Remnant-Like Particles Cholesterol Is Higher in Diabetic Patients With Coronary Artery Disease

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Diabetes mellitus (DM) has been well known to be one of the risk factors of coronary artery disease (CAD). Recently, remnant-like particles cholesterol (RLP-C) has been reported to be associated with CAD. However, few studies reported the association of RLP-C level with CAD in subjects with DM. To investigate the effects of presence or absence of DM on the association between RLP-C and CAD, we compared the RLP-C level in 142 male patients with CAD and 123 male subjects without CAD (non-CAD), including 44 and 38 DM patients, respectively. RLP-C was significantly higher in CAD than non-CAD ($P < .05$). RLP-C and RLP-C/plasma-triglyceride (TG) ratio in CAD with DM were higher than CAD without DM ($P < .01$, $P < .05$), and non-CAD with DM ($P < .001$, $P < .05$). There was positive correlation between RLP-C and plasma-TG in non-CAD without DM ($r = .44$, $P < .01$), non-CAD with DM ($r = .56$, $P < .001$), CAD without DM ($r = .81$, $P < .0001$), and CAD with DM ($r = .75$, $P < .001$). After excluding the hypertriglyceridemic patients (>200mg/dL), RLP-C/plasma-TG ratio was significantly higher in CAD with DM than CAD without DM ($P < .001$) and non-CAD with DM ($P < .05$). These results suggest that increased RLP-C to plasma-TG may be associated with CAD in middle-aged diabetic male subjects.

IT HAS BEEN REPORTED that some of triglyceride (TG) rich lipoproteins, such as intermediate density lipoprotein (IDL), and remnant lipoproteins are associated with coronary artery disease (CAD).[1-15] Remnant lipoproteins have also been thought to be one of the atherogenic lipoproteins. However, the isolation and quantity of remnant lipoproteins need some procedures, such as ultracentrifugation and electrophoresis, which have been technically complicated and difficult. Nakajima et al[16] developed the method for isolation of remnant-like particles (RLPs), which are separated from plasma as unbound fraction to immunoaffinity mixed gels containing monoclonal apolipoprotein (apo) A-I and apo B-100 antibodies. They suggested that the cholesterol content in RLPs might be a useful marker for quantification of remnant lipoproteins. In fact, some studies have been reported that the cholesterol (RLP-C) level was higher in CAD than controls.[8-14] In vitro, it has also been reported that RLPs are easily absorbed by macrophages and fibroblasts without any modifications.[17-19] Moreover, it has been demonstrated that RLPs inhibit production of nitric oxide in endothelial cells, enhance the platelet aggregation, and induce the expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and tissue factor in endothelial cells.[20-22] Therefore, it has been considered that the increase in RLPs might be one of the dyslipidemia associated with CAD.

On the other hand, diabetes mellitus (DM) increases the risk for atherosclerosis and macrovascular disease such as CAD.[23-26] It has been well known that DM is associated with varied lipid abnormalities including hypertriglyceridemia, which may cause macrovascular complications.[27,28] Although it has been reported that the RLP-C level is high in DM patients, there are few reports about the RLP-C level in CAD with DM. Song et al[13] reported that the RLP-C level was higher in CAD with DM patients compared with healthy controls, however, they did not compare the RLP-C level in CAD with DM with that in either non-CAD with DM or CAD without DM. Recently, Hirany et al[14] reported that the RLP-C level in DM patients with macrovascular complications was higher than that in DM patients without them. However, the number of their subjects was small, and they did not investigate non-DM subjects with macrovascular complications. Therefore, we consider that there is little information about the association of RLP-C with CAD in each group with or without DM. In the present study, we measured the RLP-C level in CAD with or without DM and non-CAD with or without DM to examine the association of RLP-C with CAD among middle-aged Japanese men with or without DM.

MATERIALS AND METHODS

Subjects

The majority of subjects in this study were selected from inpatients who were admitted to our hospital during 1 year for coronary angiographies (CAG). The patients with CAD (N = 142) were defined as more than 50% stenosis in the coronary artery as measured by quantitative CAG. Patients and control subjects with a fasting blood glucose level greater than 126 mg/dL, or diabetic pattern of oral glucose tolerance test, or on any oral antidiabetic medication were defined as DM. Age, body mass index (BMI), and the percentage of DM-matched subjects without CAD (N = 123) were selected as the subjects who had no detectable coronary lesion in CAG or outpatients who came to our department in our hospital for their health check and did not have clinical manifestation of CAD or abnormal sign on treadmill exercise electrocardiography. We excluded subjects over the age of 65 years, those with a history of fresh myocardial infarction (less than 2 months) or spastic angina pectoris, or those who took medication for hyperlipidemia, such as fibrates and statins, or who were on medication affecting lipid metabolism. Written informed consent for participation in this study was obtained from all subjects. The experimental protocol was approved by the Ethical Committee of the National Defense Medical College of Japan.

Blood Sampling

The EDTA-plasma of subjects was collected in the early morning after a 12- to 13 hour fasting state. RLP particles were isolated from

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other lipoproteins as the unbound fraction in immunoaffinity mixed gels containing monoclonal apo A-I and apo B-100 antibodies within 48 hours following the method of Nakajima et al.[16] In brief, 5 μL of plasma was added to the mixture of 50 μL of CNBr Sepharose 4B, which contained 125 μg of anti-apo A-I monoclonal antibody (MoAb), 125 μg anti-apo B-100 MoAb (JI-H) and 300 μL of reaction suspension. The mixture was shaken gently for 60 minutes to mix and left for 10 minutes. Thirty microliters of clear supernatant was taken, and the cholesterol level of RLP was determined enzymatically. Plasma cholesterol, triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and glucose were determined enzymatically by commercial kits (Determiner L, Kyowa, Tokyo, Japan). Low-density lipoprotein-cholesterol (LDL-C) was calculated with the use of the Freidewald formula.[29] Hemoglobin A_{1c} (HbA_{1c}) was determined by high-performance liquid chromatography.[30]

Statistical Methods

Continuous variables are presented as mean ± SD. The data were analyzed using StatView 5.0 software (Hulinks, Tokyo, Japan). Statistical comparison of variable parameters between CAD and non-CAD was conducted by using Mann-Whitney U-test. The correlation between RLP-C and plasma-TG levels was analyzed by Spearman rank correlation. $P < .05$ was considered statistically significant.

RESULTS

Clinical characteristics and lipid profiles of the study population are shown in Table 1. The age, BMI, and the distribution of diabetes, hypertension, and current smoker were similar between the CAD group and non-CAD group. Fasting blood glucose, HbA1c, plasma-total cholesterol, plasma-TG, and LDL-C levels were not different between CAD and non-CAD. The RLP-C level of CAD was significantly higher than that of non-CAD, while the HDL-C level was significantly lower in CAD than non-CAD. RLP-C/plasma-TG ratio was not significantly different between CAD and non-CAD.

Table 1. Clinical Characteristics and Lipid Profiles of Subjects

	Non-CAD (n = 123)	CAD (n = 142)
Age (yr)	55.3 ± 6.0	56.0 ± 6.8
BMI (kg/m^2)	24.7 ± 3.5	24.5 ± 2.5
DM (n)	38	44
Hypertension (n)	40	48
Current smoker (n)	45	60
Glucose (mg/dL)	110 ± 31	106 ± 29
HbA_{1c} (%)	5.8 ± 1.3	5.7 ± 1.0
Plasma-TC (mg/dL)	208 ± 32	203 ± 34
Plasma-TG (mg/dL)	159 ± 67	169 ± 73
HDL-C (mg/dL)	51 ± 13	44 ± 13*
LDL-C (mg/dL)	129 ± 34	128 ± 30
RLP-C (mg/dL)	4.8 ± 2.0	5.5 ± 2.9†
RLP-C/plasma-TG	.035 ± .023	.034 ± .012
Correlation between RLP-C and plasma-TG (*r*, *P*)	.466, < .001	.752, < .0001

NOTE. Continuous variables are shown as mean ± SD.

Abbreviations: CAD, coronary artery disease; BMI, body mass index; DM, diabetes mellitus; plasma-TC, plasma total cholesterol; plasma-TG, plasma triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RLP-C, remnant-like particles cholesterol; RLP-C/plasma-TG, the ratio of RLP-C to plasma-TG.

*†Significantly different from the value in non-CAD at $P < .05$, and $P < .001$, respectively.

The characteristics and lipid profiles in the presence or absence of DM among patients with or without CAD are shown in Table 2. In the non-CAD group, there were no significant differences in lipid profiles containing RLP-C and the RLP/plasma-TG ratio between DM and non-DM. However, in the CAD group, RLP-C was higher in DM subjects than non-DM subjects. Moreover, the RLP-C/plasma-TG ratio was significantly higher in DM than non-DM. On the other hand, in the non-DM group, RLP-C and RLP-C/plasma-TG ratios were not significantly different between CAD and non-CAD. In DM subjects, RLP-C and RLP-C/plasma-TG ratios were higher in CAD than non-CAD.

RLP-C levels were significantly correlated with plasma-TG levels in all subgroups. After excluding the hypertriglyceridemic patients (>200 mg/dL), there was no significant difference in RLP-C, plasma-TG, and RLP-C/plasma-TG ratios between CAD and non-CAD. However, in DM subjects after excluding the hypertriglyceridemic patients, the RLP-C/plasma-TG ratio was significantly higher in CAD than non-CAD (0.040 ± 0.018 *v* 0.032 ± 0.010, $P < .05$), while there was no significant difference in RLP-C or plasma-TG between CAD and non-CAD.

DISCUSSION

It has been reported that some TG-rich lipoproteins, such as IDL, and remnant lipoproteins are associated with CAD.[1-15] Nakajima et al[16] developed an easy method for the quantity of remnant lipoproteins, such as RLP, which contained chylomicron remnants and apo E-rich very–low-density lipoprotein. After developing this method, it has been demonstrated that RLP-C was associated with CAD some case-control studies and prospective studies.[8-14,31,32] Kugiyama et al[11] reported that patients with a high level of RLP-C have a high risk of coronary events among CAD patients. In our case-control study, RLP-C in CAD was higher than that in non-CAD, which is consistent with the previous studies.

It has been well known that DM is associated with varied lipid abnormalities, including increased remnant lipoproteins, which may cause macrovascular complications.[27,28] In our present study, RLP-C was higher in DM patients than that in non-DM among CAD patients, but among non-CAD patients. Song et al[13] reported that the RLP-C level was higher in DM patients, CAD patients, and CAD with DM patients compared with healthy controls. Their report showed that RLP-C in DM patients without CAD is higher than that of DM with CAD, which is different from our study. This difference may be the reason that plasma-TG level in DM was much higher than that of controls in their subjects, although there was not a significant difference in plasma-TG levels between groups in our subjects. Recently, Hirany et al[14] reported RLP-C was higher in DM patients with macrovascular complications than in DM patients without macrovascular complications. Our study is consistent with their study. Moreover, they also reported that the RLP-C/plasma-TG ratio was higher in DM with macrovascular complications than that of DM without macrovascular complications after excluding hypertriglyceridmic patients (>200 mg/

Table 2. Characteristics and Lipid Profiles Among Subgroups

	Non-CAD		CAD	
	Non-DM (n = 85)	DM (n = 38)	Non-DM (n = 98)	DM (n = 44)
Age (yr)	55.5 ± 5.0	55.2 ± 7.0	55.7 ± 7.2	56.7 ± 5.7
BMI (kg/m^2)	24.2 ± 3.4	25.1 ± 2.5	24.5 ± 2.5	24.4 ± 2.6
Hypertension (n)	29	11	33	15
Current smoker (n)	32	13	44	16
Glucose (mg/dL)	97 ± 11	135 ± 39	94 ± 12	135 ± 37
HbA_{1c} (%)	5.3 ± 0.4	7.1 ± 1.5	5.2 ± 0.4	6.8 ± 1.0
Plasma-TC (mg/dL)	205 ± 35	213 ± 28	200 ± 31	207 ± 39
Plasma-TG (mg/dL)	151 ± 69	171 ± 58	166 ± 66	173 ± 85
HDL-C (mg/dL)	51 ± 12	52 ± 12	43 ± 12*	45 ± 15†
LDL-C (mg/dL)	127 ± 36	132 ± 32	126 ± 29	131 ± 33
RLP-C (mg/dL)	4.7 ± 1.8	5.0 ± 2.4	5.1 ± 2.0	6.5 ± 4.1†‡
RLP-C/plasma-TG	.036 ± .025	.031 ± .016	.031 ± .008	.039 ± .016†§
Correlation between RLP-C and plasma-TG (r, P)	.500, <.01	.440, <.001	.755, <.0001	.813, <.0001

NOTE. Continuous variables are shown as mean ± SD.

Abbreviations: CAD, coronary artery disease; DM, diabetes mellitus; BMI, body mass index; plasma-TC, plasma total cholesterol; plasma-TG, plasma triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RLP-C, remnant-like particles cholesterol; RLP-C/TG, the ratio of RLP-C to plasma-TG.

*Significant difference from the value in non-CAD without DM at $P < .0001$.

†Significant difference from the value in non-CAD with DM at $P < .05$.

‡§Significant difference from the value in CAD without DM at $P < .01$, and $P < .001$, respectively.

dL). We also compared the values after excluding hypertriglyceridemic subjects and demonstrated that the RLP-C/plasma-TG ratio was higher in CAD with DM than CAD without DM and non-CAD with DM. Our study is consistent with their study on this point. Therefore, increased RLP to plasma-TG is associated with CAD might show a proatherogenic lipid profile.

RLPs has been thought to be one of the TG-rich lipoproteins. In fact, it has been reported that the RLP-C level is positively correlated with plasma-TG.[13-15] In the present study, RLP-C was correlated with plasma-TG levels in whole subjects, although the degree of correlation was lower in the CAD group than in non-CAD groups, and that was lower in diabetic patients than in nondiabetic patients. Interestingly, Song et al[13] reported that RLP-C was significantly correlated with plasma-TG in control ($r = .783$), CAD ($r = .610$), and DM ($r = .746$), although they did not show the correlation in CAD with DM. On the other hand, Hirany et al[14] reported that the correlation between RLP-C and plasma-TG was observed in controls, DM with macrovascular complications, and DM without macrovascular complications ($r = .37$, $r = .60$, and $r = .76$, respectively). We cannot find that the degrees of correlation between RLP-C and plasma-TG among groups has a similar tendency in these 2 studies and our study. The difference might be caused by the ranges and values of plasma-TG and RLP-C in subjects.

It is well known that RLP-C increases after a fat-rich meal. Moreover, patients with CAD have been reported to have impaired clearance of postprandial TG-rich lipoproteins.[3-7] It was reported that RLP-C after an oral fat load increases more in CAD patients than in controls, even with a similar fasting lipid profile.[7] Some studies showed that DM also exacerbates postprandial lipemia after fat load, although the association with CAD has been controversial.[33-35] We speculate that the increase in RLP-C in the fasting state might be caused by the delay of clearance of postprandial TG-rich lipoproteins. If our speculation were correct, an increase in RLP-C in the fasting state in CAD with DM might be reasonable.

In conclusion, we demonstrated that RLP-C was higher in patients with CAD than those in subjects without CAD. RLP-C was higher in CAD than non-CAD, although the plasma-TG level was similar among groups. Among DM subjects, RLP-C and RLP-C/plasma-TG ratios were also higher in CAD than that in non-CAD, although there was no difference among non-DM subjects. Moreover, after excluding the subjects with hypertriglyceridemia, RLP-C/plasma-TG ratio was higher in CAD than in non-CAD among DM subjects. Therefore, we suggest that an increase in RLPs to TG-rich lipoproteins might be associated with CAD among DM subjects, although further prospective or intervention studies should be required.

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