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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Chronic HCV infection was associated with severe insulin resistance and mild atherosclerosis: a population-based study in an HCV hyperendemic area

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Abstract
Background Hepatitis C virus (HCV) affects glucose and lipid metabolism in vitro; however, it is unclear whether HCV infection is associated with insulin resistance and atherosclerosis at the population level. We aimed to investigate this association in a Japanese cohort of the Seven Countries Study, and our investigation was conducted in Tanushimaru, an HCV hyperendemic area.
Methods A total of 1908 inhabitants of Tanushimaru were classified into 3 groups according to HCV infection status: those who were uninfected ($n = 1780$), those with transient infection ($n = 88$), and those with chronic infection ($n = 40$). Insulin resistance and atherosclerosis were evaluated by homeostasis model assessment for insulin resistance (HOMA-IR) and carotid intima–media thickness (IMT), respectively. Intergroup differences in variables were evaluated by age- and sex-matched multivariate regression analysis.
Results Significant intergroup differences were seen in fasting glucose and insulin levels. The HOMA-IR value was significantly higher in the group with chronic infection than the values in the uninfected and transiently infected groups (3.0 ± 0.39 vs. 1.3 ± 0.03 vs. 1.5 ± 0.14; $P < 0.001$). In contrast, low-density lipoprotein (LDL)-cholesterol and triglyceride levels were significantly lower in the group with chronic infection than the levels in the other groups. IMT was reduced in the group with chronic infection, with a significant intergroup difference (0.67 ± 0.02 vs. 0.71 ± 0.003 vs. 0.72 ± 0.01 mm; $P = 0.003$).
Conclusions This population-based study in an HCV hyperendemic area revealed that chronic HCV infection was associated with severe insulin resistance and with mild atherosclerosis, suggesting a unique characteristic of HCV-related metabolic abnormality.

Keywords Hepatitis C virus · Glucose metabolism · Lipid metabolism · Homeostasis model assessment for insulin resistance · Carotid intima–media thickness

Abbreviations

ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APRI	Aspartate aminotransferase to platelet ratio index
AST	Aspartate aminotransferase
HCV	Hepatitis C virus
HDL	High-density lipoprotein
HOMA-IR	Homeostasis model assessment for insulin resistance

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IBW Ideal body weight
IMT Intima–media thickness
LDL Low-density lipoprotein

Introduction

Hepatitis C virus (HCV) is globally distributed, and nearly 180 million people are infected with the virus [1]. Patients with chronic HCV infection are known to develop not only hepatitis, but also various metabolic disorders [2, 3]. We, along with other researchers, have shown that increased insulin resistance is more frequently seen in patients with chronic HCV infection than in those with other hepatobiliary disorders [4–6]. However, these studies were conducted at referral centers, and it is possible that this association could be limited to patients with more severe forms of the disease, or that the association may be caused by various environmental factors that affect the development of insulin resistance [7].

In addition to its possible influence on glucose metabolism, HCV affects lipid metabolism. HCV is associated with low-density lipoprotein (LDL)-like lipoproteins and is transported into hepatocytes [8, 9], and HCV secretion from hepatocytes is associated with triglyceride-rich very low-density lipoproteins (VLDLs) [10, 11]. Recent population-based studies have demonstrated hypolipidemia in subjects with chronic HCV infection [12, 13]. Although altered lipid metabolism is linked to atherosclerosis, the effect of HCV on atherosclerosis remains controversial [13–15].

Since 1978, we have been studying a community-based Japanese cohort of the Seven Countries Study; this cohort lives in Tanushimaru, an HCV hyperendemic area. This focus on a hyperendemic area, because of the similarity of environmental factors, provides a significant advantage in the epidemiological analysis of the impact of chronic HCV infection on metabolic disorders. . In this community, we have previously found distinctive changes in glucose and lipid metabolism [16–18]. The aim of this epidemiological study was to investigate the association of chronic HCV infection with insulin resistance and atherosclerosis in an HCV hyperendemic area.

Patients, materials, and methods

Study subjects

Since 1978, we have been conducting health examinations in an HCV hyperendemic area, where a Japanese cohort of the Seven Countries Study has been surveyed since 1978[16–18]. In 2009, a total of 1943 inhabitants each received a health examination and were enrolled in this study. After excluding 14 inhabitants who refused to undergo anti-HCV screening (Elecys® anti-HCV assay; Roche Diagnostics, Tokyo, Japan) and 21 inhabitants who were found to be positive for hepatitis B surface antigen (HBs Ag) (Elecsys HBsAg II assay; Roche Diagnostics), the remaining 1908 inhabitants were classified into 3 groups according to HCV infection status: those who had never been infected (1780 inhabitants with a negative anti-HCV result; uninfected group), those with transient infection (88 inhabitants with a positive anti-HCV result and a negative HCV-RNA result; transient group), and those with chronic infection (40 inhabitants with a positive result for both anti-HCV and HCV-RNA; chronic group) (Fig. 1).

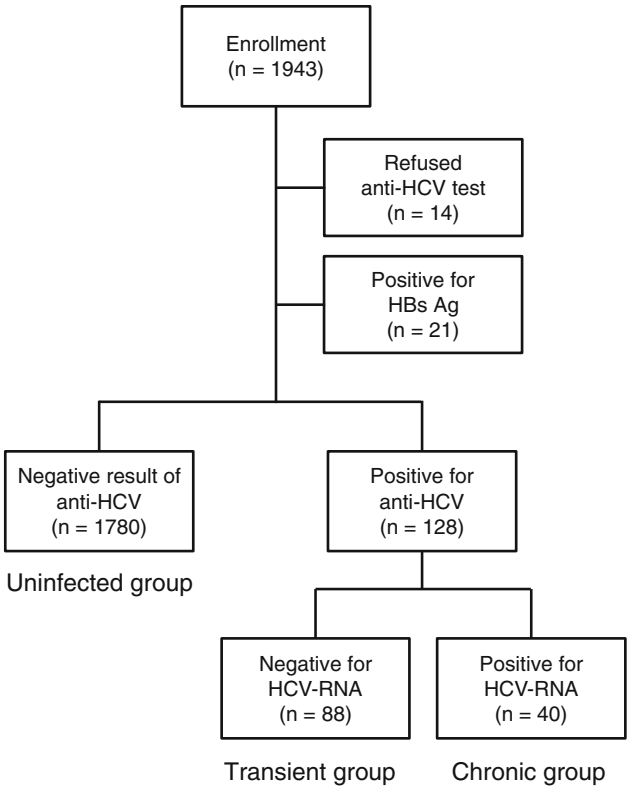

Fig. 1 Study design. *HCV* hepatitis C virus, *HBsAg* hepatitis B surface antigen

The backgrounds of each group were surveyed by interviews. None of the inhabitants in the uninfected group had been diagnosed with chronic liver disease. On the other hand, 1.1 % (1/88) and 57.5 % (23/40) of the inhabitants in the transient and chronic groups, respectively, had previously been found to be infected or had undergone treatment. The percentages of inhabitants who showed an aminotransferase (AST) to platelet ratio index (APRI) of >2, indicating the presence of advanced fibrosis [19, 20], were 0.05 % (1/1780), 0 % (0/88), and 7.5 % (3/40) in the

uninfected, transient, and chronic groups, respectively. A history of hepatocellular carcinoma was found in 0 % (0/1780), 1.1 % (1/88), and 12.5 % (5/40) of the inhabitants in the uninfected, transient, and chronic groups, respectively. A history of ischemic heart disease was found in 4.2 % (75/1780), 10.2 % (9/88), and 0 % (0/40) of inhabitants in the uninfected, transient, and chronic groups, respectively. A history of cerebrovascular disease was found in 3.9 % (69/1780), 4.5 % (4/88), and 2.5 % (1/40) of the inhabitants in the uninfected, transient, and chronic groups, respectively.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected by the approval of the Ethics Committee of the Kurume University School of Medicine. This study was also approved by the Ukiha branch of the Japan Medical Association, by the mayor of Tanushimaru town, and by the welfare section of the Tanushimaru town office. Written informed consent was obtained from each inhabitant who participated in the study.

Data collection

A cross-sectional survey of all participants was conducted in 2009 using the common protocol previously described by Keys et al. [21]. The intake levels of energy, fat, and alcohol were assessed with a validated brief-type, self-administered diet history questionnaire (BDHQ) [22–24]. The BDHQ is a four-page structured questionnaire that consists of questions regarding general dietary behaviors, major cooking methods, the quantity and the frequency of the consumption of alcoholic beverages, the quantity and frequency of consuming 58 selected food and non-alcoholic beverage items, the use of dietary supplements, the amount of rice and miso soup consumed daily, and eating speed. The food and beverage items and portion sizes used in the BDHQ were derived primarily from data in the National Nutrition Survey of Japan and several recipe books for Japanese dishes [25].

The body mass index (BMI) was used as an index of obesity and was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2). Blood pressure was measured twice with a mercury sphygmomanometer, in the right arm, after more than 5 min rest in the supine position. Venous blood samples were taken in the morning after a 12-h overnight fast and were used for complete blood counts, liver function tests, and the measurement of metabolic parameters including total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL-cholesterol, and triglycerides. The extent of liver fibrosis was assessed by APRI [19]. Serum samples were also examined for the presence of anti-HCV antibodies by using the Elecys® anti-HCV assay (Roche Diagnostics), which is a fully automated third-generation anti-HCV antibody screening test with high sensitivity and specificity [26]. In addition, for anti-HCV-positive sera, HCV-RNA was assayed using an HCV RNA PCR kit (COBAS AmpliPrep/COBAS TaqMan HCV Test; Roche Diagnostics).

Evaluation of insulin resistance and atherosclerosis

Plasma glucose levels were measured using the glucose oxidase method. Serum insulin levels were measured using an insulin chemiluminescence immunoassay kit (Chemi-lumi-insulin; Siemens Healthcare Diagnostics, Tokyo, Japan). Insulin resistance was evaluated by the homeostasis model assessment (HOMA-IR) method [27]. The formula is: HOMA-IR = fasting glucose (mg/dL) × fasting insulin (μU/mL)/405.

Atherosclerosis was evaluated by the carotid intima–media thickness (IMT). Examination was performed with an ultrasound scanner equipped with a 10.0-MHz linear-array transducer. Carotid IMT was measured as previously described [28]. For each subject, longitudinal B-mode images at the diastolic phase of the cardiac cycle were recorded. Measurements of IMT were made at 3 levels of the lateral and medial walls 1–3 cm proximal to the carotid bifurcation. These 6 combined near- and far-wall measurements were averaged.

Statistical analysis

Because we focused on the average value of each group and because the number of subjects was different in each group, we expressed all data as means ± SE, in accordance with a previous report [29]. After initial univariate analysis, variables with P values of <0.05 were selected for the initial step of logistic analysis. Finally, one-way factorial analysis of variance (ANOVA) and both age- and sex-adjusted analysis of covariance (ANCOVA) were used to identify independent variables that were related to chronic HCV infection. All statistical analyses were conducted using the SAS system (SAS Institute, Cary, NC, USA). P values of <0.05 were considered significant.

Results

One-way factorial ANOVA

Significant intergroup differences among the uninfected, transient, and chronic groups were seen in age, white blood cell counts, red blood cell counts, platelet counts, AST levels, alanine aminotransferase (ALT) levels, total protein levels, albumin levels, and APRI (Table 1). Although energy intake was not significantly different among the 3 groups, fasting glucose levels, fasting insulin levels, and

Table 1 One-way factorial analysis of variance

Variable	Reference value	Uninfected group	Transient group	Chronic group	*P*
Age (years)		65.4 ± 0.3	70.9 ± 1.1	68.5 ± 1.8	<0.0001
Female/male		1086/694	44/44	23/17	0.11
Height (cm)		156.2 ± 0.2	155.0 ± 1.1	155.9 ± 1.4	0.50
Weight (kg)		57.4 ± 0.3	57.6 ± 1.3	56.9 ± 1.3	0.95
BMI (kg/m^2)	18.5–25	23.4 ± 0.07	23.9 ± 0.4	23.4 ± 0.47	0.46
Energy intake (kcal/day)		1925.9 ± 15.3	2052.1 ± 90.4	1862.2 ± 98.3	0.16
Fat intake (% energy)		23.8 ± 0.1	24.3 ± 0.7	25.4 ± 1.0	0.22
Alcohol intake (g/day)		11.5 ± 0.6	10.8 ± 0.5	4.2 ± 1.4	0.16
Systolic blood pressure (mmHg)		133.3 ± 0.5	136.6 ± 2.0	132.8 ± 2.8	0.29
White blood cell count (/mm^3)	4000–9000	5626.0 ± 36.6	5367.9 ± 139.4	4677.2 ± 287.4	0.0002
Red blood cell count ($\times 10^4$/mm^3)	380–500	442.6 ± 1.1	433.1 ± 5.1	418.6 ± 12.9	0.002
Platelet count ($\times 10^4$/mm^3)	13–36	21.9 ± 0.1	20.4 ± 0.6	15.7 ± 1.1	<0.0001
AST (U/L)	13–33	23.7 ± 0.2	25.8 ± 1.1	48.4 ± 3.7	<0.0001
ALT (U/L)	6–30	20.1 ± 0.3	20.1 ± 1.1	45.9 ± 4.8	<0.0001
γ-Glutamyl transpeptidase (U/L)	10–47	33.8 ± 1.1	32.5 ± 6.7	43.9 ± 5.0	0.38
Total protein (g/dL)	6.70–8.30	7.4 ± 0.01	7.5 ± 0.04	7.7 ± 0.08	<0.0001
Albumin (g/dL)	4.00–5.00	4.4 ± 0.005	4.3 ± 0.02	4.1 ± 0.06	<0.0001
APRI		0.29 ± 0.004	0.35 ± 0.02	1.01 ± 0.15	<0.0001
Fasting plasma glucose (mg/dL)	80–109	95.6 ± 0.4	102.5 ± 3.1	106.7 ± 4.3	<0.0001
Fasting serum insulin (μU/mL)	5.0–15.0	5.5 ± 0.2	6.6 ± 1.1	11.9 ± 3.8	<0.0001
HOMA-IR	<2.5	1.3 ± 0.04	1.7 ± 0.4	3.2 ± 1.4	<0.0001
Total cholesterol (mg/dL)	128–219	210.1 ± 0.8	199.2 ± 3.5	166.8 ± 4.5	<0.0001
HDL cholesterol (mg/dL)	40.0–96.0	60.8 ± 0.4	59.1 ± 1.4	53.1 ± 1.6	0.005
LDL-cholesterol (mg/dL)	<139.0	128.1 ± 0.7	120.6 ± 3.3	97.1 ± 4.7	<0.0001
Triglycerides (mg/dL)	30–149	105.6 ± 1.1	93.7 ± 4.7	87.4 ± 1.4	0.008
Carotid IMT (mm)	<1.0	0.70 ± 0.004	0.75 ± 0.01	0.69 ± 0.02	0.009

Values are expressed as means ± SE. A logarithmic transformation was employed for the analysis of fasting plasma glucose, fasting serum insulin, and HOMA-IR because of skewness

BMI body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *APRI* aspartate aminotransferase to platelet ratio index, *HOMA-IR* homeostasis model assessment for insulin resistance, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein *IMT* intima–media thickness

HOMA-IR values were elevated in the chronic group, with a significant intergroup difference (Table 1). On the other hand, despite there being no significant difference in fat intake among the 3 groups, total cholesterol levels, HDL-cholesterol levels, LDL-cholesterol levels, triglyceride levels, and carotid IMT values were low in the chronic group, with a significant intergroup difference (Table 1).

Age- and sex-adjusted ANCOVA

Similar to the results of one-way factorial ANOVA, intergroup differences were seen in the white blood cell counts, red blood cell counts, platelet counts, AST levels, ALT levels, total protein levels, albumin levels, and APRI on age- and sex-adjusted ANCOVA (Table 2). Furthermore, in the chronic group, significant reductions in white blood cell counts, total protein levels, and albumin levels were seen in the chronic group compared to values in the uninfected and transient groups (Table 2). AST levels, ALT levels, and APRI were also significantly higher in the chronic group compared to values in the other groups (Table 2).

Significant intergroup differences were seen in fasting glucose levels, fasting insulin levels, and HOMA-IR values (Fig. 2a–c). Moreover, the fasting serum insulin levels and HOMA-IR values were significantly higher in the chronic group than the values in the uninfected and transient groups (Fig. 2b, c).

On the other hand, LDL-cholesterol and triglyceride levels were significantly lower in the chronic group than those in the uninfected and transient groups (Fig. 3a, b). A reduction in the carotid IMT value was seen in the chronic group, with a significant intergroup difference (Fig. 3c).

Table 2 Age- and sex-adjusted covariance analysis of differences among the groups

Variable	Reference value	Uninfected group	Transient group	Chronic group	*P*
Height (cm)		156.2 ± 0.1	155.4 ± 0.6	156.5 ± 0.9	0.14
Weight (kg)		57.3 ± 0.21	57.9 ± 0.95	57.4 ± 1.4	0.91
BMI (kg/m^2)	18.5–25	23.4 ± 0.1	23.8 ± 0.4	23.5 ± 0.5	0.46
Energy intake (kcal/day)		1929.6 ± 13.5	1986.5 ± 60.9	1844.7 ± 89.9	0.09
Fat intake (% energy)		23.8 ± 0.1	24.6 ± 0.6	25.5 ± 0.9	0.19
Alcohol intake (g/day)		11.5 ± 0.5	9.7 ± 2.2	4.3 ± 3.3*	0.09
Systolic blood pressure (mmHg)		133.5 ± 0.5	134.2 ± 2.0	131.6 ± 2.9	0.27
White blood cell count (/mm^3)	4000–9000	5626.4 ± 35.7	5355.3 ± 161.3	4686.0 ± 237.9*,**	0.0002
Red blood cell count ($\times 10^4$/mm^3)	380–500	442.4 ± 1.0	436.1 ± 4.6	420.9 ± 6.9*,**	0.0004
Platelet count ($\times 10^4$/mm^3)	13–36	21.9 ± 0.1	21.0 ± 0.6	16.0 ± 0.8*,**	<0.0001
AST (U/L)	13–33	23.7 ± 0.2	25.2 ± 1.1	48.1 ± 1.6*,**	<0.0001
ALT (U/L)	6–30	20.1 ± 0.3	20.4 ± 1.2	46.3 ± 1.8*,**	<0.0001
γ-Glutamyl transpeptidase (U/L)	10–47	33.8 ± 1.1	32.4 ± 4.8	44.4 ± 7.0	0.36
Total protein (g/dL)	6.70–8.30	7.4 ± 0.01	7.5 ± 0.05*	7.7 ± 0.07*,**	<0.0001
Albumin (g/dL)	4.00–5.00	4.4 ± 0.01	4.4 ± 0.03	4.1 ± 0.04*,**	<0.0001
APRI		0.29 ± 0.01	0.33 ± 0.02*	1.01 ± 0.03*,**	<0.0001
Total cholesterol (mg/dL)		209.8 ± 0.8	203.1 ± 3.5	168.3 ± 5.2*,**	<0.0001

Values are expressed as means ± SE. A logarithmic transformation was employed for the analysis of fasting plasma glucose, fasting serum insulin, and HOMA-IR because of skewness

* $P < 0.05$ versus the uninfected group

** $P < 0.05$ versus the transient group

Discussion

In this population-based study conducted in an HCV hyperendemic area, we found paradoxical alterations of insulin resistance and atherosclerosis in inhabitants with chronic HCV infection. Insulin resistance was more severe in this group, while atherosclerosis was milder than that in the uninfected inhabitants and those with transient HCV infection. These findings suggest a unique characteristic of HCV-related metabolic abnormality.

An association between anti-HCV positivity and the development of diabetes mellitus has been shown in population-based studies [30, 31]. In the present study, we further showed that insulin resistance was more severe in subjects with chronic HCV infection than that in uninfected subjects and those with transient infection. The reason for the increased insulin resistance remains unclear; however, in our chronic group, significant differences were seen in white blood cell counts, platelet counts, albumin levels, and APRI values compared to values in the other two groups. These findings suggest that the progression of liver disease is involved in the increased insulin resistance in the chronic group. In addition, we, along with other researchers, have recently revealed a molecular mechanism for HCV-induced insulin resistance. HCV directly inactivated insulin receptor substrate 1/2 and subsequently resulted in insulin resistance, with no pathological changes in HCV core transgenic mice livers [6, 32]. Furthermore, clearance of HCV by interferon therapy improved the hepatic expression of insulin receptor substrate 1/2 and insulin resistance [33]. Although it is difficult to assess the direct effect of chronic HCV infection on the increased insulin resistance in the present study, it is possible that HCV could be involved in the development of insulin resistance.

In general, insulin resistance is positively correlated with hypercholesterolemia and hypertriglyceridemia [34, 35]. In the present study, the chronic group exhibited significantly lower total cholesterol, LDL-cholesterol, and triglyceride levels than those in the other groups. The serum albumin level was also lower in the chronic group, suggesting that, in advanced liver disease, changes in lipid metabolism are probably due to impaired lipid synthesis. In addition, Miyazaki et al. [12] recently demonstrated that serum lipid concentrations are significantly lower in HCV carriers with normal aminotransferase levels than in subjects without HCV infection. Cholesterol is reported to be crucial for HCV maturation [36, 37] and HCV inhibits triglyceride-rich VLDL secretion [38]. Thus, the alteration in lipid parameters in our subjects with chronic HCV infection was probably caused by the progression of liver disease and partly by a metabolic process associated with HCV replication.

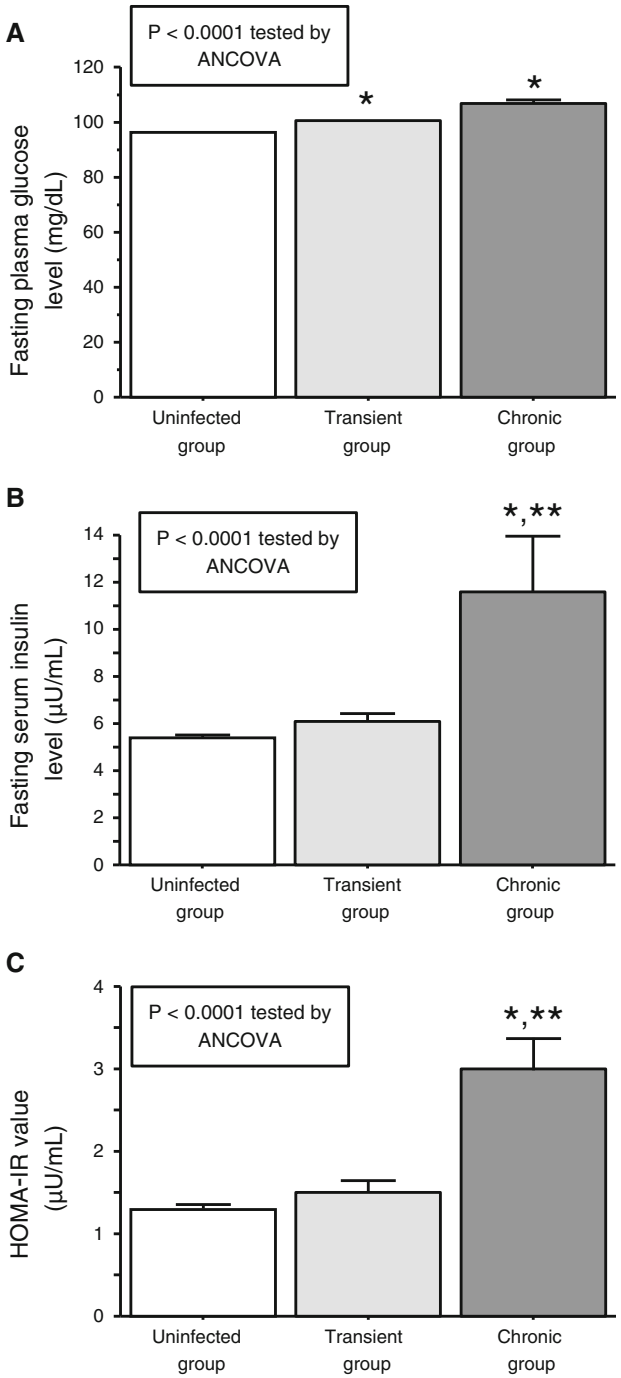

Fig. 2 Changes in fasting plasma glucose levels (**a**), fasting serum insulin levels (**b**), and homeostasis model assessment for insulin resistance (*HOMA-IR*) values (**c**) in the uninfected, transient, and chronic groups. Intergroup differences were assessed by age- and sex-adjusted analysis of covariance (*ANCOVA*). $*P < 0.05$ versus the uninfected group, $**P < 0.05$ versus the transient group

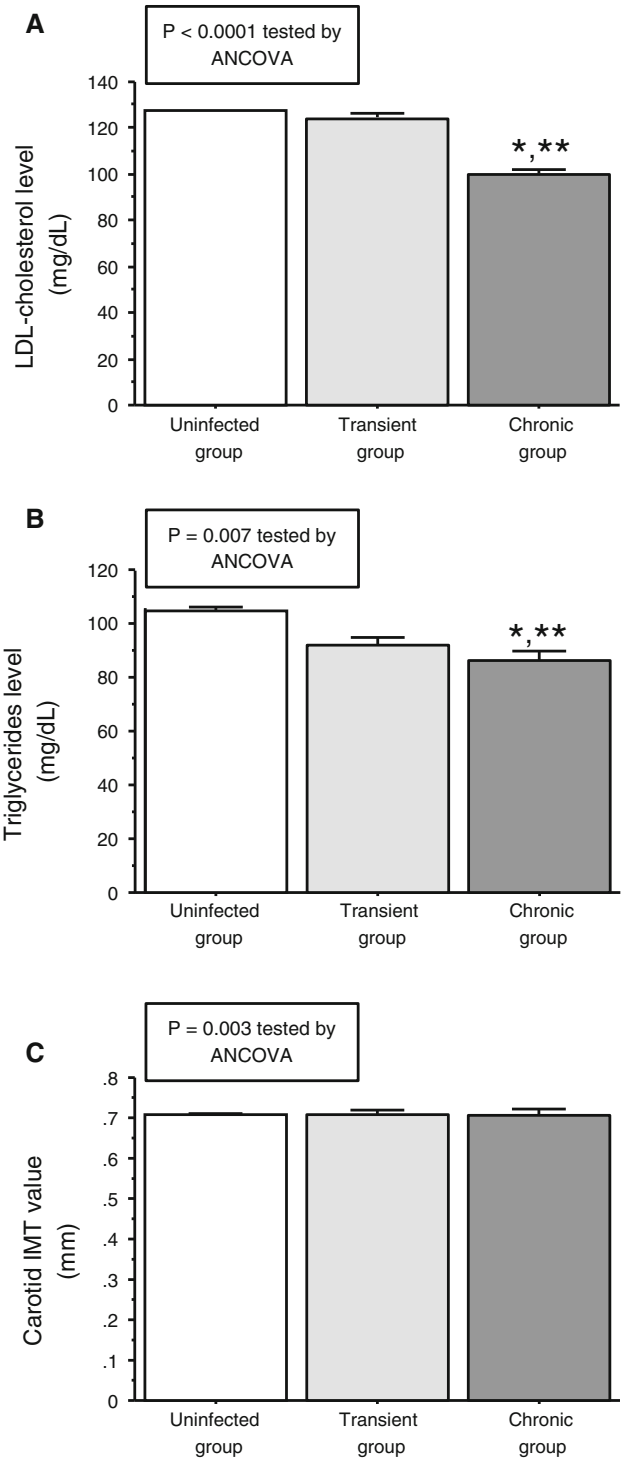

Fig. 3 Changes in serum low-density lipoprotein (*LDL*)-cholesterol levels (**a**), serum triglyceride levels (**b**), and carotid intima–media thickness (*IMT*) values (**c**) in the uninfected, transient, and chronic groups. Intergroup differences were assessed by age- and sex-adjusted ANCOVA. $*P < 0.05$ versus the uninfected group, $**P < 0.05$ versus the transient group

In the present study, we have demonstrated, for the first time, significantly lower carotid IMT values in subjects with chronic HCV infection, compared with values in uninfected subjects and those with transient disease. However, previous Japanese studies have demonstrated that chronic HCV infection increases carotid IMT [14, 39]. Although these previous studies were also designed as community-based cross-sectional studies, the mean age of the subjects was higher in our study. Serum LDL-cholesterol levels were not available in the study performed by Ishizaka et al. [39]; however, the serum triglyceride level in our study was lower than that in their study [39]. These differences suggest that the stage of liver disease in our study participants was more advanced than that in the study by Ishizaka et al. [39]. The difference in the severity of liver disease may be a possible explanation for the differences in the carotid IMT values between our study and theirs. Of note, the prevalence of atherosclerosis is known to be higher in Japanese urban areas, where previous studies were carried out [40]. As our study was conducted in a rural area, it is possible that differences in residential areas could account for the difference in the results.

In clinical settings, anti-diabetic agents are often administered to diabetic patients with HCV infection in order to prevent the development of vascular complications. However, we demonstrated a favorable effect of HCV on atherosclerosis. Notably, the incidence of diabetic retinopathy and cerebrovascular disease is reported to be low in patients with liver disease [41–43]. Moreover, exogenous insulin and sulfonylurea treatment can be harmful to patients with HCV infection because of an increased incidence of hepatocellular carcinoma [44]. We think that these findings highlight the need for distinctive therapeutic strategies that are suited to the paradoxical alterations of insulin resistance and atherosclerosis in subjects with chronic HCV infection.

In conclusion, our population-based study conducted in an HCV hyperendemic area revealed that chronic HCV infection was associated with increased insulin resistance and reduced atherosclerosis. This paradoxical metabolic profile suggests a unique characteristic of HCV-related metabolic abnormality.

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Conflict of interest Michio Sata received lecture fees from Chugai Pharmaceutical Co., Ltd. Takumi Kawaguchi and Michio Sata belongs to a department funded by donations from MSD K.K.

References

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5:558–67.
2. Weinman SA, Belalcazar LM. Hepatitis C: a metabolic liver disease. Gastroenterology. 2004;126:917–9.
3. Kawaguchi T, Izumi N, Charlton MR, Sata M. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. Hepatology. 2011;54:1063–70.
4. Allison ME, Wreghitt T, Palmer CR, Alexander GJ. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. J Hepatol. 1994;21:1135–9.
5. Eslam M, Aparcero R, Kawaguchi T, Del Campo JA, Sata M, Khattab MA, et al. Meta-analysis: insulin resistance and sustained virological response in hepatitis C. Aliment Pharmacol Ther. 2011;34:297–305.
6. Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, et al. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol. 2004;165:1499–508.
7. Voeks JH, McClure LA, Go RC, Prineas RJ, Cushman M, Kissela BM, et al. Regional differences in diabetes as a possible contributor to the geographic disparity in stroke mortality: the REasons for Geographic And Racial Differences in Stroke Study. Stroke. 2008;39:1675–80.
8. Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. Proc Natl Acad Sci USA. 1999;96:12766–71.
9. Nahmias Y, Casali M, Barbe L, Berthiaume F, Yarmush ML. Liver endothelial cells promote LDL-R expression and the uptake of HCV-like particles in primary rat and human hepatocytes. Hepatology. 2006;43:257–65.
10. Icard V, Diaz O, Scholtes C, Perrin-Cocon L, Ramiere C, Bartenschlager R, et al. Secretion of hepatitis C virus envelope glycoproteins depends on assembly of apolipoprotein B positive lipoproteins. PLoS One. 2009;4:e4233.
11. Negro F. Abnormalities of lipid metabolism in hepatitis C virus infection. Gut. 2010;59:1279–87.
12. Miyazaki T, Honda A, Ikegami T, Saitoh Y, Hirayama T, Hara T, et al. Hepatitis C virus infection causes hypolipidemia regardless of hepatic damage or nutritional state: an epidemiological survey of a large Japanese cohort. Hepatol Res. 2011;41:530–41.
13. 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;59:1135–40.
14. Ishizaka N, Ishizaka Y, Takahashi E, Tooda E, Hashimoto H, Nagai R, et al. Association between hepatitis C virus seropositivity, carotid-artery plaque, and intima–media thickening. Lancet. 2002;359:133–5.
15. Yang KC, Chen MF, Su TC, Jeng JS, Hwang BS, Lin LY, et al. Hepatitis B virus seropositivity is not associated with increased risk of carotid atherosclerosis in Taiwanese. Atherosclerosis. 2007;195:392–7.
16. Adachi H, Goetz FC, Jacobs DR, Tsuruta M, Hirai Y, Fujiura Y, et al. The role of obesity in the association of cardiovascular risk factors and glucose intolerance in small Japanese and North American communities. Diabetes Res Clin Pract. 2000;49:41–51.
17. Adachi H, Hino A. Trends in nutritional intake and serum cholesterol levels over 40 years in Tanushimaru, Japanese men. J Epidemiol. 2005;15:85–9.
18. Enomoto M, Adachi H, Fukami A, Furuki K, Satoh A, Otsuka M, et al. Serum dehydroepiandrosterone sulfate levels predict

longevity in men: 27-year follow-up study in a community-based cohort (Tanushimaru study). J Am Geriatr Soc. 2008;56:994–8.
19. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518–26.
20. Lackner C, Struber G, Liegl B, Leibl S, Ofner P, Bankuti C, et al. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. Hepatology. 2005;41:1376–82.
21. Keys A, Aravanis C, Blackburn HW, Van Buchem FS, Buzina R, Djordjevic BD, et al. Epidemiological studies related to coronary heart disease: characteristics of men aged 40–59 in seven countries. Acta Med Scand Suppl. 1966;460:1–392.
22. Kobayashi S, Honda S, Murakami K, Sasaki S, Okubo H, Hirota N, et al. Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. J Epidemiol. 2012;22:151–9.
23. Oya J, Nakagami T, Sasaki S, Jimba S, Murakami K, Kasahara T, et al. Intake of n-3 polyunsaturated fatty acids and non-alcoholic fatty liver disease: a cross-sectional study in Japanese men and women. Eur J Clin Nutr. 2010;64:1179–85.
24. Kobayashi S, Murakami K, Sasaki S, Okubo H, Hirota N, Notsu A, et al. Comparison of relative validity of food group intakes estimated by comprehensive and brief-type self-administered diet history questionnaires against 16 d dietary records in Japanese adults. Public Health Nutr. 2011;14:1200–11.
25. Sasaki S, Yanagibori R, Amano K. Self-administered diet history questionnaire developed for health education: a relative validation of the test-version by comparison with 3-day diet record in women. J Epidemiol. 1998;8:203–15.
26. Alborino F, Burighel A, Tiller FW, van Helden J, Gabriel C, Raineri A, et al. Multicenter evaluation of a fully automated third-generation anti-HCV antibody screening test with excellent sensitivity and specificity. Med Microbiol Immunol. 2011;200:77–83.
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;28:412–9.
28. Furuki K, Adachi H, Matsuoka H, Enomoto M, Satoh A, Hino A, et al. Plasma levels of asymmetric dimethylarginine (ADMA) are related to intima–media thickness of the carotid artery: an epidemiological study. Atherosclerosis. 2007;191:206–10.
29. Altman DG, Bland JM. Standard deviations and standard errors. BMJ. 2005;331:903.
30. 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 Intern Med. 2000;133:592–9.
31. Nagao Y, Kawaguchi T, Tanaka K, Kumashiro R, Sata M. Extrahepatic manifestations and insulin resistance in an HCV hyperendemic area. Int J Mol Med. 2005;16:291–6.
32. 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;126:840–8.
33. 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;102:570–6.
34. Reaven GM. Insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension. Parallels between human disease and rodent models. Diabetes Care. 1991;14:195–202.
35. Grundy SM. Hypertriglyceridemia, insulin resistance, and the metabolic syndrome. Am J Cardiol. 1999;83:25F–9F.
36. Aizaki H, Morikawa K, Fukasawa M, Hara H, Inoue Y, Tani H, et al. Critical role of virion-associated cholesterol and sphingolipid in hepatitis C virus infection. J Virol. 2008;82:5715–24.
37. Honda A, Matsuzaki Y. Cholesterol and chronic hepatitis C virus infection. Hepatol Res. 2011;41:697–710.
38. Perlemuter G, Sabile A, Letteron P, Vona G, Topilco A, Chretien Y, et al. Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. FASEB J. 2002;16:185–94.
39. Ishizaka Y, Ishizaka N, Takahashi E, Unuma T, Tooda E, Hashimoto H, et al. Association between hepatitis C virus core protein and carotid atherosclerosis. Circ J. 2003;67:26–30.
40. Konishi M, Iso H, Iida M, Naito Y, Sato S, Komachi Y, et al. Trends for coronary heart disease and its risk factors in Japan: epidemiologic and pathologic studies. Jpn Circ J. 1990;54:428–35.
41. Bianchi G, Marchesini G, Zoli M, Bugianesi E, Fabbri A, Pisi E. Prognostic significance of diabetes in patients with cirrhosis. Hepatology. 1994;20:119–25.
42. Fujiwara F, Ishii M, Taneichi H, Miura M, Toshihiro M, Takebe N, et al. Low incidence of vascular complications in patients with diabetes mellitus associated with liver cirrhosis as compared with type 2 diabetes mellitus. Tohoku J Exp Med. 2005;205:327–34.
43. Kawaguchi T, Sata M. Importance of hepatitis C virus-associated insulin resistance: therapeutic strategies for insulin sensitization. World J Gastroenterol. 2010;16:1943–52.
44. Kawaguchi T, Taniguchi E, Morita Y, Shirachi M, Tateishi I, Nagata E, et al. Association of exogenous insulin or sulphonylurea treatment with an increased incidence of hepatoma in patients with hepatitis C virus infection. Liver Int. 2010;30:479–86.