coef 55, 95%CI 19-90, p=0.0025) and presence of steatosis (Coef -44, 95%CI -86 to -3, p=0.038) were independently associated with a change in SSPG. Controlling for age or family history of diabetes did not cause a substantial change in the coefficients.

When evaluating the impact of changes in patient characteristics and serologic studies on change in SSPG, increases in AST levels (Coef 2.5 per 10 units/L, 95%CI 0.6-4.5, p=0.01), LDL levels (Coef 6.4 per 10 mg/dL, 95%CI 0.7-12.1, p=0.029), and BMI (Coef 21 per 5 kg/m^2, 95%CI 3-46, p=0.092) from baseline were associated with increases in SSPG over time. When controlling for the presence of IR at baseline, increases in BMI (Coef 30 per 5 kg/m^2, 95%CI 6-53 kg/m^2, p=0.015) and TG levels (Coef 3.5 per 10 mg/dL, 95%CI 0.3-6.7, p=0.034) were associated with an increase in SSPG over time.

DISCUSSION

This study describes the largest cohort of HCV-infected individuals to undergo comprehensive metabolic evaluation including direct measurement of IR to date. In this non-diabetic and non-cirrhotic HCV cohort, there was a wide distribution of insulin sensitivity, and overall, the degree of peripheral IR remained stable on follow-up evaluation. IR was positively associated with Latino race/ethnicity, increasing BMI, and increasing serum ferritin levels and negatively associated with male gender and higher HDL levels. Viral factors including HCV viral load and genotype did not appear to influence IR significantly. Previous studies have demonstrated an association between HCV infection and IR. Experiments have shown that HCV interacts directly with various components of the insulin signaling pathway or its regulatory factors in the liver. In particular, HCV has been reported to reduce insulin-mediated phosphorylation of insulin receptor substrate (IRS)-1 and Akt (23, 24), as well as degrade IRS-1 and IRS-2 by up-regulating suppressor of cytokine signaling 3 (SOCS3).(25) Additional studies have implicated HCV as a mediator of insulin resistance in peripheral tissues (16), with treatment of HCV reducing muscle insulin resistance.(17) Although these experimental data suggest a direct effect of HCV on glucose metabolism, the findings of the current study indicate that host factors play a more prominent role than viral factors in the development of peripheral insulin resistance in the HCV population.

We have shown that similar to the general population, there is a wide spectrum of peripheral insulin sensitivity in the HCV population. Approximately 25% of study subjects had levels of peripheral IR shown to be associated with negative clinical sequelae in the general population. This rate is lower than those observed in studies of HCV-infected non-diabetic patients with similar patient selection criteria that utilized the surrogate measurement of IR,

HOMA-IR, with reported rates ranging from 32 to 54%.(9, 26, 27) The difference in observed rates is likely due to use of low HOMA-IR cut-off values (mainly >2 or >3) to define insulin resistance that have been shown to have high misclassification rates, as well as the limited reliability of this surrogate estimate that can be influenced by degrees of obesity and ethnicity.(12, 13) The three prior studies to date utilizing the hyperinsulinemic-euglycemic clamp did not specifically assess the distribution of insulin resistance in the HCV population and were limited by small sample sizes and a lack of heterogeneity with respect to gender and race/ethnicity.(15, 16, 28)

The complex interplay between host and viral factors underlying the development of IR in HCV is poorly understood. With respect to host factors, Latinos and African Americans are known to be at increased risk for diabetes(29), and these racial/ethnic groups were associated with IR in HCV. In addition, similar to other HCV studies, increasing age, higher BMI, lower HDL levels, and the presence of metabolic syndrome were positively associated with IR.(9, 27) Interestingly, although generally there is a higher prevalence of diabetes among males(30), in this study, female gender was associated with higher IR. It is possible that varying muscle mass and levels of physical activity may account for these gender differences, though these were not assessed. Higher serum ferritin levels also predicted IR. Higher ferritin levels have been shown to correlate positively with IR and the presence of diabetes in patients with HCV.(31-33) The proposed potential pathophysiological mechanisms underlying this finding include interference with insulin inhibition of hepatic gluconeogenesis, defective hepatic uptake/metabolism of insulin with resultant hyperinsulinemia, and iron-induced oxidative stress with resultant hepatic inflammation. (31-33)

Previously reported HCV-related factors associated with IR include HCV viral load, genotype, and higher degrees of steatosis and fibrosis on histologic evaluation of the liver. (9, 27) The viral factors examined here, including HCV duration of infection, viral load, and genotype, did not appear to have much impact on IR, with small estimates that were in the biologically implausible direction. Evidence against any substantial impact, however, was not strong for genotype 1, because the confidence interval extended to a potentially important 19-point increase. Overall, these findings suggest that host factors, rather than viral factors, may play a greater role in the development of IR in this population.

Importantly, this study assessed baseline factors influencing changes in IR over time. Consistent with studies in the general population(34), average insulin sensitivity remained stable over 6 months in this HCV cohort. We found that active tobacco consumption at baseline and increases in BMI and triglyceride levels predicted an increase in IR. On the other hand, the presence of steatosis at baseline was negatively associated with change in IR. It is unclear why steatosis predicted decreases in IR over time, although most patients in this study had mild degrees of steatosis. However, cigarette smoking(35) and increasing BMI(36) are known to increase IR, and the association between triglyceride levels, a component of metabolic syndrome, and IR has been clearly demonstrated in prior studies. (37) Proposed mechanisms for a decrease in insulin sensitivity with cigarette smoking include increases in circulating levels of insulin-antagonistic hormones (such as catecholamines and cortisol) and free fatty acids by induction of lipolysis.(38) Furthermore, nicotine and other metabolites drived from smoking may also play a role in the development of insulin resistance.(38)

This study contributes significantly to our understanding of peripheral IR in HCV, including factors associated with IR and its change on follow-up testing. The main goal of this study was to accurately describe the distribution of IR in HCV, and as such, a control group of HCV-uninfected individuals were not included. Although a larger sample size may provide

additional insight, performing direct measurements of insulin sensitivity is logistically challenging and impractical in larger patient cohorts. In addition, evaluation of the natural history of IR in HCV requires a longer follow-up period, but this may not be feasible given the current availability of effective HCV anti-viral therapy and the challenge of controlling for the confounding effect of worsening liver disease itself on insulin action.

In summary, there was a wide distribution of IR in our HCV population, and approximately 25% of HCV-infected patients were significantly insulin-resistant. Host factors, rather than viral factors, appeared to more greatly influence insulin action and its change in HCV. Therefore, interventions directed towards addressing modifiable risk factors for IR are critical to the prevention of diabetes in this population.

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Abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	body mass index
HCV	hepatitis C virus
HDL	high-density lipoprotein cholesterol
HOMA-IR	homeostasis model assessment of insulin resistance
IR	insulin resistance
IST	insulin suppression test
LDL	low-density lipoprotein cholesterol
OGTT	oral glucose tolerance test
SSPG	steady-state plasma glucose determined by the insulin suppression test
TG	triglyceride

REFERENCES

1. Alter MJ, Kruszon-Moran D, Nainan OV, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med. 1999; 341:556–62. [PubMed: 10451460]
2. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. Med Clin North Am. 2004; 88:787–835, ix. [PubMed: 15308380]
3. Facchini FS, Hua N, Abbasi F, Reaven GM. Insulin resistance as a predictor of age-related diseases. J Clin Endocrinol Metab. 2001; 86:3574–8. [PubMed: 11502781]
4. Yip J, Facchini FS, Reaven GM. Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. J Clin Endocrinol Metab. 1998; 83:2773–6. [PubMed: 9709945]
5. Grasso A, Malfatti F, De Leo P, et al. Insulin resistance predicts rapid virological response in non-diabetic, non-cirrhotic genotype 1 HCV patients treated with peginterferon alpha-2b plus ribavirin. J Hepatol. 2009; 51:984–90. [PubMed: 19695729]
6. Petta S, Camma C, Di Marco V, et al. Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. Am J Gastroenterol. 2008; 103:1136–44. [PubMed: 18477344]

7. Romero-Gomez M, Del Mar Viloria M, Andrade RJ, et al. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology. 2005; 128:636–41. [PubMed: 15765399]
8. Fartoux L, Poujol-Robert A, Guechot J, Wendum D, Poupon R, Serfaty L. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. Gut. 2005; 54:1003–8. [PubMed: 15951550]
9. Moucari R, Asselah T, Cazals-Hatem D, et al. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology. 2008; 134:416–23. [PubMed: 18164296]
10. Hui JM, Sud A, Farrell GC, et al. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. Gastroenterology. 2003; 125:1695–704. [PubMed: 14724822]
11. Khalili M, Lim JW, Bass N, Ascher NL, Roberts JP, Terrault NA. New onset diabetes mellitus after liver transplantation: the critical role of hepatitis C infection. Liver Transpl. 2004; 10:349–55. [PubMed: 15004760]
12. Lam KD, Bacchetti P, Abbasi F, et al. Comparison of surrogate and direct measurement of insulin resistance in chronic hepatitis C virus infection: impact of obesity and ethnicity. Hepatology. 2010; 52:38–46. [PubMed: 20578127]
13. Yeni-Komshian H, Carantoni M, Abbasi F, Reaven GM. Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy nondiabetic volunteers. Diabetes Care. 2000; 23:171–5. [PubMed: 10868826]
14. Greenfield MS, Doberne L, Kraemer F, Tobey T, Reaven G. Assessment of insulin resistance with the insulin suppression test and the euglycemic clamp. Diabetes. 1981; 30:387–92. [PubMed: 7014307]
15. Vanni E, Abate ML, Gentilcore E, et al. Sites and mechanisms of insulin resistance in nonobese, nondiabetic patients with chronic hepatitis C. Hepatology. 2009; 50:697–706. [PubMed: 19582803]
16. Milner KL, van der Poorten D, Trenell M, et al. Chronic hepatitis C is associated with peripheral rather than hepatic insulin resistance. Gastroenterology. 2010; 138:932–41. e1–3. [PubMed: 19962985]
17. Brandman D, Bacchetti P, Ayala CE, Maher JJ, Khalili M. Impact of insulin resistance on HCV treatment response and impact of HCV treatment on insulin sensitivity using direct measurements of insulin action. Diabetes Care. 2012; 35:1090–4. [PubMed: 22399695]
18. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2003; 26(Suppl 1):S5–20. [PubMed: 12502614]
19. Marchesini G, Bianchi GP, Forlani G, et al. Insulin resistance is the main determinant of impaired glucose tolerance in patients with liver cirrhosis. Dig Dis Sci. 1987; 32:1118–24. [PubMed: 3308376]
20. Letiexhe MR, Scheen AJ, Gerard PL, et al. Insulin secretion, clearance, and action on glucose metabolism in cirrhotic patients. J Clin Endocrinol Metab. 1993; 77:1263–8. [PubMed: 8077319]
21. Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. Am J Surg Pathol. 1995; 19:1409–17. [PubMed: 7503362]
22. McLaughlin T, Deng A, Gonzales O, et al. Insulin resistance is associated with a modest increase in inflammation in subcutaneous adipose tissue of moderately obese women. Diabetologia. 2008; 51:2303–8. [PubMed: 18825363]
23. Shintani Y, Fujie H, Miyoshi H, et al. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. Gastroenterology. 2004; 126:840–8. [PubMed: 14988838]
24. Aytug S, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV: a mechanism for increased prevalence of type 2 diabetes. Hepatology. 2003; 38:1384–92. [PubMed: 14647049]
25. Kawaguchi T, Yoshida T, Harada M, et al. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol. 2004; 165:1499–508. [PubMed: 15509521]

26. Imazeki F, Yokosuka O, Fukai K, Kanda T, Kojima H, Saisho H. Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus-infected and hepatitis C virus-cleared patients. Liver Int. 2008; 28:355–62. [PubMed: 18290778]
27. Tsochatzis E, Manolakopoulos S, Papatheodoridis GV, et al. Serum HCV RNA levels and HCV genotype do not affect insulin resistance in nondiabetic patients with chronic hepatitis C: a multicentre study. Aliment Pharmacol Ther. 2009; 30:947–54. [PubMed: 19604179]
28. Yazicioglu G, Isitan F, Altunbas H, et al. Insulin resistance in chronic hepatitis C. Int J Clin Pract. 2004; 58:1020–2. [PubMed: 15605664]
29. Carter JS, Pugh JA, Monterrosa A. Non-insulin-dependent diabetes mellitus in minorities in the United States. Ann Intern Med. 1996; 125:221–32. [PubMed: 8686981]
30. Cowie CC, Rust KF, Byrd-Holt DD, et al. Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health And Nutrition Examination Survey 1999-2002. Diabetes Care. 2006; 29:1263–8. [PubMed: 16732006]
31. Furutani M, Nakashima T, Sumida Y, et al. Insulin resistance/beta-cell function and serum ferritin level in non-diabetic patients with hepatitis C virus infection. Liver Int. 2003; 23:294–9. [PubMed: 12895270]
32. D'Souza RF, Feakins R, Mears L, Sabin CA, Foster GR. Relationship between serum ferritin, hepatic iron staining, diabetes mellitus and fibrosis progression in patients with chronic hepatitis C. Aliment Pharmacol Ther. 2005; 21:519–24. [PubMed: 15740534]
33. Sumida Y, Kanemasa K, Fukumoto K, Yoshida N, Sakai K. Hepatic iron accumulation may be associated with insulin resistance in patients with chronic hepatitis C. Hepatol Res. 2007; 37:932–40. [PubMed: 17854465]
34. Facchini F, Humphreys MH, Jeppesen J, Reaven GM. Measurements of insulin-mediated glucose disposal are stable over time. J Clin Endocrinol Metab. 1999; 84:1567–9. [PubMed: 10323381]
35. Reaven G, Tsao PS. Insulin resistance and compensatory hyperinsulinemia: the key player between cigarette smoking and cardiovascular disease? J Am Coll Cardiol. 2003; 41:1044–7. [PubMed: 12651055]
36. Ryan MC, Fenster Farin HM, Abbasi F, Reaven GM. Comparison of waist circumference versus body mass index in diagnosing metabolic syndrome and identifying apparently healthy subjects at increased risk of cardiovascular disease. Am J Cardiol. 2008; 102:40–6. [PubMed: 18572033]
37. Howard BV, Mayer-Davis EJ, Goff D, et al. Relationships between insulin resistance and lipoproteins in nondiabetic African Americans, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. Metabolism. 1998; 47:1174–9. [PubMed: 9781617]
38. Cena H, Fonte ML, Turconi G. Relationship between smoking and metabolic syndrome. Nutr Rev. 2011; 69:745–53. [PubMed: 22133198]
39. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009; 120:1640–5. [PubMed: 19805654]

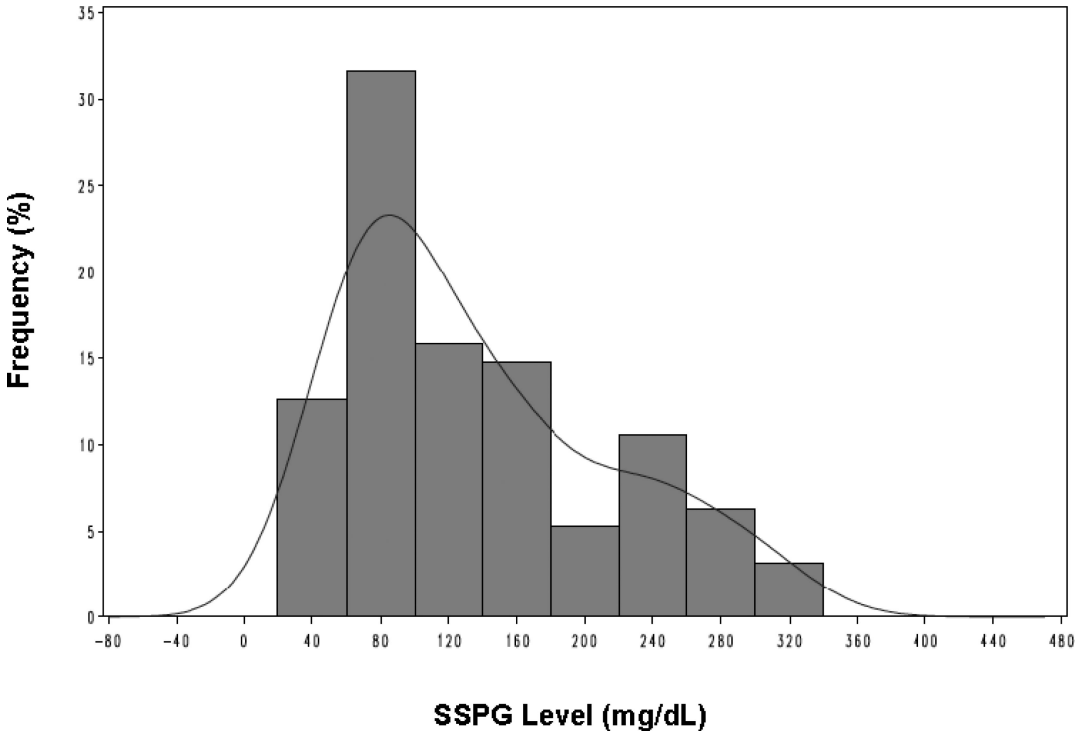

Figure 1. Distribution of Insulin Resistance (SSPG) Among HCV Subjects

Histogram depicts the proportion of subjects at each designated SSPG interval. Twenty-five percent of HCV-infected subjects had a SSPG greater than 180mg/dl.

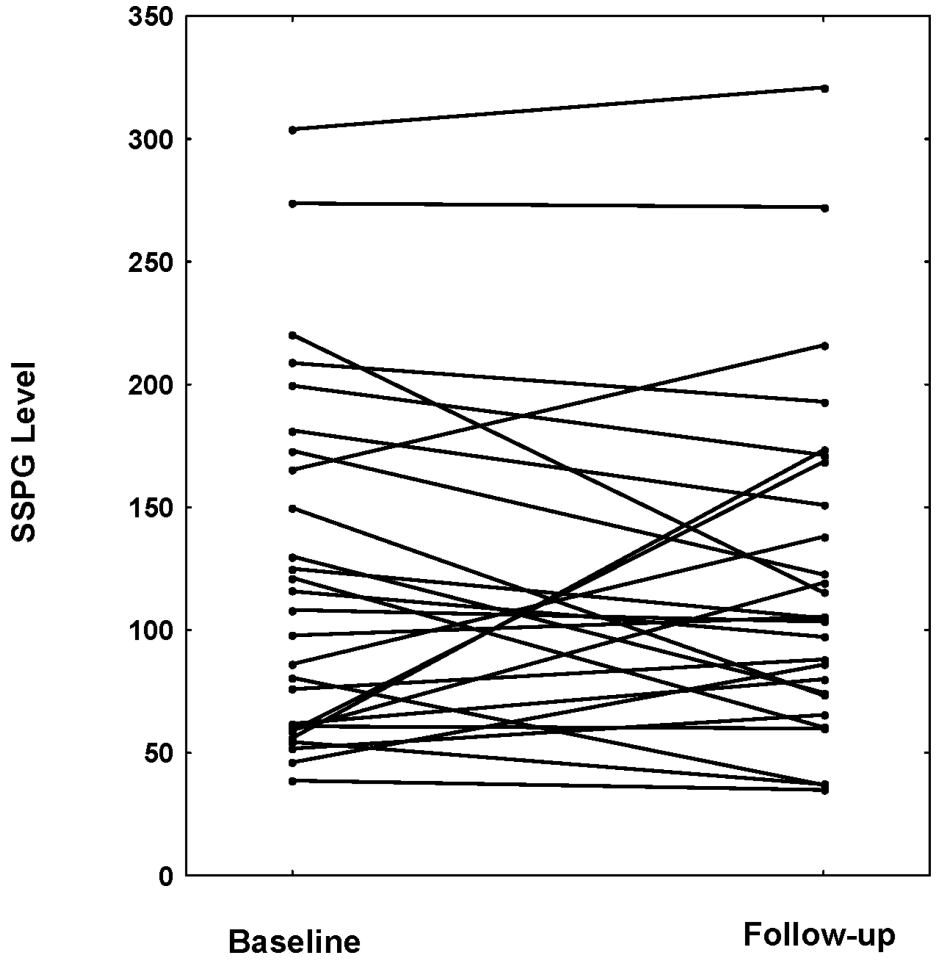

Figure 2. Change in SSPG Levels Over Time

Line plots depict changes in SSPG level over 6 months follow-up for each of 27 HCV-infected subjects.

Table 1

Comparison of Insulin Sensitivity States

Characteristic	Insulin Sensitive (N=71)	Insulin Resistant (N=24)	*P-Value
Age (mean ± SD), years	48 ± 6	47 ± 8	0.97
Male Sex (N, (%))	55 (78)	14 (58)	0.069
Race/Ethnicity (N, (%))			
Caucasian	36 (51)	5 (21)	0.0080
African-American	14 (20)	6 (25)	
Hispanic/Latino	14 (20)	13 (54)	
Other	7 (9)	--	
Education Level, College or Greater (N, (%))	40 (56)	13 (54)	0.22
BMI (mean ± SD), kg/m^2	26 ± 4	29 ± 5	0.11
Waist Circumference (mean ± SD), cm	94 ± 11	98 ± 13	0.21
Current Tobacco Use (N, (%))	45 (63)	11 (46)	0.13
Tobacco Use (mean ± SD), pack-years (median (min-max)), pack-years	18 ± 15 16 (0-74)	12 ± 14 6 (0-50)	0.028
Current Alcohol Consumption (N, (%))	20 (28)	4 (17)	0.26
Average Past Daily Alcohol Consumption (N, (%))			
< 20g	16 (23)	7 (29)	0.52
≥ 20-49g	20 (28)	6 (25)	
≥ 50g	35 (49)	11 (46)	
Duration of Alcohol Consumption (mean ± SD), years	28 ± 10	26 ± 14	0.69
Family History of Diabetes (N, (%))	29 (41)	14 (58)	0.14
History of IVDU	56 (79)	16 (67)	0.65
HCV Viral Load (mean ± SD), log_{10} IU/mL	5.9 ± 0.6	5.6 ± 1.0	0.27
HCV Genotype (N, (%))			
Genotype 1	52 (73)	13 (54)	0.34
Genotype 2	11 (16)	4 (17)	
Genotype 3	8 (11)	5 (21)	
Indeterminate	0 (0)	2 (8)	
Duration of HCV Infection (mean ± SD), years	26 ± 9	24 ± 12	0.49
Liver Biopsy Findings (N)	60	17	
Inflammation Grade ≥ 2 (N, (%))	35 (58)	13 (76)	0.086
Fibrosis Score ≥ 2 (N, (%))	24 (40)	10 (59)	0.58

Characteristic	Insulin Sensitive (N=71)	Insulin Resistant (N=24)	*P-Value
Steatosis Present (N, (%))	18 (30)	9 (53)	0.080
Steatosis Grade >1 (N, (%))	2 (3)	1 (4)	0.22
ALT (mean ± SD), units/L	98 ± 93	84 ± 53	0.93
AST (mean ± SD), units/L	73 ± 58	60 ± 29	0.74
Ferritin (mean ± SD), ng/mL	172 ± 155	170 ± 143	0.86
Total cholesterol (mean ± SD), mg/dL	176 ± 42	170 ± 39	0.57
LDL (mean ± SD), mg/dL	103 ± 36	102 ± 32	0.87
HDL (mean ± SD), mg/dL	52 ± 11	46 ± 15	0.023
Triglycerides (mean ± SD), mg/dL	104 ± 56	103 ± 43	0.73
Fasting Glucose (mean ± SD), mg/dL	91 ± 9	97 ± 11	0.014
Fasting Insulin (mean ± SD), pIU/mL	13 ± 5	24 ± 11	<.0001
Metabolic Syndrome** (N, (%))	6 (9)	10 (42)	0.0002
SSPG (mean ± SD), mg/dL (min-max), mg/dL	99 ± 39 39-177	248 ± 40 181-328	<.0001

*Statistical significance is at p-value of <0.05 (2-sided).

**The clinical diagnosis of metabolic syndrome was based on the presence of 3 or more criteria, including increased waist circumference, elevated triglycerides, reduced HDL cholesterol, elevated blood pressure, and elevated fasting glucose.(39)

Table 2

Univariate Factors Associated with SSPG

Characteristic	Coefficient	95% CI	*P-Value
Age, per 10 years	-8	-32 to 16	0.50
Male Sex	-24	-60 to 13	0.20
Race/Ethnicity (versus Caucasian)			0.0026
African-American	39	-2 to 80	0.060
Hispanic/Latino	67	32 to 102	0.0002
Other	-21	-78 to 37	0.48
Education Level, College or Greater	-44	-72 to -16	0.0022
BMI, per 5 kg/m^2	31	16 to 47	<0.0001
Waist Circumference	12	5 to 20	0.0021
Current Tobacco Use	-33	-64 to -2	0.038
Amount of Tobacco use, per 10 pack-years	-13	-23 to -3	0.012
Current Alcohol Consumption	-24	-58 to 10	0.17
Average Alcohol Consumption ≥ 50g	-11	-41 to 20	0.49
Duration of Alcohol Consumption, per 10 years	-6	-19 to 7	0.38
Family History of Diabetes	22	-9 to 53	0.17
HCV Viral Load, per 1 $\log_{10}$ IU/mL	-8	-29 to 12	0.42
HCV Genotype (versus Genotype 1)			
Genotype 2	-5	-41 to 32	0.81
Genotype 3	40	-4 to 83	0.072
Duration of HCV Infection, per 10 years	-13	-28 to 2	0.080
Liver Biopsy Findings			
Inflammation Grade ≥ 2	64	-24 to 152	0.15
Fibrosis Score ≥ 2	20	-31 to 70	0.44
Steatosis Present	36	1 to 71	0.042
ALT, per 10 units/L	0.5	-0.9 to 1.8	0.49
AST, per 10 units/L	0.02	-1.78 to 1.82	0.99
Ferritin, per 10 ng/mL	0.7	-0.4 to 1.9	0.20
Total cholesterol, per 10 mg/dL	-1	-4 to 2	0.56

Characteristic	Coefficient	95% CI	*P-Value
LDL, per 10 mg/dL	-1	-5 to 3	0.59
HDL, per 10 mg/dL	-11	-26 to 4	0.15
Triglycerides, per 10 mg/dL	2	-1 to 4	0.17

*Statistical significance is at p-value of <0.05 (2-sided).

Table 3

Multivariate Analysis of Factors Associated with SSPG and Insulin Resistance

SSPG LEVELS				
Characteristic	**Unadjusted Coefficient (95%CI)**	***P-Value**	**†Adjusted Coefficient (95%CI)**	***P-Value**
Male Sex	-47.6 (-79.6 to -15.6)	0.0036	-45.8 (-80.2 to -11.4)	0.010
Latino (vs. Caucasian)	67.0 (37.0 to 96.0)	<0.0001	61.0 (30.0 to 93.0)	0.0002
BMI, per 5 kg/m^2	18.7 (4.9 to 32.0)	0.0077	18.5 (3.1 to 33.9)	0.019
Ferritin, per 10 ng/mL	1.4 (0.2 to 2.5)	0.018	1.3 (0.2 to 2.5)	0.020
HDL, per 10 mg/dL	-16.3 (-27.5 to -5.2)	0.0041	-15.4 (-26.8 to -4.0)	0.0090

INSULIN RESISTANT STATE (SSPG > 180 mg/dL)				
Characteristic	**Unadjusted Odds Ratio (95% CI)**	***P-Value**	**†Adjusted Odds Ratio (95% CI)**	***P-Value**
Male Sex	0.2 (0.04 to 0.7)	0.011	0.2 (0.03 to 0.6)	0.0086
Race/Ethnicity (versus Caucasian)				
African-American	6.1 (1.2 to 30.3)	0.027	6.7 (1.3 to 35.6)	0.025
Hispanic/Latino	11.9 (2.7 to 51.4)	0.001	13.3 (2.8 to 62.4)	0.0011
BMI, per 5 kg/m^2	1.7 (0.9 to 3.2)	0.048	1.7 (0.9 to 3.3)	0.048
HDL, per 10 mg/dL	0.4 (0.2 to 0.7)	0.0029	0.4 (0.2 to 0.7)	0.015

*Statistical significance is at $p < 0.05$.

†Adjusted for age and family history of diabetes.