Brachial Artery Reactivity in Asymptomatic Patients With Type 2 Diabetes Mellitus and Microalbuminuria (from the Detection of Ischemia in Asymptomatic Diabetics–Brachial Artery Reactivity Study)

Georgios I. Papaioannou, MD, Richard L. Seip, PhD, Neil J. Grey, MD, Deborah Katten, RN, MPH, Amy Taylor, MS, Silvio E. Inzucchi, MD, Lawrence H. Young, MD, Deborah A. Chyun, PhD, Janice A. Davey, MSN, Frans J.Th. Wackers, MD, Ami E. Iskandrian, MD, Robert E. Ratner, MD, Evelyn C. Robinson, RN, Stella Carolan, RN, Samuel Engel, MD, and Gary V. Heller, MD, PhD

Microalbuminuria is a novel atherosclerotic risk factor in patients with type 2 diabetes mellitus (DM) and predicts future cardiovascular events. Endothelial dysfunction and systemic inflammation have been proposed as common links between microalbuminuria and cardiovascular disease. However, no study has assessed the relation between microalbuminuria and vascular dysfunction as measured by brachial artery reactivity (BAR) in DM. We evaluated 143 patients (85 men; mean age 60.0 ± 6.7 years) with DM (mean duration 8.2 ± 7.4 years) enrolled in the Detection of Ischemia in Asymptomatic Diabetics study. Subjects were categorized as those with microalbuminuria (ratio of urinary albumin to creatinine 30 to 299 μg/mg creatinine, n = 28) and those with normoalbuminuria (ratio of urinary albumin to creatinine 0 to 29.9 μg/mg creatinine, n = 115). High-resolution ultrasound BAR testing was used to measure endothelium-dependent and endothelium-independent vasodilations. C-reactive protein was measured as a marker of systemic inflammation. Patients with microalbuminuria and normoalbuminuria had similar baseline characteristics, with the exception that those with microalbuminuria had a longer duration of DM (p = 0.03). Endothelium-dependent vasodilation at 1 minute (p = 0.01) and endothelium-independent vasodilation at 3 minutes (p = 0.007) were significantly less in patients with microalbuminuria. In addition, 96% of patients with microalbuminuria and 76% of those with normoalbuminuria had impaired endothelium-dependent vasodilation (<8%, p = 0.01). Microalbuminuria was an independent predictor of endothelium-dependent vasodilation in the entire cohort (p = 0.045) and after excluding patients on hormone replacement therapy (p = 0.01). Levels of C-reactive protein were significantly higher in patients with microalbuminuria than in those with normoalbuminuria (p = 0.02). We conclude that in DM the presence of microalbuminuria is associated with impaired endothelium-dependent and endothelium-independent vasodilations of the brachial artery and a higher degree of systemic inflammation. In addition, microalbuminuria is an independent predictor of endothelial dysfunction in asymptomatic patients with DM, especially in the absence of hormone replacement therapy. ©2004 by Excerpta Medica, Inc.

(Am J Cardiol 2004;94:294–299)

Endothelial dysfunction occurs early in the atherosclerotic process[1] and has been shown to predict cardiovascular morbidity.[2] Recently, measurement of endothelium-dependent vasodilation (EDV) and endothelium-independent vasodilation (EIV) of the brachial artery has been proposed as a method to noninvasively assess endothelial function and smooth muscle function, respectively.[3] Microalbuminuria has been associated with increased cardiovascular events in the general population and in patients with type 2 diabetes mellitus (DM).[4–6] Within the framework of the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study,[7] we investigated the relation between impaired vascular reactivity and microalbuminuria in patients with type 2 DM.

METHODS

Patient population: The patient cohort consisted of subjects enrolled in the DIAD study who underwent assessment of BAR (DIAD-BAR substudy). The DIAD study is a prospective, multicenter, randomized

From the Cardiology Division, Henry Low Heart Center, Hartford Hospital, Hartford and the University of Connecticut School of Medicine, Farmington, Connecticut; the Sections of Endocrinology and Cardiovascular Medicine, Yale University School of Medicine, New Haven, Connecticut; the Cardiovascular Division, University of Alabama at Birmingham School of Medicine, Birmingham, Alabama; the Medstar Clinical Research Institute, Washington, DC; and the Section of Endocrinology, Norwalk Hospital, Norwalk, Connecticut. This study was supported by grants from the Hartford Hospital Research Administration, Hartford, Connecticut, and Bristol-Myers Squibb Medical Imaging, North Billerica, Massachusetts. Manuscript received December 12, 2003; revised manuscript received and accepted April 1, 2004.

Address for reprints: Gary V. Heller, MD, PhD, Division of Cardiology, Hartford Hospital, 80 Seymour Street, Hartford, Connecticut 06102. E-mail: gheller@harthosp.org.

0002-9149/04/$–see front matter
doi:10.1016/j.amjcard.2004.04.022

trial that evaluates the prevalence of adenosine technetium-99m sestamibi myocardial perfusion imaging abnormalities in asymptomatic patients with type 2 DM and its association with adverse clinical outcomes. Eligible patients had asymptomatic type 2 DM, were 50 to 75 years old, and had no known coronary artery disease. Exclusion criteria were (1) known coronary artery disease, as documented by a history of angina, myocardial infarction, percutaneous coronary intervention, or coronary artery bypass surgery; Q waves; new deep negative T waves or ST-segment depression at rest on electrocardiogram; positive results on noninvasive stress test, or coronary angiography with documentation of coronary artery disease before recruitment; (2) signs or symptoms that normally require cardiac evaluation, such as presence of left bundle branch block, congestive heart failure, chest pain, or ventricular tachycardia; (3) any stress test or cardiac catheterization performed within 3 years before study enrollment; (4) significant nondiabetic co-morbidity affecting life expectancy (e.g., malignancy); (5) pregnancy; (6) known medical noncompliance with follow-up; and (7) history, clinical findings, or treatment for significant bronchospasm. One hundred forty-nine patients from 5 participating centers were enrolled in the DIAD-BAR substudy. Institutional review committees from all centers approved the study, and all patients gave informed consent.

Study design: Baseline history, physical examination, and laboratory tests were obtained at patient centers. Subsequently, the 149 patients who agreed to participate underwent a baseline study of BAR. Subjects were then categorized as those with microalbuminuria (ratio of urinary albumin to creatinine 30 to 299 μg/mg creatinine, n = 28) and those with normoalbuminuria (ratio of urinary albumin to creatinine 0 to 29.9 μg/mg creatinine, n = 115). Six subjects were excluded from the final analysis (4 with microalbuminuria and 2 with unacceptable images of brachial arteries).

Clinical and laboratory measurements: The following clinical characteristics were collected for each patient: age, gender, smoking history, duration of DM, type of DM therapy, history of hypertension and therapy, history of hyperlipidemia and treatment, and body mass index. Systolic and diastolic blood pressures were defined as the mean of 3 measurements in the supine position. Blood and urine samples were obtained during morning hours after an overnight fast. This method is preferred because of the known diurnal variation in albumin excretion.[8] Blood and urine specimens were analyzed by a central laboratory (LabCorp, Raritan, New Jersey), with the exception of homocysteine and high-sensitivity C-reactive protein measurements, which were analyzed elsewhere (LipoScience, Raleigh, North Carolina). The Immulite 2000 Homocysteine assay and high-sensitivity C-reactive protein assay (Diagnostic Products Corporation, Holliston, Massachusetts) were used to determine total levels of plasma homocysteine and C-reactive protein, respectively. Patients with high levels (>10 mg/L) of C-reactive protein had repeated measurements and were excluded if there was evidence of infection or systemic inflammation. Urinary albumin and creatinine concentrations were determined by immunoturbidimetric and kinetic methods, respectively. With these methods, the coefficients of variation for albumin and creatinine were 2.7% and 3.5%, respectively.

Measurements of EDV and EIV: All measurements of BAR were obtained in the morning after an overnight fast, with medications withheld the morning of the study. Patients were also instructed to avoid caffeine-containing products, smoking, and exercise ≥12 hours before the test. Images were obtained with an Acuson 10.0-MHz linear array transducer and an Aspen cardiac ultrasound system (Acuson Corp, Elmwood Park, New Jersey) according to a standard technique for all participating centers. After initial baseline measurements of brachial artery diameter, a blood pressure cuff was placed around the forearm distal to the segment of the artery scanned and inflated 60 mm Hg above the patient's systolic blood pressure for 5 minutes. After deflation, the brachial artery diameter was recorded at 1 minute and 3 minutes after occlusion. After a 15-minute break, a second baseline measurement of brachial artery diameter was recorded, and 0.4 mg of sublingual nitroglycerin was administered. Response of brachial artery diameter was recorded at 3 and 5 minutes after administration of nitroglycerin. Ten cardiac cycles were analyzed for each scan, and measurements were averaged. Brachial artery diameter was measured at a fixed distance from an anatomic marker as the distance between the near and far intima. EDV and EIV were calculated as percent maximal increases in artery diameter 1 minute and 3 minutes after occlusion and 3 and 5 minutes after nitroglycerin administration, respectively. Examination of BAR from Hartford Hospital and Yale University were recorded with CVI acquisition software (version 2.1, Data Translation Inc., Marlboro, Massachusetts). Images from the other 3 participating centers were recorded on tape. Two different interpreters at Hartford Hospital analyzed all scans independently by using CVI Analysis software. The intra- and interobserver variabilities in our laboratory were 1.1% and 2.1%, respectively.

Statistical analysis: Results are presented as means ± SD unless otherwise stated. Chi-square or Fisher's exact test was used to compare categorical data. For continuous variables, differences between patients with microalbuminuria and those with normal albuminuria were compared with a 2-sample independent *t* test or Mann-Whitney U test. To study the correlation of urinary albumin:creatinine ratio with BAR, Spearman's rank correlation test was used. Each variable was evaluated for its association with EDV and EIV using univariate analysis. Variables with a p value <0.1 were entered into a stepwise multivariate regression model to identify independent predictors of EDV and EIV. Results were considered statistically significant when $p < 0.05$. Analysis was performed with SPSS 10.1 (SPSS Inc., Chicago, Illinois).

TABLE 1 Baseline Clinical and Biochemical Characteristics of Patients

Characteristic	Patients With Microalbuminuria (n = 28)	Patients With Normoalbuminuria (n = 115)	All Patients (n = 143)	p Value
Men	17 (60%)	68 (59%)	85 (59%)	0.88
Women	11 (40%)	47 (41%)	58 (41%)	
Age (yrs)	60.7 ± 7.4	59.9 ± 6.6	60.0 ± 6.7	0.59
White	21 (75%)	88 (77%)	109 (76%)	
Black	5 (18%)	25 (22%)	30 (21%)	0.28
Other	2 (7%)	2 (1%)	4 (3%)	
Systemic hypertension	15 (54%)	58 (50%)	73 (51%)	0.77
Hyperlipidemia requiring medication	19 (68%)	64 (56%)	83 (58%)	0.24
Current smoker	3 (11%)	9 (8%)	12 (8%)	0.62
Duration of diabetes (yrs)	11.1 ± 8.5	7.5 ± 7.0	8.2 ± 7.4	0.03
Systolic blood pressure (mm Hg)	131 ± 14	126 ± 16	127 ± 16	0.14
Diastolic blood pressure (mm Hg)	77 ± 8	77 ± 9	77 ± 9	0.91
Body mass index (kg/m^2)	33.7 ± 8.5	30.6 ± 6.4	31.2 ± 6.9	0.07
Total cholesterol (mg/dl)	188 ± 40	190 ± 38	190 ± 39	0.84
Low-density lipoprotein (mg/dl)	109 ± 35	108 ± 32	109 ± 33	0.94
High-density lipoprotein (mg/dl)	49 ± 11	51 ± 14	50 ± 14	0.57
Triglycerides (mg/dl)	165 ± 97	155 ± 103	157 ± 102	0.51
Hemoglobin A1$_c$ (%)	7.2 ± 1.2	6.7 ± 1.1	6.8 ± 1.2	0.06
Serum creatinine (mg/dl)	1.0 ± 0.3	0.9 ± 0.2	0.9 ± 0.2	0.84
Urine creatinine (mg/dl)	121 ± 69	135 ± 85	133 ± 82	0.47
Urine albumin (μg/ml)	82 ± 83	15 ± 11	28 ± 46	<0.001
Urine albumin:creatinine ratio (μg/mg creatinine)	64 ± 39	12 ± 7	22 ± 28	<0.001
C-reactive protein* (mg/L)	6.3 ± 1.4	3.9 ± 0.5	4.3 ± 0.5	0.02
Homocysteine* (μmol/L)	6.9 ± 2.6	6.7 ± 2.3	6.7 ± 2.3	0.84

± Values are means ± SD.
*Data for C-reactive protein (mean ± SEM) and homocysteine were available for 74 patients (microlbuminuria, n = 12; normoalbuminuria, n = 62).

TABLE 2 Baseline Medications of Patients

Medication	Patients With Microalbuminuria (n = 28)	Patients With Normoalbuminuria (n = 115)	All Patients (n = 143)	p Value
Angiotensin-converting enzyme inhibitors	12 (43%)	47 (41%)	59 (41%)	0.85
β Blockers	3 (10%)	6 (5%)	9 (6%)	0.29
Calcium channel blockers	2 (7%)	9 (8%)	11 (8%)	0.90
Angiotensin receptor blockers	1 (4%)	1 (1%)	2 (1%)	0.35
Diuretics	6 (21%)	13 (11%)	19 (13%)	0.16
Sulfonylureas	13 (46%)	44 (38%)	57 (41%)	0.43
Metformin	16 (57%)	60 (52%)	76 (53%)	0.64
Thiazolidinediones	12 (43%)	28 (24%)	40 (28%)	0.05
Insulin	6 (21%)	16 (14%)	22 (15%)	0.32
Aspirin	13 (46%)	51 (44%)	64 (45%)	0.84
Statins	18 (64%)	55 (48%)	73 (51%)	0.11
Hormone (estrogen) replacement therapy*	2 (18%)	22 (52%)	24 (45%)	0.04

*Hormone replacement therapy data refer to the subpopulation of 53 postmenopausal women (microalbuminuria, n = 11; normoalbuminuria, n = 42).

RESULTS

Baseline characteristics: The baseline clinical and biochemical characteristics of the entire study population are listed in Table 1. There were no significant differences between groups regarding use of medications (Table 2) with the exceptions of thiazolidinediones being used more frequently in patients with microalbuminuria ($p = 0.05$), and hormone replacement therapy was less prevalent in patients with microalbuminuria than in those with normoalbuminuria ($p = 0.04$).

Relation between microalbuminuria and brachial artery reactivity: There was a trend for larger baseline brachial artery diameter in patients with microalbuminuria ($p = 0.06$). The results of the BAR test are presented in Table 3. EDV response was significantly less in patients with microalbuminuria than in those with normoalbuminuria at 1 minute (1.9 ± 4.1% vs 4.9 ± 5.7%, $p = 0.01$) and 3 minutes (0.6 ± 5.7% vs 2.8 ± 5.1%, $p = 0.04$) after occlusion. A similar significantly attenuated response was recorded for EIV in patients with microalbuminuria compared with those with normoalbuminuria at 3 minutes (9.5 ± 5.2% vs 13.4 ± 7.2%, $p = 0.007$) and 5 minutes (12.7 ± 5.5% vs 17.2 ± 7.8%, $p = 0.005$) after nitroglycerin administration.

Increasing levels of microalbuminuria had a sig-

TABLE 3 Endothelium-dependent and Endothelium-independent Vasodilations of the Brachial Artery in Patients With Microalbuminuria Versus Those With Normal Albuminuria

Variable	Patients With Microalbuminuria (n = 28)	Patients With Normoalbuminuria (n = 115)	All Patients (n = 143)	p Value
Baseline diameter (mm)	4.1 ± 0.7	3.8 ± 0.7	3.9 ± 0.7	0.06
EDV (%) at 1 minute	1.9 ± 4.1	4.9 ± 5.7	4.3 ± 5.5	0.01
EDV (%) at 3 minutes	0.6 ± 5.7	2.8 ± 5.1	2.4 ± 5.3	0.04
Maximal EDV (%)	2.6 ± 4.5	5.6 ± 5.3	5.0 ± 5.3	0.007
EIV (%) at 3 minutes	9.5 ± 5.2	13.4 ± 7.2	12.7 ± 7.0	0.007
EIV (%) at 5 minutes	12.7 ± 5.5	17.2 ± 7.8	16.3 ± 7.6	0.005
Maximal EIV (%)	13.1 ± 5.2	17.4 ± 7.7	16.6 ± 7.4	0.006

TABLE 4 Multivariate Linear Regression Model of Endothelium-dependent Vasodilation as a Dependent Variable (n = 143, R^2 = 0.258).

Independent Variables	Coefficient β*	Standardized Coefficient β†	p Value
Baseline diameter	−3.62	−0.44	<0.001
Women	4.89	0.43	<0.001
Hormone replacement therapy	3.48	0.24	0.01
Age	−0.16	−0.20	0.009
Hemoglobin $A1_c$	−0.85	−0.18	0.02
Microalbuminuria	−1.66	−0.12	0.045

*Linear regression coefficient.
†The standardized coefficient β describes the unit-independent contribution of the independent variable to the model.
R^2 = square of the multivariate correlation coefficient.

TABLE 5 Multivariate Linear Regression Model of Endothelium-independent Vasodilation as a Dependent Variable (n = 143, R^2 = 0.247)

Independent Variables	Coefficient β	Standardized Coefficient β	p Value
Baseline diameter	−5.51	−0.52	<0.001
Women	4.59	0.32	0.001
Hemoglobin $A1_c$	−1.24	−0.20	0.007
Age	−0.16	−0.15	0.03

See Table 4 for explanations.

nificant inverse correlation with EDV at 1 minute (r = −0.20, p = 0.02) and EIV at 3 minutes (r = −0.27, p = 0.001). With stepwise multivariate regression analysis, significant independent predictors of EDV were baseline diameter, gender, current estrogen replacement therapy, age, hemoglobin $A1_c$, and presence of microalbuminuria (Table 4). Microalbuminuria (standardized β coefficient −0.22, p = 0.01) and age (standardized β coefficient −0.20, p = 0.02) were the only independent predictors of EDV after excluding patients on hormone replacement therapy (n = 119). Data concerning independent predictors of EIV are presented in Table 5. Baseline diameter (standardized β coefficient −0.30, p = 0.001) and microalbuminuria (standardized β coefficient −0.20, p = 0.026) were independent predictors of EIV after excluding patients on hormone replacement therapy.

The exact value of EDV that represents a normal endothelium-dependent response has not been established.[3] However, a cut-off value of ≥8%[9,10] has been proposed to represent a normal EDV response. When using this cut-off value, 27 of 28 patients with microalbuminuria (96%) and 87 of 115 patients with normal albuminuria (76%) had abnormal EDV at 1 minute (p = 0.01).

Microalbuminuria and C-reactive protein: For the subgroup of patients in whom C-reactive protein was measured (n = 74), C-reactive protein levels were increased in those with microalbuminuria (6.3 ± 1.4 mg/L) compared with those with normoalbuminuria (3.9 ± 0.5 mg/L, p = 0.02). After excluding women on hormone replacement therapy, because hormone replacement therapy has been shown to increase C-reactive protein levels,[11] patients with microalbuminuria still had increased C-reactive protein levels (6.4 ± 1.5 mg/L) compared with those with normal albuminuria (3.8 ± 0.6 mg/L, p = 0.027). In current clinical practice, C-reactive protein levels <1, 1 to 3, and ≥3 mg/L differentiate low-, moderate-, and high-risk groups.[12] In patients with microalbuminuria, percentages of subjects with low- (<1 mg/L), moderate- (1 to 3 mg/L), and high-risk (≥3 mg/L) C-reactive protein levels were 0% (0 of 11 patients), 18% (2 of 11 patients), and 82% (9 of 11 patients), respectively, compared with 28% (14 of 50 patients), 32% (16 of 50 patients), and 40% (20 of 50 patients), respectively, of patients with normoalbuminuria. The percentage of high-risk patients was significantly higher in the group with microalbuminuria than in the group with normoalbuminuria (82% vs 40%, p = 0.012), whereas the percentage of low-risk patients was significantly lower (0% vs 28%, p = 0.046).

DISCUSSION

The aim of this study was to investigate the relation between microalbuminuria and BAR in asymptomatic patients with DM. Our results demonstrated that patients with microalbuminuria have significant impairment of EDV and EIV compared with those with normoalbuminuria and a higher degree of systemic inflammation. These data associated the presence of microalbuminuria in patients with DM with impaired vascular reactivity secondary to smooth muscle dysfunction rather than to endothelial dysfunction alone.

Microalbuminuria and brachial artery reactivity: In patients with type 2 DM, the presence of microalbuminuria may necessitate screening for vascular disease and aggressive interventions to decrease cardiovascu-

lar risk.[4–6,8] In contrast, endothelial dysfunction, at least in the coronary circulation, predicts cardiovascular events[2] and may be an early common pathway for microalbuminuria and cardiovascular disease in DM.[13] Several studies have suggested that endothelial dysfunction precedes the onset of microalbuminuria in patients with type 1 DM.[14–16] In type 2 DM, inflammatory markers of endothelial dysfunction, microalbuminuria, and risk of death are parallel and progress with time.[17] Consistent with these results, our study correlates endothelial dysfunction with increasing levels of microalbuminuria. The attenuated EIV response in subjects with microalbuminuria also suggests vascular smooth muscle dysfunction due to increased inactivation of nitric oxide or decreased reactivity of the vascular smooth muscle to nitric oxide.[18]

The exact values of normal brachial EDV and EIV have not been established mainly due to differences in age, gender, and methods.[19] In patients with peripheral vascular disease, an EDV $<8.1\%$ was associated with ninefold increases in rates of morbidity and mortality compared with those with EDV of $\geq 8.1\%$.[9] In healthy nonsmokers, mean EDV at 1 minute has been reported to be 7.7%,[10] and in our laboratory, an EDV $\geq 8\%$ at 1 minute after lower arm occlusion was considered a normal response in healthy subjects of similar age and body size. With this cut-off point, 76% of patients with normal albuminuria had EDV of $<8\%$, thus underscoring the concept that endothelial dysfunction may precede the development of microalbuminuria in DM.

Coronary risk factors and their treatment may affect endothelium-mediated response in the brachial artery.[20] The 2 groups had no significant baseline differences, aside from a trend for higher body mass index and hemoglobin $A1_c$ in patients with microalbuminuria. Active treatments that may favor endothelial function were also similar in the 2 groups, with the exception of thiazolidinediones and hormone replacement therapy. Hormone replacement therapy has been reported to improve brachial artery endothelial function in postmenopausal women with or without coronary artery disease.[20,21] Nevertheless, in multivariate regression analysis, microalbuminuria was an independent predictor of EDV, including or excluding subjects on hormone replacement therapy.

Microalbuminuria and C-reactive protein: One important observation from our study was that subjects with microalbuminuria had significantly higher serum C-reactive protein levels compared with subjects with normal albuminuria. Microalbuminuria is associated with a greater degree of systemic inflammation as measured by various plasma indexes.[17] Increased levels of C-reactive protein are also associated with impaired endothelial vasoreactivity.[22] The results from our study with respect to C-reactive protein are consistent with the notion that the presence of microalbuminuria correlates with higher states of systemic inflammation.

Potential clinical applications: Assessment of BAR has the potential to be a preclinical marker of cardiovascular disease.[3] Ongoing studies in several large populations, including the Framingham Heart Study and the Cardiovascular Health Study, may determine whether testing of BAR identifies patients at risk for developing coronary artery disease and whether it is a practical clinical tool. Despite a lack of large epidemiologic studies, impaired endothelial function of brachial arteries is present in patients with coronary risk factors without evidence of coronary disease and improves with appropriate risk modification.[20,23] Our study suggests that impaired vascular reactivity may identify subjects with normoalbuminuria earlier in the atherosclerotic process as candidates for more aggressive medical therapy. However, such a recommendation cannot be made at the moment because testing of BAR is a research rather than a clinically available tool and requires the care and precision of a well-organized laboratory.[19]

Study limitations: Our study is limited by its cross-sectional design and the absence of a control group. In addition, the interpretation of the results of EDV should be viewed with caution because EIV was also impaired in subjects with microalbuminuria compared with those with normoalbuminuria. However, the impaired EDV in 76% of patients with normal albuminuria was associated with a "preserved" EIV response ($17.4 \pm 7.7\%$).[24]

1. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical coronary vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986;315:1046–1051.

2. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 2000;101:948–954.

3. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, et al. Guidelines for the ultrasound assessment of endothelium-dependent flow-mediated vasodilation of the brachial artery. A report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002;39:257–265.

4. Borch-Johnsen K, Feldt-Rasmussen B, Strandgaard S, Schroll M, Jensen JS. Urinary albumin excretion. An independent predictor of ischemic heart disease. *Arterioscler Thromb Vasc Biol* 1999;19:1992–1997.

5. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, Gans RO, Janssen WM, Grobbee DE, de Jong PE. Prevention of Renal and Vascular End Stage Disease (PREVEND) Study Group. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 2002;106:1777–1782.

6. Mattock MB, Morrish NJ, Viberti G, Keen H, Fitzgerald AP, Jackson G. Prospective study of microalbuminuria as predictor of mortality in NIDDM. *Diabetes* 1992;41:736–741.

7. Wackers FJTh, Young LH, Inzucchi SE, Chyun DA, Davey JA, for the DIAD Investigators. Detection of ischemia in asymptomatic diabetics: preliminary results of the DIAD study. *J Am Coll Cardiol* 2003;41(suppl A):409A.

8. American Diabetes Association. Diabetic nephropathy: position statement. *Diabetes Care* 2003;26(suppl 1):S94–S98.

9. Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, Vita JA. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol* 2003;41:1769–1775.

10. Gaenzer H, Neumayr G, Marschang P, Sturm W, Kirchmair R, Patsch JR. Flow-mediated vasodilation of the femoral and brachial artery induced by exercise in healthy nonsmoking and smoking men. *J Am Coll Cardiol* 2001;38:1313–1319.

11. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation* 2003;107:363–369.

12. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14,719 initially healthy American women. *Circulation* 2003;107:391–397.

13. Yudkin JS. Coronary heart disease in diabetes mellitus: three new risk factors and a unifying hypothesis. *J Intern Med* 1995;238:21–30.

14. Stehouwer CD, Fischer HR, van Kuijk AW, Polak BC, Donker AJ. Endothelial dysfunction precedes development of microalbuminuria in IDDM. *Diabetes* 1995;44:561–564.

15. Lekakis J, Papamichael C, Anastasiou H, Alevizaki M, Desses N, Souvatzoglou A, Stamatelopoulos S, Koutras DA. Endothelial dysfunction of conduit arteries in insulin-dependent diabetes mellitus without microalbuminuria. *Cardiovasc Res* 1997;34:164–168.
16. Dogra G, Rich L, Stanton K, Watts GF. Endothelium-dependent and independent vasodilation studied at normoglycemia in type 1 diabetes mellitus with and without microalbuminuria. *Diabetologia* 2001;44:593–601.
17. Stehouwer CD, Gall MA, Twisk JW, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. *Diabetes* 2002;51:1157–1165.
18. Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA. Impaired nitric oxide-mediated vasodilation in patients with non–insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1996;27:567–574.
19. Kuvin JT, Patel AR, Karas RH. Need for standardization of noninvasive assessment of vascular endothelial function. *Am Heart J* 2001;141:327–328.
20. Anderson TJ. Assessment and treatment of endothelial dysfunction in humans. *J Am Coll Cardiol* 1999;34:631–638.
21. Haines CJ, Yim SF, Sanderson JE. The effect of continuous combined hormone replacement on arterial reactivity in postmenopausal women with established angina pectoris. *Atherosclerosis* 2001;59:467–470.
22. Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. *Circulation* 2000;102:1000–1006.
23. Vogel RA. Coronary risk factors, endothelial function and atherosclerosis: a review. *Clin Cardiol* 1997;20:426–432.
24. Bhagat K, Hingorani A, Vallance P. Flow associated or flow mediated dilatation? More than just semantics. *Heart* 1997;78:7–8.