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Viral clearance is associated with improved insulin resistance in genotype 1 chronic hepatitis C but not genotype 2/3

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Abstract

Objectives—Genotype-specific associations between hepatitis C virus (HCV) and insulin resistance (IR) have been described, but a causal relationship remains unclear. This study investigated the association between a sustained virological response (SVR) and IR after chronic HCV therapy.

Methods—2255 treatment-naïve patients with chronic HCV genotype 1 or 2/3 were enrolled in two phase 3 trials of albinterferon alpha-2b versus pegylated interferon alpha-2a for 48 or 24 weeks, respectively. IR was measured before treatment and 12 weeks after treatment using homeostasis model assessment (HOMA)-IR.

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Competing interests No other potential conflicts of interest relevant to this article were reported.

Ethics approval This study was approved by the institutional review board of each participating site (represented by each of the authors contained in appendix 1) and informed consent was obtained from each patient.

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Results—Paired HOMA-IR measurements were available in 1038 non-diabetic patients (497 with genotype 1; 541 with genotype 2/3). At baseline the prevalence of HOMA-IR >3 was greater in patients with genotype 1 than 2/3 (33% vs 27%; $p=0.048$). There was a significant reduction in the prevalence of IR in patients with genotype 1 achieving SVR (8 10%; $p<0.001$), but not in genotype 1 non-responders or those with genotype 2/3. Multivariate analysis indicated that SVR was associated with a significant reduction in mean HOMA-IR in patients with genotype 1 ($p=0.004$), but not in those with genotype 2/3, which was independent of body mass index, alanine transaminase, γ -glutamyl transpeptidase and lipid level changes.

Conclusions—SVR is associated with a reduction in HOMA-IR in patients with HCV genotype 1 but not in those with genotype 2/3. Genotype 1 may have a direct effect on the development of IR, independent of host metabolic factors, and may be partially reversed by viral eradication.

INTRODUCTION

Approximately 170 million people are chronically infected with hepatitis C virus (HCV) globally.¹ The related complications of cirrhosis, end-stage liver disease and hepatocellular carcinoma have made HCV the most common cause of liver-related mortality. There appears to be an association between chronic HCV and metabolic disease, involving insulin resistance (IR), hepatic steatosis and modulation of lipid-cholesterol biosynthesis. Several studies have described an association between chronic HCV and increased prevalence of IR²⁻¹⁰ and type 2 diabetes mellitus.¹¹⁻¹⁷ IR results in more rapid fibrosis progression in patients with chronic HCV,^{2, 3, 18} increasing the risk of cirrhosis and hepatocellular carcinoma.^{19, 20} In addition, IR has been associated with a reduced rate of sustained virological response (SVR) in response to pegylated interferon (IFN)- α and ribavirin therapy.^{10, 21-25}

In a recent large cross-sectional French study, IR was associated with HCV genotype (Gt) 1/4 and high viral load. Furthermore, HCV Gt 1 has been implicated in the development of both IR and type 2 diabetes.^{3, 12} Studies have also suggested that viral suppression or clearance may be associated with a reduction in IR during and after IFN therapy,^{21, 26, 27} as well as a reduction in the rate of incident glucose abnormalities.^{22, 28, 29} These studies have all been limited by small cohorts, on-treatment assessment of IR (which may be confounded by the effect of IFN) or an inability to adequately adjust for treatment-related weight loss. A causal relationship between HCV and IR has therefore not yet been confirmed.

The aim of the present study was to explore the potential causal role of HCV in the development of IR by evaluating the association between SVR and IR in a large well-characterised cohort of patients with chronic HCV. The relationships between viral clearance and lipid parameters and between baseline IR and hepatic fibrosis stage were also assessed.

PATIENTS AND METHODS

A total of 2255 treatment-naïve patients with chronic HCV Gt 1 or 2/3 were enrolled in two separate phase 3 active-controlled studies of albinterferon alpha-2b versus pegylated IFN α -2a plus ribavirin for 48 weeks (ACHIEVE-1 trial; Gt 1) or 24 weeks (ACHIEVE-2/3; Gt 2/3), respectively.^{30, 31} For this substudy, patients were required to have paired pre- and post-treatment measures of IR. Patients with missing IR data at either baseline or follow-up were excluded (ACHIEVE-1: $n=683$; ACHIEVE-2/3: $n=302$); patients with known diabetes or an elevated fasting blood glucose level (>7 mmol/l) at baseline were also excluded (ACHIEVE-1: $n=99$; ACHIEVE-2/3: $n=52$). All patients had compensated liver disease and no evidence of hepatocellular carcinoma. The study was performed in accordance with the principles of the Declaration of Helsinki.

A detailed clinical and biochemical dataset was collected as part of the parent studies.^{30, 31} Clinical data recorded included age, gender, race and body mass index (BMI). Obesity was defined as BMI ≥ 30 in Caucasians and ≥ 25 in Asians.³² Fasting samples were prospectively collected for measurement of IR and lipids at baseline, weeks 12 and 24, week 48 for ACHIEVE-1 only, and 12 weeks post-treatment. Samples were not collected for IR measurement at 24 weeks post-treatment.

IR was measured in fasting serum samples using homeostasis model assessment-IR (HOMA-IR), calculated as fasting insulin ($\mu\text{U/ml}$) $\times$ fasting glucose (mmol/l)/22.5.³³ A threshold of HOMA-IR >3 was considered for the clinical definition of IR, in line with the definition used by Moucari and colleagues.² HOMA-IR was also assessed as a continuous variable.

Serum HCV RNA was measured using the COBAS Ampliprep/COBAS Taqman HCV Test (Roche Molecular Diagnostics, Pleasanton, California, USA), which has a lower limit of quantitation of 43 IU/ml and a lower limit of detection of 15 IU/ml. HCV Gt was defined using the TRUGENE HCV 5'NC genotyping assay (Bayer HealthCare LLC, Berkeley, California, USA).

Baseline liver biopsies were centrally evaluated for METAVIR fibrosis stage, METAVIR inflammatory activity and hepatic steatosis grade by a single expert histopathologist who was blinded to all clinical and laboratory data. Clinically significant fibrosis was classified as METAVIR stage F2–4.³⁴ Steatosis was graded as the percentage of hepatocytes containing macro-vesicular fat droplets: grade 0, 0–5%; grade 1, 6–30%; grade 2, 31–60%; and grade 3, $\geq 61\%$.

All statistical analyses were performed using SAS V.9.1 (SAS Institute). For descriptive statistics, continuous variables were summarised as median (25th–75th percentile) and categorical variables were described as frequency and percentage. Despite log transformation to approximate normality for skewed data, there remained a small number of significant outliers ($>2\text{SD}$ from the mean). Owing to potential confounders relating to undiagnosed diabetes or non-fasting state, these outliers were excluded from the analysis cohort (ACHIEVE-1: $n=44$; ACHIEVE-2/3: $n=37$). Comparisons between groups for baseline variables were performed using a Wilcoxon test for continuous variables. χ^2 test and Fisher exact test were used for categorical data. The association between viral clearance and IR (HOMA-IR >3) was assessed using the McNemar test to compare frequency before and after treatment. A paired t test was used to compare pre- and post-treatment quantitative HOMA-IR measures. Multivariable linear and logistic regression modelling with backwards selection was used to identify variables independently associated with baseline IR and change in IR post-treatment after adjustment for relevant covariates. Significance was defined at $p<0.05$ for all analyses.

RESULTS

Patient characteristics

The final analysis included 1038 patients (Gt 1: $n=497$, Gt 2: $n=261$, Gt 3: $n=280$). The characteristics of the study population are shown in table 1. More patients with HCV Gt 1 were Caucasian (91% vs 51%; $p<0.001$) (ACHIEVE-1 was limited to sites in North America, Europe and Australia whereas ACHIEVE-2/3 included sites in south-east Asia). Baseline BMI was higher in patients with HCV Gt 1. Fasting total cholesterol levels were lower and the frequency of hepatic steatosis ($>5\%$ hepatocytes) was higher in patients with Gt 2/3, consistent with the well-described association between Gt 3, lipid metabolism and

hepatic steatosis. No differences were found in METAVIR stage or grade according to Gt. SVR was more common in patients with Gt 2/3 than Gt 1 (84% vs 60%; $p<0.001$).

Insulin resistance

IR, defined by HOMA-IR >3 , was present in 308 patients (30%). Patients with IR were mostly older Caucasian men with an elevated BMI (table 2A). IR was more common in HCV Gt 1 than Gt 2/3 (33% vs 27%; $p=0.048$, table 1). Other factors associated with IR in a univariable analysis included elevated alanine transaminase (ALT), serum triglyceride levels, presence of hepatic steatosis, moderate to severe hepatic inflammation and significant hepatic fibrosis (F2–4). There was a modest association between IR and serum HCV RNA level $>600\,000$ IU/ml ($p=0.08$). Logistic regression modelling identified Caucasian race, obesity, elevated serum ALT and triglyceride levels, hepatic steatosis and F2–4 hepatic fibrosis to be significantly and independently associated with IR (table 2B). After adjustment for other variables, Gt was not an independent predictor of HOMA-IR >3 in this model.

HOMA-IR was also considered as a continuous variable. HCV Gt was associated with a difference in HOMA-IR levels on univariable analysis (median 2.3 (range 1.4–3.5) vs 1.9 (1.3–3.2), $p=0.006$, table 1). A weak correlation was also noted between serum HCV-RNA and HOMA-IR ($R^2=0.07$, $p=0.02$). In a multivariable linear regression model, Gt remained independently associated with HOMA-IR although the statistical significance was borderline ($p=0.04$, see material 1 in online supplement). HCV RNA level was removed by backward selection and was not independently associated with HOMA-IR after adjustment for relevant covariables. The major contributor to HOMA-IR was BMI (estimated by pseudo- R^2 values in the linear regression model: BMI=0.115; Gt=0.003), as observed in the logistic regression model (table 2A).

There was no difference in the prevalence of IR (HOMA-IR >3) or median HOMA-IR score between patients with Gt 2 HCV and those with Gt 3 HCV. The analysis of the effect of viral clearance on IR therefore compared Gt 1 with Gt 2/3 HCV.

Impact of sustained virological response on IR

For analysis of the potential impact of viral clearance on IR, the ACHIEVE-1 and ACHIEVE-2/3 cohorts were considered separately due to the differential association between SVR and IR across HCV Gt (interaction $p=0.02$). First, it was considered whether viral clearance was associated with a reduction in the number of patients with HOMA-IR >3 . In the setting of Gt 1, SVR ($n=300$) was associated with a reduction from baseline in the prevalence of IR from 29% to 19% ($p<0.001$). In contrast, no significant change in IR was detected in Gt 1 non-responders ($n=197$; figure 1). Virological response did not affect the prevalence of IR in patients with Gt 2/3 when considered as a group, nor when Gt 2 and 3 were considered separately.

To investigate the relationship between viral clearance and change in HOMA-IR, HOMA-IR was considered as a continuous variable. In patients with HCV Gt 1, SVR was associated with a significant reduction in mean HOMA-IR 12 weeks after treatment ($p=0.004$; figure 1). In contrast, there was no reduction in mean HOMA-IR for Gt 1 non-responders ($p=0.14$). Mean HOMA-IR did not change in patients with Gt 2/3 regardless of virological response. No association between SVR and IR was observed when patients with Gt 2 and Gt 3 were considered separately (data not shown).

Many patients treated with IFN-based therapy report weight loss, potentially confounding analyses relating to metabolic factors. The change in HOMA-IR (post-therapy – pre-therapy) was therefore modelled considering the following predictors: SVR and changes in

BMI, ALT, γ -glutamyl transpeptidase (GGT) and fasting lipids (total cholesterol and triglycerides). Treatment regimen (peginterferon vs albinterferon) was not associated with change in IR. Histology could not be included in this analysis as post-treatment liver biopsies were not performed. In the HCV Gt 1 cohort, HOMA-IR reduction was independently associated with SVR as well as reductions in BMI, serum GGT and serum triglyceride levels (table 3). In contrast, the only factors independently associated with HOMA-IR reduction in Gt 2/3 HCV were reduced BMI (β estimate = -0.0129; $p=0.006$) and reduced triglyceride levels (β estimate = -0.0656; $p<0.001$). Similarly, when we considered the patients with HOMA-IR score >3 at baseline (Gt 1, $n=162$; Gt 2/3, $n=146$), normalisation of HOMA-IR (<3) was independently associated with SVR in patients with Gt 1 but not in those with Gt 2/3 (see table 2 in online supplement).

Impact of viral clearance on serum lipid levels

In patients with HCV Gt 1 and 2/3, SVR was associated with a significant increase in both total cholesterol and triglyceride levels which was independent of change in BMI (figure 2). This increase from baseline was most pronounced in patients with Gt 3 (mean pretreatment vs 12-week post-treatment cholesterol: 3.90 vs 4.83 mmol/l; $p<0.001$).

IR and liver fibrosis

Significant hepatic fibrosis (METAVIR F2–4) was present in 173 patients (17%). In univariable analyses, patients with significant fibrosis were more likely to be male, older and obese (data not shown). Significant fibrosis was also associated with IR, elevated serum ALT, lower serum total cholesterol levels, hepatic steatosis and moderate to severe hepatic inflammation (METAVIR activity A2–3). By logistic regression, significant fibrosis was independently associated with age >40 years, HOMA-IR >3 and moderate to severe necroinflammation (table 4). Hepatic steatosis was also independently associated with fibrosis, but the strength of this relationship differed according to Gt, being stronger with Gt 1 and 2 than Gt 3 (interaction $p<0.001$). Significant fibrosis was negatively associated with elevated cholesterol levels.

DISCUSSION

In this well-characterised clinical trial cohort of patients with chronic HCV, SVR was independently associated with a reduction in IR in patients with HCV Gt 1 but not Gt 2/3. To date, this is the largest study to examine the Gt-specific effects of virological response on IR, and the results suggest a causal relationship between Gt 1 and IR. In addition, the results confirmed a viral-specific effect on lipid levels in patients with HCV Gt 3.

The prevalence of IR (HOMA-IR >3) in these non-diabetic patients with chronic HCV was higher with Gt 1 HCV than with Gt 2/3. Although neither Gt nor HCV viral load were observed to be independently associated with HOMA-IR >3 , Gt 1 HCV was independently associated with higher HOMA-IR level when considered as a continuous variable ($p=0.04$). The discrepancy between the logistic and linear regression models might be explained by the differential power to detect small effect sizes, where the power of the linear model is greater. We note that the contribution of viral Gt to the final linear regression model was not great, however, being responsible for only 3% of the variability in HOMA-IR. BMI was the major contributor to HOMA-IR level in both models.

More convincingly, in Gt 1 HCV, viral clearance was associated with a decreased frequency of IR as well as a reduction in quantitative HOMA-IR. Linear regression modelling confirmed that the association between IR and SVR was independent of change in BMI. The

results therefore suggest a causal relationship between Gt 1 and IR that was reversed with successful antiviral therapy.

Several small studies have previously described a relationship between viral suppression or clearance and improvement in IR in patients with chronic HCV. Romero-Gomez and colleagues²¹ observed a decline in HOMA-IR in 25 sustained responders to IFN-based therapy. Kawaguchi and colleagues²⁶ reported reduced HOMA-IR values in 29 responders to antiviral therapy; hepatic expression of insulin receptor substrate (IRS)-1/2 increased in 14 of these patients who had paired liver biopsies pre- and post-treatment. Most recently, investigators from the HALT-C study reported that on-treatment virological suppression correlated with a reduction in HOMA-IR at week 24 in 96 patients.²⁷ These studies were limited by small sample size, failure to adjust for treatment-related BMI and absence of post-treatment samples. The present study extends these observations to a much larger cohort, allowing adjustment for important metabolic covariates, and confirms that HCV-mediated IR appears to be HCV Gt-specific.

The pathogenesis of HCV-mediated IR remains poorly understood. Experimental models have implicated HCV core and NS5a proteins in direct viral interference with the insulin signalling pathway. The HCV core protein has been associated with decreased expression of IRS-1/2 in vitro by increasing levels of suppressor of cytokine signaling-3, which then promotes proteasomal degradation of IRS-1/2.^{7, 35-37} The HCV core has also been shown to inhibit post-receptor insulin signalling through an interaction with P28 γ , an inducer of late proteasomal activity which is essential for the development of IR in HCV core transgenic mice.³⁶ The HCV NS5a protein upregulates protein phosphatase 2A, which subsequently dephosphorylates and inactivates Akt to promote IR in vitro.^{37, 38} There are few data on Gt-specific effects on IR, but experimental proof of concept has recently been established. Overexpression of Gt 1b and 3a core proteins has been shown to have differential effects on insulin signalling pathways in the Huh7 hepatoma cell line.³⁵ Cells expressing Gt 1b core displayed increased phosphorylation of IRS-1 at inhibitory serine residues (636/639), as well as activating the mammalian target of rapamycin (mTOR), resulting in reduced IRS-1 signalling. In contrast, Gt 3a core promoted IRS-1 degradation and upregulated the suppressor of cytokine signal 7 (SOCS-7). Alternatively, the association between HCV and IR may arise as a non-specific consequence of intrahepatic inflammation. Interleukin-6 and tumour necrosis factor α are proinflammatory cytokines upregulated in chronic HCV that have been associated with IR. Further detailed translational studies of IR in patients with chronic HCV will be needed to resolve these issues.

The data have clinical implications. All patients with Gt 1 HCV should be screened for the presence of IR. The risk of progression to type 2 diabetes mellitus is increased in patients with chronic HCV infection. Hypoglycaemic therapy may be required to prevent the systemic complications of diabetes. Diabetes has also been associated with an increased risk of cirrhosis and hepatocellular carcinoma in patients with CHC. Improvement of IR with Gt 1 HCV eradication may be an additional benefit of antiviral therapy, potentially reducing the risk of incident glucose abnormalities and related metabolic morbidity²² as well as contributing to long-term improvement of liver-related morbidity and mortality. Finally, recent data suggest that IR reduces IFN responsiveness^{10, 21}; whether this phenomenon is Gt-specific or whether there is likely to be a long-term benefit from insulin-sensitising therapy as part of the antiviral regimen remains unclear.^{25, 39}

In conclusion, this study found that successful eradication of HCV Gt 1 was associated with reduced IR, suggesting a direct viral aetiology. This relationship was not observed in patients with Gt 2/3. The molecular mechanisms underlying this Gt-specific host/virus relationship require further exploration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance of this study

What is already known about this subject?

- ▶ Insulin resistance (IR) has been associated with progressive fibrosis, cirrhosis and hepatocellular carcinoma in patients with chronic hepatitis C virus infection (CHC).
- ▶ CHC has been associated with IR, with cross-sectional data suggesting that this association may be HCV genotype-specific.
- ▶ The impact of HCV clearance on IR in vivo has not been defined, and therefore a causal relationship between HCV infection and IR has not been confirmed.

What are the new findings?

- ▶ This is the largest study of the metabolic complications of CHC (n=2255).
- ▶ IR was more common in patients with genotype 1 HCV than in those with genotype 2/3 HCV.
- ▶ Viral eradication was associated with a reduction in IR in patients infected with genotype 1 but not in those with genotype 2/3 HCV, suggesting a causal relationship between genotype 1 HCV and IR in vivo.

How might it impact on clinical practice in the foreseeable future?

- ▶ Patients with genotype 1 CHC should be tested for IR.
- ▶ Improvement of IR with eradication of genotype 1 HCV may be an additional benefit of antiviral therapy, contributing to long-term improvements of liver-related morbidity and mortality and reducing the risk of incident diabetes and related morbidity.

Figure 1.

(A) Sustained virologic response (SVR) was associated with reduction in prevalence of insulin resistance (homeostasis model assessment-insulin resistance (HOMA-IR) >3) in patients with hepatitis C virus (HCV) genotype (Gt) 1 before treatment vs 12 weeks after treatment; p values calculated using the McNemar test. (B) Quantitative reduction in HOMA-IR in patients with Gt 1 was observed before treatment vs 12 weeks after treatment. Data presented as mean±SE; p values calculated using paired t test. (C) The prevalence of IR (HOMA-IR) did not change with viral clearance in patients with Gt 2/3. (D) No significant change in HOMA-IR was observed after treatment in patients with Gt 2/3.

Figure 2.

Mean fasting total cholesterol levels before treatment vs 12 weeks after treatment in patients with (A) hepatitis C virus (HCV) genotype (Gt) 1 and (B) Gt 2/3. Mean fasting triglyceride levels before treatment vs 12 weeks after treatment in patients with (C) Gt 1 and (D) Gt 2/3. Data presented as mean $\pm$ SE; p values calculated using paired t test.

Table 1

Baseline characteristics of study patients

Characteristic	Overall (N=1038)	Gt 1 (n=497)	Gt 2 (n=261)	Gt 3 (n=280)	p Value (Gt 1 vs 2/3)	p Value (Gt 2 vs 3)
Male sex, n (%)	569 (55)	259 (52)	131 (50)	179 (58)	0.09	0.001
Median (25th–75th centile) age (years)	46 (35–53)	47 (34–53)	50 (43–57)	40 (32–47)	0.74	<0.001
Age > 40 years, n (%)	674 (65)	328 (66)	209 (80)	137 (49)	0.49	<0.001
Ethnicity, n (%)						
Caucasian	727 (70)	450 (91)	87 (33)	190 (68)	<0.001	<0.001
Asian	254 (24)	13 (3)	157 (60)	84 (30)		
Other	57 (6)	34 (7)	17 (7)	6 (2)		
Median (25th–75th centile) BMI, kg/m ²	25 (23–28.1)	26 (23–29)	25 (22–28)	25 (22–28)	<0.001	0.29
BMI ≥ 30 kg/m ² , n (%)	190 (18)	106 (21)	36 (14)	48 (17)	0.02	0.28
Median (25th–75th centile) HOMA-IR	2.0 (1.3–3.4)	2.3 (1.4–3.5)	1.8 (1.3–3)	2 (1.3–3.4)	0.006	0.364
HOMA-IR > 3, n (%)	308 (30)	162 (33)	65 (25)	81 (29)	0.048	0.29
Median (25th–75th centile) HCV RNA, log ₁₀ IU/ml	6.1 (5.5–6.6)	6.0 (5.5–6.5)	6.1 (5.2–6.6)	6.2 (5.4–6.7)	0.06	0.06
HCV RNA > 600 000 IU/ml, n (%)	604 (58)	276 (56)	154 (59)	174 (62)	0.10	0.46
Median (25th–75th centile) ALT, IU/ml	64 (40–105)	58 (39–94)	60 (35–109)	79 (48–122)	<0.001	0.0005
Median (25th–75th centile) GGT, IU/ml	37 (21–66)	41 (23–73)	29 (17–58)	38 (22–62)	<0.001	0.003
Median total cholesterol, mmol/L (25th–75th centile)	4.3 (3.6–4.9)	4.4 (3.8–5.0)	4.5 (3.9–5.1)	3.9 (3.1–4.6)	0.001	<0.001
Median (25th–75th centile) triglycerides, mmol/l	0.98 (0.71–1.25)	0.96 (0.74–1.30)	1.0 (0.8–1.4)	0.8 (0.6–1.1)	0.08	<0.001
Liver histology, n (%) [*]						
Hepatic steatosis (>5%), n (%)	313 (30)	115 (23)	65 (25)	126 (46)	<0.001	<0.001
METAVIR activity (A2–3), n (%)	474 (46)	229 (46)	120 (47)	125 (45)	0.14	0.754
METAVIR stage [*] , n (%)						
F0	386 (38)	206 (42)	77 (30)	103 (37)	0.05	0.38
F1	461 (45)	206 (42)	130 (50)	125 (45)		
F2	72 (7)	32 (7)	20 (8)	20 (7)		
F3	52 (5)	24 (5)	13 (5)	15 (5)		
F4	49 (5)	18 (4)	18 (7)	13 (5)		
METAVIR stage F2–4, n (%)	173 (17)	74 (15)	51 (20)	48 (17)	0.80	0.471
SVR, n (%)	754 (73)	300 (60)	230 (88)	224 (80)	<0.001	0.010

* Liver histology was not available in 18 patients.

ALT, alanine transaminase; BMI, body mass index; GGT, γ -glutamyl transpeptidase; Gt, genotype; HCV, hepatitis C virus; HOMA-IR, homeostasis model assessment-insulin resistance; SVR, sustained virological response.

Table 2

Factors associated with insulin resistance (HOMA-IR >3) at baseline

(A) Univariable analysis			
	HOMA-IR 3	HOMA-IR >3	p Value
n (%)	730 (70)	308 (30)	
Male sex, n (%)	385 (53)	184 (60)	0.04
Age >40 years, n (%)	454 (62)	220 (71)	0.004
Ethnicity, n (%)			
Caucasian	486 (67)	241 (78)	<0.001
Asian	203 (28)	51 (17)	
Other	41 (6)	16 (5)	
Median (25th–75th centile)	24 (22–27)	28 (25–31)	<0.001
BMI, kg/m ²			
BMI ≥ 30 kg/m ² , n (%)	79 (11)	111 (36)	<0.001
HCV Gt, n (%)			
1	335 (46)	162 (53)	0.048
2/3	395 (54)	146 (47)	
Median (25th–75th centile) HCV RNA, log ₁₀ IU/ml	6.1 (5.4–6.5)	6.1 (5.6–6.6)	0.12
HCV RNA > 600 000 IU/ml, n (%)	412 (56)	192 (62)	0.08
Median (25th–75th centile) ALT, IU/ml	59 (38–98)	73 (48–129)	<0.001
Elevated ALT, n (%) *	154 (21)	105 (34)	<0.001
Median (25th–75th centile) total cholesterol, mmol/l	4.26 (3.64–4.99)	4.27 (3.65–4.84)	0.46
Elevated total cholesterol, n (%) *	198 (27)	71 (23)	0.18
Median (25th–75th centile) triglycerides, mmol/l	0.91 (0.67–1.18)	1.01 (0.80–1.44)	<0.001
Elevated triglycerides, n (%) *	154 (21)	107 (35)	<0.001
Liver histology, n (%)			
Hepatic steatosis (>5%)	189 (26)	124 (40)	<0.001
METAVIR activity (A2–3)	313 (43)	161 (52)	0.006
METAVIR stage (F2–4)	100 (14)	73 (24)	<0.001

(B) Multivariable logistic regression analysis

	OR (95% CI)	p Value
Caucasian vs Asian	1.889 (1.297 to 2.751)	<0.001
Obese vs non-obese †	3.835 (2.687 to 5.473)	<0.001
Elevated ALT *	1.721 (1.229 to 2.410)	0.002
Elevated fasting triglycerides *	1.865 (1.356 to 2.567)	<0.001
Hepatic steatosis (>5% vs ≤5%)	1.554 (1.135 to 2.127)	0.006
METAVIR stage (F2–4 vs F0–1)	1.569 (1.074 to 2.293)	0.02

Covariates: male sex, age >40 years, ethnicity (Caucasian/Asian/other), obesity, hepatitis C virus (HCV) genotype (Gt) 1 vs 2/3, elevated alanine transaminase (ALT), elevated γ -glutamyl transpeptidase (GGT), elevated triglycerides, hepatic steatosis (> 5% hepatocytes), METAVIR activity grades A2–3, METAVIR fibrosis grades F2–4.

* Biochemical variables were dichotomised at 75th centile.

[†] Obesity: Asian body mass index (BMI) ≥ 25 , non-Asian BMI ≥ 30 kg/m².

HOMA-IR, homeostasis model assessment-insulin resistance.

Table 3

Factors associated with change in insulin resistance (log-transformed HOMA-IR) in multivariable linear regression analysis

Multivariable analysis	Estimate	SE	p Value
Δ BMI	0.02	<0.01	0.004
Δ GGT	<0.01	<0.01	0.03
Δ triglycerides	0.08	0.02	<0.001
SVR	0.08	0.037	0.007

Covariates: sustained virological response (SVR), Δ body mass index (BMI), Δ alanine transaminase, Δ γ -glutamyl transpeptidase (GGT), Δ cholesterol, Δ triglycerides. Variables not present in the final model were removed by backward selection. Significance level of 0.05 was used for removal from the model. (Δ = delta; SE = standard error).

Table 4

Factors associated with significant hepatic fibrosis (F2–4) in multivariable logistic regression models

(A) Multivariable logistic regression for F2–4 considering predictors as continuous variables*			
Multivariable analysis	B-estimate	SE	p Value
Age (years)	1.50	0.27	<0.001
HOMA-IR >3	0.47	0.19	0.02
Serum total cholesterol	–1.20	0.26	<0.001
METAVIR A2–3	1.02	0.20	<0.001
HCV Gt × hepatic steatosis (>5%)			
Gt 1 vs 3	2.54	0.46	<0.001
Gt 2 vs 3	1.96	0.51	<0.001
(B) Multivariable logistic regression for F2–4 considering predictors as categorical variables[†]			
Multivariable analysis	OR (95% CI)		
Age >40 years	4.50 (2.67 to 7.60)		
HOMA-IR >3	1.60 (1.10 to 2.33)		
Elevated total cholesterol [‡]	0.30 (0.18 to 0.50)		
METAVIR A2–3	2.79 (1.90 to 4.09)		

* (A) Model considers predictors as continuous variables, where possible, including interaction term for hepatitis C virus (HCV) genotype (Gt) and steatosis. Covariates: male gender, age, ethnicity (Caucasian/Asian/other), obesity, homeostasis model assessment-insulin resistance (HOMA-IR) >3, HCV Gt 1 vs 2/3, total cholesterol, hepatic steatosis (>5% hepatocytes), METAVIR activity grades A2–3, METAVIR fibrosis grades F2–4, HCV Gt × hepatic steatosis interaction term. Variables not present in final model were removed by backward selection. Significance level of 0.05 was used for removal from model.

[†] (B) All predictors were dichotomised and considered as categorical.

[‡] Dichotomised at the 75th centile.

CI, confidence interval; OR, odds ratio; SE, standard error.