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Low density lipoprotein cholesterol level inversely correlated with coronary flow velocity reserve in patients with Type 2 diabetes

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Abstract

Objectives To evaluate the association of coronary artery endothelial function and plasma levels of low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) in patients with Type 2 Diabetes Mellitus (DM). **Methods** We investigated 90 participants from our institution between October 2007 to March 2010: non-DM ($n = 60$) and DM ($n = 30$). As an indicator of coronary endothelial dysfunction, we used non-invasive Doppler echocardiography to quantify coronary flow velocity reserve (CFVR) in the distal part of the left descending artery after rest and after intravenous adenosine administration. **Results** Plasma level of LDL-C was significantly higher in patients with DM than in non-DM (3.21 ± 0.64 *vs.* 2.86 ± 0.72 mmol/L, $P < 0.05$), but HDL-C level did not differ between the groups (1.01 ± 0.17 *vs.* 1.05 ± 0.19 mmo/L). Furthermore, the CFVR value was lower in DM patients than non-diabetics (2.45 ± 0.62 *vs.* 2.98 ± 0.68, $P < 0.001$). Plasma levels of LDL-C were negatively correlated with CFVR in all subjects ($r = -0.35$, $P < 0.001$; 95% confidence interval (CI): $-0.52 – -0.15$) and in the non-DM ($r = -0.29$, $P < 0.05$; 95% CI: $-0.51 – -0.05$), with an even stronger negative correlation in the DM group ($r = -0.42$, $P < 0.05$; 95% CI: $-0.68 – -0.06$). Age ($\beta = -0.019$, s = 0.007, sβ = -0.435, 95% CI: $-0.033 – -0.005$, $P = 0.008$), LDL-C ($\beta = -0.217$, s = 0.105, sβ = -0.282, 95% CI: $-0.428 – -0.005$, $P = 0.045$) remained independently correlated with CFVR in the DM group. However, we found no correlation between HDL-C level and CFVR in any group. **Conclusions** Diabetes may contribute to coronary artery disease (CAD) by inducing dysfunction of the coronary artery endothelium. Increased LDL-C level may adversely impair coronary endothelial function in DM. HDL-C may lose its endothelial-protective effects, in part as a result of pathological conditions, especially under abnormal glucose metabolism.

1 Introduction

Coronary artery disease (CAD) is the major cause of mortality in individuals with Diabetes Mellitus (DM). Individuals with diabetes often have a clustering of cardiovascular risk factors, such as hypertension and dyslipidaemia, which, in conjunction with hyperglycemia, increase the risk of endothelial dysfunction and atherosclerosis. Endothelial dysfunction has been demonstrated in Type 2 DM, in both the peripheral circulation and coronary circulation.[1,2] Diabetic dyslipidaemia is characterized by elevated levels of triglycerides and low density lipoprotein cholesterol (LDL-C) and reduced levels of high density lipoprotein cholesterol (HDL-C). LDL-C may directly impair endothelial nitric oxide (NO) synthase activity and NO bioavailability.[3] Some studies have shown a beneficial effect of lowering the LDL level on endothelial function in Type 2 DM.[4] Reduced levels of HDL-C are associated with increased risk of coronary disease and cardiovascular events, even in patients with low LDL-C levels.[5,6] The deleterious effects of oxidized LDL-C on the endothelium may be counteracted by increased HDL-C levels because of HDL-mediated vasoprotective effects. However, the effect of HDL in counteracting the impaired vascular relaxation induced by oxidized LDL-C is reduced in Type 2 DM.[7]

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Non-invasive transthoracic ultrasonography of coronary blood flow is increasingly used to assess coronary micro-circulation in epicardial coronary artery stenosis.[8] Coronary flow velocity reserve (CFVR), derived from the ratio of maximal hyperemic to basal coronary blood flow, is an accepted index for assessing coronary microcirculation and provides important information about coronary endothelial function. Pharmacological hyperemic vasodilation induced by adenosine is often used to assess microcirculatory function due to the ability to induce maximal vasodilation of coronary resistance vessels.[9] A recent study showed CFVR is significantly decreased in patients with abnormal glucose metabolism,[10] and CFVR was found to provide independent prognostic value in diabetic and non-diabetic patients.[11] Our previous study demonstrated CFVR was decreased in chronic hyperhomocysteinemic patients.[12] However, the association between LDL-C or HDL-C level and CFVR remains uncertain, especially in patients with abnormal glucose metabolism.

In the present study, we investigated whether coronary endothelial function was damaged in Type 2 DM. We also evaluated the effect of LDL-C and HDL-C levels on CFVR in diabetic and non-diabetic patients.

2 Methods

2.1 Subjects

Patients were selected from the Department of Endocrinology and Cardiology at Peking University Third Hospital from October 2007 to March 2010. We enrolled 90 participants, including 60 non-DM and 30 patients with type 2 DM. We excluded patients with acute myocardial infarction, coronary artery disease (> 50% stenosis as shown on angiography), heart failure, renal function impairment, liver function impairment, systemic inflammatory disease, infectious disease, cancer, or a serious illness, or who were undergoing treatment with nitrates or insulin that would affect their participation.

2.2 Study design

The study participants were divided into two groups: non-DM ($n = 60$) and patients with DM ($n = 30$). All patients underwent coronary Doppler echocardiography after rest and after adenosine treatment. Fasting blood samples were drawn for analysis of clinical chemical levels. Plasma samples were stored at −70ºC.

All subjects gave their written informed consent to participate in the study. The study was approved by the Ethics Committee of the Health Science Center, Peking University.

2.3 The assessment of coronary microvascular function

Assessment by transthoracic Doppler echcardiography (TTDE) is an effective method to evaluate CFVR.[9] Coronary artery endothelial function was assessed by measurement of CFVR. The peak coronary flow velocities in the distal left anterior descending coronary artery were recorded at rest and during hyperemia after intravenous infusion of adenosine [0.14 mg/(kg·min)] in all subjects. CFVR was calculated by the following formula: CFVR = peak coronary flow velocity during hyperemia/peak coronary flow velocity at rest.

2.4 Laboratory measurements

Blood samples were taken in the morning after an overnight fast and collected into vacuum tubes containing EDTA for the measurement of plasma lipid and lipoprotein levels. Levels of total cholesterol (TC), LDL-C, HDL-C and triglycerides were analyzed by colorimetric enzymatic assays with use of an Auto-Analyzer (HITACHI-7170). Fasting plasma glucose, fasting insulin and hemoglobin A1c levels were determined at the central chemistry lab of Peking University Third Hospital.

2.5 Statistical analysis

We use Prism 4.0 statistical software to analyse all data. Data are expressed as mean ± standard deviation (SD). Differences between groups were analyzed by Student's *t-* test or Chi-square test. Multivariate logistic regression analysis was used to evaluate the associations between the CFVR and metabolic parameter levels while adjusting for potential confounders. A $P < 0.05$ (two-tailed) was considered significant.

3 Results

3.1 Clinical characteristics of the study participants

The clinical characteristics of study participants are in Table 1. The non-DM and DM groups did not differ in age, sex, body mass index, or smoking habits. However, the prevalence of hypertension and dyslipidaemia was significantly higher in the DM patients than non-diabetics. Plasma levels of TC, LDL-C, fasting blood glucose, and hemoglobin A1c were significantly higher in the DM than non-DM (Table 1).

3.2 CFVR in DM

In total, 88 patients underwent assessment of CFVR by TTDE. CFVR was significantly impaired in the DM group as compared with the non-DM (2.45 ± 0.62 *vs.* 2.98 ± 0.68, $P < 0.001$) (Figure 1).

Table 1. Characteristics and metabolic variables of non-DM and DM patients.

Variables	Non-DM ($n = 60$)	DM group ($n = 30$)
Age (yr)	55.17 ± 6.55	56.80 ± 7.81
Body mass index (kg/m^2)	25.50 ± 2.95	26.55 ± 3.46
Sex (M/F)	38/22	20/10
Smoking (%)	25	30
Hypertension (%)	25.0	53.3*
Hyperlipidemia (%)	31.7	60.0*
Metabolic parameters		
Total cholesterol, mmol/L	4.95 ± 0.65	5.27 ± 0.78*
HDL-cholesterol, mmol/L	1.05 ± 0.19	1.01 ± 0.17
LDL-cholesterol, mmol/L	2.86 ± 0.72	3.21 ± 0.64 *
Triglycerides, mmol/L	2.20 ± 1.32	2.69 ± 1.41
Fasting plasma glucose, mmol/L	5.62 ± 1.20	6.92 ± 1.84#
Fasting insulin, μU/L	12.30 ± 5.11	12.62 ± 6.72
HbA1c, %	6.06 ± 0.53	7.09 ± 1.25#

*$P < 0.05$, #$P < 0.001$ compared with non-DM patients. Data are mean ± SD unless indicated. HDL: high-density lipoprotein; LDL: low-density lipoprotein; HbA1c: glycosylated hemoglobin.
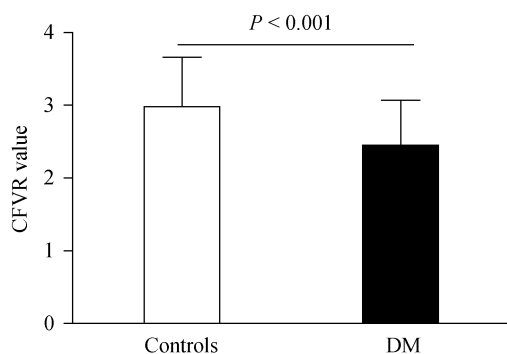

Figure 1. CFVR was significantly decreased in DM patients as compared with the non-diabetics. $n = 60$ in non-DM, $n = 30$ in DM group. Values are means ± SD. CFVR: coronary flow velocity reserve; DM: diabetes mellitus.

3.3 Correlation between CFVR and plasma levels of LDL-C and HDL-C in all subjects

To test whether LDL-C or HDL-C levels were related to impaired coronary endothelial function, we tested the correlation between CFVR and plasma levels of LDL-C and HDL-C in all subjects. The LDL-C level was negatively correlated with CFVR ($r = -0.35$, $P < 0.001$; 95% CI: $-0.48 - -0.13$) (Figure 2A), with no significant correlation between HDL-C level and CFVR ($r = -0.08$; 95% CI: $-1.07 - 0.46$) (Figure 2B).

3.4 Correlation between CFVR and plasma levels of LDL-C and HDL-C in Type 2 DM

To test whether LDL-C or HDL-C levels were related to impaired coronary endothelial function, demonstrated in
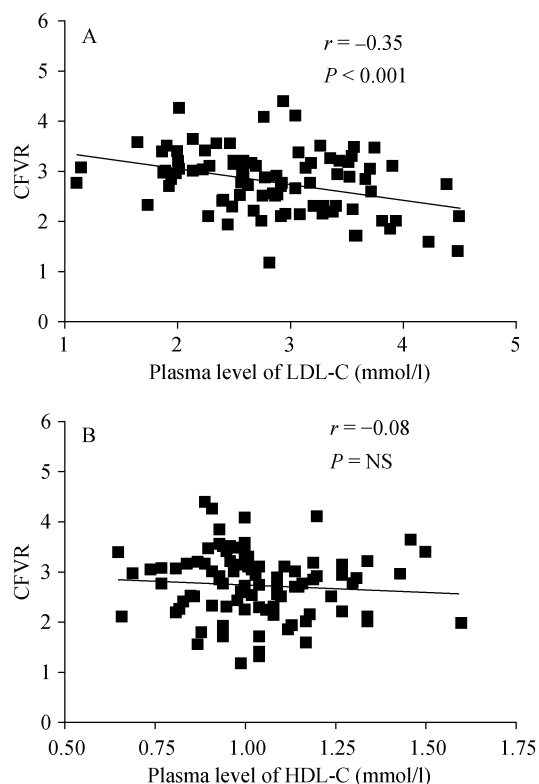

Figure 2. Correlation between CFVR and plasma levels of (A) LDL-C and (B) HDL-C in all subjects ($n = 90$). CFVR: coronary flow velocity reserve; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; NS: not significant.

Type 2 DM, we tested the correlation between CFVR and plasma levels of LDL-C or HDL-C in patients with DM. The negative correlation between LDL-C level and CFVR was determined to be stronger between LDL-C and CFVR in DM patients than in all subjects ($r = -0.42$, $P < 0.05$) (Figure 3A). The HDL-C level was not correlated with CFVR in patients with DM (Figure 3B).

3.5 Correlation between CFVR and plasma level of LDL-C in non-DM

We found a significant, but weak negative correlation between LDL-C level and CFVR in the non-DM ($r = -0.29$, $P < 0.05$; 95% CI: $-0.42 - -0.04$) but no correlation between HDL-C level and CFVR (Figure 4).

3.6 Multivariate logistic regression analysis between the CFVR and plasma level of lipids

After multiple logistic regression analysis, Age ($\beta = -0.019$, s = 0.007, s$\beta = -0.435$, 95% CI $-0.033 - -0.005$, $P = 0.008$), LDL-C ($\beta = -0.217$, s = 0.105, s$\beta = -0.282$, 95% CI: $-0.428 - -0.005$, $P = 0.045$) remained independently correlated with CFVR in DM group.
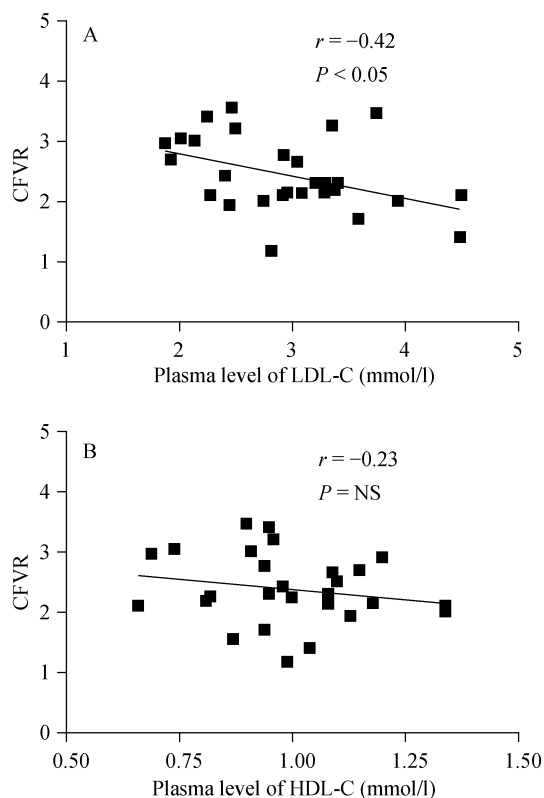
Figure 3.   Correlation between CFVR and plasma levels of (A) LDL-C and (B) HDL-C in DM patients (n = 30). CFVR: coronary flow velocity reserve; DM: diabetes mellitus; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; NS: not significant.

Figure 4.   Correlation between CFVR and plasma levels of (A) LDL and (B) HDL in non-DM patients (n = 60). CFVR: coronary flow velocity reserve; DM: diabetes mellitus; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; NS: not significant.

4   Discussion

DM has been associated with impaired vascular endothelial function and CAD. We demonstrated that CFVR was significantly impaired in patients with DM. In addition, the plasma level of LDL-C was negatively correlated with CFVR both in diabetic patients and non-DM patients. Finally, CFVR was not correlated with plasma levels of HDL-C in diabetic patients or non-DM. An increased LDL-C level may induce greater dysfunction of the coronary artery endothelium under DM than non-DM conditions.

CFVR, defined as the ratio of maximal to basal coronary blood flow, is an accepted index for assessment of the coronary microcirculation.[8] It reflects the effect on total coronary resistance in terms of epicardial coronary arteries and the vasodilator capacity of the microcirculation. The vasodilator effect of adenosine was thought to involve both the direct stimulation of vascular smooth muscle cells and the release of NO from endothelial cells.[13,14] Hence, adenosine-derived CFVR involves both endothelium dependent
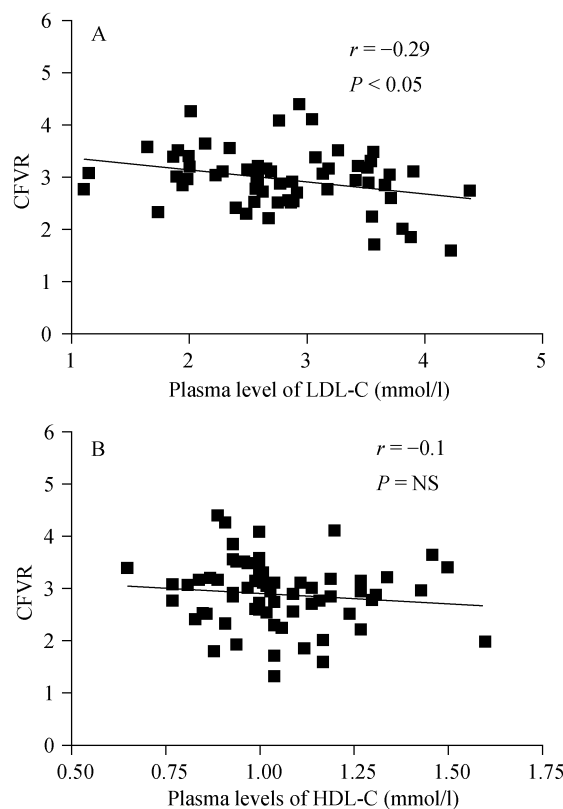
and independent microcirculatory functions. The CFVR response to adenosine reflects at least in part, endothelial function. Our previous results showed CFVR significantly lower in hyper homocysteinemic patients.[12] Our present study demonstrated CFVR is significantly lower in diabetic patients than non-DMs. Consistent with our findings, Logstrup *et al*.[10] showed that CFVR was significantly reduced in patients with known, or newly diagnosed, DM with the lowest value recorded in known diabetic patients. A prospective study showed the cardiovascular event rate markedly higher for diabetic patients with abnormal CFVR than for those with normal CFVR. In addition, a normal CFVR in diabetic patients off therapy showed better survival rates.[11] Hence, abnormal glucose metabolism may contribute to CAD and cardiovascular events by inducing dysfunction of the coronary artery endothelium.

Endothelial dysfunction is an early sign of diabetic vasculopathy and atherosclerosis. Endothelial function was markedly impaired in Type 2 DM and the risk of coronary artery disease was increased. The precise pathogenetic mechanisms underlying the development of endothelial

dysfunction in DM remain uncertain, but they probably involve dyslipidaemia and insulin resistance. Studies of humans and animals have shown LDL-C causing vascular endothelial dysfunction that leads to CAD mainly by increasing oxidative stress, impairing endothelial NOS activity, and attenuating NO bioavailability.[3,15,16] The treatments lowering LDL-C showed a beneficial effect on endothelial function and cardiovascular events.[4,17] Clinical evidence revealed an inverse correlation of LDL-C levels and CFVR in hypercholesterolemia.[18] In the present study, we found LDL-C levels negatively correlated with CFVR both in DM patients and non-diabetics, with a stronger inverse correlation in diabetic patients. These results suggest a potential serious impairment of coronary endothelial function by LDL-C levels acting on CFVR in Type 2 DM.

Reduced HDL-C level is a major risk factor for coronary disease and is associated with increased risk of coronary disease and major cardiovascular events. HDL-C can have direct endothelial-protective effects, such as stimulating endothelial cell production of NO and endothelium-dependent vasodilation.[19,20] The deleterious effects of oxidized LDL-C on the endothelium may be counteracted by HDL and has been attributed to HDL-mediated vasoprotective effects. However, the effects of HDL-C level on the endothelium could be highly heterogeneous. The beneficial effect of HDL-C level in counteracting the oxidized LDL–induced impairment of vascular relaxation was reduced under the pathological conditions of Type 2 DM.[7] HDL from patients with CAD was pro-inflammatory rather than anti-inflammatory.[21] Moreover, therapy increasing HDL had no beneficial effect on coronary atherosclerosis progression.[22] Our present study demonstrates HDL-C level was not correlated with CFVR in diabetic patients or non-diabetics. In partial agreement with our findings, Kaufmann *et al*.[18] reported a weak correlation between CFVR and HDL levels in normal subjects, but not patients with hypercholesterolemia. Why HDL-C loses its beneficial effects on coronary endothelial function under DM, hypercholesterolemia, or coronary artery disease is unknown. The mechanisms are likely complex and may include oxidative modification of HDL-C and potentially altered endothelial binding capacity of HDL-C. A recent study showed that HDL from patients with Type 2 DM lost the capacity to stimulate endothelial NO production and NO-mediated vasodilation. As well, diabetic patients showed increased lipid peroxidation of HDL.[23] Hence, the plasma level of HDL may not represent a reliable index to predict vasoprotective effects of HDL, especially in patients with DM.

In conclusion, we found age and LDL-C independently correlated with CFVR in the DM group. Furthermore,

LDL-C may contribute to CAD by impairing endothelial function, and this deleterious effect may be more serious under pathophysiological conditions, such as DM. HDL-C may partially lose its endothelial protective effects, in part, due to pathological conditions, especially in Type 2 DM.

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