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Endothelial function in individuals with coronary artery disease with and without type 2 diabetes mellitus

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Abstract

The goal of this study was to determine if individuals with coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM) had greater endothelial dysfunction (ED) than individuals with only CAD. Flow-mediated dilation (FMD), calculated as percentage increase in brachial artery diameter in response to postischemic blood flow, was measured after an overnight fast in 2 cohorts. The first cohort included 76 participants in the Northern Manhattan Study with CAD; 25 also had T2DM. The second cohort was composed of 27 individuals with both T2DM and CAD who were participants in a study of postprandial lipemia. Combined, we analyzed 103 patients with CAD: 52 with T2DM (T2DM+) and 51 without T2DM (T2DM−). The 52 CAD T2DM+ subjects had a mean FMD of 3.9% ± 3.2%, whereas the 51 CAD T2DM− subjects had a greater mean FMD of 5.5% ± 4.0% ($P < .03$). An investigation of various confounders known to affect FMD identified age and body mass index as the only significant covariates in a multiple regression model. Adjusting for age and body mass index, we found that FMD remained lower in T2DM+ subjects compared with T2DM− subjects (difference, −1.99%; $P < .03$). In patients with CAD, the concomitant presence of T2DM is independently associated with greater ED, as measured by FMD. This finding may be relevant to the greater early and late morbidity and mortality observed in patients with both CAD and T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) are closely linked, at least in part via concomitant risk factors that often manifest before the onset of T2DM [1]. Although the severity of CVD risk varies among diabetics of different ages and duration of disease, many patients have enough risk that T2DM has been considered to be a risk equivalent of coronary artery disease (CAD) [2,3]. The basis for the increase in CVD risk in patients with T2DM is multifactorial and may include the accumulation of glycated end products, cellular oxidative stress, and impaired production of nitric oxide [4]. Increased levels of inflammatory cytokines and soluble integrins, such as soluble intercellular adhesion molecule (s-ICAM) and soluble vascular cellular adhesion molecule (s-VCAM), are also present in individuals with T2DM and may contribute to a higher risk for CVD [5,6].

Endothelial dysfunction (ED) is a key early event in the development of atherosclerosis [7-9] and can be demonstrated before the onset of overt CVD [10-12]. Endothelial dysfunction is found in people with insulin resistance and may be a link between T2DM, in which insulin resistance plays a central role, and CVD [13,14]. Indeed, ED can predict the future onset of T2DM [15]. Endothelial dysfunction can be demonstrated in individuals with either type 1 diabetes mellitus [16,17] or T2DM [18-20].

In humans, endothelial function can be tested noninvasively using echocardiographic methods to measure flow-

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mediated dilation (FMD) of the brachial artery [21-23]. Flow-mediated dilation of the brachial artery correlates negatively with ED in the coronary arteries [24,25]. Several studies have shown that FMD is abnormal in patients with CAD [26,27] and correlates negatively with extent of CAD [28,29]. Importantly, FMD also predicts future CVD events in healthy populations [11,30,31] as well as long-term outcomes in people who have had CAD events [32-34]. There are few studies, however, that have examined FMD in people with both CAD and T2DM [35,36]. Because both CAD and T2DM are associated with ED, the question of whether ED is even greater in individuals with both CAD and T2DM is an important one, particularly in view of the clear demonstration of greater early and late morbidity and mortality in people with T2DM who have had a coronary event [37,38]. Therefore, we assessed FMD in people with CAD, either with T2DM (T2DM+) or without T2DM (T2DM−). Our goal was to determine if individuals diagnosed with CAD and T2DM had a greater degree of ED than people with only CAD.

2. Methods

2.1. Subjects

We enrolled 103 subjects from 2 cohorts. All subjects signed a consent form, and the studies were approved by the Columbia University Medical Center (CUMC) Institutional Review Board. One cohort was composed of 76 subjects from the Northern Manhattan Study (NOMAS) [39,40]. In brief, NOMAS is a multiethnic population-based prospective cohort study that examines risk factors for cerebrovascular disease. In NOMAS, there were 76 participants with FMD data who had CAD (diagnosed by self-report or documented history of myocardial infarction): 25 of the 76 also had T2DM (diagnosed by presence of fasting plasma glucose level >126 mg/dL, subjects' self-report, use of insulin or other hypoglycemic medications). The other 51 NOMAS subjects with CAD did not have T2DM. The second cohort was obtained from a study of postprandial lipemia in T2DM (DMPPL) [41]. In this T2DM+ cohort, 27 with FMD data also had a diagnosis of *CAD* (defined as documented prior myocardial infarction, percutaneous transluminal coronary angioplasty/stent, coronary artery bypass graft, or >70% stenosis in any vessel by coronary angiography). Both cohorts were recruited from the same neighborhood surrounding CUMC. Combining the 2 cohorts allowed us to examine a group of 103 individuals with CAD: 52 T2DM+ and 51 T2DM−.

2.2. Laboratory

Total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and glucose were measured using standard enzymatic techniques on a Hitachi 912 chemistry analyzer (Hitachi, Indianapolis, IN) [42]. Low-density lipoprotein cholesterol levels were calculated using the method of Friedewald et al [43]. C-reactive protein (CRP) levels in the DMPPL cohort were measured using a commercially available enzyme-linked immunosorbent assay kit (Diagnosis System Laboratories, Webster, TX) in the Biomarkers Laboratory of the Irving Institute for Clinical and Translational Research at CUMC. The CRP in the NOMAS cohort was measured using a BN-II nephelometer (Dade-Behring, Deerfield, IL) in the Center for Advanced Laboratory Medicine at CUMC. Complete blood count, metabolic panel, and total glycohemoglobin were measured on fasting samples by the CUMC clinical laboratory (reference range, 5.1%-8.5%).

2.3. Assessment of endothelial function

Arterial endothelial function was noninvasively assessed by FMD, which is the change in brachial artery diameter after regional ischemia. High-resolution B-Mode ultrasonography was used to measure FMD [22]. All of the studies were analyzed by one reader blinded to the subject's clinical status. After a 12-hour fast, no smoking for 12 hours, and no alcohol intake for 3 days, individuals were examined in a dark, temperature-controlled, quiet room after 20 minutes of rest. Brachial artery FMD was assessed using a 15-MHz linear array transducer (Agilent 5500, Andover, MA). The vessel was imaged above the antecubital fossa in the longitudinal plane. Flow-mediated dilation was measured as the dilatory response to reactive hyperemia induced by inflation of a blood pressure cuff on the forearm to suprasystolic levels for 3 minutes. One minute after cuff deflation, the brachial artery diameter was remeasured. Continuous image recording was performed on S-VHS tapes for 30 seconds before and 90 seconds after cuff deflation. The arterial diameter was measured using a digital caliper on the image at a comparable site at baseline and after cuff release. Flow-mediated dilation was expressed as percentage change = 100× [brachial artery diameter at peak hyperemia minus the diameter at rest]/brachial artery diameter at rest. Three measurements were averaged at baseline and after cuff deflation, and the averages were used in the analysis. The intra- and interobserver variabilities on a sample of 15 subjects were 1.3% and 2.7%, respectively [44].

2.4. Statistics

All data were analyzed using SAS software (version 9.1; SAS, Cary, NC). Data are reported as means ± standard deviations, except in Fig. 1 where standard errors are reported. Triglyceride levels, which were not normally distributed, are summarized by medians and interquartile ranges (IQRs). Group comparisons of continuous variables were by unpaired *t* tests; TG levels were log transformed to make the distributions normal before *t* tests. The relationships between FMD and other confounding factors were

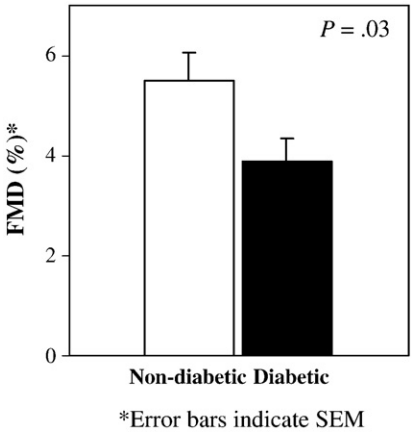

Fig. 1. Subjects with both CAD and T2DM have lower FMD than subjects with only CAD: Percentage FMD was measured in 52 participants with CAD and T2DM and in 51 participants with CAD but without T2DM. The data are presented as means and SEs of the percentage change from baseline in brachial artery diameter after 3 minutes of local ischemia. These data were not adjusted for any covariates or potential confounders.

analyzed by multiple regression to study the independent effects of multiple factors.

3. Results

We first investigated the validity of combining the 2 cohorts. Although the methodology for determining FMD was the same for the 2 cohorts, with the same physiology laboratory and the same reader used for both, the possibility existed that the cohorts differed in some way. The cohorts were different in age, with the NOMAS population older (72 ± 8 vs 60 ± 7 years, $P < .001$); they also differed in sex ratio (female, 52% in NOMAS and 19% in DMPPL). In subsequent analyses, therefore, we included cohort (NOMAS vs DMPPL) as a factor to account for any difference between the cohorts, along with other covariates (sex, age, body mass index [BMI], ethnicity, hypertension, lipid levels) in multiple regression models. Statin treatment had the same prevalence in the 2 groups (27 in T2DM+ and 27 in T2DM−). The prevalence of hypertension was high in both groups (36.5% in T2DM+ and 47% in T2DM−).

Table 1 presents basic characteristics and lipid profiles in subjects with and without T2DM. As expected, fasting glucose levels were higher in T2DM+ subjects than in T2DM− subjects (174 ± 64 vs 89 ± 12 mg/dL, $P < .0001$). Glycohemoglobin levels were available in the 27 T2DM+ and CAD+ subjects recruited from the DMPPL study. The levels were 11.9 ± 3.1, with a range from 6.8 to 18. Twenty-three of these 27 subjects were on hypoglycemic agents (sulfonylureas, 13; metformin, 13; insulin, 6; acarbose, 1; resulin, 3; and troglitazone, 1). We also observed lower HDL-C levels (35 ± 12.2 vs 44 ± 11.7 mg/dL, $P < .0001$) and higher high-sensitivity (hs)CRP levels (9.1 ± 9.3 vs 5.3 ± 7.1 ng/mL, $P = .05$) in T2DM+ subjects. Median TG levels were nominally higher in T2DM+ (135 mg/dL; IQR, 101-159 mg/dL) than in T2DM− subjects (108 mg/dL; IQR, 77-163), but this difference was not significant ($P = .17$) by t test on log-transformed data.

Fig. 1 shows FMD in the 2 groups. The 52 T2DM+ subjects with CAD had a mean FMD of 3.9% ± 3.2%, whereas the 51 T2DM− with CAD had a mean FMD of 5.5% ± 4.0% ($P < .03$). In a preliminary multiple regression model, study cohort (NOMAS vs DMPPL) and several of the variables in Table 1 (sex, race, smoking, lipid levels, systolic blood pressure, and hsCRP levels) were all nonsignificant. In particular, HDL-C and hsCRP, the 2 variables that were significantly different between T2DM+ and T2DM− (Table 1), showed no relationship to FMD in the multiple regression model. However, 2 other variables from Table 1,

Table 1
Baseline characteristics of each group

Characteristics	CAD + T2DM+ n = 52 mean ± SD	CAD + T2DM− n = 51 mean ± SD	Comparisons (P values)
Age (y)	66 ± 9.6	69 ± 8.9	NS
BMI (kg/m^2)	31 ± 4.7	29 ± 6.0	NS
SBP (mm Hg)	137 ± 21.6	143 ± 20.5	NS
DBP (mm Hg)	78 ± 13	85 ± 10	NS
TC (mmol/L)	5 ± 1.1	4.9 ± 1.1	NS
LDL-C (mmol/L)	3.2 ± 0.7	3.1 ± 0.9	NS
HDL-C (mmol/L)	0.9 ± 0.3	1.1 ± 0.3	.003
TG (mmol/L)[a]	1.5 (1.1-1.8)	1.2(0.9-1.8)	NS
Glucose (mmol/L)	9.7 ± 3.6	4.94 ± 0.7	<.0001
CRP (ng/mL)	9.1 ± 9.3	5.3 ± 7.1	.05
Male	65	53	NS
Ethnicity (%)			
Black	10	16	NS
Hispanic	63	59	NS
White	27	25	NS
Cigarette smoke (%)	50	63	NS
Use lipid medications (%)	54	47	NS

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; NS, not significant.

[a] Median and interquartile range are shown for TG.

Table 2
Flow-mediated dilation is significantly lower in CAD with T2DM compared with CAD without T2DM after adjusting for age, BMI, and study cohort

Variable	Regression coefficient estimate	SE	P value
T2DM	−1.99	0.88	.026
Age	−0.09	0.04	.042
BMI	−0.13	0.07	.055
Cohort	0.64	1.13	.57

Flow-mediated dilation was 1.99% lower in subjects with both T2DM and CAD compared with subjects with only CAD, even after adjusting for age and BMI, which were also independently associated with lower FMDs, and cohort, which was not significantly related to FMD. Each year of increasing age was associated with a reduction of 0.09% in FMD; and each unit of BMI, a reduction of 0.13% in FMD.

age ($P < .05$) and BMI ($P < .06$), were potential confounders; and they were retained in the final model. Table 2 shows that, after adjusting for the study cohort, age, and BMI, FMD remained lower in T2DM+ compared with T2DM− subjects (difference, −1.99%; $P = .026$).

4. Discussion

Type 2 diabetes mellitus and CAD are both known to affect endothelial function, and many studies have examined the association between either CAD [26] or T2DM [19,20] and endothelial function by measuring FMD. Neunteufl et al [26] examined FMD in 74 subjects without T2DM and found that subjects with CAD showed markedly impaired FMD compared with the non-CAD group. Flow-mediated dilation has also been shown to detect the severity of CAD in various studies [28,29]. Wu et al [28] found that FMD was associated with the presence and extent of coronary disease assessed by stress thallium imaging. Rossi et al [15] prospectively examined 840 healthy, nonobese, postmenopausal women and measured FMD. After a 4-year follow-up, they found 102 women who developed T2DM; there was a significant increase in the relative risk of diabetes with each unit decrease of baseline FMD [15]. In a large cohort from the HOORN study (N = 650) [45], investigators found that presence of T2DM (n = 269) was independently associated with impaired endothelium-dependent FMD; impaired glucose tolerance (n = 135) was not associated with FMD. In contrast, Su et al [46] found that FMD decreased in a stepwise fashion across the spectrum of impaired fasting glucose, glucose intolerance, and T2DM in people without CAD.

There have been only 2 studies, however, that compared FMD in subjects with both T2DM and CAD to subjects with only CAD [35,36]; and those 2 studies produced conflicting results. Kirma et al [36] examined a Turkish cohort of 150 patients with CAD, of whom 42 had T2DM. Similar to our findings, they observed that the subjects with both CAD and T2DM had lower FMD than subjects with CAD but no T2DM. In a stepwise multiple regression analysis, these authors found that age and the presence of T2DM were independent predictors of lower FMD. In our study as well, age was a potential confounder. After adjusting for both age and BMI, we found that FMD remained lower in T2DM+ compared with T2DM− subjects. Bhargava et al [35] examined FMD in 198 Indian subjects divided into 4 groups; they confirmed prior findings that, in the absence of CAD, FMD is significantly impaired in patients with T2DM compared with subjects without T2DM. However, in contrast to our findings, they observed a similar degree of ED in subjects with T2DM and CAD compared with subjects who only had CAD. In reviewing the study by Bhargava et al, we did not find any clear reason for the different outcome. However, FMD was significantly lower in their subjects with both T2DM and CAD compared with subjects with T2DM and no CAD; the group with CAD but no T2DM had an FMD between the latter 2 groups that was not significantly different from either. This stepwise gradient in FMD lowering from no T2DM and no CAD, to T2DM without CAD, to CAD without T2DM, and finally to both T2DM and CAD is not markedly different from our results, which only pertain to the last 2 groups in the Bhargava et al article. We would note, however, that the population studied by Bhargava et al (Asian Indians) was different from the triethnic population that we investigated.

Why would individuals with both T2DM and CAD have greater ED than individuals with only CAD? One possibility is that people with both CAD and T2DM have a greater number of other factors that are associated with reduced FMD, such as lower HDL-C [47], higher TG levels [48], and more hypertension [49], than people with only CAD. In the present study, HDL-C was lower and TG levels tended to be higher in the group with T2DM. On the other hand, both systolic and diastolic blood pressures were slightly but not significantly lower in the gHT2DM+ than in the T2DM− subjects, consistent with a somewhat lower prevalence of diagnosed hypertension in the T2DM+ group. Smoking status has been shown to affect endothelial function [50], and our study included similar numbers of individuals with smoking history in each group. Importantly, when these and other baseline demographic and biochemical data were included in a model to explain the association between T2DM and FMD, only age and BMI were seen to be related to FMD besides the presence of T2DM.

C-reactive protein is an acute phase reactant, and serum concentrations of hsCRP may be indicative of the presence of inflammation [51]. Whether elevated hsCRP is a marker of a proinflammatory state or plays a pathologic role in the development of CVD or T2DM is unclear [52]. Vitale et al [53] found a significant correlation between plasma hsCRP levels and endothelial function, suggesting a correlation between inflammation and the integrity of the endothelium. In that study, optimal medical therapy for CAD reduced CRP levels along with a parallel improvement in endothelial function. In our study, hsCRP levels were significantly higher in the T2DM+ group compared with the T2DM− group. However, hsCRP levels were not correlated with FMD within each group; and the difference in FMD between the 2 groups remained essentially unchanged after adjusting for hsCRP levels. We did not measure inflammatory cytokines in this study; CRP is considered by some to be a representative, if not the strongest marker, of CVD risk among the family of inflammatory markers [46,54]. We did not determine plasma levels of soluble adhesion molecules [6]. Both families of molecules are typically elevated in people with T2DM and in individuals with CVD. The value of soluble adhesion molecules for predicting future CVD events has varied by study [6].

One obvious difference in the group with both T2DM and CAD compared with the group that only had CAD was the presence of hyperglycemia in the former. Acute hyperglyce-

mia can reduce FMD in healthy subjects [55,56], which is reversed by concomitant administration of vitamin C [57] or a combination of vitamins C and E [56], suggesting a role for oxidative stress in this phenomenon. Evidence that chronic mild hyperglycemia can have detrimental effects on endothelial function comes from the NOMAS, in which fasting glucose levels were linearly and inversely related to FMD in subjects without diabetes [44]. Su et al [46] reported a similar relationship between glycemia and FMD in subjects without CAD. In addition, a single oral challenge of advanced glycation end products reduced FMD in both healthy and T2DM subjects [58]. Several groups have studied the effects of glucose-lowering therapies on FMD in patients with T2DM; most, but not all, of the studies show that improved glucose control is associated with improved endothelial function. It is not clear from those studies whether there are differences across classes of agents [59-64].

Studies of the effect of intensive glucose lowering on cardiovascular outcomes have produced mixed outcomes. The United Kingdom Diabetes Study showed a trend toward reduced events with either insulin or sulfonylurea therapy [65] and a significant reduction in events with metformin treatment [66]. The Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction study demonstrated long-term reductions in mortality after intensive insulin therapy post–myocardial infarction [67]. Most recently, 3 major clinical trials—Action to Control Cardiovascular Risk in Diabetes [68], Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation [69], and the study of glucose control and vascular complications in veterans with type 2 diabetes mellitus diabetes [70]—all failed to demonstrate reduced cardiovascular events in patients with T2DM treated to lower hemoglobin A_{1c} levels. These results suggest that markers other than glycemia may be more useful in designing new and more intensive approaches to reducing CVD in patients with T2DM. Whether changes in FMD can be used as such a measure of treatment efficacy remains to be determined.

Our study had some limitations: We did not know the angiographic severity of CAD in one of our cohorts, the duration of diabetes, or the specific type of treatment regimens for T2DM that our subjects were receiving. In addition, we did not include a group of participants with DM and no CAD. Furthermore, our study had relatively small numbers; it may have been underpowered to detect some associations with potential cofounders, including hsCRP.

5. Summary

It is well known that individuals with CAD have low FMD, probably related to the multiple risk factors that contributed to their CAD. In this study, we showed that presence of T2DM is associated with additional negative effects on endothelial function even in the presence of CAD. This could help explain why patients with T2DM and CAD have a worse prognosis, both in terms of early and late survival [37,38].

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