Glycation of Apoprotein A-I Is Associated With Coronary Artery Plaque Progression in Type 2 Diabetic Patients

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OBJECTIVE—To investigate whether glycation level of apoprotein (apo)A-I is associated with coronary artery disease (CAD) and plaque progression in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS—Among 375 consecutive type 2 diabetic patients undergoing quantitative coronary angiography (QCA) and intravascular ultrasound (IVUS), 82 patients with nonsignificant stenosis (luminal diameter narrowing <30% [group I]) and 190 patients with significant CAD (luminal diameter stenosis ≥70% [group II]) were included for analysis of apoA-I glycation level and serum activity of lecithin: cholesterol acyltransferase (LCAT). The control group had 136 healthy subjects. At the 1-year follow-up, angiography and IVUS were repeated mainly in group II patients for plaque progression assessment.

RESULTS—Relative intensity of apoA-I glycation by densitometry was increased, and serum LCAT activity was decreased stepwise across groups control, I, and II. These two measurements were associated with the number of diseased coronary arteries and extent index in group II. During 1-year follow-up, QCA detected 45 patients with plaque progression in 159 subjects, and IVUS found 38 patients with plaque progression in 127 subjects. Baseline relative intensity of apoA-I glycation was significantly increased in patients with plaque progression compared with those without, with values associated with changes in QCA and IVUS measurements. Multivariable regression analysis revealed that baseline relative intensity of apoA-I glycation was an independent determinant of CAD and plaque progression in type 2 diabetic patients.

CONCLUSIONS—ApoA-I glycation level is associated with the severity of CAD and coronary artery plaque progression in type 2 diabetic patients.

P opulation studies have consistently demonstrated an inverse association between serum HDL cholesterol levels and the risk of coronary artery disease (CAD) (1). The antiatherogenic properties of HDL and its major component apoprotein (apo)A-I are mainly related to reverse cholesterol transport process (i.e., removing excess cholesterol from the arterial wall's foam macrophages to the liver for metabolism).

Physiologically, the efficiency of reverse cholesterol transport depends on the activity of lecithin: cholesterol acyltransferase (LCAT), and apoA-I activates LCAT to facilitate reverse cholesterol transport and HDL maturation (1–3). Thus, the function of apoA-I and LCAT is essential for maintaining body cholesterol homeostasis.

The impact of advanced glycation end products has been implicated in diabetic atherogenesis (4). Since glycation of HDL significantly decreases its ability to inhibit oxidized LDL-induced monocyte–endothelial cell interaction (5,6) and infusion of reconstituted HDL obviously reduces atherosclerotic plaque volume and increases anti-inflammatory and cholesterol efflux properties of plasma HDL in patients with type 2 diabetes (7,8), we hypothesized that glycation of apoA-I might decrease LCAT activation and reverse cholesterol transport, leading to accelerated development of atherosclerotic lesions in diabetic patients.

Thus, in the current study, we investigated whether glycation level of apoA-I and serum LCAT activity were associated with CAD and plaque progression defined by quantitative coronary angiography (QCA) and intravascular ultrasound imaging (IVUS) in type 2 diabetic patients at a 1-year follow-up.

RESEARCH DESIGN AND METHODS—A total of 375 consecutive patients with type 2 diabetes undergoing coronary angiography and IVUS between January 2009 and May 2010 were screened. The patients were recruited from the database of Shanghai Rui Jin Hospital PCI Outcomes Program. This program uses clinical and angiographic information of CAD patients to estimate risk-adjusted outcomes. Data on patient demographics; clinical, angiographic, and IVUS features; and in-hospital managements were collected retrospectively, whereas clinical outcomes, repeat angiography, and IVUS characteristics during follow-up were identified prospectively. The diagnosis of type 2 diabetes was made according to the criteria of the American Diabetes Association (symptoms of diabetes with casual plasma glucose concentration ≥200 mg/dL [11.1 mmol/L] or fasting blood glucose ≥126 mg/dL [7.0 mmol/L], 2-h postprandial glucose ≥200 mg/dL [11.1 mmol/L] during an oral glucose tolerance test, and current or previous treatment with insulin and/or oral hypoglycemic agents) (9). Hypertension and

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hyperlipidemia were defined according to previously published guidelines (10,11). For the purpose of the study and for avoidance of confounding data, patients with acute coronary syndrome within 7 days, heart failure, concomitant valvular disease, congenital heart disease, or cardiomyopathy were excluded. We also excluded patients with type 1 diabetes by measurement of C-peptide (12). The patients eligible for study were categorized as two groups: group I included 82 patients with normal coronary artery or nonsignificant coronary lesions (luminal diameter stenosis <30%), and group II consisted of 190 patients with significant CAD (luminal diameter narrowing ≥70%, a severity level according to the clinical standards of the American College of Cardiology/ American Heart Association guidelines for coronary angiography [13]). Group II patients were further subdivided according to the number of diseased coronary arteries. Besides group I and group II patients, there were 103 diabetic patients with coronary artery stenosis ≥30% but <70%.

One hundred and thirty-six healthy subjects served as a control group. Detailed medical and family histories were recorded, and fasting blood samples were collected during an annual physical check-up. All had normal serum levels of glucose, lipid profiles, and hepatic and renal function. None had a history of cardiovascular diseases (including past history of angina and myocardial infarction). Their electrocardiogram and ergometer exercise tests were negative for myocardial ischemia.

All participants gave written informed consent, and the protocol was approved by the hospital ethics committee.

Follow-up

All patients were clinically followed up in a special outpatient clinic for diabetes vascular complications or by telephone conversation with patients and their family members every 3 months after initial angiography. Medical treatments (Table 1 and Supplementary Table 1), including statins, aspirin, clopidogrel, ACE inhibitors/ angiotensin receptor blockers (ARBs), and antidiabetes agents, were prescribed according to the guidelines recommended by the American Heart Association and American Diabetes Association (14,15). For meeting the treatment goal, serum levels of fasting blood glucose, 2-h postprandial blood glucose, and HbA$_{1c}$ were examined every 6

months, with instant self-monitoring of blood glucose for glycemic control and hypoglycemic prevention. A lipid profile and glycation levels of apoA-I were evaluated at 12 months. For cardiac event follow-up, the major events consisted of nonfatal myocardial infarction and cardiac death. Patients of cardiac death were not included in this study of plaque progression because of unavailable follow-up angiography (Fig. 1). Secondary events included unstable angina, heart failure, and coronary revascularization. In order

to guarantee rigorous data quality, all adverse cardiac events were reviewed by two experienced interventional cardiologists. The choice of repeat percutaneous coronary intervention or coronary artery bypass grafting was at the physician's discretion.

Follow-up coronary angiography and IVUS were performed at ~12 months to assess the therapeutic effects and progression of atherosclerotic lesions in group II patients. The patients in group I and those with coronary artery stenosis

Table 1—Baseline characteristics and biochemical assessments

Variables	Control group (no CAD or diabetes)	Group I (diabetes, no CAD)	Group II (diabetes and CAD)	P
n	136	82	190	
Male sex	80 (58.8)	52 (63.4)	124 (65.3)	0.49
Age (years)	64 ± 11	63 ± 9	67 ± 10	<0.001
Cigarette smoking	26 (19.1)	19 (23.2)	61 (32.1)	0.025
Alcohol consumption	9 (5.81)	6 (7.32)	16 (8.42)	0.827
Hypertension		40 (48.8)	118 (62.1)	0.041
Systolic BP (mmHg)	124 ± 8	132 ± 9	140 ± 11&	<0.001
Diastolic BP (mmHg)	70 ± 8	76 ± 9	82 ± 8&	<0.001
Hyperlipidemia		38 (46.3)	120 (63.2)	0.01
Total cholesterol (mmol/L)	3.68 ± 0.68	4.20 ± 0.95	4.26 ± 1.23	<0.001
HDL cholesterol (mmol/L)	1.31 ± 0.26	1.16 ± 0.27	1.06 ± 0.26	<0.001
LDL cholesterol (mmol/L)	2.34 ± 0.61	2.40 ± 0.78	2.50 ± 0.98	0.374
Triglycerides (mmol/L)	1.20 ± 0.38	1.78 ± 0.88	1.85 ± 1.05	<0.001
Lipoprotein (a) (g/L)	0.12 ± 0.06	0.16 ± 0.10	0.23 ± 0.22&	<0.001
ApoA (g/L)	1.35 ± 0.32	1.29 ± 0.25	1.16 ± 0.22#	<0.001
ApoB (g/L)	0.66 ± 0.20	0.82 ± 0.22	0.89 ± 0.35	<0.001
Fasting glucose (mmol/L)	4.12 ± 0.52	6.02 ± 1.12	7.33 ± 2.82&	<0.001
2-h postprandial glucose (mmol/L)	6.95 ± 1.38	11.07 ± 3.68	13.69 ± 4.47&	<0.001
HbA$_{1c}$ (%)	5.72 ± 0.36	6.95 ± 0.81	7.71 ± 1.45&	<0.001
Blood urea nitrogen (mmol/L)	4.69 ± 1.05	5.16 ± 1.54	5.91 ± 1.86#	<0.001
Creatinine (μmol/L)	61 ± 7	67 ± 19	80 ± 24&	<0.001
Uric acid (μmol/L)	288 ± 60	336 ± 80	338 ± 85	<0.001
Medical treatments				
ACEI or ARB		61 (74.4)	168 (88.4)	0.004
CCB		27 (32.9)	53 (27.9)	0.403
Statins*		73 (89.0)	174 (91.6)	0.503
Metformin		33 (40.2)	70 (36.8)	0.596
Sulphonylureas		27 (32.9)	78 (41.1)	0.207
α-Glucosidase		36 (43.9)	73 (38.4)	0.397
PPARγ agonist		11 (13.4)	29 (15.3)	0.693
Insulin therapy		13 (15.9)	77 (40.5)	<0.001
Antiplatelet		70 (85.4)	175 (92.1)	0.088
LCAT activity (μmol/mL × h)	0.30 ± 0.04	0.25 ± 0.04	0.20 ± 0.03&	<0.001
Relative intensity of apoA-I glycation	1.35 ± 0.41	5.50 ± 1.40	10.12 ± 4.76&	<0.001

Data are means ± SD or n (%). "No CAD" was determined as no significant coronary artery stenosis according to definition given in the text. ACEI, ACE inhibitor; BP, blood pressure; CCB, calcium channel blocker. *Statins: mainly simvastatin, pravastatin, and atorvastatin; &P <0.01 vs. Group I; #P <0.05 vs. Group I.
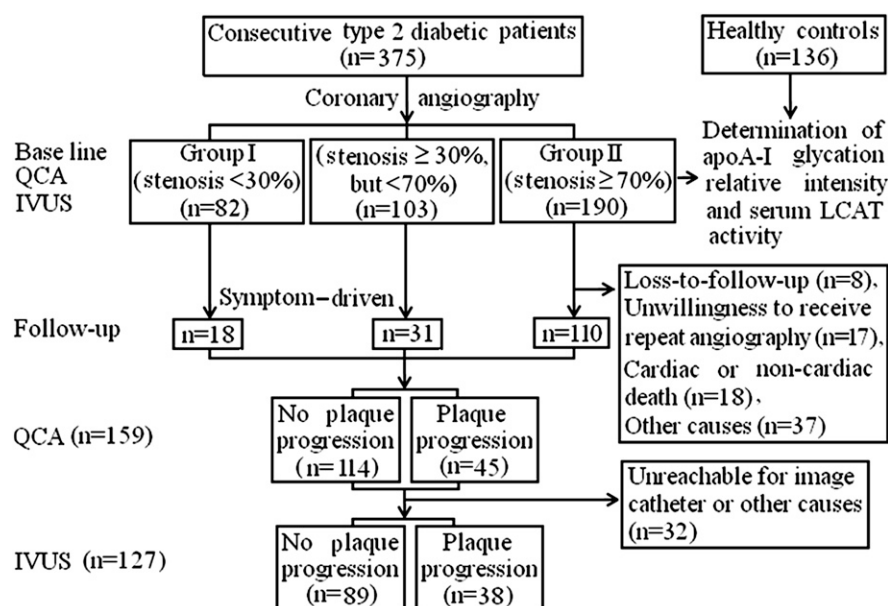
Figure 1—*Flowchart of follow-up in patients.*

≥30% but <70% were also subject to these examinations if angina symptoms were aggravated or electrocardiographic changes of myocardial ischemia occurred during follow-up. Through analysis of baseline and repeat imaging data, patients were categorized as those with plaque progression or with no plaque progression.

Angiography and quantitative analysis

Coronary angiography was performed using standard Judkins technique or through a radial approach. Significant CAD was diagnosed visually if luminal diameter narrowing was estimated as ≥70% in a major epicardial coronary artery. Left main coronary artery narrowing ≥50% was considered as two-vessel disease. Drug-eluting stents were always used in diabetic patients based upon the evidence that they are superior to bare-metal stents regarding mortality, myocardial infarction, and revascularization rates (16). QCA was performed using the Cardiovascular Measurement System software (version 3.0; Terra, GE) by two interventional cardiologists blinded to the study protocol. The extent index was calculated as the longitudinal percentage of coronary segments involved with a stenosis: $[100 \times \sum (\text{stenosis lengths})/\sum (\text{segment lengths})]$ (17). Coronary plaque progression was defined as one or more lesion with decreased minimum lumen diameter (MLD) ≥0.4 mm from baseline to follow-up angiography—a change representing approximately twice the SD for repeat measurements for lesions (18). Coronary artery score represents the per-patient average of the MLD of all the measured segments, and a higher score reflects less obstructed coronary artery lumen. Change in coronary artery score was defined as the per-patient mean of MLD changes (follow-up minus baseline angiogram) (19,20). Cumulative coronary stenosis score was calculated by adding all percent diameter stenoses in stenosis index units (50% = 0.50) (21) and used as an index of the anatomic extension and severity of CAD; a higher value reflects more severe disease.

IVUS examination and analysis

The IVUS acquisition and analysis were performed as previously described (22,23). Briefly, after intracoronary administration of nitroglycerin, the image catheter of IVUS was inserted distally within a coronary artery. The target vessel for imaging was required to have a segment of at least 30 mm in length with no lumen narrowing ≥50%, no previous revascularization performance, and not being considered a culprit vessel for a previous myocardial infarction. Continuous imaging was acquired during withdrawal of the catheter through the segment of artery at a constant rate of 0.5 mm/s. Images were stored on a floppy disc and subsequently analyzed by core laboratory personnel who were blinded to research protocol. Meticulous care was taken during follow-up IVUS so that imaging of the coronary artery segment was identical to that at the baseline examination. Percent atheroma volume (PAV) was calculated as previously described (22,23). The serial progression rate of plaque was compared with change in PAV, measured as follows: (follow-up PAV − baseline PAV)/baseline PAV × 100 (23). Substantial plaque progression was defined as at least a 5% relative increase in PAV.

Biochemical assessments

Blood samples were obtained on the day of angiography after an overnight fasting in all patients. Serum levels of glucose, blood urea nitrogen, creatinine, uric acid, total cholesterol, LDL cholesterol, HDL cholesterol, lipoprotein (a), apoA, apoB, and triglycerides were measured with standard laboratory techniques on a Hitachi 912 Analyzer (Roche Diagnostics, Mannheim, Germany). Blood concentration of HbA_{1c} was assayed using ion-exchange high-performance liquid chromatography with a Bio-Rad Variant Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA).

Isolation of lipoproteins

HDL (density 1.063 to 1.21 g/mL) was isolated from fresh plasma by sequential ultracentrifugation as previously described (24). HDL was dialyzed against 150 mmol/L NaCl and 0.01% EDTA (pH 7.4) at 4°C overnight and then stored in sealed tubes. ApoA-I mass was determined using SDS-PAGE and stained with silver nitrate.

Antibodies and Western blot analysis

Isolated HDL protein was separated on SDS–polyacrylamide gel and then transferred to polyvinylidene fluoride membrane. After blocking with 5% milk, the membrane was incubated overnight at 4°C with anti–apoA-I (Santa Cruz Biotechnology), anti–Nε-(carboxyethyl)-lysine (CEL), or anti–Nε-(carboxymethyl)-lysine (CML) antibody (Cosmo Bio, Tokyo, Japan). Enhanced chemiluminescence detection reagent (GE Healthcare, Buckinghamshire, U.K.) was used after reaction with secondary antibody. Films were scanned using an HP Scanjet Pro flatbed scanner, and images were analyzed and quantified using Adobe Photoshop CS2 software (25). A band of 28 kDa was validated to be

apoA-I protein by Western blot, and Western blot also detected glycation level of apoA-I protein in patients and control subjects, using anti-CEL and anti-CML antibodies (Fig. 2). Absolute intensity was calculated by multiplying the mean density value by pixel for each band, and relative intensity of apoA-I glycation (here, CEL level was analyzed because it is more related to nonenzymatic glycation of lipids, while CML can also form through oxidative reaction and inflammation [26]) was calculated as absolute intensity of apoA-I glycation divided by that of protein apoA-I.

Determination of serum LCAT activity

For determining serum LCAT activity, we measured the decrease in endogenous unesterified cholesterol after incubation of serum with liposomes according to methods previously described (27), with slight modification using microplate spectrophotometer (Model PowerWave XS2; BioTek Instruments).

Statistical analyses

Data are expressed as means $\pm$ SD for continuous variables and frequencies and percentages for categorical ones. χ^2 test was used to analyze dichotomous variables. Comparisons of continuous variables among groups were done by one-way ANOVA, with post hoc analysis in two-group comparisons using the Fisher least significant differences test or Dunnett T3 test. The correlations of serum relative intensity of apoA-I glycation with LCAT activity, number of diseased coronary arteries, and parameters of QCA and IUVS were determined by Pearson or Spearman rank test as appropriate. In the multivariable logistic regression analysis, conventional risk factors without (models 1, 3, and 5) and with (models 2, 4, and 6) relative intensity of apoA-I glycation were assessed for independent determinants of CAD and plaque progression defined by QCA and IVUS in diabetic patients. Receiver operating characteristic analysis of risk factors was performed. We compared the discriminatory capability between models with and without apoA-I glycation levels by using C statistics and also risk reclassification according to Pencina method for determining net reclassification improvement (28). For assessment of model calibration (or how closely the predicted probabilities reflect actual risk), the Hosmer-Lemeshow calibration was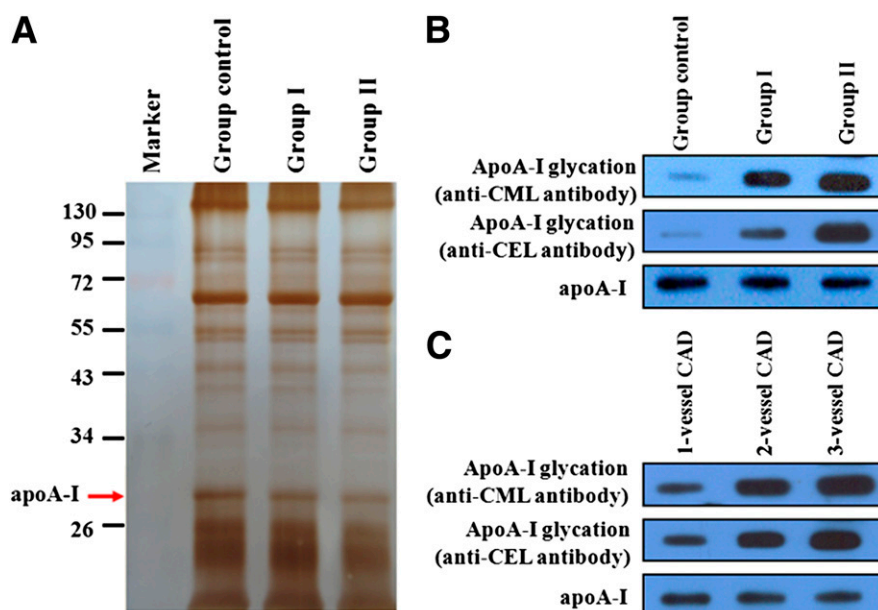 computed. SPSS 13.0 software was used for all statistical testing (SPSS, Chicago, Illinois). A two-tailed <0.05 was considered statistically significant.

RESULTS

Clinical characteristics of diabetic patients with and without CAD

Type 2 diabetic patients with CAD (group II) were more hypertensive and dyslipidemic and had significantly higher levels of lipoprotein (a), blood urea nitrogen, creatinine, fasting glucose, 2-h postprandial glucose, and HbA$_{1c}$ and lower levels of apoA than those without (group I) (Table 1).

Glycation level of apoA-I and LCAT activity in diabetic patients with and without CAD

Relative intensity of apoA-I glycation was stepwise increased from control subjects to group II and from patients with one-vessel disease to those with three-vessel disease (for all comparisons, $P < 0.01$) (Table 1 and Fig. 2). These relative intensity values correlated closely with blood HbA$_{1c}$ concentrations (Pearson $r = 0.744$, $P < 0.01$), extent index of atherosclerosis (Pearson $r = 0.596$, $P < 0.01$), and serum LCAT activity (Pearson $r = -0.644$, $P < 0.01$). However, serum LCAT activity was decreased across group II, group I, and

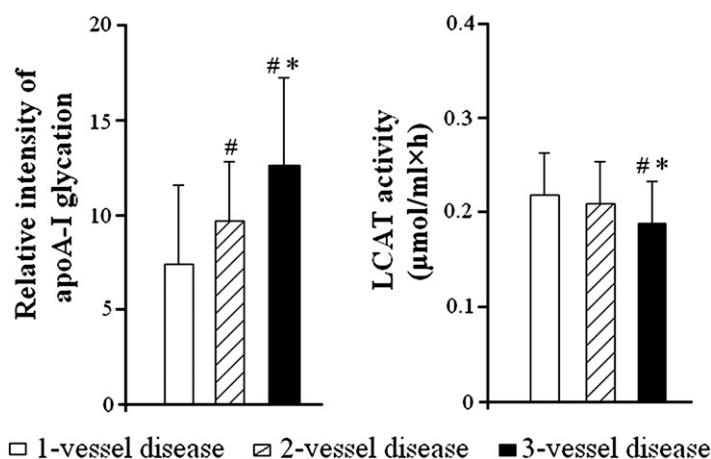Figure 2—*Detection of apoA-I glycation and apoA-I levels by Western blot.*

Figure 3—*Glycation level of apoA-I and serum LCAT activity in subgroups with a varying number of diseased coronary arteries. #P < 0.01 versus one-vessel disease subgroup; *P < 0.01 versus two-vessel subgroup.*

control subjects, with significant reduction in the three-vessel disease subgroup compared with one-vessel and two-vessel disease subgroups (for all comparison, $P < 0.01$) (Table 1 and Fig. 3).

Glycation level of apoA-I and LCAT activity in diabetic patients with and without plaque progression
Repeat angiography and IVUS examinations were performed during follow-up (mean 13.2 ± 3.6 months) in 159 and 127 patients, respectively (follow-up data and the causes for not having imaging examinations are provided in Fig. 1). Coronary artery plaque progression occurred in 45 and 38 patients according to QCA and IVUS criteria. Patients with QCA- and IVUS-defined plaque progression had higher baseline levels of fasting blood glucose, 2-h postprandial blood

glucose, HbA_{1c}, and LDL cholesterol and lower baseline levels of HDL cholesterol than those without (Table 2). During follow-up (Supplementary Table 1), readmission rate and percentage of unstable angina were obviously higher in patients with plaque progression than in those without. Consistently with baseline data, fasting and 2-h postprandial blood glucose and HbA_{1c} levels during follow-up were higher but HDL cholesterol levels were lower in patients with plaque progression than in those without. These data jointly represented poor glycemic control or brittle diabetes and more dyslipidemic status in patients with plaque progression compared with those without. There was no difference in medical treatments between the two groups during follow-up (Supplementary Table 1).

Follow-up angiography showed that patients with plaque progression had a significantly greater decrease in MLD and increase in cumulative coronary stenosis score compared with those without (for all comparisons, $P < 0.05$) (Table 3). For IVUS measurements, patients with plaque progression displayed prominent enhancement versus those without regarding change in atheroma volumes, PAV values at follow-up, and changes in PAV (for all comparisons, $P < 0.01$) (Table 3).

Notably, baseline relative intensity of apoA-I glycation was significantly increased and serum LCAT activity was reduced in patients with QCA- or IVUS-defined plaque progression compared with those without (for all comparisons, $P < 0.05$). Moreover, this relative intensity of apoA-I glycation correlated closely with changes in coronary artery score

Table 2—Baseline characteristics and biochemical assessments between patients with and without plaque progression

Variables	QCA No PP	QCA PP	P	IVUS No PP	IVUS PP	P
n	114	45		89	38	
Male sex	70 (61.4)	33 (73.3)	0.156	57 (64)	28 (73.7)	0.290
Age (years)	63 ± 9	67 ± 9	0.013	62 ± 8	66 ± 8	0.010
Cigarette smoking	32 (28.1)	14 (31.1)	0.703	24 (27)	12 (31.6)	0.597
Alcohol consumption	8 (7.02)	4 (8.89)	0.742	7 (7.87)	4 (10.53)	0.732
Hypertension	63 (55.3)	31 (68.9)	0.082	51 (57.3)	26 (68.4)	0.240
Systolic BP (mmHg)	137 ± 9	140 ± 10	0.069	138 ± 9	140 ± 9	0.271
Diastolic BP (mmHg)	79 ± 9	82 ± 8	0.053	80 ± 8	82 ± 8	0.199
Hyperlipidemia	64 (56.1)	30 (66.7)	0.167	50 (56.2)	24 (63.2)	0.465
Total cholesterol (mmol/L)	4.03 ± 1.02	4.26 ± 1.09	0.211	4.05 ± 1.02	4.27 ± 1.04	0.271
HDL cholesterol (mmol/L)	1.18 ± 0.27	1.03 ± 0.26	0.002	1.17 ± 0.26	1.01 ± 0.25	0.002
LDL cholesterol (mmol/L)	2.28 ± 0.87	2.69 ± 0.91	0.009	2.29 ± 0.85	2.71 ± 0.89	0.013
Triglycerides (mmol/L)	1.81 ± 1.02	1.86 ± 1.04	0.782	1.83 ± 1.03	1.88 ± 1.06	0.804
Lipoprotein (a) (g/L)	0.19 ± 0.20	0.24 ± 0.21	0.163	0.18 ± 0.19	0.22 ± 0.20	0.287
ApoA (g/L)	1.21 ± 0.21	1.16 ± 0.22	0.184	1.22 ± 0.21	1.17 ± 0.22	0.228
ApoB (g/L)	0.81 ± 0.21	0.87 ± 0.22	0.187	0.82 ± 0.21	0.87 ± 0.21	0.222
FBG (mmol/L)	6.85 ± 2.78	7.96 ± 2.81	0.025	6.80 ± 2.74	7.98 ± 2.78	0.029
2-h PG (mmol/L)	11.12 ± 3.56	13.81 ± 4.11	<0.001	11.05 ± 3.53	13.78 ± 4.07	<0.001
HbA_{1c} (%)	7.02 ± 1.38	7.97 ± 1.44	<0.001	7.00 ± 1.38	7.99 ± 1.41	<0.001
BUN (mmol/L)	5.76 ± 1.75	5.99 ± 1.81	0.461	5.74 ± 1.74	5.89 ± 1.80	0.660
Creatinine (μmol/L)	79 ± 23	81 ± 24	0.626	79 ± 22	80 ± 24	0.820
Uric acid (μmol/L)	334 ± 81	340 ± 85	0.679	325 ± 79	338 ± 83	0.405
Medications						
ACEI or ARB	101 (88.6)	38 (84.4)	0.477	78 (87.6)	32 (84.2)	0.603
CCB	30 (26.3)	13 (28.9)	0.742	22 (24.7)	10 (26.3)	0.849
Statins*	106 (93.0)	41 (91.1)	0.742	82 (92.1)	36 (94.7)	0.723
Metformin	37 (32.5)	17 (37.8)	0.523	28 (31.5)	14 (36.8)	0.266
Sulphonylureas	44 (38.6)	19 (42.2)	0.674	34 (38.2)	17 (44.7)	0.492
α-Glucosidase	43 (37.7)	20 (44.4)	0.435	32 (36.0)	18 (47.4)	0.228
PPARγ agonist	16 (14.0)	8 (17.8)	0.553	13 (14.6)	7 (18.4)	0.589
Insulin therapy	42 (36.8)	18 (40)	0.801	34 (38.2)	17 (44.7)	0.492
Antiplatelet	98 (86.0)	41 (91.1)	0.440	78 (87.6)	35 (92.1)	0.552

Data are means ± SD or *n* (%). ACEI, ACE inhibitor; BP, blood pressure; BUN, blood urea nitrogen; CCB, calcium channel blocker; FBG, fasting blood glucose; PP, plaque progression; 2-h PG, 2-h plasma glucose. *Statins: mainly simvastatin, pravastatin, and atorvastatin.

(Pearson $r = 0.529$, $P < 0.01$), cumulative coronary stenosis score (Pearson $r = 0.661$, $P < 0.01$), and PAV (Pearson $r = 0.618$, $P < 0.01$). Furthermore, follow-up relative intensity of apoA-I glycation was also higher in patients with plaque progression than in those without (for QCA- and IVUS-defined plaque progression, both $P < 0.001$) (Supplementary Table 1). In 15 patients who had cardiac death, baseline relative intensity of apoA-I glycation was significantly elevated (12.27 ± 4.66) compared with patients of no plaque progression ($P < 0.001$).

Multivariable analysis for the risk of CAD in diabetic patients
In multivariable logistic regression analysis, age, hypertension, hyperlipidemia, HbA_{1c}, and reduced apoA-I levels were independently associated with significant CAD in type 2 diabetic patients (model 1). When relative intensity of apoA-I glycation was included (model 2), it remained independently associated with CAD, besides the other factors in model 1. Moreover, the addition of apoA-I glycation relative intensity significantly improved the risk prediction (C statistic, from 0.768 to 0.907; $P < 0.001$; net reclassification improvement, 9.56%; $P < 0.001$). The calibration of model 1 (0.451) and model 2 (0.283) was good. The inclusion of apoA-I glycation relative intensity produced a significant improvement of 16.0% (Nagelkerke R^2 value for model 1 and model 2: 0.572 and 0.732, respectively; $P < 0.01$) in explaining the variation of dependent variables.

Multivariable analysis for the risk of plaque progression in diabetic patients
Multivariable logistic regression analysis, including all parameters in Table 2, revealed that LDL cholesterol, HbA_{1c}, and low HDL cholesterol were independent determinants of plaque progression by both QCA (model 3) and IVUS (model 5) (Table 4). When relative intensity of apoA-I glycation was included in multivariable analysis, relative intensity of apoA-I glycation and risk factors in models 3 and 5 remained significantly associated with both QCA-defined (model 4) and IVUS-defined (model 6) plaque progression (Table 4). Further statistics demonstrated that inclusion of apoA-I glycation relative intensity marginally improved risk prediction for plaque progression by QCA (C statistic, from 0.699 to 0.788, $P < 0.05$) and IVUS (C statistic, from 0.691 to 0.796, $P < 0.01$). Net reclassification improvement was also satisfactory with glycated apoA-I levels for QCA- and IVUS-defined plaque progression (7.59 and 7.88%, respectively; both $P < 0.01$).

CONCLUSIONS—The current study demonstrates that in patients with type 2 diabetes, increased apoA-I glycation level is associated with the severity of CAD and plaque progression defined by QCA and IVUS measurements. This observation supports the notion that glycation modification of apoA-I contributes to the development and progression of atherosclerotic lesions in type 2 diabetic patients, besides low apoA-I levels.

Previous studies have repeatedly reported a significant negative relationship between HDL cholesterol levels and development of coronary atherosclerosis (29). Plaque progression, a key feature derived from QCA and IVUS imaging, is recognized to be a risk for increased adverse cardiovascular events (30,31). In a small randomized trial, a 5-week infusion of an HDL-mimic drug (apoA-I Milano) induced significant regression of plaque volume in patients with acute coronary syndrome (32). Treatment with torcetrapib—an inhibitor of cholesteryl ester transfer protein—achieved a substantial increase in HDL cholesterol levels but did not obviously decrease coronary plaque progression as determined by IVUS and clinical outcome (33), suggesting that the structure perfection of HDL may be as crucial as the absolute level in point of plasma HDL function. This assertion is supported by previous studies showing that glycated apoA-I induces proatherosclerotic effects and changes of vascular structure (34–36) and by our in vitro data, which have shown that glycation-modified HDL from diabetic patients

Table 3—*Serum LCAT activity, relative intensity of apoA-I glycation, and changes in QCA and IVUS measurements in patients with and without plaque progression*

Variables	No PP	PP	P
QCA measurements			
n	114	45	
Minimum lumen diameter at baseline (mm)	1.57 ± 0.52	1.58 ± 0.56	0.915
Minimum lumen diameter at follow-up (mm)	1.39 ± 0.51	0.99 ± 0.56	<0.001
Change in coronary artery score (mm)	0.18 ± 0.10	0.59 ± 0.15	<0.001
Cumulative coronary stenosis score at baseline	0.76 ± 0.27	0.78 ± 0.28	0.678
Cumulative coronary stenosis score at follow-up	0.84 ± 0.28	0.96 ± 0.27	0.015
Change of cumulative coronary stenosis score	0.07 ± 0.05	0.18 ± 0.04	<0.001
LCAT activity (μmol/mL $\times$ h)	0.224 ± 0.030	0.196 ± 0.022	0.016
Relative intensity of apoA-I glycation	8.91 ± 4.10	12.8 ± 5.11	<0.001
IVUS measurements			
n	89	38	
Vessel volume at baseline (mm^3)	458.5 ± 206.1	455.7 ± 202.2	0.945
Vessel volume at follow-up (mm^3)	461.4 ± 205.7	459.1 ± 201.3	0.955
Change in vessel volume (mm^3)	2.90 ± 1.64	2.39 ± 1.94	0.173
Lumen volume at baseline (mm^3)	276.3 ± 123.3	266.5 ± 121.3	0.682
Lumen volume at follow-up (mm^3)	274.6 ± 123.8	253.9 ± 117.4	0.375
Change in lumen volume (mm^3)	1.66 ± 1.37	12.55 ± 4.94	<0.001
Atheroma volume at baseline (mm^3)	182.2 ± 98.5	189.2 ± 96.7	0.712
Atheroma volume at follow-up (mm^3)	186.8 ± 98.1	205.2 ± 99.3	0.336
Change in atheroma volume (mm^3)	4.56 ± 2.34	15.95 ± 4.50	<0.001
PAV at baseline (%)	38.45 ± 9.96	40.96 ± 8.21	0.173
PAV at follow-up (%)	39.46 ± 9.92	44.50 ± 8.02	0.006
Change in PAV (%)	2.91 ± 2.54	9.09 ± 4.30	<0.001
LCAT activity (μmol/mL $\times$ h)	0.221 ± 0.028	0.198 ± 0.025	<0.001
Relative intensity of apoA-I glycation	7.69 ± 3.04	11.86 ± 3.88	<0.001

Data are means $\pm$ SD. PP, plaque progression.

Table 4—*Multivariable logistic regression analysis for the risk of QCA- and IVUS-determined plaque progression*

Variables	OR	95% CI	P
QCA-defined plaque progression			
Model 3			
Male	1.03	0.49–2.17	0.94
Age	1.51	0.62–3.70	0.36
Hypertension	1.64	0.72–3.73	0.24
HbA$_{1c}$	3.11	1.45–6.66	0.004
LDL cholesterol	2.85	1.18–6.91	0.02
HDL cholesterol	0.39	0.15–0.73	0.03
Model 4			
Male	1.02	0.41–2.59	0.96
Age	1.33	0.51–3.44	0.56
Hypertension	1.46	0.62–3.45	0.39
HbA$_{1c}$	2.51	1.12–5.60	0.03
LDL cholesterol	3.6	1.42–9.14	0.007
HDL cholesterol	0.13	0.08–0.34	<0.001
Relative intensity of apoA-I glycation	1.22	1.09–1.36	<0.001
IVUS-defined plaque progression			
Model 5			
Male	1.05	0.49–2.19	0.68
Age	1.66	0.68–4.05	0.27
Hypertension	2.19	0.90–5.33	0.09
HbA$_{1c}$	2.36	1.07–5.19	0.03
LDL cholesterol	2.86	1.16–7.06	0.02
HDL cholesterol	0.33	0.10–0.65	0.013
Model 6			
Male	1.16	0.44–3.07	0.77
Age	1.39	0.55–3.47	0.49
Hypertension	1.24	0.45–3.43	0.68
HbA$_{1c}$	2.44	1.12–5.46	0.03
LDL cholesterol	4.21	1.56–11.35	0.005
HDL cholesterol	0.12	0.07–0.33	<0.001
Relative intensity of apoA-I glycation	1.32	1.17–1.48	<0.001

OR, odds ratio for plaque progression. Models 3 and 5, adjusted for conventional cardiovascular risk factors. Models 4 and 6, adjusted for conventional cardiovascular risk factors with addition of relative intensity of apoA-I glycation.

with severe CAD attenuates LCAT activation and impairs cholesterol efflux in human macrophage THP-1 cells (data not shown).

In this study, relative intensity of apoA-I glycation was significantly elevated in type 2 diabetic patients with significant CAD, particularly for those who had plaque progression during 1-year follow-up. The values of apoA-I glycation relative intensity correlated with the severity of CAD and changes of QCA and IVUS measurements. These results suggest that the more profuse glycation apoA-I has, the more substantial the alteration of apoA-I structure and HDL function (including LCAT activation) that tends to occur. Since NH$_2$-terminal portion and residues 143–164

of human apoA-I are believed to be critical in structure for LCAT activation (37,38), further studies are required to investigate whether glycation of these key residues of apoA-I could affect multiple or individual function of HDL.

It should be pointed out that although the half-life of apoA-I in plasma is not long, continuing formation of glycated apoA-I and increased glycation level of apoA-I protein in plasma and in extracellular tissue, promoted by inflammation and diabetes status, could exert long-term effects on the cardiovascular system and contribute to atherogenesis. In addition, in our study the difference in apoA-I glycation levels between diabetic patients and control subjects was much greater than that of HbA$_{1c}$. Except for an

amplifying effect of Western blot technique in detection of apoA-I glycation, this finding supports the existence of other causes in plasma for apoA-I glycation, including mainly a sustained inflammatory reaction besides hyperglycemia, while hemoglobin is located in erythrocyte and less affected by plasma milieu compared with HDL. Moreover, the difference of LCAT was not so large among control subjects and diabetic patients with or without CAD. This may be explained, at least partly, by a weak effect of serum biomeasurement in reflecting protein activity in local vascular tissue.

Limitations and concluding statements
We recognize limitations in our study. First, this study was cross-sectional for the point of diabetic CAD assessment, thereby allowing detection of association but not formulation of a causative role of apoA-I glycation for atherogenesis in diabetes. Second, the follow-up time in this study was ~1 year, which is not sufficiently long for major cardiac event evaluation. A large-scale long-term study is needed to confirm our results and to test other potential causes, such as hypoglycemia. Nevertheless, QCA and IVUS examinations have displayed substantial imaging changes of coronary arteries in a 1-year time period, and a clear association of apoA-I glycation level with changes of QCA and IVUS measurements was observed. In conclusion, increased glycation levels of apoA-I are associated with the severity of CAD and coronary artery plaque progression in type 2 diabetic patients.

responsibility for the integrity of the data and the accuracy of the data analysis.

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