

## Case Control Study

# Hepatitis C virus G1b infection decreases the number of small low-density lipoprotein particles

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## Abstract

**AIM:** To investigate how hepatitis C virus (HCV) G1b infection influences the particle number of lipoproteins.

**METHODS:** The numbers of lipoprotein particles in fasting sera from 173 Japanese subjects, 82 with active HCV G1b infection (active HCV group) and 91 with cleared HCV infection (SVR group), were examined. Serum lipoprotein was fractionated by high-performance liquid chromatography into twenty fractions. The cholesterol and triglyceride concentrations in each fraction were measured using LipoSEARCH. The number of lipoprotein particles in each fraction was calculated using a newly developed algorithm, and the relationship between chronic HCV G1b infection and the lipoprotein particle number was

determined by multiple linear regression analysis.

**RESULTS:** The median number of low-density lipoprotein (LDL) particles was significantly lower in the active HCV group [1182 nmol/L, interquartile range (IQR): 444 nmol/L] than in the SVR group (1363 nmol/L, IQR: 472 nmol/L,  $P < 0.001$ ), as was that of high-density lipoprotein (HDL) particles (14168 nmol/L *vs* 15054 nmol/L, IQR: 4114 nmol/L *vs* 3385 nmol/L,  $P = 0.042$ ). The number of very low-density lipoprotein (VLDL) particles was similar between the two groups. Among the four LDL sub-fractions, the number of large LDL particles was similar between the two groups. However, the numbers of medium (median: 533.0 nmol/L, IQR: 214.7 nmol/L *vs* median: 633.5 nmol/L, IQR: 229.6 nmol/L,  $P < 0.001$ ), small (median: 190.9 nmol/L, IQR: 152.4 nmol/L *vs* median: 263.2 nmol/L, IQR: 159.9 nmol/L;  $P < 0.001$ ), and very small LDL particles (median: 103.5 nmol/L, IQR: 66.8 nmol/L *vs* median: 139.3 nmol/L, IQR: 67.3 nmol/L,  $P < 0.001$ ) were significantly lower in the active HCV group than in the SVR group, respectively. Multiple linear regression analysis indicated an association between HCV G1b infection and the decreased numbers of medium, small, and very small LDL particles. However, active HCV infection did not affect the number of large LDL particles or any sub-fractions of VLDL and HDL particles.

**CONCLUSION:** HCV G1b infection decreases the numbers of medium, small, and very small LDL particles.

**Key words:** Chronic hepatitis C; Lipoprotein particles; Low-density lipoproteins; Very low-density lipoproteins; Triglycerides; Cholesterol; Regression analysis

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**Core tip:** Hepatitis C virus (HCV) infection was previously reported to alter the serum lipid and lipoprotein profiles. However, the effect of HCV on the number of lipoprotein particles was not previously examined because counting the number of lipoprotein particles is challenging. In this report, we investigated how HCV infection changes the particle number of lipoproteins and demonstrated that HCV infection independently decreased the numbers of medium, small, and very small low-density lipoprotein particles. This finding may provide a new perspective on the association between HCV infection and atherosclerosis.

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## INTRODUCTION

Chronic hepatitis C virus (HCV) infection causes liver cirrhosis and hepatic carcinogenesis and is one of the most important health problems in the world. Patients with chronic HCV have lower serum cholesterol levels, which is associated with liver steatosis<sup>[1,2]</sup>, indicating that HCV infection could alter host lipid metabolism. Thus, many studies have attempted to clarify the association between HCV infection and lipid metabolism<sup>[3-8]</sup>.

Lipids are required as an energy source and utilized as an essential component of cellular structure. Hydrophobic lipids are transported to peripheral tissues through blood circulation as water-soluble lipoprotein particles. Lipoproteins are sorted into five classes based on their specific gravity, which is defined by the proportion of triglyceride (TG) and cholesteryl ester (CE) in the core of lipoprotein. These classes are chylomicron (CM), very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)<sup>[9]</sup>.

The liver, as the main regulator of lipid metabolism, assembles VLDLs and secretes them into circulation, where they are catabolized by lipoprotein lipase (LPL) to IDL. In this process, TG in the core of VLDL is hydrolyzed and delivers free fatty acids to the peripheral tissues. While most IDL is cleared by the liver, some is converted to LDL by hepatic triglyceride lipase (HTGL). LDL is incorporated into the liver or the peripheral tissue through LDL receptors<sup>[10]</sup>.

Previous investigations revealed that HCV co-opts the VLDL assembly, maturation, degradation, and secretory machinery in liver cells<sup>[11,12]</sup>. HCV particles associate with lipoproteins to form lipo-viral particles (LVP) and acquire infectivity<sup>[13]</sup>. Every 24 h,  $10^{18}$  VLDL particles are produced, and  $10^{12}$  HCV virions are secreted from the liver<sup>[14,15]</sup>.

Although the number of serum lipoprotein particles must be determined to understand lipoprotein metabolism, it has not been examined in HCV infection, mainly because measuring the number of lipoprotein particles is challenging. However, very recently, it became possible to accurately estimate the number of serum lipoprotein particles by using data derived from LipoSEARCH (Skylight Biotech, Akita, Japan). In this study, we investigated whether the number of lipoprotein particles differed between patients with active HCV genotype 1b (G1b) infection and those with cleared HCV infection using this newly developed algorithm.

## MATERIALS AND METHODS

### Patient population

We enrolled 173 Japanese patients with chronic HCV G1b infection or with cleared chronic HCV infection through interferon (IFN)-based therapy who were

**Table 1** Particle sizes in each major lipoprotein class and subclass

	Particle size (nm)	Major class	Subclass
1	> 90	CM	-
2	75.0	VLDL	Large VLDL
3	64.0		
4	53.6		
5	44.5		
6	36.8	LDL	Medium VLDL
7	31.3		Small VLDL
8	28.6		Large LDL
9	25.5		Medium LDL
10	23.0	HDL	Small LDL
11	20.7		Very small LDL
12	18.6		
13	16.7		
14	15.0	HDL	Large HDL
15	13.5		Medium HDL
16	12.1		
17	10.9		
18	9.8	HDL	Small HDL
19	8.8		
20	7.6		

CM: Chylomicron; VLDL: Very low-density lipoprotein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

treated at Jikei University Katsushika Medical Center from July 2013 to June 2015. Patients who exhibited decompensated cirrhosis, hepatocellular carcinoma, other types of hepatitis, co-infection with other viruses, and habitual alcohol abuse or had been treated for dyslipidemia or diabetes were excluded from this study. In addition, patients who had received antiviral therapy within 6 mo of the time of enrollment were excluded.

Of the 173 patients, 82 were chronically infected with HCV G1b, and 91 patients were classified as sustained virological response (SVR) based on undetectable HCV-RNA using the COBAS® Ampliprep/COBAS® TaqMan® HCV Test, v2.0 (Roche Molecular Diagnostics) at least 24 wk after completing IFN-based antiviral therapy. The HCV genotypes of enrolled patients were determined using the method described by Okamoto *et al.*<sup>[16]</sup>. The HCV genotypes of the SVR patients were determined before antiviral therapy: 58 cases were designated HCV G1b, and 32 were designated HCV G2. The genotype of the one remaining patient was not known.

Written informed consent was obtained from all patients who enrolled in this study. This study was conducted with the approval of the ethics committee of the Jikei Medical University and complied with the Helsinki Declaration 2004.

### Study design

Peripheral blood was obtained after overnight fasting for at least 10 h. Basic laboratory tests and serum lipid profiles were examined. In addition, serum lipoprotein was fractionated into 20 fractions by high-performance liquid chromatography (HPLC), and the cholesterol

and TG concentration in each fraction was measured using LipoSEARCH. The number of lipoprotein particles in each fraction was calculated using the algorithm developed by Skylight Biotech, and the effect of chronic HCV G1b infection on the number of lipoprotein particles was determined by multiple linear regression analysis.

### Demographic data and basic laboratory tests

Basic laboratory tests were performed to determine the levels of clinical markers including aspartate 2-oxoglutarate aminotransferase (AST), alanine 2-oxoglutarate aminotransferase (ALT),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP), albumin, total bilirubin, platelet count, and HbA1c. Demographic data including age, gender, and body mass index (BMI) were collected from the medical record of each patient.

### Serum lipid profiles

Total cholesterol, TG, and HDL cholesterol were directly assayed using a commercially available kit (Kyowa Medex, Tokyo, Japan). The concentration of LDL cholesterol was calculated using the Friedewald equation.

### Number of lipoprotein particles in lipoprotein fractions

The number of lipoprotein particles in each HPLC fraction was calculated according to the newly developed algorithm. This program is based on the assumption that the volume ratio of the surface layer to lipid core is equivalent for lipoproteins with the same particle size, as previously described for oil droplets<sup>[17,18]</sup>. The detailed algorithm for determining the lipoprotein number is presented in the international register of patents (<https://patentscope.wipo.int/search/>; patent number: WO2015/152371).

The numbers of particles in the major lipoprotein classes were expressed as the sum of fractions 3–7 for VLDL, the sum of fractions 8–13 for LDL, and the sum of fractions 14–20 for HDL. In addition, VLDL was subdivided into three subclasses (fractions 3–5: large VLDL, fraction 6: medium VLDL, and fraction 7: small VLDL). Similarly, LDL was subdivided into four subclasses (fraction 8: large LDL, fraction 9: medium LDL, fractions 10–11: small LDL, and fractions 12–13: very small LDL), and HDL into three (fractions 14–16: large HDL, fraction 17: medium HDL, and fractions 18–20: small HDL). The particle size for each major lipoprotein class and subclass is shown in Table 1.

### Statistical analysis

We performed the  $\chi^2$  test or Mann-Whitney *U* test to compare categorical or continuous data between the two groups because the data deviated considerably from a Gaussian distribution. Although some clinical data in our study did not strongly deviate from a Gaussian distribution, other clinical data were not normally distributed. Thus, we applied a non-parametric

**Table 2** Comparison of clinical features between patients with chronic active hepatitis C virus G1b infection (active hepatitis C virus) and cleared hepatitis C virus infection (Sustained virological response)

	Active HCV ( <i>n</i> = 82)				SVR ( <i>n</i> = 91)				<i>P</i> value
	<i>n</i> (%)	Median	Q1	Q3	<i>n</i> (%)	Median	Q1	Q3	
Discrete traits									
Sex									0.500
Male	36 (44)				46 (51)				
Female	46 (56)				45 (49)				
Quantitative traits									
Age (yr)		69.0	63.0	74.8		65.0	57.0	73.0	0.048
BMI (kg/m <sup>2</sup> )		21.9	20.3	24.0		22.5	21.0	24.5	0.145
HbA1c (%)		5.5	5.2	5.8		5.6	5.4	6.0	0.105
Glucose (mg/dL)		105.0	95.0	117.0		104.0	95.0	114.5	0.800
AST (IU/L)		41.0	30.0	74.3		23.0	20.0	28.0	< 0.001
ALT (IU/L)		36.5	24.8	58.5		17.0	13.0	23.0	< 0.001
Total bilirubin (mg/dL)		0.7	0.6	0.9		0.7	0.5	0.8	0.040
γ-GTP (IU/L)		30.5	21.0	52.8		21.0	16.0	30.0	< 0.001
Albumin (g/dL)		4.2	3.8	4.3		4.4	4.2	4.6	< 0.001
Platelet (10 <sup>3</sup> /μL)		15.5	11.8	20.6		17.3	13.4	21.0	0.048
Lipid profiles									
Total cholesterol (mg/dL)		174.5	151.3	192.8		193.0	175.5	216.5	< 0.001
Triglyceride (mg/dL)		89.5	64.0	116.8		88.0	68.0	135.0	0.700
LDL cholesterol (mg/dL)		93.5	77.0	110.5		112.5	93.0	133.5	< 0.001
HDL cholesterol (mg/dL)		59.8	47.3	72.2		61.7	51.2	74.0	0.195

HCV: Hepatitis C virus; SVR: Sustained virological response; BMI: Body mass index; AST: Aspartate 2-oxoglutarate aminotransferase; ALT: Alanine 2-oxoglutarate aminotransferase; γ-GTP: γ-Glutamyl transpeptidase; LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

test to all data.

To determine the effect of chronic active HCV infection on the number of lipoprotein particles in each major class or subclass, multiple regression models were generated considering each major class (VLDL, LDL, and HDL) or subclass as a dependent variable. We selected nine values including age, gender, BMI, ALT, γ-GTP, albumin, platelet count, HbA1c, and HCV infection status (active or cleared) as candidate independent variables. Categorical data were assigned as 0 (male, cleared HCV infection) or 1 (female, active HCV infection).

All statistical analyses were performed using STATISTICA software, version 6 (StatSoft Japan Inc. Tokyo, Japan). Two-tailed  $P < 0.05$  was considered significant, while  $0.05 < P < 0.1$  was marginal.  $P$  values less than 0.001 were expressed as  $P < 0.001$ .

## RESULTS

### Features of the active HCV group and SVR group

The clinical features of the active HCV group and the SVR group are summarized in Table 2. The median age of the active HCV group was 69.0 years, which was significantly higher than that of SVR group ( $P = 0.048$ ). The difference in gender between the two groups was marginal. There were no significant differences in other demographic data including BMI. In the routine laboratory tests, AST, ALT, and γ-GTP levels were significantly higher in the active HCV group than in the SVR group, whereas albumin levels and platelet counts were lower in the active HCV group. In sera, total cholesterol and LDL cholesterol were significantly lower

in the active HCV group than in the SVR group, and there were no differences in TG and HDL cholesterol levels between the two groups (Table 2).

### Effect of active HCV G1b infection on the numbers of lipoprotein particles

The numbers of LDL particles (median and interquartile range (IQR): 1182 nmol/L and 444 nmol/L vs 1363 nmol/L and 472 nmol/L,  $P < 0.001$ ) and HDL particles (14168 nmol/L and 4114 nmol/L vs 15054 nmol/L and 3385 nmol/L,  $P = 0.042$ ) were significantly lower in the active HCV group than in the SVR group, respectively. On the other hand, the number of VLDL particles (median and IQR: 127 nmol/L and 67 nmol/L vs 132 nmol/L and 64 nmol/L,  $P = 0.477$ ) was similar between the two groups (Figure 1).

Multiple linear regression analysis of the factors that significantly influenced the number of lipoprotein particles indicated that HCV G1b infection was associated with the decreased number of LDL particles. However, HCV G1b infection did not affect the VLDL or HDL particle numbers. Other factors that affected the number of LDL particles were age, albumin, and gender. BMI, FBS, and platelet count were not associated with the LDL particle number. We did not identify a factor that significantly influenced the number of VLDL particles. For HDL particles, gender, ALT, albumin, and platelet count were identified as significantly associated factors (Table 3).

### Effect of active HCV G1b infection on the particle numbers for LDL subclasses

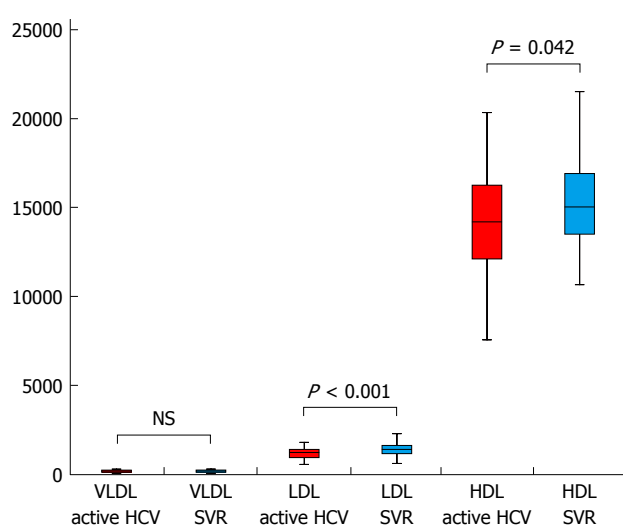
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**Table 3** Effect of chronic active hepatitis C virus infection on particle number of serum lipoproteins analyzed by multiple regression models

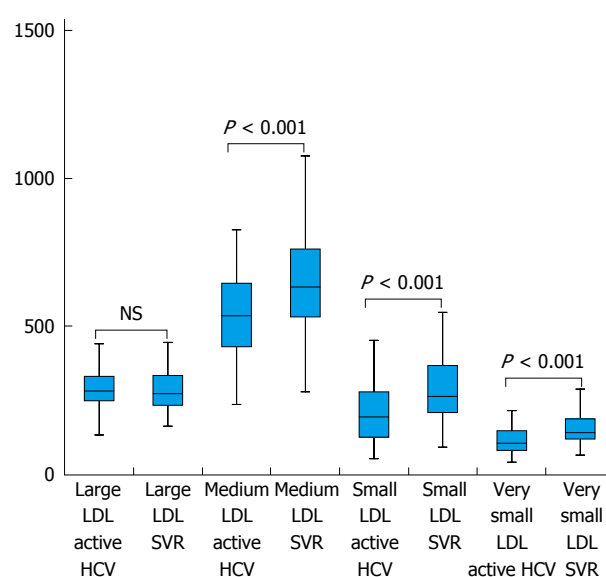
	VLDL			LDL			HDL		
	B	SE	P value	B	SE	P value	B	SE	P value
Constant	119.1	85.4	0.165	-371.5	414.5	0.371	8693.2	3114.6	0.006
HCV infection (active HCV <i>vs</i> SVR)	-7.5	12.9	0.558	-150.2	62.4	0.017	256.6	468.8	0.585
Age	0.3	0.5	0.583	4.8	2.3	0.041	-39.4	17.5	0.026
Female	-10.7	10.7	0.321	11.6	52.1	0.825	1513.1	391.8	< 0.001
BMI	2.7	1.6	0.083	11.7	7.6	0.125	-72.2	57.0	0.207
ALT	0.1	0.2	0.498	-0.2	1.0	0.815	-16.5	7.43	0.028
Albumin	-13.6	14.6	0.353	213.8	70.8	0.003	2054.6	532.2	< 0.001
Glucose	0.1	0.3	0.614	2.3	1.4	0.094	4.1	10.3	0.692
Platelet	-0.4	0.5	0.369	-0.5	2.2	0.820	37.2	16.4	0.025

VLDL: Very low-density lipoprotein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; SE: Standard error; HCV: Hepatitis C virus; SVR: Sustained virological response; BMI: Body mass index; ALT: Alanine 2-oxoglutarate aminotransferase.

**Figure 1** Comparison of the particle numbers of the three major lipoprotein classes for the active hepatitis C virus and sustained virological response groups. HCV: Hepatitis C virus; SVR: Sustained virological response.

particles in four LDL sub-fractions, the number of large LDL particles was similar for the active HCV group and SVR group (median and IQR: 281.1 nmol/L and 83.2 nmol/L *vs* 272.9 nmol/L and 101.6 nmol/L, respectively,  $P = 0.415$ ). The numbers of medium (533.0 nmol/L and 214.7 nmol/L *vs* 633.5 nmol/L and 229.6 nmol/L,  $P < 0.001$ ), small (190.9 nmol/L and 152.4 nmol/L *vs* 263.2 nmol/L and 159.9 nmol/L,  $P < 0.001$ ), and very small LDL particles (103.5 nmol/L and 66.8 nmol/L *vs* 139.3 nmol/L and 67.3 nmol/L,  $P < 0.001$ ) were significantly lower in the active HCV group than in the SVR group, respectively (Figure 2).

By multiple regression analysis, HCV G1b infection independently associated with the decreased numbers of medium, small, and very small LDL particles; however, it did not affect the number of large LDL particles. The albumin level also significantly correlated with the numbers of medium, small, and very small LDL particles. Female gender positively correlated with the number of large LDL particles (Table 4).

**Figure 2** Comparison of the particle numbers of the four low-density lipoprotein subclasses for the active hepatitis C virus and sustained virological response groups. HCV: Hepatitis C virus; SVR: Sustained virological response; LDL: Low-density lipoprotein.

### Effect of active HCV G1b infection on the particle numbers for VLDL and HDL subclasses

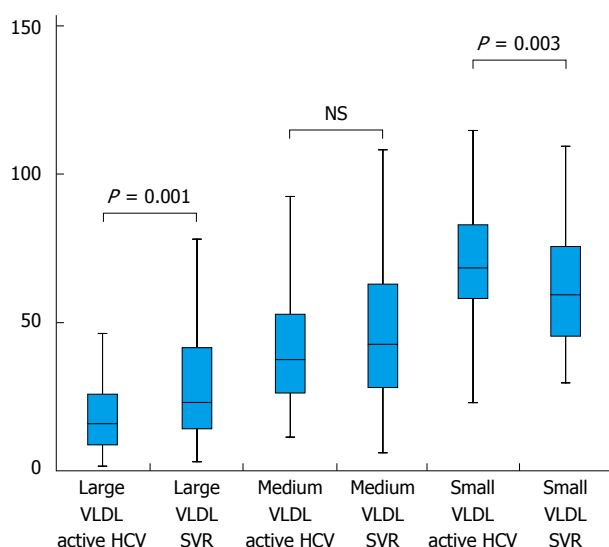
In the three VLDL sub-fractions, between the HCV group and SVR group, respectively, the number of large VLDL particles (median and IQR: 16.3 nmol/L and 16.9 nmol/L *vs* 23.2 nmol/L and 27.3 nmol/L,  $P < 0.001$ ) was lower in the HCV group, whereas that of small VLDL particles (68.3 nmol/L and 24.9 nmol/L *vs* 59.5 nmol/L and 29.8 nmol/L,  $P = 0.004$ ) was higher in the active HCV group; the number of medium VLDL particles (37.8 nmol/L and 26.5 nmol/L *vs* 43.0 nmol/L and 34.5 nmol/L,  $P = 0.168$ ) was similar for the two groups (Figure 3).

For the HDL sub-fractions, the number of large HDL particles (median and IQR: 2434 nmol/L and 1678 nmol/L *vs* 1953 nmol/L and 1522 nmol/L,  $P = 0.060$ ) tended to be higher in the HCV group than in the SVR group, respectively. The numbers of medium (3618

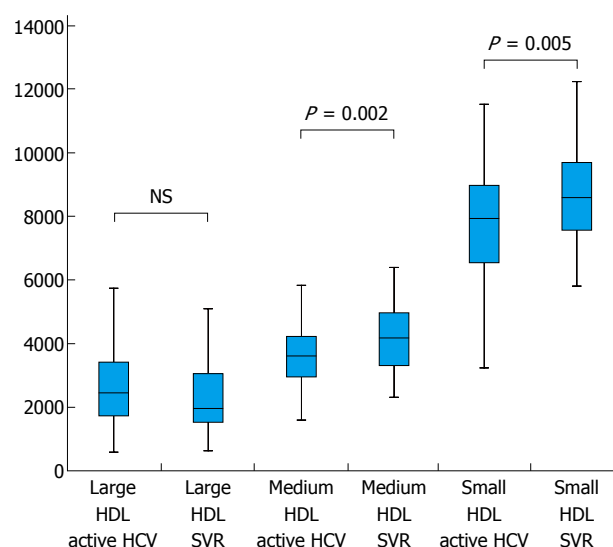
**Table 4** Effect of chronic active hepatitis C virus infection on particle number of four low-density lipoprotein sub-fractions analyzed by multiple regression models

	Large LDL			Medium LDL			Small LDL			Very small LDL		
	B	SE	P value	B	SE	P value	B	SE	P value	B	SE	P value
Constant	206.0	93.4	0.029	-263.6	202.3	0.195	-274.1	139.6	0.051	-39.8	64.6	0.538
HCV infection (Active HCV <i>vs</i> SVR)	0.4	14.1	0.978	-66.4	30.5	0.031	-55.3	21.0	0.009	-28.8	9.7	0.004
Age	0.9	0.5	0.086	2.1	1.1	0.072	1.2	0.8	0.128	0.6	0.4	0.083
Female	42.6	11.7	< 0.001	9.2	25.5	0.719	-29.7	17.6	0.093	-10.5	8.1	0.198
BMI	1.7	1.7	0.308	5.0	3.7	0.177	3.7	2.6	0.151	1.2	1.2	0.297
ALT	0.1	0.2	0.584	-0.1	0.5	0.823	-0.1	0.3	0.726	-0.1	0.2	0.404
Albumin	-14.1	16.0	0.378	121.2	34.6	0.001	80.6	23.8	0.001	26.1	11.0	0.019
Glucose	0.2	0.3	0.519	1.3	0.7	0.060	0.7	0.5	0.149	0.2	0.2	0.412
Platelet	-0.3	0.5	0.507	-0.2	1.1	0.877	0.0	0.7	0.967	-0.04	0.3	0.917

LDL: Low-density lipoprotein; SE: Standard error; HCV: Hepatitis C virus; SVR: Sustained virological response; BMI: Body mass index; ALT: Alanine 2-oxoglutarate aminotransferase.



**Figure 3** Comparison of the particle numbers of the three very low-density lipoprotein subclasses for the active hepatitis C virus and sustained virological response groups. HCV: Hepatitis C virus; SVR: Sustained virological response; VLDL: Very low-density lipoprotein.



**Figure 4** Comparison of the particle numbers of the three high-density lipoprotein subclasses for the active hepatitis C virus and Sustained virological response groups. HCV: Hepatitis C virus; SVR: Sustained virological response.

nmol/L and 1274 nmol/L *vs* 4187 nmol/L and 1657 nmol/L,  $P = 0.002$ ) and small HDL particles (7926 nmol/L and 2401 nmol/L *vs* 8586 nmol/L and 2120 nmol/L,  $P = 0.005$ ) were significantly lower in the active HCV group than in the SVR group, respectively (Figure 4).

Multiple regression analysis for the sub-fractionated VLDL particles indicated that HCV G1b did not affect the particle number of any VLDL subclass. Female gender was associated with decreased numbers of large and small VLDL particles, and albumin was positively associated with the number of small VLDL particles (Table 5). In addition, HCV G1b infection was not associated with the number of particles in any of the HDL sub-fractions. Instead, female gender was positively associated with the numbers of large and medium HDL particles. BMI and platelet count affected the number of large HDL particles, age and

ALT affected the number of medium HDL particles, and age and albumin affected the number of small HDL particles (Table 6).

## DISCUSSION

HCV infection has been associated with biased lipid metabolism<sup>[19-24]</sup>. Because lipids are transported to the peripheral tissues in the blood as lipoprotein particles and HCV acquires infectivity as LVPs, it is important to investigate the number of lipoprotein particles in the sera of patients with HCV infection. Although the number of LDL particles can be measured by SpectraCell's LPP™ test (<https://www.spectracell.com/clinicians/products/lpp>), there have been no reports about changes in lipoprotein particle numbers during HCV infection. One reason for this lack is the methodological difficulty of accurately measuring

**Table 5** Effect of chronic active hepatitis C virus infection on the particle number for three very low-density lipoprotein sub-fractions analyzed by multiple regression models

	Large VLDL			Medium VLDL			Small VLDL		
	B	SE	P value	B	SE	P value	B	SE	P value
Constant	-18.1	32.1	0.574	21.1	38.9	0.588	116.1	35.0	0.001
HCV infection (active HCV <i>vs</i> SVR)	-6.6	4.8	0.176	-4.0	5.9	0.494	3.0	5.3	0.564
Age	0.03	0.2	0.868	0.05	0.2	0.819	0.2	0.2	0.352
Female	-12.7	4.0	0.002	-7.2	4.9	0.145	9.1	4.4	0.040
BMI	0.9	0.6	0.126	1.1	0.7	0.122	0.7	0.6	0.263
ALT	0.01	0.08	0.878	0.03	0.09	0.750	0.1	0.08	0.247
Albumin	8.8	5.5	0.109	-0.7	6.6	0.912	-21.7	6.0	< 0.001
Glucose	-0.04	0.1	0.679	0.1	0.1	0.477	0.1	0.1	0.413
Platelet	-0.02	0.2	0.903	-0.2	0.2	0.355	-0.2	0.2	0.294

VLDL: Very low-density lipoprotein; SE: Standard error; HCV: Hepatitis C virus; SVR: Sustained virological response; BMI: Body mass index; ALT: Alanine 2-oxoglutarate aminotransferase.

**Table 6** Effect of chronic active hepatitis C virus infection on the particle number for three high-density lipoprotein sub-fractions analyzed by multiple regression models

	Large HDL			Medium HDL			Small HDL		
	B	SE	P value	B	SE	P value	B	SE	P value
Constant	4544.7	1510.8	0.003	3499.5	1188.8	0.004	649.0	2033.7	0.750
HCV infection (active HCV <i>vs</i> SVR)	345.1	227.4	0.131	-106.9	178.9	0.551	18.4	306.1	0.952
Age	9.4	8.5	0.272	-18.9	6.7	0.005	-29.9	11.4	< 0.001
Female	494.1	190.0	0.010	719.2	149.5	< 0.001	299.8	255.8	0.243
BMI	-96.1	27.6	0.001	-29.19	21.76	0.182	53.1	37.2	0.156
ALT	-5.6	3.6	0.122	-7.67	2.84	0.008	-3.2	4.9	0.515
Albumin	-136.8	258.2	0.597	361.7	203.1	0.077	1829.7	347.5	< 0.001
Glucose	-5.0	5.0	0.323	5.1	3.9	0.196	3.9	6.7	0.559
Platelet	19.6	8.0	0.015	9.8	6.3	0.122	7.9	10.7	0.463

HDL: High-density lipoprotein; SE: Standard error; HCV: Hepatitis C virus; SVR: Sustained virological response; BMI: Body mass index; ALT: Alanine 2-oxoglutarate aminotransferase.

the number of lipoprotein particles for all classes of lipoproteins<sup>[25]</sup>. However, a new algorithm allows exact calculation of the number of lipoprotein particles in all major classes.

Using this algorithm, we found that the number of LDL particles, specifically the numbers of medium, small, and very small LDL particles, was decreased in patients with active HCV G1b infection. As an independent factor, HCV infection significantly influenced the numbers of these particles in multiple linear regression analysis but had no concomitant effect on the number of large LDL particles or the numbers of VLDL and HDL particles.

The heterogeneity of LDL size and density is well established<sup>[26]</sup>. LDL is derived from delipidation of VLDL through IDL (by HTGL and LPL, respectively). Parallel pathways that generate LDLs with different sizes and densities are thought to occur through different VLDLs in this VLDL-IDL-LDL cascade<sup>[27,28]</sup>. In animal models, independent pathways to generate each LDL subclass were suggested<sup>[29-31]</sup>. Sufficient TG is required to sustain lipolysis and enable the metabolism of VLDL to IDL and LDL. Thus, the amount of TG in VLDL is one determinant of LDL particle size, and it was hypothesized that TG-rich large VLDL1 was a precursor

of smaller LDL<sup>[32]</sup>. A relationship between large VLDL and small, dense LDL in human was demonstrated previously<sup>[33]</sup>, potentially involving CE transfer protein (CETP) and HTGL. In hypertriglyceridemia, in which TG-rich VLDL1 is increased, CETP transports CE from LDL to VLDL1 and TG from VLDL1 to LDL. This lipid exchange results in an increase of TG-rich LDL, which is hydrolyzed by HTGL into small, dense LDL<sup>[34]</sup>. The plasma TG level is dependent on VLDL1 levels<sup>[35]</sup> and positively correlates with small, dense LDL levels<sup>[36-38]</sup>. On the other hand, decreasing the activity of HTGL resulted in increased numbers of larger LDL particles<sup>[39,40]</sup>. Thus, generation of small LDL particles may be prescribed by the level of TG in VLDL and the activities of CETP and HTGL.

Previously, LPL and HTGL were shown to reduce the infectivity of HCV<sup>[41]</sup>. Therefore, for persistent HCV infection, it is preferable to avoid catabolism of HCV-LVP by LPL and HTGL. In HCV infection, the activity of HTGL is reduced through transcriptional down-regulation<sup>[42]</sup>. Furthermore, we previously investigated serum TG profiles in HCV G1b infection and found that HCV infection is associated with a low level of large VLDL-TG<sup>[43]</sup>. Therefore, the decreased numbers of medium, small, and very small LDL particles might

be due to a decreased amount of TG in large VLDL and reduced activity of HTGL. However, HCV G1b infection did not affect the number of large VLDL particles. Therefore, HCV G1b infection might suppress the transport of TG into VLDL without impairing the secretion of VLDL particles.

It has been reported that small, dense LDL is associated with increased risk of myocardial infarction<sup>[44,45]</sup>. Atherosclerotic plaques can be formed by oxidation of LDL particles<sup>[46]</sup>. Small, dense LDL has lower affinity to LDL receptors than larger LDL particles, resulting in longer retention in the blood and increased susceptibility to oxidative stress<sup>[47]</sup>. Moreover, small, dense LDL was more easily taken up into arterial walls through binding to polyanionic proteoglycans, resulting in induction of atherosclerosis<sup>[48]</sup>. Thus, small, dense LDL is thought to be a risk factor for atherosclerosis, and the number of LDL particles is considered a better predictor of cardiovascular events than LDL-cholesterol concentrations<sup>[49,50]</sup>.

The reduced number of small LDL particles in HCV G1b infection strongly suggested that small, dense LDL do not play a major role in atherosclerosis for patients with chronic HCV G1b infection. Although the association between atherosclerosis and HCV infection has been investigated, the results are conflicting. Some studies indicated that HCV infection accelerated atherosclerosis<sup>[51-54]</sup>, whereas others found no relationship between HCV infection and atherosclerosis or cardiovascular event<sup>[55-57]</sup>. HCV infection activates T helper cells and releases a number of pro-inflammatory cytokines<sup>[58]</sup>, which might accelerate atherosclerosis, as chronic inflammation is a factor preceding atherosclerosis<sup>[59]</sup>. In addition, HCV infection induces insulin resistance and evokes diabetes mellitus and metabolic syndrome<sup>[60,61]</sup>, which could be related to atherosclerosis in HCV patients<sup>[62]</sup>. Furthermore, increased levels of remnant lipoprotein could have a role in atherosclerosis during HCV infection<sup>[43]</sup>. Further study is required to assess the effect of the reduced number of small LDL particles on atherosclerosis in chronic HCV infection.

Although the accuracy of the lipoprotein particle calculation has been demonstrated in healthy individuals, it has not been directly demonstrated in patients with chronic HCV infection. However, we believe that this calculation method would not introduce error in the accuracy of our results that would alter our conclusions. Additionally, we emphasize that we investigated only patients with HCV G1b infection, and it is unclear whether these results are extensible to patients with other HCV genotypes.

In conclusion, HCV G1b infection is associated with decreased numbers of medium, small, and very small LDL particles. To the best of our knowledge, this is the first such report of the link between HCV and lipoprotein particle numbers.

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## COMMENTS

### Background

Hepatitis C virus (HCV) infection was reported to alter host lipid metabolism based on observations that patients with chronic HCV have lower serum cholesterol levels, which is associated with liver steatosis. Furthermore, previous studies indicated that persistent HCV infection utilizes the very low-density lipoprotein (VLDL) assembly, maturation, degradation, and secretory machinery of liver cells.

### Research frontiers

Although investigation of the number of serum lipoprotein particles is required to understand lipoprotein metabolism, it has not been examined in HCV infection, mainly because of it is difficult to measure. However, very recently, accurate estimation of the number of serum lipoprotein particles became possible by using the data derived from LipoSEARCH (Skylight Biotech, Akita, Japan).

### Innovations and breakthroughs

To the best of the authors' knowledge, this is the first study to examine the number of serum lipoprotein particles in patients with chronic HCV infection. The results of the present study are indispensable for improving our understanding of HCV-induced disruption of lipoprotein metabolism.

### Applications

The decreased number of LDL particles in patients with HCV infection might help elucidate the mechanism of progressing atherosclerosis in patients with chronic HCV.

### Peer-review

The topic of the current study is very promising, the authors efficiently selected the aim of this research. Whereas, HCV infection was previously reported to alter the serum lipid and lipoprotein profiles. These changes may have a new role in the pathogenesis of HCV. This finding also may provide a new perspective on the association between HCV infection and atherosclerosis.

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