Post-load insulin resistance is an independent predictor of hepatic fibrosis in virus C chronic hepatitis and in non-alcoholic fatty liver disease

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Background: Insulin resistance is a significant risk factor for hepatic fibrosis in patients with both non-alcoholic fatty liver disease (NAFLD) and chronic hepatitis C (CHC), either directly or by favouring hepatic steatosis. Several methods are available to assess insulin resistance, but their impact on this issue has never been evaluated.

Aims: To determine the relative contribution of steatosis, metabolic abnormalities and insulin resistance, measured by different basal and post-load parameters, to hepatic fibrosis in CHC and in NAFLD patients.

Methods: In 90 patients with CHC and 90 pair-matched patients with NAFLD, the degree of basal insulin resistance (by the homeostasis model assessment, (HOMA)) and post-load insulin sensitivity (by the oral glucose insulin sensitivity (OGIS) index) was assessed, together with the features of the metabolic syndrome according to Adult Treatment Panel III definition. Data were correlated with hepatic histopathology.

Results: The prevalence of basal insulin resistance (HOMA values >75th percentile of normal) was 23.3% in CHC patients and 57.8% in NAFLD, but it increased to 28.8 and 67.8% when measured by post-load insulin resistance (OGIS <25th percentile). In a multivariate model, after adjustment for age, gender and body mass index, OGIS was a predictor of severe fibrosis in CHC and in NAFLD patients, independently of steatosis. An OGIS value below the cut-off of the 25th percentile increased the likelihood ratio of severe fibrosis by a factor of 1.5–2 and proved to be a more sensitive and generally more specific test than HOMA-R for the identification of subjects with severe fibrosis both in NAFLD and in CHC.

Conclusions: Post-load insulin resistance (OGIS <9.8 mg/kg/min) is associated with severe hepatic fibrosis in both NAFLD and CHC patients, and may help identify subjects at risk of progressive disease.

The bulk of recent data suggests that insulin resistance is a significant risk factor for hepatic fibrosis both in patients with chronic hepatitis C (CHC)^{1–8} and in those with non-alcoholic fatty liver disease (NAFLD).^{9–13} This association appears to be mediated mostly by hepatic steatosis, the histological hallmark of insulin resistance,^{1,3,4} but in some cases it has been shown to be independent of it.^{2,6,7,14} Despite the amount of available information and a very recent analysis of pooled data,¹⁵ whether metabolic abnormalities and insulin resistance/hyperinsulinaemia may directly contribute to fibrosis remains unsettled.¹⁶ The discrepancy among different studies may be explained by the different significance of steatosis according to the aetiology (metabolic vs viral) and hepatitis C virus (HCV) genotype (genotype 3 vs non-3), by differences in the demographic characteristics of patients and by the prevalence of alcohol abusers. Also the techniques used to assess insulin resistance are expected to affect the results, but their impact has never been evaluated.

Several methods are available to determine insulin sensitivity/resistance, each with its own advantages and limitations.¹⁷ The most widely used are those based on fasting glucose and insulin concentrations (homeostasis model assessment (HOMA-R)),¹⁸ Quantitative Insulin Sensitivity Check Index (QUICKI)¹⁹, which are suitable for epidemiological studies, where the large number of cases compensates for the limited precision of insulin measurements when compared with dynamic tests. Fasting insulin determinations strongly depend upon the precision of the assay, and small errors greatly affect these indices, especially when calculated on a single determination. The HOMA index has been extensively used to

investigate insulin resistance in NAFLD and represents the only method used so far in CHC.

Oral glucose tolerance test (OGTT)-derived indexes are cumbersome and expensive but, due to multiple blood samples, the values they provide are less biased by the precision of the insulin assay.^{20–22} The oral glucose insulin sensitivity (OGIS) is a good correlate of the insulin sensitivity measured by the clamp, with correlation coefficients ranging from 0.7 to 0.8.²² In a population of NAFLD patients,¹⁰ OGIS was an independent predictor of hepatic fibrosis, whereas its usefulness in CHC patients has never been tested.

We aimed to determine the relative contribution of steatosis, metabolic abnormalities and insulin resistance, assessed by parameters derived from both basal and post-load tests, to hepatic fibrosis in a comparative analysis of matched CHC and NAFLD patients.

PATIENTS AND METHODS

Subjects

From March 2003 to June 2005, 90 consecutive patients with CHC who underwent a liver biopsy at the Ancona, Senigallia

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, Adult Treatment Panel; BMI, body mass index; CHC, chronic hepatitis C; CV, coefficient of variation; FBG, fasting blood glucose; GGT, γ -glutamyltransferase; HCV, hepatitis C virus; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; HSC, hepatic stellate cell; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OGIS, oral glucose insulin sensitivity; OGTT, oral glucose tolerance test

and Pesaro Hospitals were prospectively enrolled. CHC was defined by high transaminase values for more than 6 months and by the presence of serum HCV RNA in the absence of co-infection with hepatitis B virus (hepatitis B surface antigen and core antibody-positive), autoimmune hepatitis, cholestatic (primary biliary cirrhosis, sclerosing cholangitis) or genetic (haemochromatosis, α 1-antitrypsin deficiency, Wilson disease) liver disease. Subjects with pharmacologically treated diabetes or previous antiviral treatment were also excluded. The habitual alcohol intake in the last 6 months was assessed by interviews extended to family members and general practitioners, and patients with alcohol consumption > 40 g/day were excluded.

This CHC cohort was pair-matched for age (± 4 years), body mass index (BMI; ± 2 kg/m²) and glucose regulation (normal/impaired fasting glucose or impaired glucose tolerance/diabetes) (see below)²³ with 90 NAFLD patients observed either at the Gastroenterology Division of the University Hospital of Turin or at the Unit of Metabolic Diseases, University Hospital of Bologna. For each CHC patient, the first NAFLD patient was selected from a list of over 400 NAFLD cases. The diagnosis of NAFLD was based on chronically elevated aminotransferase levels (alanine aminotransferase (ALT) > 1.5 times the upper normal limit for 6 months or more), negative hepatitis B (hepatitis B surface antigen and core antibody) and C (anti-hepatitis C virus) viral markers, absence of autoimmune hepatitis or coeliac disease, no evidence of genetic, drug-induced or cholestatic liver disease and alcohol consumption ≤ 20 g/day (corresponding to two glasses of wine, four glasses of beer and 1/2 a measure of spirit). Patients with clinical evidence of cirrhosis were excluded from both groups.

The protocol of data collection was part of the common clinical practice in the different hospital units. All subjects were requested to give their informed consent to the use of personal data, analyses and liver biopsy at time of admission. This specific study was approved by the institutional review boards of the participating centres, regulating non-interventional studies, and by the Italian Ministry of Health.

Anthropometric and laboratory evaluations

Body weight was measured in light clothing and without shoes to the nearest 0.5 kg. Height was measured to the nearest 0.5 cm. BMI was calculated as weight (in kg) divided by height squared (m²). Subjects in the BMI range 25–29.9 kg/m² and ≥ 30 kg/m² were considered overweight and obese, respectively. Waist circumference was measured to the nearest 0.5 cm at the shortest point below the lower rib margin and the iliac crest, whereas hip circumference was obtained similarly at the widest point between hip and buttocks.

Blood pressure measurements were obtained according to the Guidelines of the International Society of Hypertension.²⁴ The components of metabolic syndrome were classified according to the Adult Treatment Panel (ATP)III proposals,²⁵ and subjects having three or more positive criteria were labelled as having metabolic syndrome.

Laboratory investigation included serum levels of ALT, aspartate aminotransferase (AST), γ -glutamyltransferase (GGT), total and high-density lipoprotein (HDL) cholesterol, triglycerides, serum glucose and insulin concentrations. An OGTT, with glucose and insulin determinations every 30 min for 120 min, was also performed.

Venous plasma glucose was measured in duplicate with an automated analyser (Beckman Instruments, Fullerton CA, USA; inter-assay coefficient of variation (CV) $< 4\%$). Basal and glucose-stimulated insulin was measured by an immunoenzymometric assay (AIA-PACK IRI, AIA-1200 system, Tosoh Co., Tokyo, Japan) with intra- and inter-assay CVs for quality control $< 7\%$. Insulin determination in the two centres was

standardised. Fasting serum liver function tests and lipid levels were determined by routine laboratory techniques. HCV RNA was quantified by Quantiplex HCV RNA 2.0 assay (Chiron Diagnostics, Emeryville, CA, USA). HCV genotyping was performed with a second-generation reverse hybridisation line probe assay (Inno-Lipa HCVII; Innogenetics, Zwijndrecht, Belgium).

All subjects were divided into three groups according to their fasting blood glucose (FBG) levels: normal fasting glucose (FBG < 110 mg/dl), impaired fasting glucose (FBG between 110 and 125/dl) and diabetes mellitus (FBG > 125 mg/dl).²³ The 120-min blood glucose after OGTT was used to classify patients further into normal glucose tolerance (< 140 mg/dl), impaired glucose tolerance (140–199 mg/dl) and diabetes mellitus (≥ 200 mg/dl).

Insulin resistance/sensitivity was calculated by means of the HOMA-R method¹⁸ and the OGIS calculator during OGTT.²² Patients were considered to have basal insulin resistance in the presence of HOMA-R values > 2.7 ; this cut-off corresponds to the upper quartile of a previously published control population.²⁶ Similarly, patients were considered to have post-load insulin resistance in the presence of OGIS < 9.8 mg/kg/min; this limit corresponds to the 25th percentile of a population of 527 healthy subjects with normal fasting glucose and normal glucose tolerance (G Pacini, personal communication).

Histopathology

Liver biopsies were available in all subjects and were scored in a blinded manner by two pathologists at the University of Ancona and at the University of Turin for CHC and NAFLD patients, respectively. In both groups, steatosis was scored according to the criteria proposed by Brunt et al²⁷ as mild (5–33% of hepatocytes affected), moderate (34–66%) and severe ($> 66\%$). In CHC, the degree of necroinflammatory activity was scored 0–15 and the stage of fibrosis 0–6 according to Ishak.²⁸ In NAFLD, Brunt classification was used to score necroinflammation from 0 to 3 and fibrosis from 0 to 4. Non-alcoholic steatohepatitis (NASH) was defined on the basis of the presence of fibrosis (stage 1 or more) or necroinflammation (grade 2 or more).

Statistical analysis

Data were processed on a personal computer and analysed using StatView 5.0 (SAS Institute, Inc., Cary, NC, USA). Unpaired t test (two tailed), χ^2 contingency test, Fisher's exact test and linear regression analysis were used whenever appropriate. Non-parametric methods (Mann-Whitney and Spearman rank correlation) were also used for non-normally distributed values. Spearman correlation analysis was used to test the association of age, sex and BMI with the severity of steatosis and fibrosis. Initially, we tested the association of insulin resistance with steatosis. For this purpose, age, sex and BMI, together with HOMA and OGIS values, dichotomised using the cut-offs of upper quartiles of normal, were used as independent variables. Multivariate logistic regression analysis was used to identify factors significantly associated with severe fibrosis (dependent variables) in the different cohorts in three separate analyses (all cases, NAFLD and CHC). The CHC group was also split into CHC-3 and CHC-non-3 in post-hoc analyses. All analyses were adjusted for age, sex and BMI. In order to pool the data of CHC and NAFLD where different classifications had been used, fibrosis was categorised as mild (stage 1–2 in NAFLD and 1–3 in CHC) and severe (stage 3–4 in NAFLD and 4–6 in CHC), whereas necroinflammation was scored as mild (grade 1–2 in NAFLD and 1–5 in CHC) and severe (stage 3–4 in NAFLD and > 5 in CHC). Independent variables were the parameters of insulin resistance (HOMA and OGIS values,

categorised as above), the ATPIII score, the aspartate to alanine ratio (a correlate of disease stage associated with fibrosis⁹) and fat grading. The type of disease (NAFLD vs CHC) was also included among independent variables in the whole group, whereas in CHC we also considered the log of viral load. Results were expressed as odds ratios (95% CI). All data in the text and in the tables are given as means (SD), when not otherwise indicated. Values of $p < 0.05$ were considered statistically significant.

RESULTS

In the 90 CHC patients, the infection was due to genotype 1 in 46 cases, to genotype 2 in 20, to genotype 3 in 23 and to genotype 4 in a single case. For the purpose of the present study, 67 subjects were considered infected by genotype non-3 (CHC-non-3) and 23 by genotype 3 (CHC-3). The clinical and biochemical characteristics of the two groups of patients are given in table 1.

Matching between NAFLD and CHC was highly effective, but NAFLD patients had a moderately higher BMI (by approximately 1 kg/m^2), generated by higher visceral adiposity, and higher fasting glucose and insulin, triglycerides and arterial pressure. Insulin resistance, both in the fasting state and after load, was also present in a larger proportion of NAFLD patients. The prevalence of the metabolic syndrome in this NAFLD cohort of non-obese non-diabetic subjects was very low, but fourfold higher than in CHC. Notably, the prevalence of insulin resistance was higher in NAFLD than in CHC, and was particularly elevated in CHC-non-3, particularly when assessed in response to glucose load (OGIS $< 9.8 \text{ mg/kg/min}$: CHC-non-3, 33% vs 17% in CHC-3), compared with the fasting HOMA-R assessment (HOMA $> 2.7 \text{ mg/kg/min}$: CHC-non-3, 25% vs 17% in CHC-3).

Histological grading and staging are reported in fig 1. Steatosis was present in 49% of CHC, and was more common and severe in CHC-3 (mild in 43% of cases, moderate in 13%, severe in 13%) compared with CHC-non-3 cases (21, 6 and 13%, respectively). Necroinflammation was more common and severe in CHC than in NAFLD, and particularly in CHC-3 (mild, 73%; severe, 27%). Finally, fibrosis was more common in CHC, without differences in relation to viral genotype.

Figure 1 Prevalence of histological alteration in the whole cohort of liver patients, in subjects with non-alcoholic fatty liver disease (NAFLD) and in subjects with chronic hepatitis C (CHC), in relation to viral genotype. Note that necroinflammation and fibrosis were scored only as absent, mild and severe, as reported in the Patients and methods section. The significance of differences between NAFLD and CHC is reported.

Table 1 Clinical and biochemical characteristics of patients with non-alcoholic fatty liver and chronic hepatitis C

	NAFLD	CHC	p Value
Male gender (%)	80.0	68.9	0.123
Age (years)	44 (12)	47 (13)	0.060
BM (kg/m^2)	26.5 (2.9)	25.2 (3.9)	0.017
Waist circumference (cm)	95 (8)	90 (12)	0.056
Alanine aminotransferase (U/l)*	63 (50)	100 (72)	< 0.0001
Aspartate aminotransferase (U/l)*	40 (26)	56 (46)	0.0002
γ -Glutamyltransferase (U/l)*	56 (81)	47 (59)	0.332
Fasting glucose (mg/dl)	98 (18)	88 (17)	0.0003
120-min glucose (mg/dl)*	121 (72)	124 (62)	0.361
Fasting HDL-cholesterol (mg/dl)	48 (12)	46 (20)	0.503
Fasting triglycerides (mg/dl)	122 (72)	124 (62)	< 0.0001
Systolic pressure (mmHg)	130 (16)	118 (12)	< 0.0001
Diastolic pressure (mmHg)	81 (9)	76 (6)	< 0.0001
Normal/overweight/obese (%)	31/57/12	45/47/8	0.120
Normal/IFG+IGT/diabetes (%)	84/2/13	84/2/13	–
Enlarged waist circumference (%)†	28.9	8.9	0.001
Low HDL-cholesterol (%)†	21.1	25.6	0.597
High triglycerides (%)†	36.7	11.1	< 0.0001
Raised arterial pressure (%)*	55.6	15.6	< 0.0001
Raised fasting glucose (%)*	14.4	8.9	0.496
Metabolic syndrome (%)*	22.2	4.4	0.0007
Fasting insulin ($\mu\text{U/ml}$)*	14.0 (9.2)	9.4 (7.6)	< 0.0001
HOMA (%)*	3.35 (2.89)	1.98 (1.81)	0.003
HOMA > 2.7 (%)	57.8	23.3	< 0.0001
OGIS (mg/kg/min)*	8.83 (2.20)	10.92 (3.17)	< 0.0001
OGIS $< 9.8 \text{ mg/kg/min}$ (%)	67.8	28.8	< 0.0001

BMI, body mass index; CHC, chronic hepatitis C; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NAFLD, non-alcoholic fatty liver disease; OGIS, oral glucose insulin sensitivity

All data are means (SD), median (interquartile range) or prevalence (%)

*Differences are analysed by Mann-Whitney test.

†Scored according to Adult Treatment Panel III criteria.

Association of insulin resistance with steatosis

In the whole data set, steatosis was significantly associated with age ($r_s = -0.149$; $p = 0.047$) and BMI ($r_s = 0.189$; $p = 0.012$), but not with sex. After adjustment for age, sex, BMI and the ATPIII score, insulin resistance, measured by both HOMA-R and OGIS, was significantly associated with steatosis in the whole group (table 2)—particularly with moderate to severe steatosis—and the association was maintained in the NAFLD cohort, but not in CHC.

Association of insulin resistance with severe fibrosis

By Spearman correlation analysis, the severity of fibrosis was significantly associated with age ($r_s = 0.185$; $p = 0.013$) in the whole data set, but not with sex or BMI ($r_s = 0.041$; $p = 0.588$).

In the multivariate analysis, after adjustment for age, gender and BMI (table 3), severe fibrosis was associated with post-load insulin resistance (OGIS) and with the degree of liver fat infiltration in the whole data set, and separately in the CHC cohort, while in NAFLD cases OGIS was the sole predictor of severe fibrosis.

In particular, an OGIS value below the cut-off of the 25th percentile proved to be more sensitive and generally more specific than HOMA-R to identify subjects with severe fibrosis both in the NAFLD and in the CHC group (table 3). This cut-off value increased the likelihood ratio of severe fibrosis by a factor of 1.5–2.

When the distribution of fibrosis was tested according to OGIS (fig 2), the degree of fibrosis was negatively associated with post-load insulin sensitivity in the whole data set and in NAFLD, and not in the global cohort of CHC, but it was again significantly related to OGIS in CHC-non-3 ($p = 0.047$).

In a separate analysis, both HOMA-R and OGIS were entered into the regression as continuous variables. In this case, both in the whole data set and separately in NAFLD cases, the ATPIII score was the only parameter significantly associated with severe fibrosis (not reported in detail).

DISCUSSION

Insulin-resistant subjects with both NAFLD and CHC are more likely to have severe fibrosis, but previous studies failed to reach a consensus as to whether this association is mediated by hepatic steatosis or is independent of it.^{1 3 9–13 16} The results of the present study suggest that insulin resistance is indeed a predictor of liver fibrosis, independent of age, gender, BMI, presence of the metabolic syndrome (ATPIII criteria) and steatosis, but also indicate that the method used to measure insulin sensitivity/resistance is critical to unveil this association.

The gold standard for the determination of insulin sensitivity is the euglycaemic hyperinsulinaemic clamp.^{29 30} HOMA-R is a simplified predictor of insulin resistance when the sole fasting measurements are available, and is mainly used in population studies for its simplicity and wide applicability. HOMA-R represents insulin resistance, not insulin sensitivity, and the correlation with the clamp is inverse and curvilinear. In previous studies, such a correlation was reported to range from strong³¹ to weak,³² or even not significant.²²

Conversely, OGIS is based on glucose load, which activates the insulin–glucose homeostatic processes, and mostly reflects glucose uptake by muscle tissue—that is, peripheral insulin resistance. For this reason, it correlates significantly with glucose clearance measured by the clamp technique, and is considered a more sensitive measure of insulin sensitivity than HOMA-R.²² In this study, we specifically wished to test the relative effectiveness of HOMA-R and OGIS in demonstrating an association between insulin resistance and fibrosis, and to provide a cut-off value for each method that could be used to identify patients with fibrosis. Accordingly, we tested the cut-off identifying the upper/lower quartile of reference populations, validated in previous studies and used to define insulin resistance. In keeping with what was observed in much larger cohorts,¹ HOMA-R failed to identify severe fibrosis in CHC, whereas OGIS predicted severe fibrosis. OGIS was previously shown to be an independent predictor of hepatic fibrosis in NAFLD,¹⁰ but this is the first time it has been tested in CHC patients. The correlation between post-load insulin resistance (OGIS <9.8 mg/kg/min) and fibrosis in CHC patients is in keeping with the higher sensitivity and specificity of this index compared with HOMA-R, specifically in a population with a mild to moderate degree of insulin resistance. In CHC, insulin resistance can also be induced or facilitated by the virus itself, independent of the presence of steatosis.³³ Thus, discrepancies with other studies in which insulin resistance measured by HOMA-R was not directly correlated with hepatic fibrosis may simply depend on the method used.^{1 3 4}

Several mechanisms may explain the correlation between insulin resistance and/or hyperinsulinaemia and hepatic fibrosis. Insulin resistance is a low-grade chronic inflammatory state and the liver is exposed to the entire spectrum of cytokines released into the circulation by the adipose tissue and to the hormonal changes that occur in response to these cytokines. Hypoadiponectinaemia is known to influence liver fibrosis in animal models.³⁴ Leptin is involved in hepatic fibrogenesis through hepatic stellate cell (HSC) activation,³⁵ and a correlation between plasma leptin levels and the degree of fibrosis has

Table 2 Association of steatosis with insulin resistance, assessed as HOMA-R >2.7 or OGIS <9.8 mg/kg/min (odds ratio and 95% CI)

	HOMA-R >2.7			OGIS <9.8		
	OR	95% CI	p Value	OR	95% CI	p Value
All cases						
Steatosis absent	1	–		1	–	
Steatosis mild	1.94	0.71 to 5.36	0.198	3.91	1.40 to 10.92	0.009
Steatosis moderate/severe	5.05	1.81 to 14.11	0.002	8.11	2.79 to 22.55	<0.001
NAFLD						
Steatosis mild	1	–		1	–	
Steatosis moderate/severe	3.38	1.30 to 8.84	0.013	1.71	1.16 to 2.53	0.006
CHC						
Steatosis absent	1	–		1	–	
Steatosis mild	1.44	0.39 to 5.24	0.583	2.02	0.56 to 7.25	0.281
Steatosis moderate/severe	2.28	0.57 to 9.16	0.243	3.53	0.88 to 14.21	0.076

CHC, chronic hepatitis C; HOMA, homeostasis model assessment; NAFLD, non-alcoholic fatty liver disease; OGIS, oral glucose insulin sensitivity; OR, odds ratio.
The reference is steatosis absent/mild in the whole data set and in patients with CHC, and mild steatosis in NAFLD. All data are corrected for age, gender, body mass index and Adult Treatment Panel III score.

Table 3 Factors associated with severe fibrosis in the whole data set and separately in patients with chronic hepatitis C and non-alcoholic fatty liver disease at multivariate analysis, after adjustment for age, gender and body mass index (odds ratio and 95% confidence interval)

	Severe fibrosis			No. +ve	SENS	SPEC	LR+
	OR	95% CI	p Value				
All cases (n = 180)							
AST/ALT	0.79	0.38 to 1.65	0.534				
ATP/III score	1.44	0.93 to 2.22	0.100				
OGIS (<9.8 mg/kg/min)	4.51	1.71 to 11.87	0.002	87/180	73.9	60.4	1.87
HOMA-R (>2.7%)	1.13	0.46 to 2.76	0.785	77/180	60.9	63.4	1.66
Fat grading	1.72	1.13 to 2.62	0.011				
Type: NAFLD	0.43	0.17 to 1.09	0.075				
NAFLD (n = 90)							
AST/ALT	0.75	0.31 to 1.81	0.525				
ATP/III score	1.71	0.93 to 3.15	0.083				
OGIS (<9.8 mg/kg/min)	5.75	1.09 to 30.49	0.040	61/90	92.0	41.5	1.57
HOMA-R (>2.7%)	2.30	0.60 to 8.86	0.225	53/90	84.0	50.8	1.71
Fat grading	1.70	0.84 to 3.47	0.141				
CHC (n = 90)							
AST/ALT	0.70	0.07 to 7.12	0.764				
ATP/III score	0.99	0.47 to 2.10	0.980				
OGIS (<9.8 mg/kg/min)	8.78	1.79 to 43.07	0.007	26/90	52.4	78.3	2.41
HOMA-R (>2.7%)	0.36	0.06 to 2.05	0.248	24/90	33.3	75.4	1.35
Fat grading	1.99	1.11 to 3.55	0.020				
Log viral load	1.05	0.48 to 2.31	0.902				

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP, Adult Treatment Panel; CHC, chronic hepatitis C; HOMA, homeostasis model assessment; LR, likelihood ratio; NAFLD, non-alcoholic fatty liver disease; OGIS, oral glucose insulin sensitivity; SENS, sensitivity; SPEC, specificity
The reference value is fibrosis absent+mild. The sensitivity, specificity and the likelihood ratio of OGIS and HOMA-R in the identification of severe fibrosis are reported.

Figure 2 Insulin sensitivity, measured by oral glucose insulin sensitivity (OGIS), in all patients (all cases) and in patients with non-alcoholic fatty liver disease (NAFLD) or chronic hepatitis C (CHC) according to the severity of fibrosis. In this "box and whiskers" plot, the boxes identify the median and the 25th and 75th percentiles, while the "whiskers" stretch to the 5th and 95th percentiles. Note that the degree of fibrosis was negatively associated with post-load insulin sensitivity in the whole data set and in NAFLD. The association was not significant in the global cohort of CHC but it was again significant in CHC-non-3 ($p = 0.047$).

recently been shown in patients with hepatitis C,³⁶ although the role of leptin in humans is questioned.^{37,38} Insulin receptors had been identified on HSCs in liver sections from patients with chronic HCV, and hyperinsulinaemia induces HSC proliferation and collagen synthesis.^{39,40} Furthermore, hyperglycaemia per se can up-regulate the expression of pro-fibrogenic cytokines, such as connective tissue growth factor,³⁹ and activated HSCs express functional receptors for advanced glycosylated end-products, resulting from long-standing hyperglycaemia.⁴¹

Notably, insulin resistance has been related to a reduced response to antiviral treatment in non-diabetic CHC patients,⁴² and successful antiviral treatment has been associated with an improvement in glucose tolerance.⁴³ Whether insulin resistance is a marker for difficult to treat patients and whether it plays a role in interferon plus ribavirin resistance remains unclear. Anyway, insulin resistance, either primary or secondary to host-related and viral factors, represents an important player in NAFLD as well as in CHC. In both diseases, the cut-off of OGIS we propose as a correlate of insulin resistance appears to be an easy and reliable tool to identify patients with insulin resistance-associated fibrosis, with implications for prognosis and treatment, and should be part of the general screening in these patients.

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REFERENCES

- 1 **Cammà C**, Bruno S, Di Marco V, *et al*. Insulin resistance is associated with steatosis in nondiabetic patients with genotype 1 chronic hepatitis C. *Hepatology* 2006;**43**:64–71.
- 2 **D'Souza R**, Sabin CA, Foster GR. Insulin resistance plays a significant role in liver fibrosis in chronic hepatitis C and in the response to antiviral therapy. *Am J Gastroenterol* 2005;**100**:1509–15.
- 3 **Fartoux L**, Poujol-Robert A, Guechot J, *et al*. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. *Gut* 2005;**54**:1003–8.
- 4 **Hourigan LF**, Macdonald GA, Purdie D, *et al*. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999;**29**:1215–9.
- 5 **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.
- 6 **Muzzi A**, Leandro G, Rubbia-Brandt L, *et al*. Insulin resistance is associated with liver fibrosis in non-diabetic chronic hepatitis C patients. *J Hepatol* 2005;**42**:41–6.
- 7 **Ratziu V**, Munteanu M, Charlotte F, *et al*. Fibrogenic impact of high serum glucose in chronic hepatitis C. *J Hepatol* 2003;**39**:1049–55.
- 8 **Zekry A**, McHutchison JG, Diehl AM. Insulin resistance and steatosis in hepatitis C virus infection. *Gut* 2005;**54**:903–6.
- 9 **Angulo P**, Keach JC, Batts KP, *et al*. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999;**30**:1356–62.
- 10 **Bugianesi E**, Manzini P, D'Antico S, *et al*. Relative contribution of iron burden, HFE mutations and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004;**39**:179–87.
- 11 **Haukeland JW**, Konopski Z, Linnestad P, *et al*. Abnormal glucose tolerance is a predictor of steatohepatitis and fibrosis in patients with non-alcoholic fatty liver disease. *Scand J Gastroenterol* 2005;**40**:1469–77.
- 12 **Marchesini G**, Bugianesi E, Forlani G, *et al*. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003;**37**:917–23.
- 13 **Willner IR**, Waters B, Patil SR, *et al*. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001;**96**:2957–61.
- 14 **Hui JM**, Hodge A, Farrell GC, *et al*. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004;**40**:46–54.
- 15 **Leandro G**, Mangia A, Hui J, *et al*. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. *Gastroenterology* 2006;**130**:1346–62.
- 16 **Merchante N**, Macias J, Ramayo E, *et al*. Insulin resistance is not associated with liver fibrosis progression in HIV/hepatitis C virus-coinfected patients. *J Viral Hepat* 2006;**13**:449–56.
- 17 **Pacini G**, Mari A. Methods for clinical assessment of insulin sensitivity and beta-cell function. *Best Pract Res Clin Endocrinol Metab* 2003;**17**:305–22.
- 18 **Matthews DR**, Hosker JP, Rudenski AS, *et al*. Homeostasis model assessment: insulin resistance and beta-cell function from plasma fasting glucose and insulin concentrations in man. *Diabetologia* 1985;**28**:412–9.
- 19 **Katz A**, Nambi SS, Mather K, *et al*. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000;**85**:2402–10.
- 20 **Matsuda M**, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;**22**:1462–70.
- 21 **Stumvoll M**, Mitrakou A, Pimenta W, *et al*. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care* 2000;**23**:295–301.
- 22 **Mari A**, Pacini G, Murphy E, *et al*. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care* 2001;**24**:539–48.
- 23 **Alberti KG**, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabet Med* 1998;**15**:539–53.
- 24 **Guidelines Committee**. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens* 2003;**21**:1011–53.
- 25 **Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults**. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001;**285**:2486–97.
- 26 **Bugianesi E**, Pagotto U, Manini R, *et al*. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J Clin Endocrinol Metab* 2005;**90**:3498–504.
- 27 **Brunt EM**, Janney CG, Di Bisceglie AM, *et al*. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;**94**:2467–74.
- 28 **Ishak KG**. Chronic hepatitis: morphology and nomenclature. *Mod Pathol* 1994;**7**:690–713.
- 29 **Bergman RN**, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. *Endocr Rev* 1985;**6**:45–86.
- 30 **Ferrannini E**, Mari A. How to measure insulin sensitivity. *J Hypertens* 1998;**16**:895–906.
- 31 **Bonora E**, Targher G, Alberiche M, *et al*. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000;**23**:57–63.
- 32 **Ferrannini E**, Balkau B. Insulin: in search of a syndrome. *Diabet Med* 2002;**19**:724–9.
- 33 **Bugianesi E**, Marchesini G, Gentilecore E, *et al*. Fibrosis in genotype 3 chronic hepatitis C and nonalcoholic fatty liver disease: role of insulin resistance and hepatic steatosis. *Hepatology* 2006;**44**:1648–55.
- 34 **Kamada Y**, Tamura S, Kiso S, *et al*. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. *Gastroenterology* 2003;**125**:1796–807.
- 35 **Marra F**. Leptin and liver fibrosis: a matter of fat. *Gastroenterology* 2002;**122**:1529–32.
- 36 **Crespo J**, Rivero M, Fabrega E, *et al*. Plasma leptin and TNF-alpha levels in chronic hepatitis C patients and their relationship to hepatic fibrosis. *Dig Dis Sci* 2002;**47**:1604–10.
- 37 **Giannini E**, Ceppa P, Botta F, *et al*. Leptin has no role in determining severity of steatosis and fibrosis in patients with chronic hepatitis C. *Am J Gastroenterol* 2000;**95**:3211–7.
- 38 **Chitturi S**, Farrell G, Frost L, *et al*. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology* 2002;**36**:403–9.
- 39 **Paradis V**, Perlemuter G, Bonvoust F, *et al*. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. *Hepatology* 2001;**34**:738–44.
- 40 **Svegliati-Baroni G**, Ridolfi F, Di Sario A, *et al*. Insulin and insulin-like growth factor-1 stimulate proliferation and type I collagen accumulation by human hepatic stellate cells: differential effects on signal transduction pathways. *Hepatology* 1999;**29**:1743–51.
- 41 **Fehrenbach H**, Weiskirchen R, Kasper M, *et al*. Up-regulated expression of the receptor for advanced glycation end products in cultured rat hepatic stellate cells during transdifferentiation to myofibroblasts. *Hepatology* 2001;**34**:943–52.
- 42 **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.
- 43 **Konrad T**, Zeuzem S, Vicini P, *et al*. Evaluation of factors controlling glucose tolerance in patients with HCV infection before and after 4 months therapy with interferon-alpha. *Eur J Clin Invest* 2000;**30**:111–21.