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Eradicating hepatitis C virus ameliorates insulin resistance without change in adipose depots

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SUMMARY. Chronic hepatitis C (CHC) is associated with lipid-related changes and insulin resistance; the latter predicts response to antiviral therapy, liver disease progression and the risk of diabetes. We sought to determine whether insulin sensitivity improves following CHC viral eradication after antiviral therapy and whether this is accompanied by changes in fat depots or adipokine levels. We compared 8 normoglycaemic men with CHC (genotype 1 or 3) before and at least 6 months post viral eradication and 15 hepatitis C antibody negative controls using an intravenous glucose tolerance test and two-step hyperinsulinaemic–euglycaemic clamp with [6,6-$^2H_2$] glucose to assess peripheral and hepatic insulin sensitivity. Magnetic resonance imaging and spectroscopy quantified abdominal fat compartments, liver and intramyocellular lipid. Peripheral insulin sensitivity improved (glucose infusion rate during high-dose insulin increased from 10.1 ± 1.6 to 12 ± 2.1 mg/kg/min/, $P = 0.025$), with no change in hepatic insulin response following successful viral eradication, without any accompanying change in muscle, liver or abdominal fat depots. There was corresponding improvement in incremental glycaemic response to intravenous glucose (pretreatment: 62.1 ± 8.3 *vs* post-treatment: 56.1 ± 8.5 mM, $P = 0.008$). Insulin sensitivity after viral clearance was comparable to matched controls without CHC. Post therapy, liver enzyme levels decreased but, interestingly, levels of glucagon, fatty acid–binding protein and lipocalin-2 remained elevated. Eradication of the hepatitis C virus improves insulin sensitivity without alteration in fat depots, adipokine or glucagon levels, consistent with a direct link of the virus with insulin resistance.

Keywords: adipokines, chronic hepatitis C, hyperinsulinaemic–euglycaemic clamp, insulin resistance, liver steatosis, magnetic resonance spectroscopy.

INTRODUCTION

Chronic hepatitis C (CHC) infection is associated with insulin resistance, type 2 diabetes, hepatic steatosis and lipid abnormalities, with insulin resistance predicting a poor response to antiviral therapy [1,2] and liver disease progression [3]. An intricate symbiotic relationship exists between the hepatitis C virus and lipid metabolism, which induces insulin resistance by an unexplained mechanism. Recent data by our group [4] and Vanni *et al.* [5] have confirmed the association between CHC and insulin resistance using the gold standard measurement of insulin resistance (the hyperinsulinaemic–euglycaemic clamp). Our data indicated that the insulin resistance is predominantly peripheral (related to muscle) rather than hepatic, is similar in genotype 1 and 3 infection, is not related to hepatic lipid and is associated with viral load and subcutaneous fat. Our data also indicated that insulin secretion is not impaired. Previous studies [6–8] showed an improvement in glucose handling with viral clearance,

Abbreviations: AFABP, adipocyte fatty acid-binding protein; BMI, body mass index; CHC, chronic hepatitis C; GC-MS, gas chromatography–mass spectrometry; GIR, glucose infusion rate; GLP-1, glucagon-like peptide-1; HOMA-IR, homeostasis model assessment of insulin resistance; IMCL, intramyocellular lipid; IVGTT, intravenous glucose tolerance test; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

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using an oral glucose tolerance test, homeostasis model assessment of insulin resistance (HOMA-IR) or very recently, the steady-state plasma glucose (SSPG) technique [9]. However, improvement in insulin resistance with viral clearance has not been studied by the hyperinsulinaemic–euglycaemic clamp. Moreover, it is unclear whether improvements in insulin resistance after viral eradication relate to improvement in liver or muscle insulin response or are related to changes in abdominal, liver or muscle fat depots or adipokine levels. Using a metabolically well-characterized cohort, we aimed to determine whether insulin resistance is diminished following treatment-induced viral clearance and if so, whether this related to improvement in hepatic or peripheral insulin sensitivity and whether the improvement correlated with changes in abdominal, liver or intramyocellular lipid (IMCL); or with reduced levels of glucagon, adipocyte fatty acid-binding protein (AFABP) or lipocalin-2, which are elevated in hepatitis C [4]. Results were compared to an age-, body mass index- (BMI) and gender-matched control (non-hepatitis C) group.

PATIENTS AND METHODS

Participants

We studied subjects both before and after successful eradication of their hepatitis C infection. These subjects were part of a previously published pretherapy study [4], where 29 subjects with CHC were recruited from liver clinics at several hospitals prior to antiviral therapy. Of these 29 subjects, we were able to restudy eight subjects (genotype 1, $n = 2$; genotype 3, $n = 6$) who cleared the virus with antiviral treatment (Fig. 1).

We included subjects with genotype 1 and 3 (despite their differing propensity to generate hepatic lipid) because of their similar insulin sensitivity and the lack of association of hepatic lipid with insulin sensitivity in our previous study [4]. We also studied 15 controls (part of the previous pretherapy study) [4] recruited from the community with normal liver function tests and negative antibody tests for hepatitis C at baseline. Controls were matched by age, ethnicity, gender and BMI to the subjects with CHC.

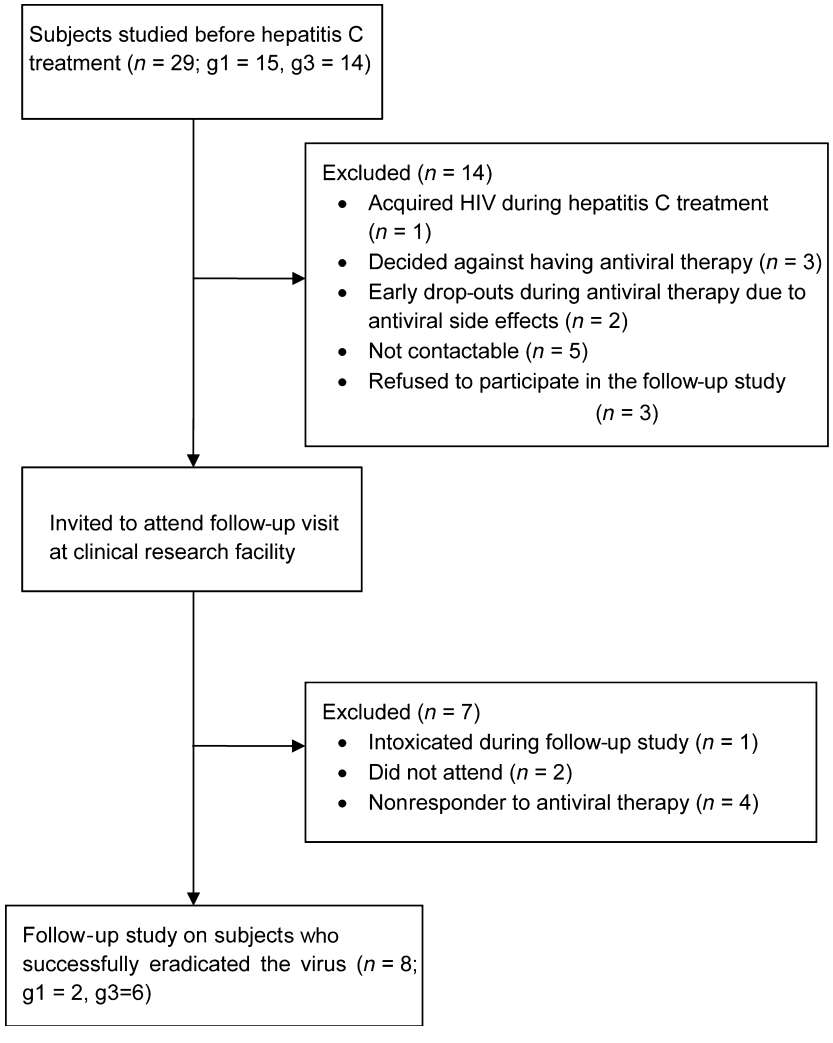

Fig. 1 Flow chart of hepatitis C subjects included in the follow-up study. g1, genotype 1; g3, genotype 3

To reduce possible confounders, all subjects were Caucasian men with a BMI < 30kg/m^2, fasting blood glucose level < 5.5 mM and absent or minimal fibrosis (fibrosis score ≤ F2) on liver biopsy (seven subjects had a liver biopsy) or a Fibrosis Probability Index score <= 0.3 [10] and Forns index < 6.9 [11]. Patients with alcohol consumption > 20 g per day were excluded (assessed by two separate interviews). Other exclusion criteria included concurrent HIV or other causes of liver disease. Chronic hepatitis C subjects with genotype 1 and 3 infections were treated for 12 and 6 months, respectively, with pegylated interferon and ribavirin. Subjects who cleared the virus had no detectable virus at the end of treatment and 6 months later. Subjects were studied at least 6 months after cessation of antiviral therapy to minimize the effects of treatment (especially interferon) and weight loss, directly or indirectly, on insulin resistance [12]. The study was registered at the clinical trials.gov website (NCT-00707603). The protocols were approved by the human research ethics committees of St. Vincent's Hospital, St. George Hospital and Westmead Hospital and conform to the ethical guidelines of the World Medical Association Declaration of Helsinki. All subjects provided written informed consent.

All examinations were undertaken following a 10-h fast with abstinence from exercise, alcohol or the use of addictive drugs for 48 h prior to the study. Routine physical activity (exercise/home duties) was assessed using a standardized validated questionnaire [13].

Magnetic resonance spectroscopy and imaging

All magnetic resonance examinations were undertaken on a 1.5 tesla scanner (General Electric Medical Systems, Milwaukee, WI, USA). Image-localized water-suppressed and water-unsuppressed ^{1}H-magnetic resonance spectra were collected from a 1.5 × 1.5 × 2 cm^2 voxel within the soleus muscle, avoiding blood vessels and fatty tissue. Image-localized ^{1}H-MR spectra of the liver were obtained using a 12 × 14 cm ^{1}H-tuned butterfly coil (General Electric Medical Systems, Milwaukee, WI, USA) placed over the lateral aspect of the abdomen. Spectra were collected from a 2 × 2 × 2cm^2 voxel, avoiding blood vessels, ducts and fat tissue using a modified PRESS pulse sequence (TR = 1500 ms, TE = 125 ms, 12 averages). Interpretation of ^{1}H-magnetic resonance spectra was performed with the java-based magnetic resonance user interface (JMRUI version 2.0) [14] using the AMARES algorithm [15]. Intramyocellular lipid concentrations were calculated as previously described [16]. Hepatic lipid content was calculated as previously described [17] and expressed relative to water content of the liver (ratio of signal from fat (f) to total signal from fat and water (w) (f/(f+w)%). To quantify abdominal adipose tissue, five T1-weighted axial magnetic resonance images (10 mm thick, 2 mm gap) were collected during a breath-hold at the level of the fourth lumbar vertebra. Images were analysed using HIPPO FAT software for the determination of abdominal subcutaneous and visceral adipose tissue areas [18,19].

Following imaging, weight, height and waist circumference (midpoint between lower border of the rib cage and iliac crest) were measured and BMI was calculated as weight (kg) divided by square of the height (m^2). Teflon catheters were placed in the antecubital vein for infusions and into a contralateral dorsal hand vein, heated using a warming device placed over the hand to achieve arterialisation of venous blood, for blood sampling. A small volume of normal saline was infused in both lines to maintain patency.

Intravenous glucose tolerance test (IVGTT)

An IVGTT was performed to assess changes in B-cell function before and after viral clearance, and to compare the subjects post treatment with controls. A bolus of 50% dextrose (300 mg/kg^1, max 24 g) was administered intravenously over 1 minute through an antecubital vein with blood sampling for glucose (YSI 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH) and insulin levels at 1, 3, 5, 7 and 10 min. The acute insulin response was calculated as the ratio of the increment of serum insulin (mU/L) to that of blood glucose (mM) during the 10 min of the IVGTT. The incremental insulin ($AUC_{insulin}$) and glucose ($AUC_{glucose}$) areas under the curve were calculated by the trapezoidal method.

Hyperinsulinaemic–euglycaemic clamp

A 2-h-primed (5 mg/kg body weight) continuous infusion (3 mg/kg/h) of [6,6-^{2}H] glucose (Cambridge Isotope Laboratories, Andover, MA, USA) was begun through an antecubital vein followed by a two-step (15 mU/m^2/min^1 and 80 mU/m^2/min^1) hyperinsulinaemic–euglycaemic clamp as previously described [4]. The plasma glucose level was maintained at 5 mM with a variable-rate infusion of dextrose (25 g/100 ml enriched to approximately 2.5% with di-deuterated glucose) adjusted according to 10-min results. The steady-state glucose infusion rate represented the whole-body glucose disposal rate, an estimate of insulin sensitivity, calculated as a mean at 10-min intervals during the last 40 min of the low-dose and high-dose insulin clamp. On conclusion of the clamp, the subjects received a meal and glucose infusion was continued until blood glucose stabilized. Blood samples were immediately cold-centrifuged at +4 °C. Serum was stored at −80 °C until analyses. Insulin sensitivity was also assessed by HOMA-IR [insulin*glucose/22.5] [20].

Analyses

Commercial radioimmunoassays (LincoResearch, Inc., St. Charles, MO, USA) were used to analyse serum insulin and

glucagon. Lipid and liver function tests were assessed using a conventional automated analyser within the Department of Clinical Chemistry at St. Vincent's Hospital. Gas chromatography–mass spectrometry (GC-MS) (Agilent Technologies 6890 gas chromatograph interfaced to an Agilent 5973 Mass Selective Detector; Agilent Technologies, Ryde, NSW, Australia) analysis of enrichments of [6,6-^{2}H] glucose in plasma and infusates were performed as previously described [4]. Adipocyte fatty acid-binding protein [21] and lipocalin-2 [22] were measured by sandwich ELISA. Hepatitis C viral load was measured by the polymerase chain reaction method (Amplicor HCV; Roche Diagnostics, Branchburg, NJ) and hepatitis C virus (HCV) antibodies (Monolisa anti-HCV; Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) were measured using an enzyme-linked immunoassay. HCV genotype was performed using a second-generation reverse hybridization line probe assay (inno-Lipa HCV II; Immunogenetics, Belgium).

Calculations

Isotopic enrichment determined by GC-MS was corrected for the constant background contribution of naturally occurring [6, 6-^{2}H] glucose, and the values obtained were smoothed by linear regression against time within each measurement period [23]. The rates of endogenous glucose production (Ra) and glucose utilization (Rd) were estimated during the basal and insulin-stimulated 30-min steady-state periods using Steele's one-compartment fixed-volume model [24], as modified by Finegood *et al.* [25] to account for the added infusion of exogenous glucose. In the calculations, the distribution volume of glucose was taken as 20% of body weight and the pool fraction was 0.65. Endogenous glucose output during the clamp was calculated by subtracting the glucose infusion rate from the rate of glucose appearance measured with the isotope tracer method.

Statistical methods

Statistical analysis was carried out using SPSS version 15.0 (SPSS Inc., Chicago, IL). Two-tailed tests with a significance level of 5% were used throughout. Variables exhibiting skewed distribution were log(ln)-transformed to approximate normality prior to analysis. For comparison, before and after treatment, paired t-tests were used for normally distributed variables. For comparison between subjects with CHC and controls, 2-sample Student's t-tests were used for normally distributed variables. Chi-squared or Fisher's exact tests were used to compare the distribution of categorical variables by group. Strength of association between continuous variables was quantified using Spearman's rank correlations. Repeated-measures analysis of variance analysed the relationship between the effects of treatment on variables of interest. Data are expressed as mean ± SD in the text.

RESULTS

Subject characteristics

Eight subjects with CHC were studied before and at least 6 months after therapy. One subject was on methadone at the first visit and was off methadone at the post-treatment visit. His insulin sensitivity improved post viral clearance (from 11 to 12.39 mg/kg/min), even though methadone, as shown in our previous study [4], was an insulin sensitizer (Table 1). Physical activity was unchanged between visits (data not shown).

Glucoregulatory hormones and lipids

There were no differences in fasting insulin levels, insulin levels and $AUC_{insulin}$ post IVGTT in the subjects with CHC before and after therapy. There were no differences in fasting glucose levels; however, $AUC_{glucose}$ post IVGTT was significantly reduced post therapy. Homeostasis model assessment of insulin resistance was unaltered before and after therapy (1.86 ± 0.9 *vs* 1.96 ± 0.5, $P = 0.7$). Fasting glucagon levels were unchanged before and after therapy, remaining elevated compared to controls. Post treatment, there was a nonsignificant increase in cholesterol (4.03 ± 1.04 to 4.84 ± 0.6 mM, $P = 0.064$) and low-density lipoprotein (LDL) levels (2.6 ± 0.7 to 3.3 ± 0.5 mM, $P = 0.054$), while triglyceride levels were unchanged (Table 1).

Fat depots

Subcutaneous abdominal fat volumes were similar before and after therapy, and there was no significant reduction in visceral fat. When the two subjects with genotype 1 were excluded from the liver fat analysis, there was no significant reduction in liver fat in the subjects with genotype 3 ($n = 6$) (liver fat, 12.98 ± 12.6 to 7.4 ± 2.2 F/(F+H2O)%, $P = 0.7$), which was unchanged when subjects with genotype 1 were included. Liver or intramyocellular lipid was unchanged before and after therapy (Table 1).

Hyperinsulinaemic–euglycaemic clamp

Basal rates of endogenous glucose production were unchanged in subjects before and after therapy (1.6 ± 0.2 *vs* 1.8 ± 0.3 mg/kg/min, $P = 0.12$) and suppressed during low dose insulin by 70% before treatment and 80% post treatment (to 0.6 ± 0.6 *vs* 0.3 ± 0.4 mg/kg/min, $P = 0.3$) (Fig. 2). At high-dose insulin, hepatic glucose production was completely suppressed.

Insulin levels increased during the clamp before and after therapy to 33.8 ± 5.9 and 34.9 ± 7.9 mU/L ($P = 0.8$) at low dose and to 252 ± 95 and

Table 1 Subject characteristics before and after therapy

	Before (n = 8)	After (n = 8)	$P^{\S}$	Controls (n = 15)	$P^{\P}$
Age (y)	40.1 (8.5)	41.5 (8.5)	<0.001	37.40 (6.6)	NS
BMI (kg/m²)	25 (3.5)	24.5 (2.5)	NS	25.2 (2.6)	NS
Weight (kg)	75.4 (7.5)	73.9 (4.8)	NS	80 (8.7)	0.08
Waist (cm)	89 (7.7)	88.3 (3.8)	NS	89.5 (7.1)	NS
Subcut fat volume (kg)*,†	6.8 (3)	6.3 (1.5)	NS	6.7 (2.7)†	NS
Visceral fat volume (kg)*,†	4.2 (2.2)	3.3 (1.9)	NS	3.3 (1.8)†	NS
IMCL (mM/kg wet wt)*	2.04 (0.6)	2.7 (1.2)	NS	2.1 (0.6)	NS
Liver fat (F/(F+H2O)%)*	10.6 (11.6)	11.6 (8.8)	NS	4.5 (2.1)	0.001
Smokers (n,%)	6 (75)	5 (62.5)	NS	1 (7)	0.009
Methadone users (n,%)	1 (12.5)	0 (0)	NS	0 (0)	NS
Alcohol intake (g/week)	28.9 (49.2)	22.5 (49.5)	NS	49	NS
Fasting glucose (mM)	4.43 (0.3)	4.37 (0.41)	NS	4.56 (0.3)	NS
Fasting insulin (mU/L)*	9.3 (4.02)	10.4 (3)	NS	8.3 (2.7)	NS
HOMA-IR*	1.86 (0.89)	1.96 (0.49)	NS	1.7 (0.6)	NS
$AUC_{glucose}$ IVGTT (mM)	62.1 (8.3)	56.1 (8.5)	.008	56.7 (7.2)	NS
$AUC_{insulin}$ IVGTT (mU/L)	374.1 (198.5)	450.1 (232.9)	NS	388.4 (196)	NS
FH T2DM (n,%)	3 (37.5)	3 (37.5)	NS	8 (53.3)	NS
Glucagon (ng/mL)*,†	78.8 (28)	77.3 (14.4)	NS	52.8 (12)	0.002
Cholesterol (mM)†	4.03 (1.04)	4.84 (0.61)	0.064	4.8 (1)	NS
LDL (mM)†	2.6 (0.7)	3.3 (0.5)	0.054	3.0 (0.9)	NS
Triglycerides (mM)*,†	0.9 (0.3)	0.9 (0.2)	NS	0.97 (0.5)	NS
ALT (U/L)*,†	102.1 (65.8)	13.9 (4.6)	<0.001	20.7 (5.5)	0.002
Lipocalin-2 (ng/mL)*	41.66 (11.87)	41.12 (13.3)	NS	29.16 (5.8)	0.009
AFABP (ng/mL)*	23.16 (11.98)	28.88 (11.8)	NS	16.2 (9.2)	0.02
HCV RNA Log_{10} (IU/mL)	6.28 (6.5)	undetectable			

NS, not significant; BMI, body mass index; WHR, waist/hip ratio; HOMA-IR, homeostasis model assessment of insulin resistance; ALT, alanine aminotransferase; subcut, subcutaneous; FH T2DM, family history of type 2 diabetes; AFABP, adipocyte fatty acid–binding protein; IMCL, intramyocellular fat; AUC_{gluc}IVGTT, incremental glucose area under the curve during the intravenous glucose tolerance test; $AUC_{insulin}$IVGTT, incremental insulin area under the curve during the intravenous glucose tolerance test. Results expressed as mean (SD) or frequency (percentage). *These have been log(ln)-transformed prior to analysis (skewed distribution); †Data missing in one subject; §P value between hepatitis C subjects before and after therapy; ¶P value between hepatitis C subjects post treatment and controls.

229 ± 37.8 mU/L (P = 0.6) at high dose, respectively. Glucose infusion rate [GIR] at low-dose insulin improved nonsignificantly before and after therapy from 3.1 ± 1.4 to 4.7 ± 1.6 mg/kg/min (P = 0.065) and post-treatment levels were similar to controls (3.9 ± 1.2 mg/kg/min, P = 0.16). GIR at high-dose insulin improved from 10.1 ± 1.6 to 12 ± 2.1 mg/kg/min, P = 0.025 (Fig. 3) and post-treatment levels were similar to the control group (12.8 ± 2.8 mg/kg/min P = 0.5). When subjects with genotype 1 were excluded, GIR at high-dose insulin in subjects with genotype 3 still showed a significant improvement post therapy (10.2 ± 1.2 to 12.4 ± 2.3 mg/kg/min, P = 0.02).

Adipokines

AFABP and lipocalin-2 levels did not change post viral clearance and remained significantly higher in the subjects with CHC compared to the controls (Table 1).

DISCUSSION

This study demonstrates that chronic hepatitis C infection (and the virus itself) is intricately associated with insulin resistance. Once the virus is eradicated, insulin sensitivity improves and is no different to that in matched controls. The improvement in insulin sensitivity was not accompanied by alterations in weight or in abdominal or intramyocellular fat depots. Subjects with genotype 3 considered alone also had improved insulin resistance post treatment consistent with our previous data [4] that the hepatitis C virus, irrespective of genotype, causes insulin resistance. The subjects affected by CHC in this cohort were slightly less insulin resistant than our initial cohort [4] (this is expected as insulin resistance is a predictor of poor response to antiviral therapy) but they were still more insulin resistant than controls. Subjects were studied at least 6 months following viral eradication, as pegylated interferon has been shown to alter insulin sensitivity both

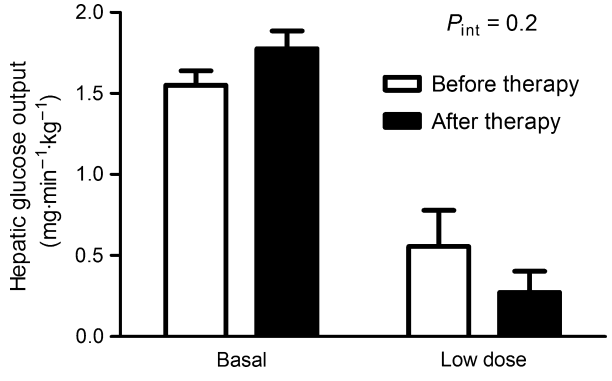

Fig. 2 Mean (± SE) hepatic glucose output in hepatitis C subjects before and after antiviral therapy. Hepatic glucose output (HGO) at high-dose insulin not shown as this was not significantly different from zero in all groups. Change in HGO (Δ HGO) suppression not significant before and after therapy ($P = 0.53$)

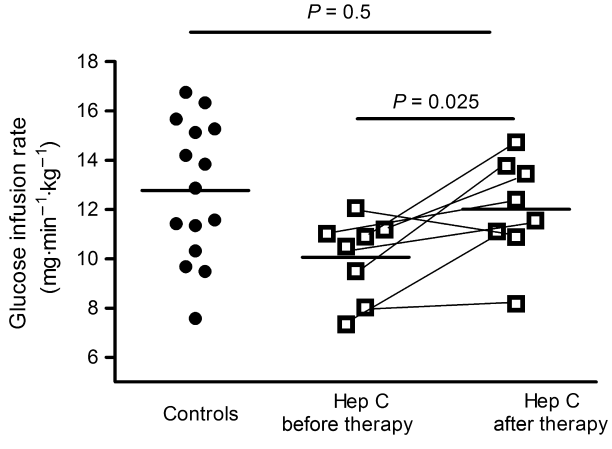

Fig. 3 Glucose infusion rates (during high-dose hyperinsulinaemic–euglycaemic clamp) both before and after antiviral therapy.

in vivo and *in vitro* [12,26]. Further, treatment typically induces weight loss and reduced food intake, both of which can alter insulin sensitivity. As expected, weight returned to baseline levels 6 months following the therapy [27].

This study was not designed to assess the molecular mechanisms of insulin resistance in CHC; however, this is the only study that examined liver, muscle, abdominal fat depots, adipokines and glucoregulatory hormones before and after viral clearance. It further demonstrates improved insulin sensitivity in the periphery (muscle and fat) with no significant difference in basal and insulin-suppressed hepatic glucose output between subjects before and after treatment at low-dose insulin. This is consistent with previous evidence [4] that the insulin resistance of CHC is predominantly in the periphery. The nonsignificant improvement in insulin sensitivity at low-dose insulin can be attributed to the higher variability of glucose turnover at lower insulin doses. Surprisingly, in subjects with genotype 3, there was no reduction in liver fat with viral clearance; this may relate to small subject numbers and lower prevalence of hepatic steatosis in this cohort at baseline. However, this finding agrees with our previous report of lack of association between liver fat and insulin sensitivity in CHC. Low-density lipoprotein levels increased post treatment, as previously shown [28], consistent with an interaction between the virus and lipid pathways. The virus has been postulated to reduce the activity of microsomal triglyceride transfer protein [29], more so in genotype 3 [30], resulting in low apolipoprotein B levels [31] and decreased export of very-low-density lipoprotein (VLDL) (precursor of LDL) from hepatocytes with intracellular accumulation of lipids. This may explain the hepatic steatosis and low LDL levels, found particularly with genotype 3. Interestingly, elevated LDL levels predict a sustained virological response to antiviral therapy [32] and are associated with the IFN-lambda variant encoding IL28 [33], which predicts response to interferon [34].

Adipocytokines have been implicated in insulin resistance and inflammation in obesity. We are unaware of previous studies besides ours [4] examining the relationship between AFABP and lipocalin-2 to insulin resistance in CHC. AFABP (expressed in adipocytes and macrophages associated with insulin resistance in nonalcoholic fatty liver disease) [35] and lipocalin-2 (expressed in adipose tissue, liver and multiple other organs) [22] were elevated in the subjects with CHC compared to controls; however, there was no alteration in levels post viral clearance and improvements in insulin sensitivity. Thus AFABP and lipocalin-2 may not be involved in the insulin resistance of CHC. Lipocalin-2 levels are higher in smokers compared to nonsmokers [22] so this may reflect the higher levels found in the hepatitis C subjects compared to controls. Although adiponectin may play a role in obesity-related insulin resistance, we have not previously shown a difference in levels between subjects with CHC and controls [4] or an association with insulin resistance in CHC [36], so we have not measured this adipokine post therapy.

Most previous studies have used surrogate measures of insulin resistance when assessing changes in insulin sensitivity following viral clearance. HOMA-IR is imprecise, even in subjects without CHC, with significant within-patient and between-patients variability [20,37]. In our study, as previously reported [8], HOMA-IR was unchanged before and after therapy; this may reflect the fact that HOMA-IR largely indicates hepatic insulin resistance, whereas the insulin resistance in CHC is predominantly peripheral. Further, in CHC, HOMA-IR has limitations in nondiabetic populations, especially in nonobese patients [4,38]. One very recent report [9] used the reliable SSPG technique to document an improvement in insulin sensitivity after HCV eradication, but the response

was greatly influenced by BMI changes and interferon therapy and a difference from subjects who did not clear the virus was uncertain; also, SSPG does not allow differentiation between hepatic versus peripheral insulin action.

Interestingly, glucagon levels remained elevated following viral clearance despite improved insulin sensitivity. We are unable to distinguish whether this is related to hypersecretion or reduced clearance and do not have liver histology post treatment to assess the relationship of glucagon levels with degrees of fibrosis although we would not expect improvements in fibrosis in our follow-up study performed only 6 months post viral clearance. Sustained virological response in long-term studies has been associated with slow fibrosis regression and it would be interesting to determine whether glucagon levels normalize over time. Glucagon hypersecretion has been noted in liver cirrhosis [39], but the literature is scanty on milder degrees of liver fibrosis. Another possible cause of elevated glucagon is the reported reduction in glucagon-like peptide-1 (GLP-1) secretion in HCV [40] as GLP-1 suppresses glucagon secretion.

The main limitation of this study is the subject numbers, but despite this, we were able to discern a significant improvement in insulin resistance. It is difficult to recruit this group of subjects for detailed metabolic studies because of patient time, convenience and compliance. This is even more problematic for repeat studies in subjects who have just been through the rigors of antiviral therapy. To date, evaluation of insulin resistance by clamp has been performed in subjects with CHC [4,5], but to our knowledge there is no clamp study assessing improved insulin resistance post viral eradication.

The mechanism for the generation of insulin resistance by the hepatitis C virus remains unclear from this study; yet, this study demonstrates improved metabolic changes accompanying viral eradication. Further studies are needed, particularly with regard to the potential interaction between the virus and adipose tissue in generating insulin resistance, which is principally in muscle [4].

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REFERENCES

1 Poustchi H, Negro F, Hui J *et al.* Insulin resistance and response to therapy in patients infected with chronic hepatitis C virus genotypes 2 and 3. *J Hepatol* 2008; 48: 28–34.
2 Romero-Gomez M, Del MVM, Andrade RJ *et al.* Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; 128: 636–641.
3 Hui JM, Sud A, Farrell GC *et al.* Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. *Gastroenterology* 2003; 125: 1695–1704.
4 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: 932–941 e1-3.
5 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.
6 Kawaguchi T, Ide T, Taniguchi E *et al.* Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2. *Am J Gastroenterol* 2007; 102: 570–576.
7 Conjeevaram HS, Wahed AS, Afdhal N, Howell CD, Everhart JE, Hoofnagle JH. Changes in insulin sensitivity and body weight during and after peginterferon and ribavirin therapy for hepatitis C. *Gastroenterology* 2011; 140: 469–477.
8 Kawaguchi Y, Mizuta T, Oza N *et al.* Eradication of hepatitis C virus by interferon improves whole-body insulin resistance and hyperinsulinaemia in patients with chronic hepatitis C. *Liver Int* 2009; 29: 871–877.
9 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: 1090–1094.
10 Sud A, Hui JM, Farrell GC *et al.* Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. *Hepatology* 2004; 39: 1239–1247.
11 Forns X, Ampurdanes S, Llovet JM *et al.* Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; 36: 986–992.
12 Imano E, Kanda T, Ishigami Y *et al.* Interferon induces insulin resistance in patients with chronic active hepatitis C. *J Hepatol* 1998; 28: 189–193.
13 Kannel WB, Sorlie P. Some health benefits of physical activity. The Framingham Study. *Arch Intern Med* 1979; 139: 857–861.

14 Naressi A, Couturier C, Castang I, de Beer R, Graveron-Demilly D. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. *Comput Biol Med* 2001; 31: 269–286.
15 Vanhamme L, Van Huffel S, Van Hecke P, van Ormondt D. Time-domain quantification of series of biomedical magnetic resonance spectroscopy signals. *J Magn Reson* 1999; 140: 120–130.
16 Szczepaniak LS, Babcock EE, Schick F *et al.* Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. *Am J Physiol* 1999; 276: E977–E989.
17 Szczepaniak LS, Nurenberg P, Leonard D *et al.* Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol* 2005; 288: E462–E468.
18 Positano V, Gastaldelli A, Sironi AM, Santarelli MF, Lombardi M, Landini L. An accurate and robust method for unsupervised assessment of abdominal fat by MRI. *J Magn Reson Imaging* 2004; 20: 684–689.
19 Demerath EW, Ritter KJ, Couch WA *et al.* Validity of a new automated software program for visceral adipose tissue estimation. *Int J Obes (Lond)* 2007; 31: 285–291.
20 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412–419.
21 Xu A, Wang Y, Xu JY *et al.* Adipocyte fatty acid-binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin Chem* 2006; 52: 405–413.
22 Wang Y, Lam KS, Kraegen EW *et al.* Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. *Clin Chem* 2007; 53: 34–41.
23 Finegood DT, Bergman RN. Optimal segments: a method for smoothing tracer data to calculate metabolic fluxes. *Am J Physiol* 1983; 244: E472–E479.
24 Steele R, Wall JS, De Bodo RC, Altszuler N. Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 1956; 187: 15–24.
25 Finegood DT, Bergman RN, Vranic M. Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps. Comparison of unlabeled and labeled exogenous glucose infusates. *Diabetes* 1987; 36: 914–924.
26 Platanias LC, Uddin S, Yetter A, Sun XJ, White MF. The type I interferon receptor mediates tyrosine phosphorylation of insulin receptor substrate 2. *J Biol Chem* 1996; 271: 278–282.
27 Seyam MS, Freshwater DA, O'Donnell K, Mutimer DJ. Weight loss during pegylated interferon and ribavirin treatment of chronic hepatitis C. *J Viral Hepat* 2005; 12: 531–535.
28 Siagris D, Christofidou M, Theocharis GJ *et al.* Serum lipid pattern in chronic hepatitis C: histological and virological correlations. *J Viral Hepat* 2006; 13: 56–61.
29 Perlemuter G, Sabile A, Letteron P *et al.* Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; 16: 185–194.
30 Mirandola S, Realdon S, Iqbal J *et al.* Liver microsomal triglyceride transfer protein is involved in hepatitis C liver steatosis. *Gastroenterology* 2006; 130: 1661–1669.
31 Serfaty L, Andreani T, Giral P, Carbonell N, Chazouilleres O, Poupon R. Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. *J Hepatol* 2001; 34: 428–434.
32 Mawatari H, Yoneda M, Fujita K *et al.* Association between lipoprotein subfraction profile and the response to hepatitis C treatment in Japanese patients with genotype 1b. *J Viral Hepat* 2010; 17: 274–279.
33 Li JH, Lao XQ, Tillmann HL *et al.* Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection. *Hepatology* 2010; 51: 1904–1911.
34 Suppiah V, Moldovan M, Ahlenstiel G *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; 41: 1100–1104.
35 Milner KL, van der Poorten D, Xu A *et al.* Adipocyte fatty acid binding protein levels relate to inflammation and fibrosis in nonalcoholic fatty liver disease. *Hepatology* 2009; 49: 1926–1934.
36 Cua IH, Hui JM, Bandara P *et al.* Insulin resistance and liver injury in hepatitis C is not associated with virus-specific changes in adipocytokines. *Hepatology* 2007; 46: 66–73.
37 Chiu KC, Chuang LM, Yoon C. Comparison of measured and estimated indices of insulin sensitivity and beta cell function: impact of ethnicity on insulin sensitivity and beta cell function in glucose-tolerant and normotensive subjects. *J Clin Endocrinol Metab* 2001; 86: 1620–1625.
38 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.
39 Alford FP, Dudley FJ, Chisholm DJ, Findlay DM. Glucagon metabolism in normal subjects and in cirrhotic patients before and after portasystemic venous shunt surgery. *Clin Endocrinol (Oxf)* 1979; 11: 413–424.
40 Itou M, Kawaguchi T, Taniguchi E *et al.* Altered expression of glucagon-like peptide-1 and dipeptidyl peptidase IV in patients with HCV-related glucose intolerance. *J Gastroenterol Hepatol* 2008; 23: 244–251.