Hepatitis C virus (HCV) infection is associated with insulin resistance (IR), which is a condition known to influence the progression of liver fibrosis and the response to pegylated interferon (PEG-IFN)/ribavirin (RBV) therapy. We aimed to assess whether a sustained virological response (SVR) after antiviral therapy prevents the development of IR in the long term. Members of the Milan Safety Tolerability study cohort, who received PEG-IFNα2a/RBV or PEG-IFNα2b/RBV, underwent a homeostasis model assessment (HOMA) at the baseline and 24 months after treatment completion. For all patients (n = 431), a liver biopsy sample was scored for grading, staging (Ishak), and steatosis. At the baseline, IR (HOMA value > 2) was detected in 48 patients (12%), and it was associated with body weight (P = 0.03), an HCV load < 0.6 × 10^6 IU/L (P = 0.006), fibrosis staging ≥ 4 (P = 0.01), and moderate to severe steatosis (P = 0.03). IR did not influence the rates of end-of-treatment response (75% versus 69%, P = 0.4), SVR (63% versus 60%, P = 0.8), or relapse (19% versus 24%, P = 0.5). After treatment, IR developed in 49 of the 384 non-diabetic patients (14%). Although the mean baseline and posttreatment HOMA values were similar in SVR patients (1.11 ± 0.8 versus 1.18 ± 1.1, P = 0.25), patients experiencing treatment failure showed a significant increase in the mean HOMA value at the follow-up visit (1.20 ± 0.85 versus 1.49 ± 1.3, P = 0.007), and there was an increased rate of de novo IR in non-SVR patients versus SVR patients (17% versus 7%, P = 0.007). According to a logistic regression analysis, treatment failure (odds ratio = 2.81, 95% confidence interval = 1.39-5.67, P = 0.004) and a 10% body mass index increase (odds ratio = 6.42, 95% confidence interval = 1.69-24.3, P = 0.006) were significantly associated with the development of de novo IR. Conclusion: In nondiabetic patients with chronic HCV, the achievement of SVR with PEG-IFN and RBV prevents the development of de novo IR. (HEPATOLOGY 2012;56:1681-1687)
supporting a direct role for HCV, studies have shown an increase in the liver expression of IRS after successful treatment with pegylated interferon (PEG-IFN) plus ribavirin (RBV), and there have been several reports of significant benefits in terms of IR reduction [as assessed by a comparison of homeostasis model assessment (HOMA) scores before and 24 weeks after treatment completion] once a sustained virological response (SVR) is achieved.\textsuperscript{10-14} Although this in theory provides an additional rationale for extending anti-HCV therapy to all infected patients, most of these studies have not assessed the long-term benefits of SVR with respect to IR, so they have failed to undisputedly demonstrate that the achievement of SVR reduces the IR development rate. By the same token, a large retrospective study from Japan demonstrating an association between SVR and reduced rates of T2DM development did not provide conclusive evidence for a prophyllactic effect of HCV clearance on IR because the patients were not studied with respect to baseline IR: this negatively influences SVR rates and, therefore, represents a potential bias in the interpretation of the study.\textsuperscript{15} All in all, a prospective validation study of the prophyllactic effects of SVR on IR in HCV-infected patients has never been performed. The cohort of patients enrolled in the Milan Safety Tolerability (MIST) protocol offered us a unique opportunity for assessing the long-term effects of SVR on the development of IR in prospectively followed patients (who were selected solely on the basis of their eligibility for interferon therapy) because the study protocol included HOMA score quantification before PEG-IFN/RBV therapy and 24 months after treatment completion.\textsuperscript{16}

**Patients and Methods**

**Aims.** This study was an extended follow-up substudy of the MIST study, which was originally designed to compare the safety and effectiveness of PEG-IFN\textsubscript{2a} and PEG-IFN\textsubscript{2b} therapy with RBV. Patients who were enrolled in the original study also consented to HOMA testing at the baseline and 24 months after treatment completion. This study was approved by the institutional review board of the Department of Internal Medicine.

The primary aim of this study was to assess the effects of the achievement of SVR on IR in terms of absolute HOMA values and IR onset. The secondary aims were to identify the baseline characteristics associated with IR and to assess the impact of IR on SVR rates.

**Patients.** Patients who were enrolled in the MIST study were randomized by a computer-generated allocation list (stratified by the HCV genotype) to receive a combination of PEG-IFN\textsubscript{2a} (Pegasys, Roche, Basel Switzerland; 180 \(\mu\)g/week) and daily RBV (Rebetol, Schering Plough Corp., Kenilworth, NJ; 800-1200 mg) or a combination of PEG-IFN\textsubscript{2b} (PegIntron, Schering Plough; 1.5 \(\mu\)g/kg/week) and daily RBV (800-1200 mg) for a standard duration based on the HCV genotype. Patients with HCV1 or HCV4 were treated for 48 weeks. PEG-IFN\textsubscript{2a} was associated with 1000 to 1200 mg of RBV per day (patients were divided into those weighing <75 kg and those weighing >75 kg); PEG-IFN\textsubscript{2b} was associated with 800 mg of RBV for patients weighing <65 kg, with 1000 mg of RBV for patients weighing 65 to 85 kg, and with 1200 mg of RBV for patients weighing >85 kg. HCV2 and HCV3 patients were treated for 24 weeks. PEG-IFN\textsubscript{2a} was associated with 800 mg of RBV per day; PEG-IFN\textsubscript{2b} was associated with 800 mg of RBV for patients weighing <65 kg, with 1000 mg of RBV for patients weighing 65 to 85 kg, and with 1200 mg of RBV for patients weighing >85 kg.

**Clinical and Laboratory Measurements.** Baseline anthropometric measurements, including the height and weight for calculating the body mass index (BMI), were recorded. Overweight was defined as a BMI in the range of 25 to 30 kg/m\(^2\), and obesity was defined as a BMI \(\geq30\) kg/m\(^2\). Blood pressure measurements were obtained according to the guidelines of the International Society of Hypertension.\textsuperscript{17} A patient’s history of diabetes, arterial hypertension, and hyperlipidemia...

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was obtained at the baseline. An overnight (12-hour) fasting blood sample was taken for routine analyses (e.g., alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transpeptidase, cholesterol, triglycerides, glucose, and insulin). Serum insulin levels were measured by an electrochemiluminescence immunoassay with an autoanalyzer (Elecsys 1010/2010 and Elecsys Modular Analytics E170, Roche, Basel, Switzerland). IR, which was assessed at the baseline and 24 months after treatment completion, was determined by HOMA according to the HOMA model formula:

\[
\text{HOMA score} = \frac{\text{Fasting insulin(mIU/L)}}{\text{Fasting glucose(mmol/L)}}/22.5
\]

In agreement with previous literature, IR was defined as a HOMA score \(>2\)\textsuperscript{13,14,18} \textit{De novo} IR was defined as a HOMA score \(<2\) at the baseline and as a HOMA score \(>2\) at the 24 month follow-up visit.

Efficacy was assessed with SVR, which was defined as the undetectability of HCV RNA by real-time polymerase chain reaction (COBAS Amplicor HCV test version 2.0, Roche Diagnostics; detection limit = 50 IU/mL) at week 24 of the posttreatment follow-up. The clearance of serum HCV RNA by real-time polymerase chain reaction was assessed at week 4 [rapid virological response (RVR)], at week 12 [early virological response (EVR)], at week 24, and at week 48 of treatment [end-of-treatment response (ETR)].

Patients with an ETR who were HCV RNA–positive during follow-up were classified as relapsers. Patients who had a virological breakthrough were considered nonresponders. Therapy was discontinued for HCV1 and HCV4 patients if the HCV RNA level (according to quantitative testing) had dropped less than 2 log by week 12 versus the baseline values (the week 12 stopping rule) and if HCV RNA was still detectable at week 24 in patients in whom the HCV RNA level had dropped \(>2\) log by week 12 (the week 24 stopping rule).

Baseline percutaneous liver biopsy was performed in all patients. The severity of hepatic inflammation was evaluated by the Ishak score with separate reports for grading and staging.\textsuperscript{19} Liver steatosis was recorded and classified as absent or minimal (<5%), mild (5%-33%), moderate (33%-66%), or severe (>66%).

**Statistical Analysis.** Comparisons between groups were made with the Mann-Whitney U test or the Student \(t\) test for continuous variables and with the \(\chi^2\) test or Fisher’s exact probability test for categorical data. \(P < 0.05\) was considered statistically significant.

A logistic regression analysis was performed to identify the variables associated with IR occurrence. All variables with statistical significance in the univariate analysis were included in the final model, and odds ratios and corresponding 95% confidence intervals were computed. Calculations were performed with the Stata 10.0 statistical package (StataCorp, College Station, TX).

**Results**

Thirty-two of the 431 Caucasian patients enrolled in the MIST study (7%) had T2DM and were excluded from the analysis. The baseline characteristics of the remaining 399 patients are presented in Table 1.

At the baseline, IR was present in 48 patients (12%), and this was independent of the HCV genotype (17 patients [11%] with HCV1, 16 patients [12%] with HCV2, 9 patients [14%] with HCV3, and 6 patients [15%] with HCV4, \(P = 0.8\)). However, it was associated with age (\(P = 0.02\)), a BMI > 25 kg/m\(^2\) (\(P = 0.005\)), a liver fibrosis stage \(\geq 4\) (\(P = 0.0001\)), an HCV viral load < 0.6 \(\times 10^6\) IU/L (\(P = 0.005\)), and moderate to severe steatosis (\(P = 0.009\)).

The SVR rates were not influenced by the presence of IR at the baseline [60% (29/48) in patients with IR at the baseline versus 63% (220/351) in patients without IR at the baseline, \(P = 0.8\)]. IR at the baseline did not influence the rates of ETR [69% (33/48) versus 75% (262/351), \(P = 0.4\)] or relapse [24% (8/33) versus 19% (49/262), \(P = 0.5\); Fig. 1]. SVR and on-treatment virological response rates (i.e., RVR, EVR, and ETR) were also not influenced by IR in an HCV genotype subgroup analysis (Fig. 2).

**Impact of SVR on IR.** HOMA was measured 24 months after treatment completion in 354 patients.
Follow-up HOMA testing was not performed in 45 patients because 20 were lost to follow-up, 2 developed hepatocellular carcinoma, 3 developed T2DM, and 20 were re-treated with PEG-IFN/RBV before the scheduled 24-month follow-up visit. At the 24 month follow-up visit, IR was found in 49 patients (14%) and was more frequently detected in patients with treatment failure versus patients with an SVR (19% versus 11%, \(P = 0.07\)). When we assessed the impact of treatment outcomes on HOMA-IR, we found the prevalence of a HOMA score > 2 at the baseline and during follow-up to be similar in SVR and non-SVR patients (12% versus 11%), whereas an increased prevalence during the follow-up visit was observed for patients experiencing treatment failure (10% versus 19%). In the overall population, the mean HOMA value at the 24 month follow-up visit was 1.29 (range = 0.9-7.0), which was significantly higher than the baseline value of 1.14 (range = 0.3-6.3, \(P = 0.03\)). An increase in HOMA values was not seen in SVR patients because the mean baseline and 24-month follow-up HOMA values were similar (1.11 ± 0.8 versus 1.18 ± 1.1, \(P = 0.25\)). On the other hand, patients experiencing treatment failure showed a significant increase in the mean HOMA value at the follow-up visit (1.20 ± 0.85 versus 1.49 ± 1.3, \(P = 0.007\); Fig. 3). This pattern was seen in HCV1 and HCV4 patients and in HCV2 and HCV3 patients, although it reached statistical significance only in the former group (Fig. 3).

Overall, 38 patients (11%) developed de novo IR by the 24 month follow-up assessment, whereas 3 (1%) developed T2DM. De novo IR occurred significantly more frequently in non-SVR patients versus SVR patients [17% (21/124) versus 7% (17/230), \(P = 0.007\)], and there was a trend toward statistical significance for HCV1 and HCV4 patients [16% (15/94) versus 8% (6/78), \(P = 0.1\)] and for HCV2 and HCV3 patients [20% (6/30) versus 7% (11/152), \(P = 0.04\); Fig. 4]. Patients with de novo IR, in comparison with patients who did not develop IR, more often had elevated triglyceride levels (21% versus 9%, \(P = 0.04\)), had a ≥10% BMI increase (11% versus 2%, \(P = 0.01\)), and failed to respond to PEG-IFN/RBV (55% versus 33%, \(P = 0.007\)). According to a logistic regression analysis, treatment failure (odds ratio = 2.81, 95% confidence interval = 1.39-5.67, \(P = 0.004\)) and a ≥10% BMI increase (odds ratio = 6.42,
95% confidence interval = 1.69-24.3, \( P = 0.006 \) were significantly associated with the development of IR.

**Discussion**

In the MIST cohort, treatment failure after PEG-IFN/RBV therapy was associated with a significant increase in HOMA values and with an increased incidence of IR development 24 months after treatment completion, which occurred regardless of the infecting genotype. These results once again highlight the interplay between HCV and metabolic factors and extend the range of potential clinical benefits from a response to antiviral therapy beyond the interruption or attenuation of HCV-related liver disease. Indeed, HCV is known to co-opt and interfere with the host’s lipid metabolism to facilitate cell entry, assembly, replication, and secretion. HCV is also known to interfere with glucose homeostasis and ultimately induce T2DM. Although the exact reasons for this are still not fully understood, it is generally accepted that the development of IR is the first step in the process. Several studies have shown a direct role of the virus and particularly the core protein in promoting IR, and others have shown that HCV may promote IR indirectly by triggering the production of proinflammatory cytokines such as interleukin-18 and tumor necrosis factor \( \alpha \). A close relationship between IR and liver fibrosis has been shown in many studies, with patients with bridging fibrosis/cirrhosis having increased HOMA values; this suggests that IR is a driving force behind accelerated fibrosis progression in patients chronically infected with HCV. Our study confirms this liaison because patients with IR at the baseline were more likely to have \( S > 3 \) at liver biopsy than patients without IR.

Whatever the exact mechanisms behind HCV-induced IR might be, our study has important clinical implications because it adds to the plethora of data showing the benefits—not just in terms of liver disease but also in terms of extrahepatic manifestations of HCV—associated with the achievement of SVR. With respect to the dangerous liaison between HCV and IR, our study fits well with previous reports of SVR being associated with a reduction in the number of patients with IR 24 weeks after treatment completion, and it adds the benefits of an extended post-treatment follow-up period. In fact, this allowed us to effectively prevent the potential bias represented by weight loss during PEG-IFN/RBV treatment, which is known to only transiently reduce HOMA values and improve insulin sensitivity.

Unlike many previous studies (including a meta-analysis of 14 trials), we could not demonstrate any negative impact of baseline IR on treatment outcomes. One possible explanation is the low prevalence of baseline IR in our cohort. This is analogous to a recent study of patients from northern Italy with similar clinical and epidemiological characteristics: no role of IR in PEG-IFN/RBV treatment outcomes was reported. 

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**Fig. 3.** Mean HOMA values before and after PEG-IFN/RBV treatment according to the treatment response and the HCV genotype.

**Fig. 4.** Rates of de novo IR according to the HCV genotype and the treatment outcome.
Interestingly, a recent meta-analysis has shown that in all studies failing to demonstrate an impact of the HOMA score on SVR rates, the mean HOMA values were <3.33 This suggests not only that the HOMA score has a predictive value for treatment outcomes mainly in patients enriched with concomitant metabolic comorbidities but also that metabolic IR (rather than HCV-induced IR) may be the actual reason that more patients with IR have failed to achieve SVR in many studies.24 This would explain why we failed to observe any role of baseline IR in the treatment outcomes of our patients, who were characterized by low rates of overweight, hypertension, and increased triglyceride counts. Moreover, it would also at least in part explain why two randomized studies using metformin and pioglitazone as an adjuvant therapy with PEG-IFN and RBV in IR patients failed to show any improvement in SVR rates versus the rates of placebo-treated patients, even with a significant on-treatment reduction in HOMA levels.34,35

We do acknowledge that our study has several limitations. First, our findings need external validation, possibly in other ethnic groups enriched by patients with metabolic risk factors. Also, because of the relatively small sample size of this study, which was initially powered to compare the efficacy of the two PEG-IFNs, we are unable to provide any information about clinical or genetic factors associated with IR development in the presence of SVR. Moreover, we are aware that the HOMA score has some limitations and provides only an indirect, surrogate measurement of IR and that the gold standard for the determination of IR is the hyperinsulinemic-euglycemic clamp.36,37 The limits of the testing in our study were highlighted by the relatively high rate of IR development that we observed during the posttreatment follow-up visit (14%) versus the baseline rate of IR that had developed over a mean of 16 years of HCV infection (12%). This was likely the result of defining IR by a predefined HOMA score cutoff (≥2), which might have misclassified some patients because of physiological fluctuations in serum insulin levels. Moreover, we had no means of excluding a potential selection bias from the original study because it could be that the patients enrolled in the MIST study were those most likely to develop IR and severe fibrosis during the natural course of the disease, and this contributed to the high rate of IR development in patients experiencing treatment failure.

Still, we think that the major drawback of our study is that our demonstration of SVR preventing IR development does not by default translate into any clinically meaningful benefit for patients. In fact, because of the relatively short follow-up period, we cannot demonstrate a positive impact of SVR on strong endpoints such as a reduction in the incidence of T2DM or cardiovascular events because only three patients developed T2DM and no cardiovascular events were observed in the follow-up period. However, conducting an ad hoc study for these endpoints would imply keeping HCV patients who are experiencing treatment failure under observation for many years; this is unethical because of the high SVR rates that can be currently achieved in these patients with regimens based on directly acting antiviral agents.38,39

In conclusion, this demonstration of reduced rates of IR after successful treatment with PEG-IFN/RBV in patients with chronic HCV provides further evidence that SVR is not merely a surrogate marker of efficacy but is an actual aim to pursue in most (if not all) HCV-infected patients.

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