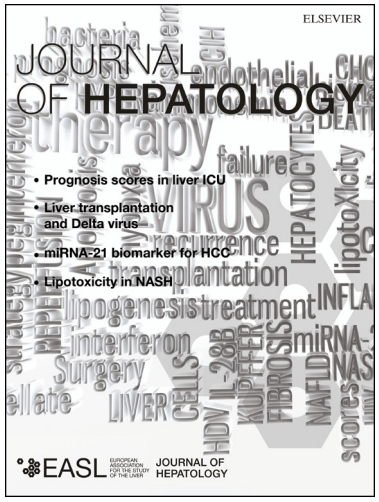
Accepted Manuscript



Chronic HCV Infection Affects Cardiac Mass in Normotensives

Perticone Maria, Miceli Sofia, Maio Raffaele, Caroleo Benedetto, Sciacqua Angela, Tassone Eliezer Joseph, Greco Laura, Staltari Orietta, Sesti Giorgio, Perticone Francesco

PII: S0168-8278(14)00379-1
DOI: http://dx.doi.org/10.1016/j.jhep.2014.05.032
Reference: JHEPAT 5184

To appear in: *Journal of Hepatology*

Received Date: 10 March 2014
Revised Date: 19 May 2014
Accepted Date: 20 May 2014

Please cite this article as: Maria, P., Sofia, M., Raffaele, M., Benedetto, C., Angela, S., Joseph, T.E., Laura, G., Orietta, S., Giorgio, S., Francesco, P., Chronic HCV Infection Affects Cardiac Mass in Normotensives, *Journal of Hepatology* (2014), doi: http://dx.doi.org/10.1016/j.jhep.2014.05.032

CHRONIC HCV INFECTION AFFECTS CARDIAC MASS IN NORMOTENSIVES

*[1]Perticone Maria, MD, *[2]Miceli Sofia, MD, [2]Maio Raffaele, MD, [2]Caroleo Benedetto, MD, [2]Sciacqua Angela, MD, [2]Tassone Eliezer Joseph, MD, [2]Greco Laura, MD, [3]Staltari Orietta, MD, [2]Sesti Giorgio, MD, [2]Perticone Francesco, MD

* These Authors equally contributed to the work

[1]Department of Experimental and Clinical Medicine
University *Magna Græcia* of Catanzaro, Italy

[2]Department of Medical and Surgical Sciences
University *Magna Græcia* of Catanzaro, Italy

[3]Department of Health Sciences
University *Magna Græcia* of Catanzaro, Italy

Address correspondence to:
Francesco Perticone, MD
Department of Medical and Surgical Sciences
Campus Universitario di Germaneto, V.le Europa
88100 - Catanzaro (Italy)
Phone: +39.0961.3647149 Fax: +39.0961.3647634 e-mail: perticone@unicz.it

Word count:4252

Number of figures: 2

Number of tables: 3

List of abbreviations

LVH = Left ventricular hypertrophy

HCV = Hepatitis C Virus

LVM = Left ventricular mass

HOMA = homeostasis model assessment

LVMI = left ventricular mass index

IR = insulin resistance

T2DM = type-2 diabetes mellitus

BP = blood pressure

SBP = systolic blood pressure

DBP = diastolic blood pressure

LDL = low density lipoprotein

HDL = high density lipoprotein

CV = coefficient of variation

Authors have no conflict of interest to disclose

Authors have no received financial support

Keywords: chronic C hepatitis, left ventricular mass, insulin resistance, cardiovascular risk, hypertension.

Abstract

Background and Aims: Left ventricular hypertrophy (LVH), is an independent predictor for cardiovascular events. We investigated if chronic hepatitis C virus (HCV) infection and the related insulin resistance (IR)/hyperinsulinemia could influence the increase of left ventricular mass (LVM).

Methods: We enrolled 260 outpatients matched for age, body mass index, gender, ethnicity: 52 with never-treated uncomplicated chronic HCV infection (HCV^+), 104 never-treated hypertensives (HT) and 104 healthy subjects (NT). LVM was calculated according to the Devereux formula and indexed for body surface area. The following laboratory parameters were measured: fasting plasma glucose and insulin, total, LDL- and HDL-cholesterol, triglyceride, creatinine, e-GFR-EPI, HOMA. Quantitative HCV-RNA was assessed by PCR.

Results: HCV^+ patients with respect to healthy normotensive subjects had an increased LVMI (100 ± 23 vs 83 ± 15 g/m^2; P<0.0001), similar to that observed in HT group (103 ± 25 g/m^2). Regarding biochemical variables, HCV^+ patients, in comparison with normotensive healthy subjects, had higher triglyceride, creatinine, fasting insulin and HOMA (3.2 ± 1.3 vs 2.5 ± 1.0; P<0.0001). At linear regression analysis, the correlation between LVMI and HOMA was similar in HT (r= 0.528, P<0,0001) and HCV^+ (r= 0.489, P<0,0001) groups. At multiple regression analysis, HOMA resulted the major determinant of LMVI in all groups, explaining respectively 21.8%, 27.8% and 23.9% of its variation in NT, HT and HCV^+. At correlational analysis HCV-RNA and HOMA demonstrated a strong and linear relationship between them, explaining the 72.4% of their variation (P=0.022).

Conclusions: We demonstrated a significant and direct correlation between HOMA and LVMI in patients with chronic HCV infection, similar to that observed in hypertensives.

Introduction

Hepatitis C virus (HCV) infection is one of the major causes associated with chronic liver disease, affecting over 3% of world population. The majority of these subjects (90%) progress to chronic hepatitis C inducing both liver fibrosis and cirrhosis [1]. In addition, there are several evidences demonstrating that HCV infection is associated with some metabolic alterations, such as insulin resistance (IR) and new onset of type-2 diabetes mellitus (T2DM). In fact, several epidemiological and experimental data clearly demonstrates that HCV, operating by different pathogenetic mechanisms, is able to alter glucose metabolism [2-10]. In keeping with this, IR is already increased in the early stages of HCV-related liver disease [3]. A possible explanation of this consists in the fact that HCV infection is able to alter glucose homeostasis through some direct and indirect mechanisms, leading to both hepatic and extra-hepatic IR [11,12].

Consequently, despite a favourable lipid profile, the cardiovascular risk of HCV^+ patients is moderately increased, as a consequence of the presence of subclinical atherosclerotic organ damage [6,13-16]. On the other hand, there are several evidences demonstrating that insulin signalling influences, through an interaction with the renin-angiotensin-aldosterone system [17-19], cardiac growth and the development of LVH [20-22] that is recognized as an independent predictor for cardiovascular events in such conditions as hypertension [23], diabetes [20], chronic kidney disease [24], as well as in general population [25].

At present, no information exists regarding a possible association between HCV infection and cardiac mass increase. Therefore, we designed the present study with the aim to investigate the effects of IR/hyperinsulinemia HCV-related on the development of cardiac hypertrophy in a group of subjects with a history of never-treated uncomplicated chronic HCV infection (HCV^+) in comparison with both never-treated hypertensives (HT) and healthy subjects (NT).

Methods

Study population

To test our hypothesis we designed a case-control study involving patients evaluated at the University Hospital of Catanzaro. We recruited 52 HCV^+ normotensive Caucasian outpatients (40 males and 12 females, mean age 48.73±10.4 years). They were matched for age, body mass index and gender in a 1:2:2 ratio with 208 subjects participating to the CAtanzaro MEtabolic RIsk factors Study (CATAMERIS) (26), 104 never treated HT (77 males and 27 females, mean age 48.5±9.7 years) and 104 NT (79 males and 25 females, mean age 48.8±11.2 years). At the time of the first evaluation, both HCV^+ and hypertensive patients were untreated with antiviral therapy or antihypertensive drugs. Secondary forms of hypertension were excluded by systematic testing by a standard clinical protocol including renal ultrasound studies, computed tomography, renal scan, catecholamine, plasma renin activity and aldosterone measurements. Other exclusion criteria were T2DM detected by an oral glucose tolerance test, according to ADA guidelines; history or clinical evidence of angina, myocardial infarction, valvular heart disease, cardiomyopathy, heart failure or peripheral vascular disease; administration of any drugs interfering with glucose metabolism; kidney, thyroid, endocrine and advanced liver diseases, transplanted patients, history of malignant disease. We collected measurements of height and weight according to a standardized protocol, and body mass index was calculated as kilograms per square meter. The Ethical Committee approved the protocol and informed written consent was obtained from all participants. All the investigations were performed in accordance with the principles of the Declaration of Helsinki.

Blood pressure measurements

Readings of clinic blood pressure (BP) were obtained in the left arm of the supine patients, after 5 min of quiet rest, with a mercury sphygmomanometer. Minimum three BP readings were taken on three separate occasions at least 2 weeks apart. Systolic and diastolic BP was recorded at the first appearance (phase I) and the disappearance (phase V) of Korotkoff sounds. Baseline BP values were the average of the last two of the three consecutive measurements obtained at intervals of 3

minutes. Patients with a clinic systolic BP (SBP) >140mmHg and/or diastolic BP (DBP) >90mmHg were defined as hypertensive.

Laboratory determinations

All laboratory measurements were performed after 12 h of fasting. Plasma glucose was determined immediately by the glucose oxidation method [Glucose analyzer, Beckman Coulter, Milan; intra-assay coefficient of variation (CV) 2.2%, inter-assay CV 3.8%]. Serum insulin was determined in duplicate by a highly specific radioimmunoassay using two monoclonal antibodies; intra-assay CV 2.1%, inter-assay CV 2.9%. IR was estimated by homeostasis model assessment ($HOMA_{IR}$) according to the following equation: HOMA=[insulin (μU/ml * glucose (mmol/l)]/22.5 [27]. Total, low-density lipoprotein- (LDL), and high-density lipoprotein- (HDL) cholesterol and triglyceride concentrations were measured by enzymatic methods (Roche Diagnostics GmbH, Mannheim, Germany). Creatinine was measured by using Jaffe methodology. Values of estimated glomerular filtration rate (mL/min/1.73m^2) were calculated by using the equation proposed by investigators in the chronic kidney disease epidemiology (CKD-EPI) collaboration [28]. Quantitative HCV-RNA was assayed by a real-time polymerase chain reaction (PCR) assay.

Echocardiographic measurements

Tracings were taken 24-48 hours after laboratory/clinical determinations with patients in a partial left decubitus position using a VIVID-7 Pro ultrasound machine (GE Technologies, Milwaukee, WI) with an annular phased array 2.5-MHz transducer. Echocardiographic readings were made in random order by the investigator, who had no knowledge of patients' BP and other clinical data. Only frames with optimal visualization of cardiac structures were considered for reading. The mean values from at least five measurements of each parameter for each patient were computed. Having the same experienced sonographer (SM) performing all studies in a dimly lit and quiet room optimized the reproducibility of measurements. In our laboratory, the CVs were 3.85% for posterior

wall thickness, 3.70% for interventricular septum thickness, 1.50% for left ventricular internal diameter, and 5.10% for LVM.

M-mode measurements

Tracings were recorded under two-dimensional guidance, and M-mode measurements were taken at the tip of the mitral valve or just below. Measurements of interventricular septum thickness, posterior wall thickness, and left ventricular internal diameter were made at end-diastole and end-systole. LVM was calculated using the Devereux equation [29] and normalized by body surface area [LVM index (LVMI)].

Statistical analysis

ANOVA for continuous clinical and biological data was performed to test the differences among groups, and the Bonferroni post-hoc test for multiple comparisons was further performed; for dichotomic variables we used the χ^2 test. Data are expressed as mean±SD, and binary data as percent frequency. Correlation coefficients were calculated according to Pearson's method. The independent relationship between LVMI and HOMA was investigated by univariate and multiple linear regression analysis, in the whole study population and in the three groups separately. In the multivariate model we inserted only HOMA to avoid a possible colinearity with fasting glucose and insulin. To compare the effect of a fixed increase in HOMA (1 unit) on LVMI in NT, HT and HCV^+ patients, we performed a covariance analysis, crude and adjusted for all variables significantly different among groups (Table 1). The effect of the patient's status, on HOMA-LVMI relationship, was assessed adding into the same linear regression model HOMA, patient's status (NT, HT and HCV^+), the interaction term of these two variables, and all variables significantly different in the study groups. The estimated increase in LVMI, indicated by a fixed increase in HOMA, was then derived by the slope of the regression line of the HOMA -LVMI link fitted to the three study groups. The multiple linear regression analysis of LVMI in the three study groups separately was

183 performed by a stepwise approach in order to construct parsimonious models. Differences were
184 assumed to be significant at $P<0.05$. All calculations were done with a standard statistical package
185 (SPSS for Windows version 16.0, Chicago, IL, USA).
186
187 **Results**
188 *Study population*
189 Clinical and laboratory characteristics of the study population are reported in Table 1. Notably,
190 HCV^+ patients, with respect to healthy normotensive subjects, had an increased LVMI (100 ± 23 vs
191 83 ± 15 g/m^2; $P<0.0001$), similar to that observed in HT group (103 ± 25 g/m^2) (Figure 1). In addition,
192 regarding biochemical variables, HCV^+ patients, in comparison with NT healthy subjects, had
193 higher triglyceride, creatinine, fasting insulin and HOMA. Of interest, no differences were found in
194 HOMA values between HT and HCV^+(3.2 ± 1.3 vs 3.3 ± 1.3; $P=0.651$) patients. The mean value of
195 HCV-RNA was 3868 ± 2963 x 10 ^3 (UI/ml) in HCV^+.
196
197 *Correlational analysis*
198 A linear regression analysis was performed to test the correlation between LMVI and different
199 covariates in the whole study population and in the three groups (Table 2). LVMI, in the whole
200 study population, was significantly correlated with SBP, DBP, pulse pressure, triglyceride, fasting
201 insulin, HOMA, and inversely correlated with HDL-cholesterol. In HT and HCV^+ groups LVMI
202 resulted statistically correlated with HOMA and fasting insulin. In addition, in the HT group, as
203 expected, the other covariates correlated with LVMI were SBP, pulse pressure, creatinine and
204 estimated glomerular filtration rate. In HCV^+ patients, instead, only viral load ($r=0.378$; $P=0.003$)
205 and triglyceride were significantly correlated with cardiac mass. Finally, the correlational analysis
206 between HCV-RNA and HOMA demonstrated a strong and linear relationship between them,
207 explaining the 72.4% of their variation ($P=0.022$).

A stepwise multivariate linear regression model was performed to evaluate the independent predictors of LVMI in all population, in which we also added the HCV status as independent covariate, and in the three groups separately (Table 3). In the whole population, as well as in HT and HCV^+ groups, HOMA was the major predictor of LVMI, explaining 21.8%, 27.8% and 23.9% of its variation, respectively. In the whole population, other independent predictors were SBP and HCV status, explaining respectively another 4.9% and 2.9% of LMVI variation; in HT group, SBP adds another 6.9% in the LVMI variation.

HOMA index and LVMI: a covariance analysis in the three study groups

To compare the effect of a fixed increase in HOMA (1 unit of increase) on LVMI in NT, HT and HCV^+ patients, we performed a covariance analysis, either crude or adjusted for all variables that significantly differed among the three study groups (see Table 1). On crude analysis (Figure 2, left panel), there was no relationship between the HOMA and LVMI in NT (r=0.08), so that 1 unit of increase in HOMA was associated to a very low and largely not significant increase in LVMI (+1.1 g/m^2; P=0.42). Notably, the same increase in HOMA (1 unit) was related to a marked increase in LVMI in HT (+9.6 g/m^2; P<0.001), a value higher than that observed in NT (P<0.01) but very close to that observed in HCV^+ patients (+8.1 g/m^2; P=0.001), suggesting that IR is a common pathogenetic pathway leading to left ventricular increase in HT and HCV^+ patients. A covariance analysis adjusted for all variables that significantly differed among the three study groups provided similar results (Figure 2, right panel). In fact, 1 unit of increase in HOMA was not associated to LVMI in NT (+2.0 g/m^2; P=0.18) but again the effect on LVMI of such an increase was very close in HT (+8.9 g/m^2; P<0.001) and HCV^+ (+6.4 g/m^2; P=0.008) patients.

Discussion

The results of this study demonstrate, for the first time, that HCV^+ normotensive patients, in comparison with healthy normotensive subjects, have a significant increase in echocardiographic

234 cardiac mass, totally similar to that observed in hypertensive patients. These findings have clinical
235 relevance because contribute to expand previous knowledge about the pathogenetic mechanisms
236 underlying the high prevalence of cardiovascular morbidity and mortality in this setting of patients.
237 In fact, there are consolidated evidences demonstrating that LVH is a powerful and independent
238 predictor of fatal and non-fatal cardiovascular events in general population [25], as well as in other
239 clinical conditions [18,23,24]. Obviously, since cardiovascular events were not prospectively
240 evaluated in this study, our results do not consent to consider LVM as an independent predictor of
241 cardiovascular events also in patients with liver disease.
242 Present data are not surprising because cardiac mass growth recognizes, beyond pressure overload
243 that explains only 15-20% of its increase, other several pathogenetic mechanisms interacting
244 between them in a multiplicative manner. In keeping with this, growing evidences attribute to the
245 proliferative effects of insulin, as demonstrated also by us [19,30], an important role in the
246 development of LVH [30]. Thus, it is plausible to consider IR/hyperinsulinemia, detected in HCV^+
247 patients, as the most important pathogenetic mechanism operating in the increase of LVM, similar
248 to what observed in hypertensive patients. In fact, previous findings have clearly demonstrated that
249 patients with high BP show a condition of IR, mainly attributable to the activation of the renin-
250 angiotensin-aldosterone system and sympathetic activity [18,19,32]. The biological plausibility of
251 this finding is supported by present results demonstrating that HOMA was retained in the muliple
252 regression analysis as the major determinant of LVM increase in HT and HCV^+ patients, explaining
253 the 21.8% and 27.8% of its variation, respectively. Previously published data demonstrated a strict
254 relationship between HCV infection and the development of IR, through a direct interaction
255 between viral products and insulin signaling pathway via IRS-1-PI3-kinase-Akt [4,6,33].
256 Interestingly, eradication of HCV by antiviral therapy induced a significant improvement in insulin
257 sensitivity reducing the risk of incident T2DM [6,34,35], emphasizing the importance of viral
258 infection. In this context, another important open question remains the association of HCV viral
259 load and the severity of IR [36]. Remarkably, our data demonstrate a significant strong relationship

between viral load and both IR and LVM, thus confirming the close association among HCV infection, metabolic alterations and subclinical organ damage. Moreover, it is important to remark the extra-hepatic effects of HCV-related IR/hyperinsulinemia, that are able to activate proinflammatory and proliferative pathways promoting LVM increase [31,37]. In fact, insulin is able to suppress, in subjects with normal insulin sensitivity, several pro-inflammatory transcription factors and activating protein-1 (AP-1) and the corresponding genes regulated by them, which mediate inflammation; on the contrary, the condition of IR results in the activation of these pro-inflammatory transcription factors and an increase in the expression of the corresponding genes [19,38]. Thus, in patients with chronic hepatitis C, the direct interactions between viral products and insulin-signaling pathways contributes to the development of IR, so leading to the activation of proliferative mechanisms resulting in the increase of LVM.

The effects of insulin on cardiac mass are multiple. It binds and activates the IGF-1 receptor resulting in increased DNA, protein synthesis and cell proliferation [39-41], stimulates sympathetic nervous system activity [32] which affects ventricular structure directly and indirectly, by increasing heart rate and BP. Finally, the activation of the renin-angiotensin-aldosterone system worsens insulin sensitivity promoting, via sympathetic system stimulation, the production of angiotensin II that, in turn, amplifies the proliferative effects of both systems. In our study, the trophic effects of insulin as well as its effects on sodium reabsorption, mediated by the renin-angiotensin-aldosterone system [42], are probably responsible for the increase of end-diastolic left ventricular internal diameter present in HCV^+ patients in comparison with normotensive health subjects, and similar to that observed in the hypertensive group.

In conclusion, the most relevant finding of this study is the evidence of a significant and direct correlation between HOMA and LVMI in patients with chronic HCV infection, similar to what observed in hypertensive patients. Thus, IR/hyperinsulinemia HCV-related, by affecting cardiac remodeling, suggests considering chronic HCV infection as a possible new factor in the global cardiovascular risk burden. At this point arises the question whether it is necessary to

treat IR in HCV^+ patients not only to prevent progression to chronic fibrosis, T2DM and/or hepatocellular carcinoma and/or to improve the response to antiviral treatment, but also to prevent cardiac damage. So it seems reasonable to stratify cardiovascular risk in HCV^+ subjects not only with an oral glucose tolerance test and/or fasting insulin but also investiganting the possible subclinical organ damage. In future, large prospective studies should be performed in order to confirm the association of LVM with cardiovascular events in HCV^+ patients. This point is of clinical relevance because in the multivariate regression analysis, performed in the whole population, HCV infection was retained as third independent predictor of cardiac mass increase, in addition to HOMA and systolic BP, explaining about a 3% of its variation.

Study limitations

Even if there are evidences demonstrating an association between liver fibrosis and insulin sensitivity (43,44), in this study we did not perform liver biopsy because the diagnosis of HCV infection has been made on the basis of clinical and laboratory data, as recommended by the Guidelines. In addition, the purpose of our study was not to investigate whether different degrees of liver disease are associated with different degrees of insulin resistance; rather, the purpose was to identify markers of subclinical organ damage contributing to explain the high cardiovascular morbidity and mortality documented in these patients.

Acknowledgements: Authors have no conflict of interest to disclose

References

[1] Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41-52.

[2] Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann Internal Medicine 2000;133:592–599.

[3] Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, et al. Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol 2001;35:279-283.

[4] Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, et al. Hepatitis C virus infection and diabetes: Direct involvement of the virus in the development of insulin resistance. Gastroenterology 2004;126:840–848.

[5] Kaddai V, Negro F. Current understanding of insulin resistance in hepatitis C. Expert Rev Gastroenterol Hepatol 2011;5:503–516.

[6] Bugianesi E, Salamone F, Negro F. The interaction of metabolic factors with HCV infection: does it matter? J Hepatol 2012;56 (suppl 1):S56-65.

[7] Hsu CS, Liu CJ, Liu CH, Wang CC, Chen CL, Lai MY, et al. High hepatitis C viral load is associated with insulin resistance in patients with chronic hepatitis C. Liver Int 2008;28:271-277

[8] Douglas MW, George J. Molecular mechanisms of insulin resistance in chronic hepatitis C. World J Gastroenterol 2009;15:4356-4364

[9] Del Campo JA, Romero-Gómez M. Steatosis and insulin resistance in hepatitis C: a way out for the virus? World J Gastroenterol 2009;15:5014-5019.

[10] Parvaiz F, Manzoor S, Tariq H, Javed F, Fatima K, Qadri I. Hepatitis C virus infection: molecular pathways to insulin resistance. Virology Journal 2011;8:474.

[11] Vanni E, Abate ML, Gentilcore E, Hickman I, Gambino R, Cassader M, et al. Sites and mechanisms of insulin resistance in nonobese, nondiabetic patients with chronic hepatitis C. Hepatology 2009;50(3):697-706

[12] Milner KL, van der Poorten D, Trenell M, Jenkins AB, Xu A, Smythe G, et al. Chronic hepatitis C is associated with peripheral rather than hepatic insulin resistance. Gastroenterology 2010;138(3):932-941.

[13] Butt AA, Xiaoqiang W, Budoff M, Leaf D, Kuller LH, Justice AC. Hepatitis C virus infection and the risk of coronary disease. Clin Infect Dis 2009;49:225-232.

[14] Völzke H, Schwahn C, Wolff B, Mentel R, Robinson DM, Kleine V, et al. Hepatitis B and C virus infection and the risk of atherosclerosis in a general population. Atherosclerosis 2004;174:99–103.

[15] Targher G, Bertolini L, Padovani R, Rodella S, Arcaro G, Day C. Differences and similarities in early atherosclerosis between patients with non-alcoholic steatohepatitis and chronic hepatitis B and C. J Hepatol 2007;46:1126– 1132.

[16] Mostafa A, Mohamed MK, Saeed M, Hasan A, Fontanet A, Godsland I, et al. Hepatitis C infection and clearance: impact on atherosclerosis and cardiometabolic risk factors. Gut 2010;59:1135–1140.

[17] Samuelsson AM, Bollano E, Mobini R, Larsson BM, Omerovic E, Fu M, et al. Hyperinsulinemia: effect on cardiac mass/function, angiotensin II receptor expression, and insulin signaling pathways. Am J Physiol Heart Circ Physiol. 2006;291:H787-796.

[18] Perticone F, Ceravolo R, Iacopino S, Cloro C, Ventura G, Maio R, et al. Relationship between angiotensin-converting enzyme gene polymorphism and insulin resistance in never-treated hypertensive patients. J Clin Endocrinol Metab 2001;86:172-178.

[19] Ceravolo R, Maio R, Cuda G, Scozzafava A, Sciacqua A, Vatrano M, et al. Relation of fasting insulin related to insertion/deletion polymorphism of angiotensin-converting enzyme-gene and cardiac mass in never-treated patients with systemic hypertension. Am J Cardiol 2003;92:1234-1237.

[20] Ilercil A, Devereux RB, Roman MJ, Paranicas M, O'grady MJ, Welty TK, et al. Relationship of impaired glucose tolerance to left ventricular structure and function: The Strong Heart Study. Am Heart J 2001;141:992-998.

[21] Yu W, Chen C, Fu Y, Wang X, Wang W. Insulin signaling: a possible pathogenesis of cardiac hypertrophy. Cardiovasc Ther 2010;28:101-105.

[22] Miceli S, Maio R, Perticone M, Tripepi G, Sciacqua A, Mazzaferro D, et al. Creatinine and insulin predict cardiac mass in drug-naïve hypertensive patients. Int J Cardiol. 2013; 167:519-524.

[23] Schillaci G, Verdecchia P, Porcellati C, Cuccurullo O, Cosco C, Perticone F. Continuous relation between left ventricular mass and cardiovascular risk in essential hypertension. Hypertension. 2000;35:580-586.

[24] Perticone F, Maio R, Ruberto C, Cassano S, Tripepi G, Perticone M, et al. Kidney function and risk factors for left ventricular hypertrophy in untreated uncomplicated essential hypertension. Am J Kidney Dis. 2008;52:74-84.

[25] Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. N Engl J Med 1990;322:1561–1566.

[26] Succurro E, Marini MA, Arturi F, Grembiale A, Lugarà M, Andreozzi F, et al. Elevated one-hour post-load plasma glucose levels identifies subjects with normal glucose tolerance but early carotid atherosclerosis. Atherosclerosis 2009;207:245-9.

[27] 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.

[28] Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604-612.

[29] Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986;57:450-458.

[30] Sesti G, Sciacqua A, Scozzafava A, Scozzafava A, Vatrano M, Angotti E, et al. Effects of growth hormone and insulin-like growth factor-1 on cardiac hypertrophy of hypertensive patients. J Hypertens 2007;25:471-477.

[31] Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome. A comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 2005;111:1448-1454.

[32] Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities. The role of insulin resistance and the sympathoadrenal system. N Engl J Med 1996;334:374-381.

[33] Biddinger SB, Kahn CR. From mice to men: insights into the insulin resistance syndromes. Ann Rev Physiol 2006;68:123–158.

[34] Kawaguchi T, Ide T, Taniguchi E, Hirano E, Itou M, Sumie S, 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.

[35] Arase Y, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Kawamura Y, et al. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. Hepatology 2009;49:739–744.

[36] Alaei A, Negro F. Hepatitis C virus and glucose and lipid metabolism. Diabetes Metabol 2008;34:692-700.

[37] Takimoto E, Kass DA. Role of oxidative stress in cardiac hypertrophy. Hypertension 2007;49:241-248.

[38] Aljada A, Ghanim H, Mohanty P, Kapur N, Dandona P. Insulin inhibits the pro-inflammatory transcription factor early growth response gene-1 (Egr)-1 expression in mononuclear cells

(MNC) and reduces plasma tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) concentrations. J Clin Endocrinol Metab 2002;87:1419–1422.

[39] Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. Endocr Rev 2007;28:463-91.

[40] Ito H, Hiroe M, Hirata Y, Tsujino M, Schirichi M. Insulin-like growth factor-I induces cardiac hypertrophy with enhanced expression of muscle-specific genes in cultured rat cardiomyocytes. Circulation 1993;87:1715.

[41] Rannels DE, Kao R, Morgan HE. Effect of insulin on protein turnover in heart muscle. J Biol Chem 1975;250:1694–701.

[42] De Fronzo RA. The effect of insulin on renal sodium metabolism. A review with clinical implications. Diabetologia 1981;21:165-171.

[43] Sud A, Hui JM, Farrell GC, Bandara P, Kench JG, Fung C, et al. Improved prediction of fibrosis in chronic hepatitis C using measures of insulin resistance in a probability index. Hepatology 2004;39:1239-47.

[44] Younossi Z, Negro F, Serfaty L, Pol S, Diago M, Zeuzem S ei al. Homeostasis model assessment of insulin resistance does not seem to predict response to telaprevir in chronic hepatitis C in the REALIZE trial. Hepatology 2013;58:1897-906.

425 **Table 1 – Demographic, clinical, humoral and echocardiographic data of the study population**

426 **and of the three groups separately**

	All (n = 260)	NT (n = 104)	HT (n = 104)	HCV+ (n = 52)	P value
Age, *years*	48.6±10.4	48.8±11.1	48.5±9.7	48.7±10.4	0.972
Sex, *M/F*	196/64	79/25	77/27	40/12	0.883**
BMI, *Kg/m²*	27.8±4.5	28.0±5.5	27.8±4.3	27.4±2.6	0.742
WC, *cm*	95.8±8.3	95.0±9.4	95.34±8.4	96.0±5.3	0.347
Current smokers, *n (%)*	73 (28)	15 (14)	29 (28)	29 (56)	0.0001**
Systolic BP, *mmHg*	134±15	126±12	147±9*	124±8§	0.0001
Diastolic BP, *mmHg*	81±10	77±8	90±6*	74±7#§	0.0001
PP, *mmHg*	52±11	49±10	57±11*	50±8§	0.0001
Heart rate, *bpm*	68±9	69±10	69±8	66±7	0.086
Total cholesterol, *mg/dl*	190±32	187±30	195±31	187±33	0.206
LDL-cholesterol, *mg/dl*	122±32	115±29	131±31*	118±35§	0.0001
HDL-cholesterol, *mg/dl*	44±10	49±10	40±9*	40±8#	0.466
Triglyceride, *mg/dl*	121±36	115±38	115±33	143±30#§	0.0001
Creatinine, *mg/dl*	0.72±0.1	0.72±0.1	0.76±0.1	0.81±0.1#§	0.0001
e-GFR, *ml/min/1.73 m²*	106±11	107±11	105±12	103±10	0.101
Fasting glucose, *mg/dl*	93±10	92±10	92±11	94±11	0.479
Fasting insulin, *U/ml*	13.1±5.4	11.2±4.5	14.5±5.6*	14.1±5.5#	0.0001
HOMA	3.0±1.3	2.5±1.0	3.3±1.3*	3.2±1.3#	0.0001
EDLVD, *cm*	4.8±0.4	4.70±0.4	4.9±0.5*	4.9±0.4#	0.006
LVMI, *g/m²*	94±23	83±15	103±25*	100±23#	0.0001

427 * = P<0.05 by Bonferroni HT vs NT; # = P<0.05 by Bonferroni HCV+ vs NT; § = P<0.05 by

428 Bonferroni HCV+ vs HT; ** chi-square test was used for dicotomic variables

BMI = body mass index; **WC** = waist circumference; **BP** = blood pressure; **PP** = pulse pressure; **LDL** = low density liporpotein; **HDL** = high density lipoprotein; **e-GFR** = estimated glomerular filtration rate; **HOMA** = homeostasis model assessment; **LVMI** = left ventricular mass index; **EDLVD** = end-diastole left ventricular diameter.

Table 2 - Linear regression analysis between LVMI and different covariates in the whole study population and in the three groups

	All n=260		**NT n=104**		**HT n=104**		**HCV$^+$ n=52**	
	R	*P*	*R*	*P*	*r*	*P*	*r*	*P*
Age, years	-0.012	0.426	-0.012	0.426	0.282	0.672	0.151	0.143
BMI, *kg/m^2*	0.005	0.468	0.005	0.468	-0.158	0.054	0.173	0.078
Systolic BP, *mmHg*	0.289	<0.0001	-0.117	0.118	0.326	<0.0001	0.153	0.139
Diastolic BP, *mmHg*	0.220	<0.0001	0.57	0.283	-0.008	0.469	0.203	0.075
PP, *mmHg*	0.187	0.001	-0.175	0.038	0.255	0.005	0.018	0.450
Heart rate, *bpm*	0.088	0.080	0.154	0.059	0.169	0.043	-0.039	0.393
Total Cholesterol, *mg/dl*	-0.036	0.281	-0.123	0.108	-0.114	0.124	0.042	0.384
LDL-Cholesterol, *mg/dl*	-0.014	0.408	-0.182	0.032	-0.141	0.076	0.042	0.384
HDL-Cholesterol, *mg/dl*	-0.193	0.001	0.053	0.296	-0.008	0.469	-0.201	0.076
Triglyceride, *mg/dl*	0.170	0.003	0.133	0.89	0.127	0.099	0.257	0.033
Creatinine, *mg/dl*	-0.017	0.393	0.005	0.482	-0.259	0.004	0.087	0,270
e-GFR, *ml/min/1.73 m^2*	0.076	0.110	0.237	0.008	0.198	0.022	-0.209	0,069
Glucose, *mg/dl*	-0.021	0.167	-0.095	0.169	-0.068	0.246	0.107	0.226
Insulin, *UI/ml*	*0,501*	<0.0001	0.111	0.131	0.588	0.001	0.479	<0.0001
HOMA	0.467	<0.0001	0.079	0.212	0.528	<0.0001	0.489	<0.0001

BMI = body mass index; **BP** = blood pressure; **PP** = pulse pressure; **LDL** = low density liporpotein; **HDL** = high density lipoprotein; **e-GFR** = estimated glomerular filtration rate; **HOMA** = homeostasis model assessment.

1. Table 3 - Multivariate Analysis of LVMI in the three groups

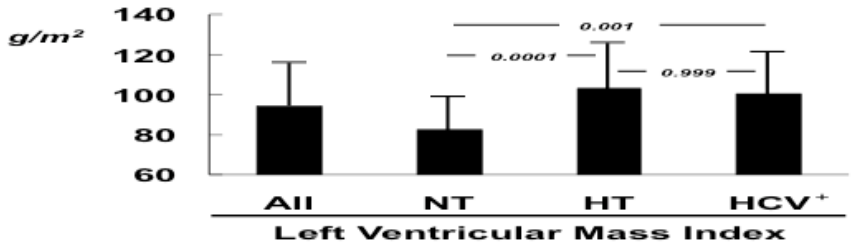
	r^2 partial	r^2 total	*P*
All			
HOMA	21.8	21.8	0.0001
Systolic BP, *mm Hg*	4.9	26.7	0.0001
HCV status, *yes/no*	2.9	29.5	0.001
NT			
e-GFR, *ml/min/1.73 m²*	5.6	5.6	0.015
HT			
HOMA	27.8	27.8	0.0001
Systolic BP, *mm Hg*	6.9	34.7	0.001
HCV⁺			
HOMA	23.9	23.9	0.0001

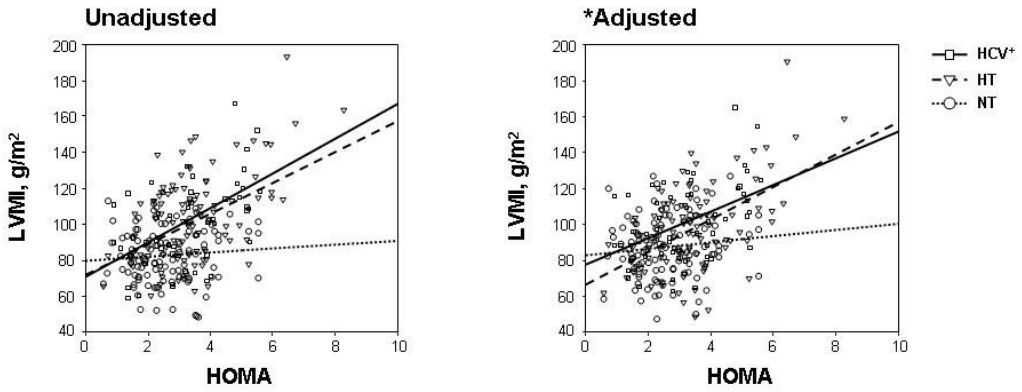
HOMA = homeostasis model assessment; **BP** = blood pressure

FIGURE LEGENDS

Fig 1-LVMI adjusted for SBP, PP, e-GFR, HDL cholesterol and triglyceride

Fig.2- Crude and adjusted analysis for relationship between insulin resistance, expressed as HOMA, and left ventricular mass index (LVMI) in normotensives (NT), hypertensive (HT) and HCV^+ patients.





*Adjusted for smoking, systolic and diastolic blood pressure, LDL- and HDL-cholesterol, and triglyceride