Relationship between Albuminuria and Cardiovascular Disease in Type 2 Diabetes

Barry I. Freedman,* Carl D. Langefeld,‡ Kurt K. Lohman,‡ Donald W. Bowden,*‡ J. Jeffrey Carr,*§ Stephen S. Rich,‡ and Lynne E. Wagenknecht†

Departments of *Internal Medicine, †Public Health Sciences, ‡Biochemistry, and §Radiology, Wake Forest University School of Medicine, Winston-Salem, North Carolina

Impaired renal function and albuminuria, common among people with type 2 diabetes, are strong predictors of atherosclerotic cardiovascular events. However, the relationships among albuminuria and measures of calcified atherosclerotic plaque are unknown. Coronary and carotid artery calcified plaque were measured using fast-gated helical computed tomography, and B-mode ultrasonography measured common carotid artery intima-medial thickness (IMT) in 588 white participants with type 2 diabetes from 325 families ascertained for the presence of multiple siblings with type 2 diabetes. Measured risk factors included age, gender, BP, body mass index, GFR, glycosylated hemoglobin, LDL cholesterol, HDL cholesterol, smoking, and medications that affect urine albumin:creatinine ratio (ACR). Generalized estimating equations with exchangeable correlation and the sandwich estimator of the variance were used to test for an association among coronary artery calcified plaque, carotid artery calcified plaque, carotid IMT, and ACR while adjusting for measured risk factors. Participants had a mean ± SD (median) age of 61.2 ± 9.2 yr (61.0 yr), ACR of 106.2 ± 590 mg/g (12.9 mg/g), GFR of 93.3 ± 33.2 ml/min (87.4 ml/min), coronary artery calcium mass score of 1394 ± 2685 (323), carotid artery calcium mass score of 295 ± 652 (51), and IMT of 0.66 ± 0.12 mm (0.65 mm). Adjusting for the measured covariates, ACR was strongly and positively associated with coronary artery calcium (P = 0.004) and carotid artery calcium (P = 0.0004). Albuminuria is strongly associated with calcified plaque in the coronary and carotid arteries in white individuals with type 2 diabetes and relatively preserved renal function.

C Cardiovascular disease (CVD) is the major cause of morbidity and mortality in people with impaired renal function and ESRD (1,2). Individuals with diabetes-associated nephropathy typically have long periods of excessive albuminuria with gradual reductions in creatinine clearance as they approach end stage. There is a graded increase in risk for cardiovascular and total mortality with incremental increases in urine albumin:creatinine ratio (ACR) among high-risk individuals with hypertension and diabetes (3). There seems to be no threshold level at which this increase in risk starts, as even minor increases in ACR are associated with higher mortality rates.

Coronary artery calcified plaque is a marker of the presence and the quantity of coronary atherosclerosis. The amount of calcium deposited in the coronary arteries, as measured with computed tomography, correlates with the pathologic extent of atherosclerosis and the presence of stenosis as identified by coronary angiography (4–6). Its ability to predict future CVD events independent of CVD risk factors has been reported in short-term studies (7–9). Coronary calcium scores have been shown to be high in people who are on dialysis and, in particular, higher than in nonuremic individuals with severe CAD (10,11). Explanations for this may include the chronic elevation in calcium-phosphate product that is often observed in people with renal failure, the use of calcium-containing phosphate binders and potent vitamin D compounds (12,13), and the presence of cells that express an osteoblastic phenotype in the media of vessel walls (14).

It is not known, however, whether calcified atherosclerotic plaque is present in patients with incident nephropathy or in those with mild renal insufficiency that is not treated with calcium-containing phosphate binders or vitamin D analogues. The aim of this study was to determine the relationship between calcified atherosclerotic plaque in the coronary and carotid arteries with albuminuria in white individuals with type 2 diabetes and relatively preserved renal function.

Materials and Methods

Study Population

Siblings who were concordant for type 2 diabetes (diabetes diagnosed after the age of 34 yr in the absence of historical evidence of diabetic ketoacidosis) were recruited from internal medicine clinics and community advertising in the Diabetes Heart Study (DHS) (15,16). We excluded family members when they had an elevated serum creatinine concentration (≥1.4 mg/dl in women, ≥1.6 mg/dl in men) or previous coronary artery bypass surgery or carotid endarterectomy. Individuals who had had a previous myocardial infarction or stroke were included.
as it was not believed that the calcium score in the relevant arteries would be significantly increased by these events. The study was approved by the Institutional Review Board at the Wake Forest University School of Medicine, and all participants gave written informed consent.

Participant examinations were conducted in the General Clinical Research Center of the Wake Forest University School of Medicine and included interviews for medical history, current medications, and health behaviors; measurements of body size and resting BP; 12-lead electrocardiogram; fasting blood draw; and morning spot urine collection. Laboratory assays included urine albumin and creatinine (for ACR), total cholesterol, LDL, HDL, triglycerides, hemoglobin A1c (HbA1c), and fasting serum glucose. Renal function was assessed by serum creatinine concentration and Modification of Diet in Renal Disease estimation of GFR (17). History of cardiovascular disease was provided by self-report. Hypertension was defined as self-report of a physician’s diagnosis, BP ≥140/90 mmHg, or use of antihypertensive medications. Antihypertensive medications were grouped into drug classes; of particular interest to this report are angiotensin-converting enzyme inhibitors (ACEi) and angiotensin-receptor blockers (ARB), medicines that are known to reduce urinary albumin excretion.

**Vascular Imaging**

Estimates of coronary and carotid artery calcium were made using fast-gated helical computed tomography (CT). Cardiac CT examinations were performed on a single-slice second generation CT or a four-channel multidetector CT both with cardiac gating and capable of 500-ms temporal resolution (HiSpeed LX and LightSpeed QXi with the SmartScore Cardiac scan package; General Electric Medical Systems, Waukesha, WI). Participants were placed in the supine position on the CT couch over a quality-control calibration phantom (Image Analysis, Inc, Columbia, KY). After a scout image of the chest, the heart was imaged during suspended respiration at end inspiration. Scan parameters were 3-mm slice thickness, 26-cm display field of view, retrospectively cardiac gating, 120 kV, 240 mA, and CT scan pitch adjusted to heart rate as described previously (18) for the single-slice system and 2.5-mm slice thickness in four-slice mode, 26-cm display field of view, prospective cardiac gating at 50% of the RR interval, 120 kV, and 240 mA for the multidetector CT. A second scan of the heart using the same parameters was performed after a 2- to 3-min rest period.

For the carotid examination, an unenhanced CT scan was performed through the neck after instructing the participant to swallow. The start and end locations were the C2-3 and C6-7 disc levels. A helical acquisition using a 3-mm (single slice) or a 2.5-mm (multidetector) slice collimation, a 120-kv, 280-mA, 0.8-s gantry rotation, 360-degree scan reconstruction, and standard reconstruction kernel was performed. The display field of view was 18 cm, resulting in pixel dimensions of 0.35 × 0.35 mm. A second scan of the carotid bifurcation using the same parameters was performed after a 2- to 3-min rest period.

CT examinations of the coronary and carotid arteries were analyzed on a GE Advantage Windows Workstation using the research version SmartScores software package (General Electric Medical Systems), which allows calculation of a calcium mass score of the amount of calcified plaque. The reproducibility of the coronary and carotid calcium mass scores obtained from the duplicate scans was 0.98 for each. Two trained readers who were blinded to the clinical characteristics of the participants analyzed the CT images. The scanning methods and quality-control procedures used, including calibration scans every 2 wk, were identical to the techniques used in the Multi-Ethnic Study of Atherosclerosis, whose scans have been performed concurrently with this study and whose methods have been published previously (19).

Common carotid artery intima-media thickness (IMT) was measured using B-mode ultrasound, as reported previously (16). Briefly, high-resolution B-mode carotid ultrasonography was performed using a 7.5-MHz transducer and a Biosound Esaote (AUS) machine. Scans were performed of the near and far walls of the distal 10-mm portion of the common carotid artery at five predefined interrogation angles on each side. The mean value of up to 20 common carotid artery IMT values is reported here.

**Statistical Analyses**

A series of generalized estimating equations assuming exchangeable correlation and using the empirical estimate of the variance (20) to adjust for familial correlation was computed to test for an association between ACR and vascular calcified plaque. The natural log of (urine ACR + 1) was calculated to minimize the influence of extremely large covariate values on parameter estimates in these models. To better approximate the distributional assumptions of conditional normality and homogeneity of variance, we analyzed the natural log of coronary calcium plus one and carotid calcium plus one. For coronary and carotid artery calcification plaque and carotid artery IMT, models were adjusted for age, gender, GFR, body mass index (BMI), log HbA1c, systolic BP, diastolic BP, use of ACEi or ARB, HDL cholesterol, LDL cholesterol, and smoking. Standard regression diagnostics for collinearity and influence were computed for each model reported. Because we were adjusting for GFR, we chose to Winsorize the small number of estimated GFR values that were >190 ml/min to prevent spuriously high estimates from excessively influencing the results.

**Results**

A total of 749 white individuals with type 2 diabetes from 325 families had complete clinical and phenotypic data and were available for analysis. A total of 161 of these individuals were excluded for elevated serum creatinine concentration (n = 50), coronary artery bypass grafting (n = 120), or carotid endarterectomy (n = 15), leaving a total of 588 participants in the final multivariate analysis. The mean ± SD (median) age in the study group was 61.2 ± 9.2 yr (61.0 yr), 58% of participants were female, and the average duration of diabetes among all participants was 9.8 ± 7.1 yr (7.0 yr). Overall, 83.9% of white DHS participants were hypertensive (86.2% of men and 81.3% of women). Table 1 contains demographic data and Table 2 contains clinical laboratory data on participants (both tables contain overall data and data grouped by level of ACR). Urine ACR was <30 mg/g in 71% of participants, 30 to 299 mg/g in 24% of participants, and ≥300 mg/g in 4% of participants (Table 2). The mean ± SD (median) Modification of Diet in Renal Disease estimated GFR ranged from 95.7 ± 32.8 ml/min (89.8 ml/min) in those with normoalbuminuria to 74.4 ± 25.8 ml/min (69.7 ml/min) in those with overt proteinuria, with intermediate values in microalbuminuric individuals (Table 2). Median serum creatinine concentrations were 1.0, 1.1, and 1.2 mg/dl in normo-, micro-, and macroalbuminuric individuals, respectively (Table 2).

Coronary artery calcium was detectable in 93.6% and 100% of normoalbuminuric and overt proteinuric participants, respectively (Table 2). Because the distributions of carotid and coronary calcium scores were highly skewed, mean scores should be interpreted cautiously. The median carotid calcium score was 51 (mean 295), median coronary artery calcium score was 323 (mean 1394), median carotid artery IMT was 0.65 mm (0.66 mean), and median ACR was 12.8 mg/g (mean 106.2). Graded
increases in median vascular calcium score and carotid artery IMT were observed with increasing level of ACR (Table 2).

ACR was significantly associated with coronary artery calcified plaque ($P = 0.004$) and carotid artery calcified plaque ($P = 0.0004$; Table 3). The initial positive association that was observed between ACR and carotid artery IMT ($P = 0.048$) was weakened when GFR was added as a covariate ($P = 0.12$; Table 3). Older age, male gender, and current smoking were also statistically significant correlates of all three measures of subclinical CVD (coronary artery and carotid artery calcified plaque and carotid IMT). BMI and systolic and diastolic BP were significantly associated with measures of subclinical disease in the carotid arteries (calcified plaque and IMT) but not with coronary artery calcified plaque.

**Discussion**

This report characterized the relationship between calcified atherosclerotic plaque and albuminuria in individuals with
type 2 diabetes and relatively preserved renal function from the DHS. After covariates were adjusted, including GFR, a strongly positive relationship was observed between ACR and mass of calcified plaque in the coronary and carotid arteries. Several other reports have noted the association between various measures of renal impairment and coronary artery calcium in individuals with type 2 (21) and type 1 diabetes (22–24). This study examined diabetic individuals with fairly well-preserved renal function, intentionally excluding those who were taking vitamin D analogues and calcium-containing phosphate binders, which may have a direct impact on the relationship between albuminuria and CVD.

These findings are consistent with accumulating evidence that albuminuria greatly increases the risk for CVD events in individuals with type 2 diabetes. Dinneen and Gerstein (25) conducted a meta-analysis of 11 cohort studies that comprised 2138 individuals with type 2 diabetes. The average follow-up period was 6.4 yr. The overall relative risk for mortality associated with microalbuminuria was 2.4 and for cardiovascular morbidity or mortality was 2.0. The authors commented that these relative risks are similar to or greater than risks associated with other, more established risk factors, which emphasizes the importance of microalbuminuria as a risk factor for CVD and death in individuals with type 2 diabetes. In the Heart Outcomes Prevention Evaluation and Losartan Intervention for End-Point Reduction studies, ACR levels below the cut point for microalbuminuria increased the risk for major cardiovascular events in individuals with or without diabetes (3). The Heart Outcomes Prevention Evaluation investigators reported a continuous relationship between ACR and outcomes: For every 0.4-mg/mmol increase in ACR, the hazard increased by 6%. Renal impairment may be an even more potent risk factor for subsequent events in individuals with diabetes relative to those without (26). Our results support these findings by demonstrating the association between ACR and vascular calcium in a purely diabetic cohort with ACR values predominantly <30 mg/g. The relationship that we observed between ACR and vascular calcified plaque did not substantially change upon removing GFR or hypertension from the model.

The mechanisms underlying the association between increased ACR and vascular calcification remain unclear. Microalbuminuria may reflect a generalized defect in vascular permeability and a concomitant atherosclerotic milieu (24). Recent observations in vascular calcification describe the presence of cells that express an osteoblastic phenotype and synthesizing osteocalcin in the vasculature (27). Histologic specimens have demonstrated bone and bone marrow in calcified vessels. This calcium is of the hydroxyapatite form, i.e., the form present in bone (28). Davies and Hruska (14) suggested that the chronic vascular injury that is observed in renal failure such as hypertension, chronic fluid overload, and systemic upregulation of the renin-angiotensin system is important to the initiation of this calcification process. A review on accelerated atherosclerosis in ESRD stressed the role of inflammation and the complex interplay between T lymphocytes with local and circulating C-reactive protein, reactive oxygen species, matrix metalloproteinases, oxidized LDL, angiotensin 2, and vascular cell adhesion molecule (29). Whether these factors are involved in vascular calcification in those with relatively well-preserved kidney function and albuminuria is unclear but warrants further analysis.

We observed several other important similarities and differences in the correlates of carotid versus coronary artery atherosclerosis. Older age, smoking, and male gender were consistently associated with carotid and coronary calcified plaque and with carotid IMT. However, use of ACEI and/or ARB was associated only with carotid artery calcified plaque. Increasing BMI was independently associated with carotid artery calcified plaque and carotid artery IMT, and both increasing LDL cholesterol and lower GFR were associated with carotid artery IMT. These findings suggest differential effects of factors that advance or improve atherosclerosis in select vascular beds.

### Table 3. Generalized estimating equations for subclinical CVD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coronary Calcium</th>
<th>Carotid Calcium</th>
<th>Carotid IMT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
<td>P Value</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>0.0999</td>
<td>0.0146</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>0.0271</td>
<td>0.0204</td>
<td>0.1834</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>-1.3229</td>
<td>0.2309</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>GFR</strong></td>
<td>-0.0027</td>
<td>0.0054</td>
<td>0.6123</td>
</tr>
<tr>
<td><strong>Log ACR</strong></td>
<td>0.1716</td>
<td>0.0592</td>
<td>0.0037</td>
</tr>
<tr>
<td><strong>ACEi/Arb</strong></td>
<td>0.2512</td>
<td>0.1870</td>
<td>0.1791</td>
</tr>
<tr>
<td><strong>LDL cholesterol</strong></td>
<td>-0.0022</td>
<td>0.0030</td>
<td>0.4625</td>
</tr>
<tr>
<td><strong>HDL cholesterol</strong></td>
<td>-0.0131</td>
<td>0.0082</td>
<td>0.1110</td>
</tr>
<tr>
<td><strong>Log HbA1c</strong></td>
<td>0.8331</td>
<td>0.4741</td>
<td>0.0789</td>
</tr>
<tr>
<td><strong>Current smoker</strong></td>
<td>1.0559</td>
<td>0.2711</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Past smoker</strong></td>
<td>0.3589</td>
<td>0.2199</td>
<td>0.1026</td>
</tr>
<tr>
<td><strong>Systolic BP</strong></td>
<td>-0.0022</td>
<td>0.0059</td>
<td>0.7093</td>
</tr>
<tr>
<td><strong>Diastolic BP</strong></td>
<td>-0.0053</td>
<td>0.0118</td>
<td>0.6496</td>
</tr>
</tbody>
</table>

*CVD, cardiovascular disease.*
Diastolic BP was negatively associated and systolic BP was positively associated with carotid artery calcium and IMT. Similar results have been described previously (30) and may indicate that pulse pressure is a better predictor of vascular calcium and carotid IMT than is systolic or diastolic BP alone.

We restricted the analyses to white DHS participants because of the increased statistical power afforded by their larger sample (currently 85% of DHS families are white and 15% are black). In addition, racial differences in the between CT measures of calcified vascular plaque and ultrasound measures of carotid IMT in type 2 diabetes suggest that race-specific analyses should be performed (31). Among white individuals with type 2 diabetes, we observed a significant correlation between carotid artery IMT and carotid/coronary artery calcified plaque. However, there was no relationship between carotid IMT and calcified plaque in black individuals with diabetes. These striking racial differences will require further analysis with the evolving evidence that black individuals have less coronary artery calcified plaque than do white individuals (31–36).

In conclusion, ACR is strongly associated with carotid and coronary artery calcified plaque in white individuals with type 2 diabetes and preserved renal function. The potential impact of this finding is great: 29% of individuals with type 2 diabetes in this sample had abnormal albuminuria despite normal serum creatinine concentrations, similar to other reports (24). Interventions that reduce albuminuria and preserve renal function should be studied to determine whether they can prevent the development of calcified vascular plaque and carotid artery wall thickening and reduce subsequent CVD events.

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References
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See related editorial, “Albuminuria, Just a Marker for Cardiovascular Disease, Or Is It More?,” on pages 1883–1885.